Proceedings of the First International Conference for Meconium Aspiration Syndrome and Meconium-induced Lung Injury

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REVIEW

Introduction: Proceedings of the First International Conference for Meconium Aspiration Syndrome and Meconium-induced Lung Injury

GI Martin and D Vidyasagar

The history of the word ‘meconium’ holds interest for all of us involved in perinatal care. It is believed that Aristotle coined this term, which has been anglicized to mean ‘opium like.’ As opium is derived from poppy seeds and when processed the resulting poppy seed sludge is black and tarry the word was an appropriate description.

Meconium-stained amniotic fluid occurs in approximately 13% of live births. Meconium aspiration syndrome (MAS) occurs in 5 to 10% of infants born through meconium-stained amniotic fluid. When MAS occurs, there is an increase in neonatal mortality and morbidity. Great progress has been made in the improvement of survival of infants with MAS. Great progress in management has been made since first description of the pathophysiology and poor outcome of infants with MAS in 1975.1 These include improved intrapartum and post-delivery management of MAS. Although there is a significant decrease in the occurrence of MAS and associated mortality in developed countries MAS remains a major problem in developing countries.

The physiological effects of MAS owing to airway obstruction and associated increased pulmonary vascular resistance are well studied. However, the mechanism of meconium-induced inflammation and subsequent meconium-induced lung injury (MILI) are just being investigated. In recent years, there is an accumulation of evidence from investigators from different institutions that MAS also causes inflammatory reaction and cellular injury. These are new findings; the impact of these findings on management is yet to be determined.

The First International Meeting for MILI was held on 23 and 24 March 2007 in Chicago. The organizing committee included: Vidyasagar, Bhat, Bhutani, Wiswell, Gadzinowski, Saugsted and Zagariya. The conference was partially funded by a grant (to DV) from NHLBI (Grant no. HL090484-01) to promote the international collaboration of researchers in the field. The symposium provided a forum for discussion of new findings and also identified knowledge gaps in management and pathophysiology of MILI. Clinical and laboratory investigators around the world were invited to discuss their experiences and research in this field. The Editorial Board of the Journal of Perinatology kindly agreed to publish the articles from the speakers at the symposium in this special supplement.

A summary of conclusions from the presentations at the conference is presented below.

The conference was divided into four sessions. The first session was devoted to discussions regarding the history of MAS and the global burden of MAS. Developed countries have noticed a steady decrease in mortality and morbidity from MAS, but developing countries such as India and Africa share a major burden of birth asphyxia and MAS. To reduce unacceptably high infant mortality rate in developing countries, it was concluded that more research is needed both in clinical management and in understanding of MAS and MILI.

The second session was devoted to understand the mechanisms of in utero passage of meconium in the fetus and potential pathways that can be blocked to reduce MAS. Mechanisms of vascular remodeling in the fetus that may contribute to severe persistent pulmonary hypertension of newborn was described in infants with severe MAS. Speakers identified several potential areas of research in the future.

The third session was devoted to review the current practices of perinatal, intrapartum and post-neonatal management of MAS. It was concluded that the existing evidence from the literature indicates that amnioinfusion in the presence of meconium-stained amniotic fluid or oropharyngeal suction of fetus being delivered and routine post-delivery endotracheal suctioning have no beneficial effects in preventing or reducing MAS. There were questions in developing countries, where resources are limited for monitoring the fetus to the above therapies. Therefore, there is a need for studies in developing countries to evaluate the effectiveness of these practices.

The investigators from India and South Africa stated that developing countries with limited resources of fetal monitoring and delivery room care should develop strategies appropriate for their
circumstance. They expressed that there is a need for collaborative studies to improve the efficacy of clinical management of MAS babies.

In the fourth session, research data regarding MILI and apoptosis was presented. It was clear that meconium is a strong inducer of inflammation and cellular apoptosis. Several pathways were identified for the induction of apoptosis. This session laid a firm foundation for future studies and potential collaboration among interested investigators. The last session was devoted to reviewing the newer treatment modalities (iNO therapy, ECMO) and long-term outcomes. It was stressed that iNO therapy successfully decreases PPHV.

ECMO was ultimate therapy for non-responding patients. It was noted that in developed countries the need for ECMO in MAS has decreased. Other discussions included the use of room air resuscitation of MAS (particularly in developing countries), use of antibiotics, steroids and other supportive pharmacologic treatments. These modalities are of significance in developing countries and need to be studied.

The long-term outcome of infants with MAS was reviewed. Existing reports consist of diverse population and mixed therapies. It was noted that there was an increased incidence of neurodevelopmental delay in babies with MSAF and MAS. These observations indicate the need for larger follow-up studies of infants with MSAF and MAS.

In summary, the conference, which was the first of its kind on MAS, brought together established investigators in the field around the globe. The conference allowed free exchange of ideas among the researchers. In this supplement of the *Journal of Perinatology* we are publishing the articles submitted by the speakers. The articles are arranged into two groups: the first 15 articles deal with history, epidemiology and clinical aspects of MAS. The next seven articles deal with the cellular mechanisms of MILI. We hope the readers of the *Journal of Perinatology* will find the information timely and useful.

**Acknowledgments**

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**Reference**

The meconium aspiration syndrome (MAS) is a common problem that continues to concern perinatologists and neonatologists. MAS is defined as respiratory distress in an infant born through meconium-stained amniotic fluid (MSAF) whose symptoms cannot be otherwise explained. This disorder may be life threatening, complicated by respiratory failure, pulmonary air leaks and persistent pulmonary hypertension. Approaches to the prevention of MAS have changed over time with collaboration between obstetricians and pediatricians forming the foundations for care. This report details the management of babies delivered with associated MSAF before the accumulation of evidence for best practice through appropriately powered, prospective randomized controlled trials.


Background

Meconium, the fecal material that accumulates in the fetal colon throughout gestation, is a term derived from the Greek mekoni, meaning poppy juice or opium. Commencing with Aristotle’s observation of the association between meconium staining of the amniotic fluid and a sleepy state or neonatal depression, obstetricians have been concerned about fetal well being in the presence of meconium-stained amniotic fluid (MSAF). Fetal hypoxic stress may stimulate colonic activity, resulting in the passage of meconium, and also may stimulate fetal gasping movements that result in meconium aspiration.

Meconium is a sterile, thick, black-green, odorless material first observed in the fetal intestine during the third month of gestation. Meconium results from the accumulation of debris, including desquamated cells from the intestine and skin, gastrointestinal mucin, lanugo hair, fatty material from the vernix caseosa, amniotic fluid and intestinal secretions. It comprises 72 to 80% water and contains cholesterol and its precursors, lipids, enzymes including pancreatic phospholipase A2, mucopolysaccharides, protein, bile acids and salts as well as drug metabolites. The black-green color results from bile pigments. When aspirated into the lung, either in the fetus or newly born infant, meconium may stimulate the release of cytokines and other vasoactive substances that lead to cardiovascular and inflammatory responses.

In the fetus, passage of meconium occurs physiologically early in gestation, when it contributes to alkaline phosphatase in amniotic fluid. Abramovich et al. noted that fetal defecation diminishes after 16 weeks and ceases by 20 weeks, concurrent with innervation of the anal sphincter. At that time, the rectum appears to be filled with meconium. From approximately 20 to 34 weeks, fetal passage of meconium was infrequent. Most newborn infants who pass meconium are mature (term). Many are postmature and the babies may exhibit peeling skin, long fingernails and decreased vernix. The vernix, umbilical cord and nails may be meconium stained, depending upon how long the infant has been exposed in utero. In general, nails will become stained after 6 h and vernix after 12 to 14 h of exposure.

The passage of meconium normally occurs within the first 24 to 48 h after birth. However, the passage of fetal meconium, resulting in MSAF, occurs in approximately 8 to 25% of all deliveries, primarily in situations of advanced fetal maturity or fetal stress. Most infants who are delivered with MSAF are beyond the 37 weeks of gestation and meconium rarely appears in amniotic fluid before 32 weeks of gestation. The premature infant lacks ‘motilin,’ while assists in moving meconium through the lower gastrointestinal tract. The meconium aspiration syndrome (MAS), associated with aspiration or perhaps diffusion of meconium into the fetal airways, occurs in about 5% of these infants.3,4

Meconium aspiration syndrome is defined as respiratory distress in an infant born through MSAF whose symptoms cannot be otherwise explained. Aspirated meconium can interfere with normal breathing by several mechanisms. They include airway obstruction, chemical irritation, infection and surfactant inactivation (see Figure 1). When aspirated into fetal lungs, meconium particles mechanically obstruct the small airways. Meconium or the chemical pneumonitis it causes inhibits surfactant function, and inflammation of lung tissue contributes further to small airway obstruction. The chest
contribute to the pulmonary symptoms. Aspiration (for example, chronic intrauterine hypoxemia) may mask the pulmonary hypertensive response, with hypertension. However, evidence of a long-term process of muscularization of distal pulmonary arterioles in infants with the MAS who died suggests that factors other than meconium aspiration (for example, chronic intrauterine hypoxemia) may contribute to the pulmonary symptoms.

Figure 1 Pathophysiology of meconium aspiration syndrome. From Fanaroff and Martin’s *Neonatal Perinatal Medicine, Diseases of the Fetus and Infant* 8th edition, edited by R Martin, A Fanaroff and M Walsh, Mosby Elsevier, 2006.

typically appears barrel-shaped, with an increased anterior—posterior diameter caused by overinflation. Auscultation reveals rales and rhonchi. These signs usually are seen immediately after birth.

The respiratory manifestations include respiratory distress, tachypnea, cyanosis, and air trapping together with reduced pulmonary compliance. Cleary and Wiswell have proposed severity criteria to define MAS: (1) mild MAS is disease that requires less than 40% oxygen for less than 48 h, (2) moderate MAS is disease that requires more than 40% oxygen for more than 48 h with no air leak and (3) severe MAS is disease that requires assisted ventilation for more than 48 h and is often associated with persistent pulmonary hypertension. Of infants in whom the MAS develops, more than 4% die, accounting for 2% of all perinatal deaths.

An estimated 25,000 to 30,000 cases and 1000 deaths related to MAS occur annually in the United States with many more cases in developing countries. However Yoder et al. documented a decline in the incidence of MAS in the United States (from 5.8 to 1.5%——almost a fourfold reduction) during the period 1990 to 1997, which they attributed to a 33% reduction in the prevalence of MSAF, the nature of the MSAF (thick or watery, thick but still liquid, particulate) and the incidence of respiratory distress when there was meconium present. In a prospective collection of data they reported MSAF in 8.8% of 1000 consecutive newborn infants over a 6 month time period. They aspired the upper and lower airways as soon as possible ‘sometimes before the first breath’ in 80 of the 88 infants with MSAF. If the trachea contained meconium, intubation and suctioning were repeated until the airway had been cleared. Fifty-seven infants had meconium in their mouth, 62 had meconium in the stomach and 8 had meconium in the trachea but none in the mouth or larynx. Half of the infants with meconium in the airway (23 of 46) had abnormal chest X-rays and 16 (70%) of these were symptomatic. They concluded that ‘all infants born through thick, particulate or ‘pea soup’ meconium should have their trachea aspirated immediately after birth by the most experienced person in the delivery room and receive thoracic physical therapy and postural drainage for the first 8 h of life, if the meconium is recovered from the trachea and/or the chest X-ray is consistent with aspiration.’ Ting and Brady a year later following a retrospective chart review noted that of 97 infants with thick meconium who received endotracheal suctioning at birth, 27 became symptomatic and only one died. On the other hand, 28 infants with thick meconium who were not suctioned, 16 became symptomatic and 7 died. Soon thereafter Carson et al. published their combined obstetric and pediatric approach to prevent MAS. In a study originally intended to examine the effectiveness of pulmonary lavage for MAS, they performed routine intrapartum pharyngeal suctioning with a DeLee catheter with the infants head on the perineum, before the onset of respirations, thereafter inspecting the larynx when the baby delivered and intubating and suctioning if meconium was present. Their treatment group was compared to historical controls, MAS occurred in 1 of 273 (0.4%) with routine suctioning and 18 of 947 (1.9%) (P = 0.07) in the non-suctioned group. They noted that routine suctioning of the trachea under direct vision after delivery is rarely necessary but should be performed if meconium is visualized at the vocal cords, and tracheobronchial lavage with saline may add to the respiratory morbidity. They indicated that ‘No deaths or severe cases of MAS have occurred since institution of the obstetric suctioning procedure’ and concluded that ‘routine intrapartum pharyngeal suctioning with a DeLee catheter of infants with meconium staining has significantly reduced the incidence and
severity of MAS despite a P-value not supporting such a conclusion.

On the basis of these studies by Gregory, Ting and Carson, the commonest approach to MSAS, whether thick, watery or particulate, was for the obstetrician to immediately suction the nasopharynx as soon as the head appeared on the perineum. Thereafter, visualization of the cords by direct laryngoscopy and suctioning of the trachea through the endotracheal tube if meconium was detected was the routine adopted at most centers. This was performed before stimulation of the infant or administration of positive pressure ventilation. This approach remained the standard of care until challenged by a series of prospective randomized trials.

Corticosteroids for MAS

Yeh et al. evaluated the efficacy of glucocorticoids in the treatment of infants with MAS. Using a ‘double blind’ technique they randomized 35 infants with MAS to hydrocortisone or a lactose placebo. No significant differences in arterial PO2, PCO2, pH, A-aDO2 gradients, requirement for assisted ventilation, or in survival were observed between the groups. In the placebo group, a significant decrease in respiratory distress score occurred at 48 h of age (P<0.01); in hydrocortisone-treated infants, it was seen only after 72 h. The infants in the steroid group took a significantly longer period of time to wean to room air than those in the control group (68.9 ± 9.6 vs 36.6 ± 6.9 h) (P<0.01). They concluded that hydrocortisone is not recommended for the treatment of MAS.

MAS and pulmonary hypertension

Pulmonary hypertension was a significant comorbidity in infants with MAS and frequently the cause of death. The management of pulmonary hypertension was haphazard and the outcomes unpredictable. Fox et al. reported on 10 patients clinically diagnosed as having perinatal aspiration syndromes together with pulmonary hypertension. These infants were either term or postmature babies and at catheterization had the following characteristics: (1) systemic or supra-systemic levels of pulmonary artery pressure (PAP) (range, 50 to 117 mm Hg); (2) a degree of pulmonary hypertension not related to the degree of aspiration evident on chest roentgenograms; (3) evidence of right-to-left shunting at the ductal or foramen ovale level and (4) sustained severe hypoxemia despite 100% inspired oxygen concentration. Indwelling pulmonary artery catheters were used for continuous monitoring of PAP in these 10 infants with severe persistent pulmonary hypertension of the newborn. The labile nature of PAP, with changes up to 50 mm Hg, was documented. PAP in the eight infants with supra-systemic pulmonary hypertension was analyzed at the time of maximum decrease in pressure (mean 36.1 mm Hg) and physiologic measurements were compared over an 8-h period.

During the study period when the infants were hyperventilated, as the PaCO2 decreased from 48.9 to 28.3 mm Hg (P<0.02), the mean PAP decreased by 36 mm Hg (P<0.001) to sub-systemic pressure levels, and the mean alveolar/arterial oxygen difference decreased by 146 mm Hg (P<0.001). Following the decrease in PAP, patients were mechanically ventilated to maintain PaCO2 in the range of 25 to 30 mm Hg until pulmonary hypertension gradually resolved in the six survivors. On the basis of this small uncontrolled study, hyperventilation to lower pCO2 levels became the standard of care, and in light of present knowledge probably contributed to a number of deaf survivors.

Extracorporeal membrane oxygenation and MAS

The history of the introduction of extracorporeal membrane oxygenation (ECMO) into neonatal perinatal medicine is closely aligned with the management of MAS. Bartlett et al. documented 16 moribund newborn infants with respiratory failure who were treated with ECMO for 1 to 8 days. Their diagnoses and outcomes included respiratory distress syndrome, four patients (two improved, one survived); MAS, eight patients (four improved, three survived); persistent fetal circulation (some with diaphragmatic hernia), four patients (three improved, two survived). Intracranial bleeding occurred in 43% accounting for most of the deaths. In a parallel series of 21 infants treated with conventional ventilator therapy, the mortality rate was 90% and intracranial bleeding occurred in 57%. They modestly concluded that ECMO provided life support and gains time in newborn respiratory failure. In high mortality risk infants, the rate of survival is higher and that of intracranial bleeding is lower with ECMO than with optimal ventilator management. So dawned the ECMO era, and ultimately the survival of many moribund infants with pulmonary hypertension and respiratory failure, including those with MAS. Indeed, in the ECMO registry, the highest survival rates (>90%) were seen in the patients with MAS who qualified for ECMO. With the evidence-based approach to prevention of MAS and the initiation of better less-invasive therapies, including inhaled nitric oxide, surfactant therapy, surfactant lavage and various modes of mechanical ventilation, including high-frequency ventilation, it is rare for an infant to require ECMO for MAS.13–16

Evidence-based practice to prevent MAS

On the basis of the evidence from non-randomized studies, to reduce the incidence and severity of MAS the recommendation was to intubate and suction all babies born through thick meconium. However, for term babies who are vigorous at birth endotracheal intubation may be both difficult and unnecessary. This concept of routine intubation was challenged first by Faciglia using a concurrent observational study. He showed no differences in outcome with and without routine intubation. Linder et al. in a
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prospective controlled trial, perhaps inadequately randomized, assessed the need for intubation of vigorous term meconium-stained infants. Of 572 infants, meconium-stained infants, delivered vaginally, with 1-min Apgar score of 9 or 10, none of 264 expectantly managed infants developed MAS and 2 of 308 intubated infants developed MAS. They concluded that intubation may be superfluous in a vigorous infant born through MSAF and practice started to change.

A decade later in international prospective, randomized, controlled trial assessing need for intubation of apparently vigorous meconium-stained infants, Wiswell et al.23 enrolled 2094 babies born with MSAF to either intubation and suctioning or to expectant management. They found no significant differences in either MAS or any respiratory disorders supporting the concept that it was not necessary to intubate vigorous term infants irrespective of the nature of the MSAF (thick or thin). Halliday21 accumulated four randomized controlled trials of endotracheal intubation at birth in vigorous term meconium-stained babies, which were identified. Meta-analysis of these trials does not support routine use of endotracheal intubation at birth in vigorous meconium-stained babies to reduce mortality, other respiratory symptoms or disorders, pneumothorax, oxygen need, stridor, HIE and convulsions. However, the event rates of many of these outcomes is low in the reported trials making reliable estimates of treatment effect impossible.

Vain et al.22 finally shattered all the anecdotal data when they reported on their prospective randomized trial that routine intrapartum oropharyngeal and nasopharyngeal suctioning of term-gestation infants born through MSAF does not prevent MAS.

Amnioinfusion

Amnioinfusion has been advocated as a technique to reduce the incidence of meconium aspiration and to improve neonatal outcome. However, meconium passage may predate labor so that a large proportion of women with MSAF have infants with meconium in the trachea or bronchioles before meconium passage has been noted clinically and before amnioinfusion can be performed.23 Fraser et al.24 reported that for women in labor who have thick meconium staining of the amniotic fluid, amnioinfusion did not reduce the risk of moderate or severe MAS, perinatal death, or other major maternal or neonatal disorders.25 Xu et al.26 summarized the evidence for the use of amnioinfusion to prevent MAS. They concluded that in clinical settings with standard peripartum surveillance, the evidence does not support the use of amnioinfusion for MSAF. In settings with limited peripartum surveillance, where complications of MSAF are common, amnioinfusion appears to reduce the risk of MAS. As always they noted that further studies were needed.

Long-term consequences

The initial follow-up report on follow-up of MAS by Marshall et al.27 in 1978 included a 1-year cohort of 17 patients, representing 3.7% of all admissions to their unit. Four patients (23.2%) died of acute respiratory failure and two patients with MAS, and persistence of the fetal circulation required cardiac catheterization to exclude cyanotic congenital heart disease. None of the survivors had persistent chronic lung disease but two of three patients with MAS and seizures had significant psychomotor retardation at follow-up examination. The current data indicate that there is an increased prevalence of asthmatic symptoms and abnormal bronchial reactivity among survivors of the MAS.

Conclusion

Meconium aspiration syndrome remains a challenging condition most notably in developing countries. In the USA avoidance of post-term pregnancies, improved intrapartum monitoring and amnioinfusion have dramatically reduced the incidence of MAS. Whereas there is inconclusive evidence as to the optimal ventilator strategy for MAS, surfactant replacement therapy has been beneficial and surfactant lavage is still being explored. Inhaled nitric oxide has significantly enhanced the treatment of pulmonary hypertension and substantially reduced the need for ECMO. Despite optimal perinatal management, infants born through MSAF may still develop MAS and have morbid or even fatal outcomes.

Disclosure

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References


Mechanism(s) of in utero meconium passage

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To use sheep and rat models and demonstrate that stressors activate fetal glucocorticoid (GC) system, corticotrophin-releasing factor (CRF) system and cholinergic neurotransmitter system (ChNS) leading to propulsive colonic motility and in utero meconium passage. Immunohistochemical studies (IHS) were performed to localize GC-Receptors, CRF-receptors and key molecules of ChNS in sheep fetal distal colon. CRF expression in placenta and enteric endocrine cells in fetal rat system were examined and the effects of acute hypoxia on in utero meconium passage was tested. IHS confirmed localization and gestation dependent changes in GC-Rs, CRF-Rs and cholinergic markers in sheep fetal colon. Rat placenta and enteric endocrine cells express CRF and gastrointestinal tract express CRF-Rs. Hypoxia is a potent inducer of meconium passage in term fetal rats. Stress is a risk factor for in utero meconium passage and laboratory animal models can be used to develop pharmacotherapy to prevent stress-induced in utero meconium passage.

Introduction

Newborn meconium passage, a developmentally programmed event, normally occurs within the first 24–48 h after birth, although little is known of the regulatory process. Clinically, a delay in newborn meconium passage has been observed in infants born with Hirschsprung disease, a defect involving the absence of intrinsic ganglia in colon. In contrast, we have no similar anatomical or neurochemical explanations for the meconium staining observed in 7–22% of all term deliveries. Greater than 90% of cases of clinically observed meconium-stained amniotic fluid are noted in fetuses at or following 37 weeks gestation, being uncommon in preterm deliveries. Passage of meconium occurs most often in deliveries beyond 40 weeks of gestation. An increased incidence of meconium passage in amniotic fluid is noted in the presence of fetomaternal stress factors such as hypoxia and infection, independent of fetal maturation. On the basis of the clinical observations, fetal stress and gastrointestinal maturation have been attributed as risk factors responsible for meconium passage as well as the potential meconium aspiration in utero. Despite these risk factors, presently very little is known of the cascade of events that leads to meconium passage in the newborn immediately after birth or to the mechanisms contributing ‘premature’ meconium passage in utero. There are no small or large animal models that could be extrapolated to humans. We review here recent studies gathered in our laboratory in rat and sheep models directed toward understanding the cellular and molecular basis of stress-induced in utero meconium passage.

Stress and gut motility: lessons learned from laboratory animals

Although impact of emotional stress on gastric and colonic motor activity has long been known in humans only during the past two decades, much attention has been paid to understand the neural circuitry and hormonal effectors that mediate effects of stress on gut functions in animal models (see review 7,8). The rat is a widely used animal model for stress-related alteration on gut motor functions, although limited investigation has also been performed in mouse models. Inhibition of gastric emptying, gastric secretion, gastric contraction and stimulation of colonic motility and defecation have now been well established in adult rats as the hallmarks of neurovisceral motor responses to stressors of psychological, physical, chemical and immunological origin. Members of stress hormone family, more specifically corticotrophin-releasing factor (CRF) and urocortin, have been shown to mimic multifaceted acute responses to stress. Both hormones mediate their actions through CRF-receptor subtype 1 (CRF-R1) and CRF-receptor subtype 2 (CRF-R2). Both receptors are encoded by distinct genes and both are G protein-coupled receptors linked to multiple intracellular signaling pathways. Of the two CRF receptor subtypes, CRF-R1 stimulates colonic motility and defecation, whereas CRF-R2 inhibits gastric emptying and gastric contraction at times of stress. Anatomical, physiological, pharmacological and biochemical studies have provided unambiguous evidence that the CRF system...
Hypothosis: in utero meconium passage is a fetal neurovisceral motor response to stress

We hypothesize that in utero meconium passage is a neurovisceral motor response to fetal stress and that the CRF system plays a critical role in the stimulation of cholinergically mediated fetal colonic propulsive motor functions that trigger meconium passage. An integrated model proposed for fetal in utero meconium passage is shown in Figure 1. The salient points of our hypothesis are as follows: fetal–maternal stress leads to increased fetal plasma glucocorticoids, which stimulate the synthesis of placental CRF. Stress-induced increase in glucocorticoids are also speculated to upregulate the CRF-R1 density on the myenteric neurons as well as the expression of peripheral choline acetyltransferase, the enzyme that catalyzes the synthesis of acetylcholine, the principle neurotransmitter that regulates the motor activity of gastrointestinal system. In conditions of stress, increased maternal and/or fetal sympathetic tone will lead to increased circulating levels of catecholamines, which are known stimulators of placental CRF release. Thus, stress-induced activation of the CRF–CRF-R1 system at the level of myenteric neurons will evoke release of acetylcholine, increased colonic smooth muscle motility and in utero meconium passage.

Stress is a potent stimulator of in utero meconium passage in term fetal rats

Although clinical signs of stress due to hypoxia are frequently observed in human neonates born with meconium staining, there is no direct evidence to support the fact that hypoxic stress is a risk factor for in utero meconium passage. We recently developed a novel hypoxic stress paradigm to demonstrate that hypoxic stress at term will provoke in utero meconium passage. The experimental design includes exposure of term pregnant rats (term = 22 days) at 22 days of gestation to decreasing concentration of oxygen in a stepwise manner as illustrated in Figure 2a. Fetuses removed from uterine horn at the end of hypoxic exposure were alive and exhibited thick meconium staining as shown in Figure 2b, whereas control fetuses did not exhibit meconium.

Examination of fetal and maternal plasma revealed a marked elevation in CRF levels, suggesting that the peripheral CRF pathway may mediate the stimulation of fetal colonic motility and meconium passage. As acute hypoxia-induced meconium passage has a rapid onset, the mechanisms underlying the peripheral pathway most likely involve the activation (directly and neurally) of fetal adrenal epinephrine and norepinephrine secretion as well as sympathetic nervous system norepinephrine release, which in turn stimulate placental CRF exocytosis. Recent studies in our laboratory indicate that rat placenta, similar to human placenta, is a site of CRF synthesis. In addition, enteric endocrine cells expressing CRF in term rat fetal gut could also function as a potential local source, as speculated in humans. Increased peripheral CRF in the fetus stimulates colonic motility through myenteric CRF-R1 and rapid meconium passage (Figure 3a).

The central CRF pathway involves activation of CRF neurons in paraventricular brain nuclei directly innervating colonic segments, as has been demonstrated in adult rats (Figure 3b). Thus, acute hypoxic stress-induced in utero meconium passage involves release of stored CRF protein pool from peripheral and/or central tissues. Ongoing studies in our laboratory also support the expression of CRF-R1 in smooth muscle and enteric neuronal circuitry in term rat fetal gastrointestinal tract, lending strong anatomical support for our hypothesis that intrauterine stress may impact gut motility through CRF-R1 in a manner analogous to stress-induced stimulation of colonic motility and defecation in adult rats.

Lessons learned from sheep model in support of stress-induced in utero meconium passage

Sheep are used in our laboratory as a large animal model for understanding the molecular mechanism of in utero meconium passage. The larger size of the gut and longer gestational age permit in vivo studies exploring gestation- and hormone-dependent changes in gastrointestinal motility, and in vitro organ bath studies examining smooth muscle contractility. The large fetal size permits withdrawal of blood in sufficient quantities for evaluating circulating hormone and cytokine levels. In the following section, we summarize some of our investigations relevant to hormonal regulation of sheep fetal distal colonic motility as well as recent progress made on the ontogeny of glucocorticoid receptor system, CRF system and cholinergic...
synaptic circuitry system in fetal distal colonic segments. In addition, we discuss our recent advances in support of knowledge of CRF signaling system in the prevention of in utero meconium passage before term.

Sheep fetal colon is a glucocorticoid target organ

As the incidence of meconium-stained amniotic fluid increases with increase in gestational ages and fetal stress, we hypothesized that fetal colon could be a glucocorticoid target organ with fetal colonic motility function regulated by glucocorticoids. We examined in vivo effects of betamethasone (a synthetic glucocorticoid) in the presence and absence of thyroxine on in vitro colonic smooth muscle cholinergic contractility. Muscle strips prepared from sheep fetuses that received intramuscular injection of betamethasone alone or in combination with thyroxine exhibited greater contractile responsiveness to bethanechol (a cholinergic agonist) than muscle strips prepared from vehicle-treated fetuses. These glucocorticoid-specific changes were noticed 48 h after intramuscular injections of betamethasone, suggesting that glucocorticoids regulate cholinergic contractile maturation through ‘genomic’ actions. The findings obtained following glucocorticoid administration has provided partial support for our hypothesis that stress-mediated glucocorticoid release may stimulate colonic motility and meconium passage.

We also examined the in vivo effect of bethanechol on colonic muscular contractile and electromyogram activity in the sheep fetus. We noticed increased strain gauze activity in response to bethanechol infusion in transverse but not in distal colon at 0.9 gestation (135 days gestation, term = 150 days). This finding suggested the presence of a local inhibitor system for cholinergic-dependent contractility in the distal colon before term, perhaps serving as an intrinsic mechanism to prevent premature meconium passage.

Localization and gestation-dependent changes in glucocorticoid receptor expression in sheep fetal distal colon

To validate our hypothesis that sheep fetal colon is glucocorticoid target organ, we undertook studies to evaluate the cellular
localization and gestation-dependent changes in glucocorticoid receptors in sheep fetuses at very preterm (118–120 days), preterm (130–132 days), near term (140–142 days) and term (146–147 days) gestation.30 Glucocorticoid receptor antibody elicited positive staining in smooth muscle layers as well as in myenteric and submucosal neurons at all stages of development with maximal expression noticed at near term, suggesting the profound changes in nuclear transcription machineries begin to occur at near term. We also noticed a robust change in fetal plasma cortisol levels at term, suggesting that the expression of glucocorticoid receptors could be turned ‘off’ by glucocorticoids at term, or immediately after birth, as circulating levels of glucocorticoids reach basal levels within 24 h after birth in many species including humans.

Evidence for prereceptor metabolism of glucocorticoids in fetal sheep distal colon
Glucocorticoid actions at the cellular level are controlled by 11-beta hydroxysteroid dehydrogenase (11bHSD) type 1 and type 2, enzymes that regulate the interconversion of active glucocorticoid and their inactive 11-keto metabolites. We recently examined the presence of 11bHSD-1 and 11bHSD-2 in sheep fetal colonic enteric nervous system as evidence that these enzymes provide a novel means for regulation of glucocorticoid-mediated ‘genomic’ activities.31 The antibodies to 11bHSD-1 and 11bHSD-2 elicited positive immunostaining of both myenteric and submucosal ganglia at all gestation ages. The percentage of myenteric ganglia expressing 11bHSD-1 and 11bHSD-2 were maximal at term. The percentage of submucosal ganglia expressing 11bHSD-1 was maximal at term, whereas the expression of 11bHSD-2 remained constant throughout gestation. We speculate that increasing levels of 11bHSD-1, and thus increased active glucocorticoid levels, at term could contribute to glucocorticoid-dependent colonic contractile maturation.

CRF system
Identification and gestation-dependent changes in CRF system in sheep fetal distal colon
We recently undertook a systematic investigation to obtain anatomical support that CRF system is an integral component of distal colonic contractility apparatus.32 Our findings can be summarized as follows: CRF-R1- and CRF-R2-specific antibodies strongly immunostained longitudinal and circular smooth muscles in addition to muscularis mucosa in colonic segments at all gestational ages. A marked decrease in CRF-R2 immunostaining occurred in smooth muscle layers close to term. CRF-R1 antibody immunostained neuronal somas both in myenteric and submucosal ganglia, and the percentage of ganglia expressing CRF-R1 were maximal at term gestation. On the basis of the reciprocal changes in CRF-R1 and CRF-R2 expression in the smooth muscle-enteric unit, we speculate that CRF-R1 could be hyper-responsive to stressors that stimulate the release of endogenous CRF leading to increased motor activity and meconium passage.

Cellular localization of CRF-binding protein in sheep distal colon
Corticotrophin-releasing factor-binding protein is a protein known to compete with CRF-R1 and CRF-R2 for CRF and urocortin.33 Our immunohistochemical investigation revealed expression of CRF-binding protein in the smooth muscle-enteric unit.34 We observed a marked decrease in smooth muscle expression of CRF-binding protein and a simultaneous increase in myenteric neuronal somas at term, indicating that this protein is a critical regulator of CRF and urocortin functions at the level of smooth muscle-enteric unit in sheep fetal distal colon.

CRF signaling system may play a role in preventing in utero meconium passage before term
We performed in vitro study of sheep fetal colonic contractility to assess the significance of CRF receptor system.29 In these studies, colonic smooth muscle strips prepared from preterm sheep fetal distal colonic segments were subjected to cholinergically dependent contractility in an organ bath system following pre-incubation with CRF or urocortin. Both neuropeptides significantly decreased the cholinergically stimulated contractility. Immunohistochemical studies confirmed the expression of muscarinic receptor subtype 3 and CRF-R2 in preterm sheep fetal colon. Additional studies revealed positive immunostaining for CRF and urocortin in smooth muscle and enteric neurons, indicating the local availability of neuropeptides to inhibit smooth muscle motility during the time of stress.30 All in all, stress hormones (CRF and urocortin) and stress hormone receptors (CRF-R1 and CRF-R2) appear to function in parallel or series pathways, and CRF-R2 plays a central role in preventing stress-induced in utero meconium passage before term.

Cholinergic circuitry system
Maturational changes in sheep fetal colonic cholinergic circuitry system parallels plasma glucocorticoid surge
We utilized antibodies specific to vesicular acetylcholine transporter, a high-affinity choline transporter, and peripheral choline acetyl transferase as markers for cholinergic synaptic circuitry system to examine the maturation of cholinergic system in distal colon of sheep fetuses from very preterm to term gestation.35 The percentage of myenteric ganglia expressing cholinergic markers in term fetuses were significantly higher than those observed in preterm. The marked increase observed in cholinergic markers in myenteric ganglia in fetal distal colon at term is possibly mediated by effects of endogenous glucocorticoid levels, which peak at term. We speculate that glucocorticoid is the physiological hormone that regulates enteric cholinergic neural synaptic circuitry system similar to their actions in central nervous system regions.
Localization of inhibitory and stimulatory muscarinic receptor subtypes in sheep fetal distal colon: implication for meconium passage

Acetylcholine mediates gastrointestinal motility through muscarinic receptors. To date, five muscarinic receptors (M1–M5) have been cloned, with M3 confirmed as the prominent mediator of smooth muscle contraction. Of the other subtypes, M1, M2 and M4 have been shown to function as potent autoinhibitor (that is, inhibitory presynaptic muscarinic receptor) of Ach release from myenteric plexus. We examined the maturation and topographical distribution of muscarinic receptor subtypes in distal colon from very preterm to term.\(^{36}\) M1–M5 antibodies elicited positive staining in colonic sections at all gestational ages. M4 is expressed most abundantly in circular and longitudinal smooth muscle layers, whereas M3 is expressed in rather low levels. Positive immunostaining for both M5 and M1 in smooth muscle layers are similar, but their immunostaining intensity was significantly lower compared with M3, M4 and M2. The percentage of neurons expressing inhibitory autoreceptors (M1, M2 and M4) in the enteric ganglia was markedly higher in distal colonic sections of preterm and near-term fetuses, with expression declining rapidly and markedly at term. Our results indicate that before term, muscarinic autoreceptors may inhibit presynaptic release of Ach and consequently limit the cholinergically mediated smooth muscle contractility. This could represent a physiological mechanism analogous to CRF-R2 functions in preventing stress-induced gut motor function.\(^{29}\)

In summary, we have established for the first time a rat model for stress-induced in utero meconium passage. We have further obtained anatomical support for the expression of key molecules of glucocorticoid system, CRF system and cholinergic circuitry system in sheep fetal distal colon. All three systems seem positioned in distal colon readily to respond to stress. Our studies unexpectedly revealed the presence of potential mechanisms to prevent the in utero meconium passage before term, including the expression of CRF-R2. Future studies should improve our understanding of the mechanisms of in utero meconium passage and possibly to develop pharmacotherapy to prevent stress-induced in utero meconium passage and consequences of meconium aspiration.

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Disclosure

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Meconium aspiration syndrome (MAS) is associated with increased risk for perinatal mortality and morbidities. To provide an overview of the advances in our knowledge concerning the obstetric approaches to the prevention of MAS. The evidence of the effectiveness of intrapartum surveillance, amnioinfusion, and delivery room management in the prevention of MAS are reviewed in the present paper. Meconium aspiration syndrome remains one of the most common but challenging conditions for obstetricians and pediatricians. The available evidence did not demonstrate a beneficial effect of either of obstetric strategies in the prevention of MAS.

**Introduction**

Meconium staining of the amniotic fluid (MSAF) occurs in <5% of preterm, 7 to 22% of term deliveries, increasing to between 23 and 52% of births at >42 weeks. The mechanisms influencing meconium passage are complex, involving hormonal and neuroregulatory functions, chronic hypoxia or reflecting maturation of the fetal gastrointestinal system. MSAF is associated with an increased risk of neonatal morbidity and mortality. Meconium aspiration syndrome (MAS) is the most serious neonatal pathology associated with MSAF. It has been reported to occur in 1.7 to 35.8% of cases complicated with MSAF, and 1 to 3% of liveborn infants. The case fatality rate of MAS has been reported to be high, ranging from 5 to 40%. Approximately one-third of babies with MAS require intubation and mechanical ventilation, and other new therapies such as high frequency ventilation, inhaled nitric oxide and surfactant administration, although the effectiveness of certain of these technologies remain controversial. Serious complications resulting from MAS include pneumothorax, convulsion and death.

**Definition of meconium aspiration syndrome**

Rossi et al. has defined MAS as respiratory distress in the first 4 h after birth with oxygen requirement and chest roentgenogram showing characteristic features of MAS. Recently, Cleary and Wiswell have defined MAS as respiratory distress in an infant born through MSAF, which cannot be otherwise explained. Mild MAS has been defined as requiring <40% oxygen for <48 h, moderate MAS as requiring >40% concentration of oxygen therapy for at least 48 h, and severe MAS if requiring assisted mechanical ventilation, that is, often associated with persistent pulmonary hypertension. They further pointed out that the severity of MAS does not necessarily correspond to the degree of chest radiographic abnormality. MAS is associated with a range of radiographic features including coarse, patchy infiltrates, consolidation, atelectasis, pleural effusions, air leaks, hyperinflation, a wet-lung picture and hypovascularity. In some cases, the chest film may be interpreted as normal.

**Pathophysiology and risk factors**

The pathophysiology of MAS is complex and remains controversial. Many factors, such as airway obstruction, alveolar or parenchymal inflammation, impaired surfactant production and function and direct toxicity of meconium constituents could be involved in the pathophysiology of the MAS. It has been suggested that the extent of lung destruction is not closely correlated to the quantity of meconium in lung tissue but rather to the degree of hypoxia and acidosis present at delivery. Ghidini and Spong postulated that the pathologic events leading to mild, moderate or severe cases of MAS may be different. Severe MAS may not be in fact causally related to the aspiration of meconium but rather may be caused by other pathologic processes occurring in utero, such as chronic asphyxia, infection or persistent pulmonary hypertension. The hypothesis is in fact supported by the lack of evidence that the severity of MAS directly correlates with the amount of meconium aspirated, the consistency of meconium and the duration of exposure to meconium. It is still unclear whether obstruction of airways because of aspiration of meconium has a pivotal role in the progress of MAS. MAS can occur before delivery,
even in the absence of labor, being reported in infants delivered by elective cesarean section.24

Although the presence of meconium during labor is known to be associated with an increased risk of perinatal morbidity and mortality, most babies have favorable outcomes. Early recognition of infants at the highest risk for the development of MAS could be essential for optimizing the clinical preventive strategies. A vast array of risk factors for the occurrence of MAS have been identified either using unselected obstetrical populations or infants born through MSAF. Those factors are heavy MSAF, nulliparity, postterm delivery, fetal heart rate (FHR) abnormalities during labor, presence of meconium below the vocal cords, cesarean delivery and the low Apgar scores.13,14,20,25–30 It has been reported that there is an apparent relationship between maternal ethnicity and risk of MSAF, and the risk of MAS has been observed to be increased in black Americans, Africans and Pacific Islanders.30–32

**Intrapartum fetal monitoring**

The goal of continuous electronic fetal heart rate monitoring (EFM) is to detect fetal hypoxemia and therefore reduce the risk of adverse neonatal outcomes. However, the effectiveness of this approach to care has been questioned. Randomized trials of EFM, with or without fetal blood gas and acid–base assessment, which were conducted in the unselected obstetrical population, have found no evidence that this approach to care reduces the risk of fetal or neonatal mortality or morbidity.33–39

Intrapartum monitoring has been recommended to screen for early signs of fetal hypoxia, a risk factor for MAS. Several authors have noted an increase in the frequency of FHR abnormalities in association with MSAF.15,20,40,41 It has been reported that, in the presence of MSAF, fetal tachycardia, variable and late decelerations and decreased long-term variability are risk factors for MAS. Certain authors investigated the relationships among abnormal cardiotocograms in labor, MSAF and adverse neonatal outcomes such as low arterial cord blood pH, and low Apgar scores. The authors did not find that the presence of abnormal FHR patterns increased the overall correlation between MSAF and adverse outcome.7,42 In contrast, Umstad et al.43 investigated the predictive value of abnormal FHR patterns in early labor and found that the presence of meconium in the amniotic fluid improved the predictive properties of the test.

**Amnioinfusion**

Amnioinfusion (AI), or transcervical infusion of saline into the amniotic cavity, was used first to relieve persistent variable FHR decelerations during labor or to prevent the occurrence of decelerations in presence of oligohydramnios.44 Results of randomized controlled trials, including a meta-analysis indicate that, in the presence of oligohydramnios, prophylactic intrapartum AI significantly reduces the risk of FHR decelerations and cesarean section.45–52

AI has been also proposed as a method to reduce MAS. Potential mechanisms through which AI could act include mechanical cushioning of the umbilical cord, which could correct or prevent recurrent umbilical compressions that lead to fetal acidemia, a condition predisposing to MAS; and dilution of meconium that could reduce its mechanical and inflammatory effects in the pathogenesis of MAS.

To date, more than 15 randomized or quasi-randomized trials of AI for MSAF have been reported, with conflicting results.53,54 The methodological quality varied across studies. The largest trial (over 1998 participants) was an international trial performed in 56 centers where EFM and neonatal intubation and suctioning for babies with respiratory difficulty were routinely available.55 Analysis was by intention to treat. The primary outcome was a composite indicator that included the occurrence of perinatal death and/or moderate or severe MAS. The results indicated that AI showed no effect on the primary outcome (relative risk; RR 1.13, 95% confidence interval; CI 0.82 to 1.95). Furthermore, the frequencies of oropharyngeal suctioning, laryngoscopy or intubation in the delivery room were similar between groups, as were the proportion of babies with meconium visualized below the vocal cords. There were no differences between groups in the occurrence of the combined outcome of perinatal mortality and/or serious morbidity (RR 1.13, 95% CI 0.88 to 1.47). In addition, an analysis that stratified for the presence or absence of variable FHR decelerations before randomization found no effect on the primary outcome either, although the study was underpowered to detect such effects within strata.

We recently conducted a systematic review, integrating the results of the largest trial.56 Studies were included if they were randomized controlled trials that evaluated the effect of prophylactic AI during labor with MSAF; treatment was randomly allocated (AI versus controls). All included studies were further subjected to a score-based quality assessment for randomized studies that was adapted from the Jadad score. The main analysis was based on the studies that were considered to be of high quality. The meta-analysis included a total of 4030 women, 1999 allocated to AI and 2031 allocated to control. Of these, 3178 women were recruited in clinical settings with standard peripartum surveillance and 852 women were randomized in centers with limited peripartum surveillance, defined as the nonavailability of EFM during labor. The methodological quality varied across studies. Heterogeneity was noted across studies with respect to the AI protocols and the end points evaluated. In the setting of standard peripartum surveillance, the results failed to demonstrate a reduction in the risk of MAS (RR 0.59, 95% CI 0.28 to 1.25), Apgar-5 <7 (RR 0.90, 95% CI 0.58 to 1.41) or caesarean delivery (RR 0.89, 95% CI 0.73 to 1.10). However, in clinical settings with limited peripartum surveillance, AI appeared to reduce the risk of
MAS (RR 0.25, 95% CI 0.13 to 0.47). Several findings from this meta-analysis are worthy of comment. Firstly, the observed heterogeneity in the stratum of studies conducted in centers with standard surveillance is largely attributable to our recent large trial. The source of this heterogeneity remains largely unexplained. The most significant finding of the meta-analysis is the apparent discordance in the observed effect of AI on MAS between centers with standard and limited peripartum surveillance. Continuous EFM is a key component in the prevention of asphyxia in patients with MSAF. Therefore, the application of this technology may reduce the contribution of severe asphyxia to the risk of occurrence of MAS. It would appear that in settings where this technology is routinely used, AI confers no additional benefit over EFM in terms of prevention of MAS. However, in settings where continuous EFM is not routinely available, AI may be beneficial for the reduction of MAS. Further studies in such settings are warranted to confirm this hypothesis.

AI may not be without risk. The use of AI has been reported to be associated with adverse events. Complications including uterine overdistension and hypertonia, uterine rupture in association with previous uterine scar, FHR abnormality, umbilical cord prolapse and chorioamnionitis have been reported. Four cases of maternal deaths have been reported associated with the AI. Several authors have reported the occurrence of excessive uterine contractions or unusually rapid labor progress related to AI. In the AI group of Fraser et al.'s trial, 10 women (1.1%) experienced bleeding, and in 63 (6.9%) women, hypertonicity, hydramnios or uterine overdistension was diagnosed during the procedure whereas the incidence of other maternal complications were comparable between AI and control groups.

American College of Obstetricians and Gynecologists has recently published a Committee Opinion that concludes that routine prophylactic AI for the dilution of MSAF should be carried out only in the setting of additional clinical trials. However, they state that AI remains a reasonable approach to the treatment of repetitive variable decelerations, regardless of amniotic fluid meconium status.

**Delivery room management**

Routine oropharyngeal suctioning before delivery of the infants' shoulders has long been involved in preventing MAS. The findings of observational studies remain conflicting. Falciglia et al. compared infants with meconium-stained fluid who underwent 'early' oronasalopharyngeal DeLee suctioning with a similar group of infants whose airways were suctioned 'late' (after chest delivery). They found no evidence of benefit of oropharyngeal suctioning in the prevention of MAS. Rossi et al. also reported the similar rates of meconium visualized in the vocal cords despite early oropharyngeal suctioning. In contrast, several authors reported that intrapartum pharyngeal suctioning reduced the severity of MAS and the risk of respiratory distress. They suggested that combined approach of intrapartum oropharyngeal suctioning and endotracheal suctioning was effective in the reduction of MAS.

Vain et al. conducted a multicenter international trial to assess the effectiveness of oropharyngeal and nasopharyngeal suctioning before delivery of the shoulders for the prevention of MAS. They found that the incidence of MAS, need for mechanical ventilation, and neonatal mortality was similar between groups (suction versus no suction). In addition, they found no evidence of a benefit of intrapartum suctioning on the occurrence of MAS, MAS requiring mechanical ventilation, or mortality.

Regarding the postdelivery management such as routine endotracheal suctioning and intubation, reports from observational studies suggested that intratracheal suctioning could prevent the occurrence of MAS for meconium-stained neonates and significantly decreased the mortality subsequent to that disorder. Intubation is not without risk and has been associated with hypoxia, bradycardia and laryngeal stridor. Should endotracheal suctioning and intubation be applied universally in infants born through MSAF or selectively reserved for those who are depressed after birth is another topic of controversy. Some investigators suggested that a selective approach may be useful and justified, whereas other suggested that universal intubation and suctioning was the best strategy to prevent potential morbidity and mortality related to meconium staining.

Linder et al. suggested that nondepressed meconium-stained infants did not benefit from immediate intatracheal suctioning and such intervention could be harmful. Liu and Harrington conducted a randomized trial to assess if intubation of the low-risk newborn with thin meconium affects the incidence of respiratory symptoms. They were unable to demonstrate the beneficial effect of intubation and intratracheal suctioning in the infant with thin meconium and an otherwise low-risk pregnancy. To address this question, Wiswell et al. conducted a multicenter randomized trial, involving a total of 2094 neonates, to investigate whether intubation and suctioning of apparently vigorous, meconium-stained neonates would reduce the risk of MAS. There were no significant differences between intubation and expectant management groups in the rates of MAS or other respiratory disorders. Moreover, intratracheal suctioning showed no benefit over expectant management even for infants born through the thickest consistency MSAF. They further identified the independent risk factors for the development of MAS using stepwise logistic regression. The results indicated that the use of oropharyngeal suctioning lead to a decreased risk of MAS (8.5% in infants who did not have intrapartum suction versus 2.7% in infants with intrapartum suctioning). The authors concluded that endotracheal intubation and suctioning still be performed in infants born through MSAF, if they are not vigorous, if they need positive pressure ventilation or they develop symptoms of respiratory
distress. Furthermore, a recently published meta-analysis of four randomized trials demonstrated no significant benefit of routine endotracheal intubation and suctioning at birth over routine resuscitation including oropharyngeal suction of vigorous, meconium-stained infants born at term.75 The authors recommended that intubation and suctioning be restricted to depressed newborns, that is, with a heart rate of <100 beats per min, poor respiratory effort and poor tone.

Conclusion

MAS remains a challenging condition for obstetricians and neonatologists. Despite the decreased risk of MAS and related mortality and morbidity, the available evidence did not demonstrate a beneficial effect of either of obstetric strategies in the prevention of MAS. The suggested apparent disparity in the effect of AI on MAS between centers with standard and limited peripartum surveillance is worthy of attentions for clinicians. Additional well-designed randomized controlled trials in settings of limited peripartum surveillance are required to elucidate the optimal management of MAS in this context.

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References


REVIEW

Delivery room management of the meconium-stained newborn

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Review of all medical literature dealing with delivery room management of meconium-stained infants. Additionally, the author contacted multiple individuals involved historically or clinically with the published studies or the persons who developed treatment guidelines. Although many therapies have been suggested as being effective, none have been definitively proven efficacious by the gold standard: a large, randomized, controlled trial (RCT). Further adequate investigations (RCTs) need to be performed to assess whether proposed management schemes are of benefit in the care of meconium-stained newborn infants.


Introduction

Meconium-stained amniotic fluid (MSAF) is noted in 10 to 15% of all deliveries (≈ 500,000 annually in the United States). Approximately 3 to 4% of meconium-stained infants will develop the meconium aspiration syndrome (MAS). Neonates that are depressed and are born through thick-consistency MSAF are at the highest risk for developing MAS. A considerable proportion of infants with MAS require mechanical ventilation, whereas many develop pulmonary air leaks. In addition, MAS is frequently associated with persistent pulmonary hypertension of the newborn. The mortality rate among infants with the disorder may be as high as 5 to 10%. Humans are not the only species affected by MAS; it is seen frequently and is a leading cause of death in domestic farm animals (pigs, cattle, and so on).

In an effort to prevent or mitigate the course of MAS, many methods have been suggested to remove aspirated meconium from the airways. Historically, farmers would grasp meconium-stained animals by their hindquarters and then swing them around their heads. Centrifugal forces would move the meconium-stained fluid toward the head of the rotating animal and the material would then be manually extracted. In 1871, Dr Bernard Schultze described a resuscitation maneuver, one of the benefits of which purportedly included the removal of meconium from the airways. Caregivers would grasp affected infants by the shoulders vertically at the level of the adult’s knees. The caregiver would then sweep the baby upward inverting them by 180° and then swinging them back down to their original position. This maneuver would be repeated as many as 10 to 12 times. This untested therapy was widely practiced through the 1920s.

Intratracheal suctioning

This author has not found any written descriptions concerning intubation and suctioning of meconium-stained infants before the year 1960. In a textbook of neonatal resuscitation published that year, Dr L Stanley James (Figure 1) stated that if meconium had been aspirated into the trachea, it should be suctioned out. He then suggested using an endotracheal tube as a suction device. Dr Jack Sinclair (personal communication) states that when he arrived as a resident in Pediatrics at Columbia Hospital for Children in New York City in 1961, both Dr James and Dr Virginia Apgar were teaching and advocating intubation and suctioning of meconium-stained babies. The latter individuals even made a teaching movie of this procedure. Dr Lillian Blackmon (personal communication), while training at Columbia Hospital for Children, did an elective with Dr William Tooley at the University of California at San Francisco (UCSF) in 1966. At that time, physicians at UCSF were not suctioning the airways of meconium-stained neonates. Dr Blackmon subsequently returned to UCSF in 1968. At virtually the same time, Dr William Silverman (Figure 2), the long-time director of Neonatology at Columbia Hospital for Children, also moved west and became chief of neonatology of San Francisco Children’s Hospital. Dr Blackmon states that the two of them began training residents and clinicians in San Francisco to intubate and suction meconium-stained neonates.

Dr George Gregory (Figure 3) and colleagues are given credit for the wide dissemination of the intubation and suctioning technique in their seminal 1974 publication. These individuals described their prospective management of meconium-stained infants at UCSF over a 6-month period. Eighty such infants were intubated and had their tracheas suctioned. In addition, the infants subsequently were treated with aerosolized distilled water, chest physiotherapy (CPT) and postural drainage (positional therapy alternating between head up and head down, as well as side to side). Sixteen (20%) of the infants were deemed to be ‘sick’, six (38%) of whom had thin-consistency meconium suctioned from...
their airways. Of the 80 infants, 2 required mechanical ventilation, but none were treated with continuous positive airway pressure. Seven neonates developed pneumothorax and/or pneumomediastinum. The authors stated that none of the 35 meconium-stained babies managed in this manner subsequent to the study period and admitted to their Newborn Intensive Care Unit required ventilation. The authors also presented data from 15 infants with MAS transferred to their unit subsequent to the study period. Of the 15 infants, 5 (33%) required either mechanical ventilation or continuous positive airway pressure, whereas 6 (40%) developed a pneumothorax. On the basis of their experience, the authors recommended intratracheal suctioning of all infants born through thick, particulate meconium (despite almost 40% of their ‘sick’ population having only thin-consistency meconium retrieved). They also recommended subsequent CPT and postural drainage for those infants from whom meconium-stained material is retrieved from the trachea, as well as for those with abnormal chest roentgenograms.

Although Gregory and colleagues are frequently cited as the original investigators who showed benefits of intubation and suctioning, Burke-Strickland and Edwards6 (Figure 4) published the results of their prospective trial of the technique a full year earlier. The latter authors intubated and suctioned 84 meconium-stained infants over a 2-year period and compared their outcomes with 17 such infants who did not receive tracheal cleansing. Overall 70% of intubated infants also had saline lavage. The non-intubated infants had more prolonged respiratory distress and longer hospital stays compared with their intubated counterparts. Burke-Strickland and Edwards recommended that all infants born through MSAF of any consistency should be intubated and suctioned.

Interestingly, 1 year after the Gregory publication, the results of another study also based out of San Francisco were published. Ting (Figure 5) and Brady7 did a retrospective review of outcomes of 125 meconium-stained infants born over a 3-year period; of these 28 babies were not intubated. Of these, 16 (57%) developed MAS and 7 (25%) died. The remaining 97 infants were intubated and suctioned; of these 27 (28%) developed MAS and 1 (1%) died. These investigators concluded that all infants born through MSAF of any consistency should be intubated and suctioned.

On the basis of their anecdotal experience, Carson et al.8 (Figure 6) suggested a different approach. They proposed that
Figure 3  Dr George A Gregory (photograph courtesy of Dr George Gregory).

Figure 4  Dr Nancy Edwards Dow (photograph courtesy of Dr Dow).

Figure 5  Dr Pauline Ting (photograph courtesy of Dr Ting).

Figure 6  Dr Bonita Carson (photograph courtesy of Dr Carson).
clinicians should visualize the hypopharynx and vocal cords of meconium-stained infants after oropharyngeal suctioning. These authors recommended that tracheal suctioning should be performed only if meconium could be visualized at the vocal cords.

Subsequent to these publications, there was widespread acceptance of the practice of intubation and suctioning of the trachea of all meconium-stained infants. This approach was recommended in numerous textbooks and journals. The most common technique (‘straw method’) consisted of providing negative pressure at the hub of the endotracheal tube using one’s mouth (Figure 7). Essentially, this practice was how residents in pediatrics gained and improved their intubation skills. Tasting meconium became a rite of passage for pediatric trainees.

Fortunately, most clinicians ultimately realized that a face mask...
could be interposed between one’s mouth and the endotracheal tube and obviated this culinary delight. In the decade following the aforementioned publications, the incidence of MAS and deaths attributable to the disorder appeared to decline significantly.

It was recognized that there was a wide variation in the amount of negative pressure produced by different individuals when directly suctioning the endotracheal tube. Moreover, the infectious risks of this practice were obvious. Some chose to directly place a standard suction catheter or a de Lee suction catheter into the larynx. Many devices (at least 20) were commercially developed as alternatives to the straw method. Bent (Figure 8) et al. assessed the various methods and found one to be consistently the best.

In 1988, Linder (Figure 9) and colleagues reported that a selective approach might be more appropriate than a universal one. They performed a trial in which apparently vigorous meconium-stained infants were quasi-randomized to either intratracheal suctioning or expectant management. Their results were questioned because of major design flaws in the trial. Although some suggested that a selective approach could be deleterious, others found it to result in good outcomes. In the late 1990s, Wiswell et al. (Figure 10) spearheaded a large international prospective, randomized controlled trial to assess a selective approach. Almost 2100 meconium-stained neonates were enrolled. During the 10 to 15 s after delivery, these babies had to be apparently vigorous (defined as having a heart rate >100 beats per min, having spontaneous respirations and having reasonable tone). They were randomized either to be intubated and suctioned or to expectant management. The results of this approach are given in the Table 1. There appeared to be no benefit to tracheal cleansing of this population. Subsequent to this publication, the Neonatal Resuscitation Program (NRP) changed its recommendations and advised that apparently vigorous meconium-stained infants do not need intubation and intratracheal suctioning.

A future question that needs to be answered is whether or not depressed meconium-stained infants actually benefit from having their airways intubated and suctioned. As many clinicians believe that most cases of MAS are due to in utero aspiration of MSAF, the potential efficacy of tracheal cleansing needs to be assessed in a well-conducted randomized, controlled trial. Another issue that needs to be addressed is the training of pediatric residents in intubation skills. Historically, most residents honed their skills during training by intubating virtually all meconium-stained infants. However, by eliminating the need to intubate the majority of the latter population, many believe that residents are no longer gaining or maintaining these skills.

**Intrapartum suctioning of the nasopharynx and oropharynx**

Intrapartum suctioning consists of using either a suction catheter or a bulb syringe to remove material from a meconium-stained infant’s oropharynx and nasopharynx after the delivery of baby’s head, but prior to delivery of the thorax. In the mid-1970s, Carson et al. performed the previously mentioned study, the original goal of which was to assess the value of tracheobronchial saline lavage (Dr Carson, personal communication). They compared meconium-stained infants managed differently during three separate time periods. Group 1 infants (n = 947) had no intrapartum suctioning, no saline lavage and inconsistent intratracheal suctioning. Group 2 infants (n = 381) had...
intrapartum suctioning, intubation and tracheal suctioning, and saline lavage. Group 3 infants \((n = 273)\) had intrapartum suctioning and selective tracheal intubation/suctioning (if meconium was present at the vocal cords). The authors intended to randomize those babies in group 3 who required intubation to saline lavage or no lavage. However, only 2 of 273 group 3 infants had meconium noted at the cords and were eligible to be lavaged. Hence, the authors were unable to assess whether saline lavage was of benefit. The incidence of MAS in these populations was 1.9% (group 1), 1.8% (group 2) and 0.4% (group 3). These differences were not statistically significant! Nonetheless, there was a widespread assumption that the lower incidence of MAS in group 3 babies was due to the intrapartum suctioning. There was worldwide acceptance of this hypothesis and it became the standard of care for almost three decades.

Subsequent studies were unable to replicate Carson group’s remarkably low incidence of MAS after intrapartum suctioning. Most notable were the reports of Falciglia (Figure 11) and colleagues. Initially, he performed a historical comparison evaluation.\(^{13}\) He assessed the outcomes of meconium-stained infants born in two different years (1975 and 1983, respectively). During the earlier year, no intrapartum suctioning was customarily performed, in contrast with the latter year when it was performed routinely. There was no difference in the incidence of MAS in 1975 (2.0% of 742) compared with 1983 (2.1% of 755). Subsequently, Falciglia et al.\(^{14}\) performed a concurrent observational study during which an independent observer (unknown to the obstetrician) documented whether or not obstetricians performed ‘early’ oronasopharyngeal suctioning while the baby’s head was at the perineum (prior to delivery of the child’s thorax) compared with those that received ‘later’ suctioning subsequent to delivery. MAS was actually more common in the group that underwent ‘early’ suctioning (23/221, 10.4%) compared with those undergoing ‘later’ suctioning (15/227, 6.9%).

To definitively assess whether or not this procedure was of benefit to neonates, Vain (Figure 12) and colleagues\(^{15}\) performed an international prospective, randomized controlled trial. The population consisted of 2514 infants born through MSAF of any consistency, gestational age \(\geq 37\) weeks and vertex (cephalic) presentation. Subjects were randomized either to (1) suctioning of the oropharynx and nasopharynx prior to delivery of the thorax or to (2) no suctioning prior to delivery. No differences were found in the incidence of MAS between suctioned (4.1%) and non-suctioned (3.8%) infants. Moreover, there were no differences in other key outcome variables including mortality, the need for mechanical ventilation, duration of mechanical ventilation and duration of supplemental oxygen use. Subsequent to this publication, both the NRP and the American College of Obstetricians and Gynecologists (ACOG) changed their stances and no longer recommend intrapartum oropharyngeal and nasopharyngeal suctioning prior to delivery when MSAF is present.

### Table 1 Incidence of MAS among 2094 apparently vigorous meconium-stained infants randomized to either intubation and suctioning or expectant management

<table>
<thead>
<tr>
<th>Consistency of MSAF</th>
<th>Intubation and suction group ((n = 1051)) (%)</th>
<th>Expectant management group ((n = 1043)) (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin</td>
<td>5/447 (1.1)</td>
<td>2/453 (0.4)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Moderately thick</td>
<td>7/301 (2.3)</td>
<td>6/307 (2.0)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Thick</td>
<td>22/303 (7.3)</td>
<td>20/283 (7.1)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Overall</td>
<td>34/1051 (3.2)</td>
<td>28/1043 (2.7)</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

*Abbreviations: MAS, meconium aspiration syndrome; MSAF, meconium-stained amniotic fluid.*

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**Figure 11** Dr Horatio Falciglia (photograph available from the following website: http://www.cincinnatichildrens.org/research/div/neonatology/fs/fac/horacio-falciglia.htm?pres=Hrs).
Other proposed delivery room therapies

CPT
Chest physiotherapy is performed in an effort to prevent accumulation of debris and to improve mobilization of airway secretions. CPT consists of techniques such as percussion, vibration, postural drainage, saline administration and suctioning. Theoretically, CPT should help remove meconium from the airways, prevent its consequences and improve gas exchange. It is widely performed on infants born through MSAF, as well as those with MAS. Nevertheless, there are virtually no data to support this therapy in meconium-stained neonates, either in the delivery room or thereafter. One must remember that there are potential complications of CPT (pneumothorax, hypoxemia, arrhythmia, airway perforation and tissue damage).

Cricoid pressure, epiglottal blockage and thorax compression
Several other maneuvers have been suggested as being of benefit in preventing MAS. Cricoid pressure involves the application of pressure to the neonate’s airway in an effort to compress it and prevent intratracheal meconium from migrating distally. Epiglottal blockage entails the placement of one or more fingers into the infant’s hypopharynx in an attempt to apply pressure on the epiglottis and block the meconium passage downward. The latter two therapies have the potential to traumatize the airway. Both are likely to produce a vagal response in a potentially compromised infant. Thorax compression consists of manually encircling a meconium-stained infant’s chest and applying pressure in an effort to prevent the child from inhaling deeply and aspirating fluid. None of the aforementioned maneuvers have ever been assessed in clinical trials. All are potentially dangerous and none are recommended.

Gastric suctioning
Karlowicz suggested suctioning of the stomachs of all meconium-stained neonates soon after delivery. He hypothesized that some cases of MAS could potentially be caused by the postnatal reflux of gastric contents into the oropharynx. The material could then potentially be aspirated into the airways. Suctioning of the stomach within minutes of delivery would obviate this from occurring, although an intriguing hypothesis, gastric suctioning, has never been evaluated for efficacy in preventing MAS.

Summary
Multiple delivery room therapies have been proposed as being effective in preventing or ameliorating MAS. However, to date, none of the therapies assessed by randomized, controlled trials have been definitively proven to be effective. An important question remains: do depressed, meconium-stained neonates benefit by being intubated and suctioned?

Disclosure
TE Wiswell has declared no financial interests.
References

I have been asked to describe the process used by the American Academy of Pediatrics (AAP) and American Heart Association (AHA) to evaluate the evidence for the purpose of developing recommendations for the Neonatal Resuscitation Program. This symposium is primarily interested in the recommendations regarding management of meconium during neonatal resuscitation, but I will first describe the neonatal program and the process used in development of all AHA and AAP resuscitation recommendations.

The Neonatal Resuscitation Program (NRP) is a standardized instruction in neonatal resuscitation. It includes a self-instructional textbook and a set of skills taught by instructors. The instructors first involved national faculty who were given the responsibility of training regional instructors in their respective states, who then trained hospital-based instructors, who in turn trained participants. The program was developed by collaboration of the AHA and AAP and is managed and revised by the NRP Steering Committee administered by the AAP. There have been over 2.2 million participants in the United States since inception of the NRP nearly 20 years ago, and the program has also been implemented in 106 other countries. Revisions are made approximately every 5 years.

The revision process involves six steps: define the issues, review the literature, debate the evidence, publish guidelines, produce educational materials, and deliver the program. The first three steps are conducted in collaboration with the International Liaison Committee on Resuscitation (ILCOR), which then publishes a document entitled Consensus on Science and Treatment Recommendations (COSTR). Each of the resuscitation councils represented on ILCOR then develops separate resuscitation guidelines appropriate for their respective countries.

The neonatal resuscitation issues considered during the most recent evidence evaluation process included topics such as the use of 100% oxygen versus room air, various issues related to temperature management, ventilation strategies during resuscitation, components and timing associated with volume expansion, glucose management following intensive resuscitation, use of CO2 detectors for confirmation of tube placement, epinephrine dosing and routes of administration, ethical considerations regarding initiating and discontinuing resuscitation, as well as various aspects of managing meconium prior to, during and following delivery.

After the issues are defined, ILCOR representatives and consultants are asked to begin the evidence evaluation process by preparing worksheets on each topic. Worksheet development begins with an intensive review of the literature. Over 30,000 abstracted and critiqued references are cataloged in an Endnotes database maintained at the AHA. Pertinent studies are then classified according to their level of evidence, ranging from level 1 (randomized controlled trial), through levels 4 (historic, nonrandomized cohort study) and 6 (animal or mechanical model study), to level 8 (rational conjecture or common practice). Each study is also ranked as to the quality of evidence (fair, good or excellent) and whether the study is supportive, neutral or opposes the evidence. The neonatal resuscitation worksheets are available for review by accessing the NRP website (http://www.aap.org/nrp/nrpmain), clicking on ‘Science’ and ‘Evidence-Based Guidelines’, and then accessing the link to the worksheets maintained on the AHA website. There are three worksheets addressing issues about meconium.

The worksheets serve as the focus for a series of ILCOR debates that take place over approximately 3 years, which culminates in the Evidence Evaluation (E-2) conference during which consensus is reached on the science. This conference forms the basis for the COSTR document, the most recent version of which was jointly published in Circulation and Resuscitation and the pediatric and neonatal portions republished in Pediatrics. The NRP Steering Committee then considered the practical implications of COSTR in drafting the NRP Resuscitation Guidelines that were also published in Circulation and Pediatrics.

Three resuscitation-associated meconium issues were examined during the most recent evidence evaluation process.
Does amnioinfusion reduce the incidence or severity of meconium aspiration syndrome?

It has been hypothesized that infusion of saline into meconium-contaminated amniotic fluid will dilute the meconium and decrease the potential for developing obstruction of airways and development of meconium aspiration syndrome. Review of the literature revealed 23 reports supporting the hypothesis: amnioinfusion significantly reduced the frequency of meconium aspiration syndrome, of meconium below the cords and neonatal acidemia, and was associated with a lower overall cesarean section rate. Nine studies showed no significant benefit of amnioinfusion. Three reports suggested that any demonstrated benefit of amnioinfusion may have been a reflection of reversing fetal heart rate decelerations rather than due to dilution of meconium. There were also case reports of uterine rupture when amnioinfusion was administered during a trial of labor. In view of the conflicting data, insufficient input from the obstetrics profession, and knowledge of an ongoing randomized control trial, the Committee decided to take no position on amnioinfusion during this evidence evaluation cycle. Although the Guidelines were in press, the Fraser et al.7 trial was published (as also reported at this conference), and the American College of Obstetrics and Gynecology released a Committee Opinion that ‘routine prophylactic amnioinfusion for the dilution of meconium-stained amniotic fluid is not recommended.’8

Should the current recommendation, to always perform intrapartum suctioning, be continued?

Since the Carson et al.9 study in 1976, there has been a generally accepted recommendation that babies born with meconium-stained amniotic fluid should have their nose, mouth and pharynx suctioned after delivery of the head, but before delivery of the shoulders (intrapartum suctioning). The recommendation was based on a probably erroneous assumption that most meconium aspiration syndrome (MAS) aspiration occurred during the establishment of air breathing and that removal of the meconium while the chest was still compressed in the birth canal could prevent MAS. The Carson et al. study reviewed retrospectively all births that occurred at the University of Colorado during three time periods (Table 1).

In total, 12 to 16% of the babies born in 1970 to 1975 had meconium in their amniotic fluids. For the first 46 months (period 1), the standard had been not to perform intrapartum suctioning, but to perform direct endotracheal suctioning of all meconium-stained babies after delivery. This post-delivery practice had been initiated following the observation of Gregory et al.10 in 1974 that 56% of meconium-stained neonates had meconium recovered from below the cords. For the next 10 months (period 2), Carson et al. performed intrapartum suctioning, continued to suction the trachea immediately following birth, and then performed a tracheobronchial lavage with normal saline. During the third epoch of 8 months (period 3), the intrapartum suctioning continued to be performed, but neonatal suction and lavage were less common. Even though there were no significant differences in the incidence of meconium aspiration syndrome among any of the epochs, the practice of intrapartum suctioning was recommended and adopted by the vast majority of clinicians for the next 30 years, probably because the practice appeared to be noninvasive and seemed logical.

However, the practice of intrapartum suctioning continued to be questioned by various obstetricians and neonatologists, and in 2000 to 2001 Vain et al.11 conducted a randomized, multicenter, control trial of 2514 meconium-stained deliveries occurring in 10 centers in Argentina and 1 in the United States. Meconium-stained births were randomized to receive either pharyngeal suctioning of the fetus before delivery of the shoulders or no intrapartum suctioning. Although there were 5% protocol deviations, an intent-to-treat analysis revealed no significant difference in the incidence of meconium aspiration syndrome between the two groups. The relative risk was 0.9, with 95% confidence limits of 0.6 to 1.3. Primarily as a result of the Vain study, ILCOR and the latest edition of NRP have stated the following: ‘Current recommendations no longer advise routine intrapartum oropharyngeal and nasopharyngeal suctioning for infants born to mothers with meconium staining of amniotic fluid (class I).’ It should be noted that, although there was some disagreement during development of the recommendation, the NRP Steering Committee is on record as emphasizing that the new recommendation should not be interpreted to mean that intrapartum suctioning of meconium-stained babies is contraindicated, but merely that the previously recommended practice of routinely performing suctioning in such babies is no longer recommended. Several members felt that clearing of the oropharynx to facilitate the possible need for subsequent visualization of the trachea for direct suctioning, still seems reasonable.

Table 1 Experience with meconium births, as reported by Carson et al.9

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Births</td>
<td>7385</td>
<td>2320</td>
<td>1681</td>
</tr>
<tr>
<td>Meconium in amniotic fluid</td>
<td>12.5%</td>
<td>16.4%</td>
<td>16.2%</td>
</tr>
<tr>
<td>MAS</td>
<td>18*</td>
<td>7**</td>
<td>1***</td>
</tr>
<tr>
<td>MAS deaths</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: MAS, meconium aspiration syndrome.
*P = 0.18; **P = 0.25.
Is the current recommendation, to limit endotracheal suctioning only to the nonvigorous newborn, still valid?

Traditional teaching has recommended that meconium-stained infants have endotracheal intubation immediately following birth and that suction be applied to the endotracheal tube as it is withdrawn. As noted above, this recommendation stemmed from the observation of Gregory et al.10 who reported on experience with 1000 births, of whom 8.8% were meconium stained and 91% of those 88 had received direct endotracheal suction. Meconium was recovered from the trachea in 56% (46 babies) and 17% (8 patients) of those had had none in ‘mouth or larynx’ during laryngoscopy. Although this was not a control trial, from these observations, these clinician investigators recommended that, ‘all infants born through thick or ‘pea soup’ meconium should have their trachea aspirated immediately after birth…’. Again, the recommendation was universally accepted and became the standard until Wiswell et al.12 conducted a randomized multicenter control trial comparing the practice of performing endotracheal suctioning on all babies, versus only suctioning those babies who were nonvigorous. Vigorous was defined as strong respiratory effort, good muscle tone, and a heart rate greater than 100 beats per minute. Over 26 months, at 12 centers, 2094 meconium-stained neonates who were classified as ‘vigorous’ at birth, were randomized to receive endotracheal suctioning versus conservative therapy. There was no significant difference in the incidence of meconium aspiration syndrome or other lung disease between those suctioned versus those not suctioned. As a result, ILCOR and the NRP revised their recommendation in 2000 to limit endotracheal suctioning only to those babies who were meconium stained and not vigorous at birth. We finally stopped the practice of chasing the healthy baby around the delivery room with a laryngoscope.

There are still several unanswered questions regarding the appropriate management of the meconium-stained baby at birth that have not received definitive answers in the most recent COSTR document and the 2005 NRP Guidelines. First, is amnioinfusion an effective procedure for diluting meconium and reducing meconium aspiration syndrome? The above-mentioned Fraser et al.7 trial and American College of Obstetrics and Gynecology statement8 appear to have answered that question and will likely influence the next evidence evaluation documents. Second, is intrapartum suctioning contraindicated, rather than simply not recommended as a routine procedure? It seems unlikely that another larger control trial will be conducted to answer this question and there appears to be little evidence suggesting that intrapartum oropharyngeal suctioning is particularly hazardous. And finally, is direct endotracheal suctioning of the newborn of any description indicated following birth? As the original observation that formed the basis for the procedure was uncontrolled and was published over 30 years ago, and as there is increasing evidence that much of meconium aspiration occurs well before birth and as essentially every report of endotracheal suctioning since the report of Gregory et al. reports some morbidity associated with the procedure, it would seem well justified for there to be a modern-day large, randomized, multicenter trial comparing endotracheal suctioning versus no endotracheal suctioning of the nonvigorous meconium-stained newborn immediately following birth. Until such a study is performed, it is likely that subsequent NRP guidelines will continue to recommend that the procedure be performed.

Disclosure

J Kattwinkel has received grant support from the American Academy of Pediatrics and the National Institutes of Health.

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4 The International Liaison Committee on Resuscitation (ILCOR) consensus on science with treatment recommendations for pediatric and neonatal patients: neonatal resuscitation. Pediatrics 2006; 117: e978–e988.
REVIEW

Developing a systems approach to prevent meconium aspiration syndrome: lessons learned from multinational studies

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Introduction

Optimizing the management of infants born with meconium staining, relevant to the Indian macro- and micro-health environment, may prevent adverse outcomes owing to perinatal asphyxia, meconium aspiration syndrome (MAS), and ensuing respiratory failure. The predictive risk factors for MAS in infants delivered through meconium-stained amniotic fluid (MSAF) have not been ascertained prospectively. On the basis of a retrospective study reported by Usta et al.,

odd ratio >3 was identified when an infant’s delivery was associated with the (a) induction for non-reassuring fetal heart rate (FHR) pattern, (b) need for endotracheal intubation (ET), (c) Apgar <3 at 1 min or (d) need for a Cesarean section (Table 1). This study informed and influenced clinical delivery room practice for several years. In addition, the management of meconium at the time of birthing has been examined from two perspectives: (i) suctioning of the meconium from an infant’s upper airway after delivery of the head but before delivery of the shoulders (intrapartum suctioning, oronasopharyngeal suctioning) and (ii) suctioning of an infant’s trachea immediately after birth with an ET. Recommendations for intrapartum suctioning for meconium had been based on consensus of studies that have yielded conflicting results about the value of intrapartum oronasopharyngeal suctioning of infants born with MSAF. A more recent large multicenter randomized trial found that intrapartum suctioning of meconium does not reduce the incidence of MAS. The latter study has led the International Liaison
Table 1  Clinical risk factors for MAS in infants born through MSAF

<table>
<thead>
<tr>
<th>Clinical risk factors</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Induction for non-reassuring fetal heart rate</td>
<td>6.9</td>
</tr>
<tr>
<td>2 Need for intubation</td>
<td>4.9</td>
</tr>
<tr>
<td>3 Apgar ≤ 5 at age 1 min</td>
<td>3.1</td>
</tr>
<tr>
<td>4 Cesarean section</td>
<td>3.0</td>
</tr>
<tr>
<td>5 Previous cesarean section</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Abbreviations: MAS, meconium aspiration syndrome; MSAF, meconium-stained amniotic fluid.

Based on live births from 1990 to 1993 (Memphis, TN, USA) with MASF n = 937 and MAS in n = 39 (4.2%).

Adapted from Usta et al.1

Committee on Resuscitation2 or ILCOR to recommend against routine intrapartum oronasopharyngeal suctioning for infants born with MASF. On the other hand, the current recommendation for ET suctioning is based on a pivotal randomized, controlled trial that showed that ET intubation and suctioning for vigorous infants at birth offers no benefit. It is also known that the benefit of ET suctioning in meconium-stained, depressed infants has not been systematically studied. However, the current ILCOR treatment recommendation is that meconium-stained, depressed infants should receive ET suctioning immediately after birth and before stimulation, presuming that the equipment and expertise is available and that ET suctioning is not necessary for infants with MAS who are vigorous. The relevance for application of ILCOR recommendations to a population with diverse access to perinatal health care has been questioned. The studies and recommendations cited by ILCOR are reported from those conducted in developed nations and nations with sophisticated medicalized health-care systems. These recommendations may not be applicable in areas with low income resources, births conducted at home or limited perinatal access to evidence-based medical care.3 Dramatic improvements in the overall birthing conditions and the continuing advances in perinatal-neonatal practices during the last decade have yet to universally affect the unacceptably high risk of morbidity and mortality in the neonatal population. Earlier experiences have led us to believe that building an interdisciplinary leadership approach that lends itself to a systems approach would provide innovative strategies to bridge the existing access barriers in micro- and macro-health environments of diverse social and cultural backgrounds.

Global perspective of births with MASF

Meconium-stained amniotic fluid is found in 7 to 20% of pregnancies at the time of delivery.3–7 MSAF is associated with fetal acidosis, abnormalities in FHRs and low Apgar scores, suggesting hypoxia as the stimulant of passage of meconium in utero.8,9 The most severe condition associated with meconium passage in utero, MAS, occurs in 2 to 9% of neonates born through MASF and has a high mortality rate of 40%.5,7,10–13 At delivery, meconium is found below the vocal cords in 20 to 45% of neonates born through MSAF.4,10,13,14 If it has not been aspirated into the lungs, the removal of meconium from the airways before the first breath can reduce the incidence of MAS. This preemptive removal of meconium from the airways can be performed at the time of delivery (intrapartum suctioning) or immediately after delivery (postpartum suctioning). Intrapartum suctioning consists of clearing the mouth, pharynx and nose with either a large-bore suction catheter (12 to 14 mm caliber) or a bulb syringe as soon as the head is delivered but before delivery of the shoulders. Postpartum suctioning consists of intubating and suctioning the trachea before performing the other steps of resuscitation.

Respiratory failure is a major cause of morbidity and mortality in the neonatal population. Infants with hypoxemia develop respiratory failure because of MAS, persistent pulmonary hypertension of the newborn and pneumonia/sepsis. Recently, Bhutani et al.15 suggested a system-based strategy comprising the prenatal, natal and postnatal management of babies delivered through MASF, so that the adverse outcomes are minimized and the least number of babies require innovative ventilatory support. At an urban perinatal center in Pennsylvania, over a 6-year period (1995 to 2000), 14.5% (3370/23 175 of live-birth babies) were delivered with MSAF (Figure 1). These data show that 4.6% of babies (155/3370) with MSAF sustained MAS (Figure 2). Overall, 26% (40/155) of babies with MAS needed ventilatory support (0.17% of all live births) (Figure 3); of these, only 20% (8/40 or 0.035% of live births) needed innovative ventilatory support. Infants with low Apgar scores were more likely to need ventilatory support (Figure 4). None died or needed extracorporeal life support.

Components of a systems approach to prevent MAS

Interdisciplinary health-care perspectives

Depending on the likelihood of MAS as a potential but significant neonatal morbidity, the development of a ‘rapid response team’ was envisioned as an institutional policy (prevalent in most US birthing facilities) with the goal of modulating an intensive care nursery census and also the risk of adverse neonatal outcomes. The potential reduction in neonatal mortality and cost containment would be the anticipated advantages of team-based interventions. The team would constitute the obstetrician, pediatrician (and/or neonatologist), the perinatal nurses (obstetrical and neonatal) and the family, supported by the maternal child health administrators.

Although the obstetrician has a task-oriented responsibility to take care of the mother and the family through the performance and delivery of quality of care services in the delivery room, a personal agenda is to strive for a ‘HAPPY’ family experience. This is achieved through minimallyatraumatic birthing with the prevention of fetal hypoxemia and acidosis, and an effective
management of maternal and personnel stress. The perinatal birthing nurse provides support, educates and cares for the family during the changing birthing environment. A seemingly benign process has the potential of disintegration as erstwhile birthing plans are shelved. Participation in clinical management includes fetal monitoring and appropriate availability and documentation of instrumentation. A pediatrician needs to be accessible in a timely manner for unpredictable and ‘stat’ calls and have the skills and ability to perform (without anxiety) in an acute stressful response. In addition, the pediatrician needs to clarify any confusion on the amount of meconium staining and the status of fetal well-being, and to make a decision of the likelihood of an elective neonatal intubation. The family perspectives are overwhelmed by the sudden medicalization of the birthing process and are aggravated by anxiety, stress, interruption of bonding and loss of privacy with unanticipated instrumentations and participation of often unselected and unsolicited providers.

Performance and judgment of the ‘rapid response team’
Besides being awake and alert, the team needs to have a physiologic understanding and risk assessment ability of the clinical progression of MAS. Credentialing for neonatal resuscitation, performance standards and intubation skills of the team are now routine at all birthing facilities. The team needs to acquire the prenatal history and the evolving perinatal events, directly observe the natal events and render as well as execute postnatal management decisions. The relevant prenatal history acquired through the attending obstetrician and nurse would include the intensity of meconium staining, status of fetal well-being and FHR patterns and fetal pH (if measured) data on the use of amnioinfusion, and risk factors for perinatal infections and for any maternal-placental complications. The latter include a prolonged second stage of labor, abruption of the placenta, placenta previa or cord accidents (nuchal cords, true knots, cord compression and cord prolapse), abnormal fetal presentations, maternal hypertension, pre-eclampsia, intrauterine growth retardation, post-maturity or placental calcifications. FHR monitoring is a predominant method to assess fetal oxygenation in labor; however, it is a nonspecific, indirect and inaccurate measure of fetal hypoxia and acidosis. Electronic fetal monitoring is associated with increased Cesarean section because of ‘non-reassuring’ FHR patterns. These include FHR between 100 and 120 with no accelerations, FHR <100 beats/min with accelerations, increased heart rate variability of >25 beats/min for...
Equivocal studies et al. trials by Falciglia with MAS incidence of 0.4% (when only postpartum suctioning was performed, as compared vigorous to postpartum suctioning. The effectiveness of intrapartum when intrapartum suctioning was also performed in addition P = 0.013) and no deaths when intrapartum suctioning was also performed in addition to postpartum suctioning. The effectiveness of intrapartum suctioning was confirmed in a subset of patients enrolled in a multicenter randomized controlled trial looking into the efficacy of postpartum suctioning in vigorous newborns.17 In this study by Wiswell et al., the incidence of MAS was 8.5% in infants who did not have intrapartum suctioning (n = 94), as compared with 2.7% in infants who had intrapartum suctioning (n = 54; P = 0.013).

Equivocal studies. Two prospective and non-randomized clinical trials by Falciglia et al.13 compared early suctioning (suctioning by the obstetrician before delivery of the thorax) and late suctioning (suctioning by the obstetrician after delivery of the thorax) and showed no difference in the incidence of MAS. In the first study, no differences in the rate of meconium below the cords (36 vs 37%) or the incidence of MAS (20% in each group) between early and late suctioning were noted.8 The second study reported a higher rate of meconium below the cords (53%) among the early suctioning group compared with the late suctioning group, which had a rate of 56% (P < 0.001), but there was no difference in the incidence of MAS between the two groups (P > 0.05).13 Rossi et al.13 reported that despite intrapartum suctioning, meconium was present in the trachea in 37% (n = 238) of neonates born through MSAF, and MAS developed in 9.2% of cases.

Studies that do not recommend intrapartum suctioning. A large multicenter, randomized controlled clinical trial enrolled 2514 patients with MSAF with the desired goal of assessing the effectiveness of intrapartum suctioning for the prevention of MAS.18 This study also examined the effect of intrapartum suctioning in high-risk subgroup infants (those with thick MSAF) with abnormal FHR patterns, infants with delivery by Caesarian section and infants needing resuscitation in the delivery room. This study showed that intrapartum suctioning does not reduce the incidence of MAS (relative risk (RR): 0.9, 95% confidence interval (CI): 0.6 to 1.3), need for mechanical ventilation (RR: 0.8, 95% CI: 0.4 to 1.4) and mortality (RR: 0.4, 95% CI: 0.1 to 1.5).

Current ILCOR recommendations. On the basis of these large studies, the current guidelines of the American Academy of Pediatrics and the American Heart Association through the Neonatal Resuscitation Program and Pediatric Working Group of the ILCOR14 no longer recommend routine intrapartum oronasopharyngeal suctioning by an obstetrician before delivery of the shoulder. Discretionary intrapartum suction also remains a standard of care. This decision is not based on a meta-analysis, but, on the basis of consensus of opinions that are most relevant to the care of infants already affected, improved the dating of pregnancy by a lowering of the gestational age at birth (fewer deliveries for pregnancies >41 weeks), earlier recognition of fetal distress and improved access to sophisticated birthing facilities in developed nations. The other currently recommended approach to prevent MAS is immediate postnatal ET suctioning in non-vigorous (depressed) babies born through MSAF. In nations and communities with improved perinatal health-care access, an observational and non-intervention strategy is indicative of eminent and cautious practice. Mandatory intrapartum suctioning in babies born through MSAF is being discontinued, largely on the basis of a single large randomized controlled study conducted in a setting with facilities for intensive fetal monitoring, prompt response to fetal distress and 24 h availability of personnel trained in neonatal resuscitation, including postnatal intubation and ET meconium suctioning.

Impact of different clinical settings. Most of the deliveries in these countries take place either at home or at ill-equipped and understaffed hospitals. The profile of women and their neonates born in a developing country setting contrasts sharply with that of developed countries. Even in the study by Vain et al.,18 which included 11 sites in Argentina, a middle-income country, the clinico-epidemiologic profile was not comparable with the diverse environment and communities encountered in India (see Table 2). The Indian experiences document high rates of
Table 2 Comparison of data from an Indian Database\textsuperscript{5} reported by Vain et al.\textsuperscript{18}

<table>
<thead>
<tr>
<th>Variable</th>
<th>NNPD\textsuperscript{3}</th>
<th>Vain et al.\textsuperscript{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{18 sites in India}</td>
<td>\textit{11 sites in Argentina and 1 in the United States}</td>
</tr>
<tr>
<td>Babies with MSAF (n = 12 156)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (g, mean ± s.d.)</td>
<td>2646 ± 552</td>
<td>3413 ± 485</td>
</tr>
<tr>
<td>Small for gestation</td>
<td>1566 (12.9%)</td>
<td>39 (3%)</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>5208 (42.8%)</td>
<td>401 (32%)</td>
</tr>
<tr>
<td>Pregnancy-induced hypertension</td>
<td>961 (7.9%)</td>
<td>65 (5%)</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>177 (1.5%)</td>
<td></td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>360 (3.0%)</td>
<td>17 (1%)</td>
</tr>
<tr>
<td>Fetal bradycardia (&lt;120 b.p.m.)</td>
<td>1523 (12.5%)</td>
<td>145 (11%)</td>
</tr>
<tr>
<td>Fetal tachycardia (&gt;160 b.p.m.)</td>
<td>369 (3.0%)</td>
<td></td>
</tr>
<tr>
<td>Meconium aspiration syndrome</td>
<td>1896 (15.6%)</td>
<td>52 (4%)</td>
</tr>
</tbody>
</table>

Abbreviations: MSAF, meconium-stained amniotic fluid; NNPD, National Neonatal Perinatal Database.

pregnancy-related complications such as pregnancy-induced hypertension, oligohydramnios, intrauterine growth retardation and post-maturity. In addition, in the Indian settings, postnatal intubation and ET suctioning of non-vigorous infants could be jeopardized if discretionary recommendations are made without specific, practical and easily implemented algorithms. Low- and middle-income nations account for the majority of the 130 million live births and 4 million neonatal deaths occurring each year.\textsuperscript{19} Therefore, we speculate that the relatively simpler and easier techniques to perform intrapartum suctioning and the availability of appropriate biotechnologies, such as large-bore, portable, disposable and easily manipulated suction devices for the mouth, nose and/or trachea that are non-traumatic, sterile and independent of wall suction, may still have a role in the prevention of MAS, whereas improvements of perinatal health-care access are initiated and implemented.

Understanding immediate postnatal tachypnea and clinical signs

The most frequent benign etiology for tachypnea is respiratory compensation for metabolic acidosis as evidenced by hypocapnia and normoxemia. On the other hand, onset of respiratory disease would be evident by hypercapnia and hypoxemia. Worrisome tachypnea could be due to air leaks (pneumothoraces and pneumomediastinum), atelectasis, aspiration, pneumonia and, most of all, concern for incipient persistent pulmonary hypertension. Peripheral cyanosis (manifested by blue toes) would require continuous pulse oximetry to document normoxemia and observation for expectant care. Central cyanosis (as manifested by blue lips) would require an evaluation for hemodynamic changes with handling, evaluation of response to inspired oxygen and continued observation in a nursery environment for continued cardiorespiratory environment. Crystalloid infusion may be helpful for infants with ‘benign tachypnea’ if the capillary perfusion is compromised. In such conditions, the measurements of arterial blood gases or chest radiographs may not be necessary. If metabolic acidosis is documented for persistent tachypnea with hypoxemia, base correction with bicarbonate is not essential and crystalloid infusion is likely to be sufficient. Persistent tachypnea-associated hypoxemia and hypercapnia would be indications for a chest radiograph evaluation. When an infant is stabilized, postnatal gastric suction, presumably to prevent postnatal aspiration, is not evidence-based and, similarly, gastric lavage is not necessary. Infants do need to be monitored for airway stability and subsequent choking/bradycardia.

Subsequent postnatal management

Infants are likely to need close glucose monitoring for hypoglycemia because of concomitant risk factors. Intravenous dextrose infusions and delayed enteral nutrition need to be considered for persistent tachypnea (owing to hypoxemia and/or hypercapnia), low Apgar scores and hypoglycemia. Screening for sepsis would be based on the presence of known maternal risk factors or an abnormal chest radiograph. The need for continued cardiorespiratory monitoring, parenteral nutrition and concerns for sepsis are the most common reasons for continued neonatal observation and management. Within about 30 min, age, and color changes should have stabilized. Similarly, within about 6 h, age, any transient grunting, flaring and retractions should stabilize. Deviations from these time frames should initiate a process of a more intense evaluation. The absence of any signs of sepsis or cardiorespiratory distress beyond 24 h of age is likely to exclude the MAS.

Conclusions

A systems approach that provides for a well-coordinated and trained ‘rapid response team’ for meconium-stained births is likely to implement an evidence-based intervention and effectively manage
a potential catastrophic outcome for the infant, the family and the staff. Classic practices of maintaining equipoise in the delivery room require working (and communicating) with the attending obstetrician and nursing staff and supporting the education of the family. The team’s approach is to be expectant but prepared to intubate the infant. The team’s role is to monitor the progress of labor, fetal status, fetal data and family status and to be prepared to interact, interpret, intervene and initiate support as needed. These data from a single US center also illustrate the complex interactions of technologies used during a birthing practice that could have both potentially useful (airway clearance) and/or deleterious effects because of the combinations of trauma, introduction or aggravation of infection and/or interference of gas exchange. Several of the components of clinical practice are based on evidence, and their implementation in practice may need further validation or resolution by consensus. There is a health and societal need to establish guidelines for practice at birthing facilities in the United States. Consistency in practice may lead to cost containment, even though the cost-effectiveness of such an approach has not been validated. Methodological inquiry, evidentiary analysis and reassessment of both clinical and biotechnological options would better inform the development of a practical stepwise algorithm to optimize bedside care.

Disclosure
The author has declared no financial interests.

References
Meconium aspiration syndrome requiring assisted ventilation: perspective in a setting with limited resources

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To determine characteristics, management, complications and outcome of neonates with meconium aspiration syndrome (MAS) requiring mechanical ventilation (MV). A retrospective review of clinical data of neonates with MAS who were admitted to a public hospital for MV between January 2004 and December 2006. Eighty-eight neonates were ventilated for MAS. Thirty-one percent were postdates and 51% had no electronic fetal monitoring. Postnatal suctioning of meconium was not performed according to protocol in 47% of nonvigorous infants. High-frequency ventilation and surfactant were used in 32 and 14% of cases, respectively. Persistent pulmonary hypertension of the newborn (PPHN) and pneumothorax occurred in 57 and 24% of cases, respectively. Overall mortality rate was 33%. Neonates suffering from MAS with PPHN had higher mortality rate of 48% compared with 13% in those suffering from MAS without PPHN. Factors associated with mortality were peak inspiratory pressure \( (P < 0.001) \), pneumothorax \( (P < 0.001) \) and PPHN \( (P = 0.001) \). Postdates, inadequate intrapartum monitoring and limited use of adjunct respiratory therapies were common. Severe MAS is associated with adverse outcome.

Introduction

Meconium aspiration syndrome (MAS) is associated with significant morbidity and mortality. Common complications associated with MAS include interstitial emphysema, pneumothorax, pneumonia and persistent pulmonary hypertension of the newborn (PPHN). Pulmonary air leaks have been observed to occur in 15 to 33% of cases of MAS. Although MAS is still associated with significant mortality, mortality rates seem to have decreased over the years. Studies from 1970s and 1980s reported high mortality rates ranging from 28 to 40%. Recent literature has reported mortality rates less than 15%. This reduction in mortality rates appears to be related (1) to reduction in incidence of MAS, which is related to changes in obstetric practice and (2) to use of adjunct respiratory therapies, such as exogenous surfactant, nitric oxide and high-frequency ventilation in the management of neonates with MAS. Yoder et al. reported that MAS decreased nearly fourfold between 1990 to 1992 and 1997 to 1998, and this was associated with 33% reduction in births more than 41 weeks gestation, more frequent diagnosis of nonreassuring fetal heart rate patterns and greater use of amnioinfusion. Approximately 50% of patients with MAS will have severe MAS defined as the need for mechanical ventilation (MV). The reports on the outcome of patients with MAS, especially those who require MV, originate mainly from developed countries. Factors such as poor monitoring during labor and low Apgar scores that are associated with the need for MV among infants with MAS occur commonly in developing countries; therefore, patients with MAS in developing countries might have worse outcomes compared with those from developed countries. Secondly, the adjunct respiratory therapies that have been associated with reduction in mortality are not easily available in developing countries. The limitations of assessing the outcomes of MAS in developing countries are related to limited resources resulting in difficulties in making a diagnosis of MAS, which requires the presence of X-ray changes. In developing countries, patients with possible MAS might die before a chest X-ray (CXR) can be taken or might improve and be discharged without having had a CXR unless they are admitted to the neonatal intensive care unit (NICU) for MV. Therefore, patients who are admitted to NICU are more likely to have CXR. The aim of this study was to describe characteristics, management and outcome of neonates with MAS in a setting where resources are limited. Owing to difficulties in reliably diagnosing MAS in patients who do not require MV, this study was limited to those who required MV.

Methods

Infants with severe MAS were identified by review of discharge diagnoses and hospital records of all patients who weighed \( \geq 2000 \text{ g} \) and were admitted to the NICU of Chris Hani Baragwanath Hospital, Johannesburg, South Africa, between January 2004 and December 2006. Maternal and neonatal hospital charts were reviewed for confirmation of the diagnosis and pertinent clinical data.

Severe MAS was diagnosed if infants met the three following criteria: meconium-stained amniotic fluid (MSAF), need for MV and radiographic abnormalities consistent with MAS (Figure 1).
Patients were excluded from the study if there was no documentation of the presence of MSAF, if records were incomplete, if CXR was not available from records and no comment was present on CXR findings in doctor’s notes or if the baby had congenital abnormalities.

During the study period, the protocol on postnatal management of infants born through MSAF was according to the guidelines of the International Liaison Committee on Resuscitation. The guidelines stated that for all infants born through MSAF with absent or depressed respiration, direct laryngoscopy should be conducted immediately after birth for suctioning of residual meconium from the hypopharynx and for intubation and suctioning of the trachea. All patients who were admitted with respiratory distress were started on antibiotics; patients who required MV were started on synchronized intermittent mandatory ventilation using a peak inspiratory pressure of 20 to 25 cm H₂O, positive end expiratory pressure of 4 to 5 cm H₂O, inspiratory time of 0.4 s and flow of 5 to 10 l/min. There were two models of conventional ventilators used in the unit: the Bear Cub 750PSV infant ventilator and Newport E100M ventilator. The mode of control for the conventional ventilators was pressure limited and time cycled. There were two ventilators available for high-frequency ventilation, both of them were Sensor Medics 3100A oscillatory ventilators. All patients had CXRs ordered immediately postintubation or after insertion of umbilical arterial/venous catheters or when patient deteriorated. Sometimes, there were delays in having X-rays taken especially at night or over the weekends because of staff (radiographers) shortages. Availability of surfactant was limited to 5 vials per month in 2004, and this was increased to 10 vials per month in 2005. Nitric oxide was made available to the unit at the end of 2004. We are limited to use two tanks of nitric oxide per year, with each tank of 10 l containing 900 parts per million of nitric oxide. Both the gas and the delivery and monitoring system were bought from Sidewinder Medical, Cape Town, South Africa. It was only used in patients who had hypoxia thought to be secondary to persistent pulmonary hypertension of the newborn, and it was stopped if there was no response in 60 min. PPHN was diagnosed either with a preductal versus postductal pulse-oximeter oxygen saturation gradient of >10% or on echocardiography.

Demographic characteristics, clinical data and neonatal outcomes to NICU discharge were recorded. Comparison between survivors and nonsurvivors was performed using χ² or Fisher exact test for categorical variables and Student’s t-test for continuous variables. Permission to conduct the study was received from the University of the Witwatersrand Human Research Ethics Committee.

Results

Among the 2157 infants who required MV from January 2004 to December 2006, 800 weighed ≥ 2000 g. Of these 800 infants, 143 had a diagnosis of MAS or had an abnormal CXR with changes suggestive of MAS and were admitted within 48 h of life. Among the 143 patients, 55 were excluded. The reasons for exclusion were absence of a history of MSAF, incomplete hospital records, and therefore inability to confirm or exclude the presence of MSAF, and CXR not found or no comment in patient’s file regarding CXR findings (Figure 2). There were 88 patients who had complete records, with history of MSAF in mother’s or infant’s hospital file, changes on CXR and were ventilated; these were included in the study. These 88 patients accounted for 4% of all admissions requiring MV and 11% of NICU admissions weighing ≥ 2000 g. There were 61 399 live births over this 3-year period. The incidence of severe MAS or of infants with MAS requiring MV was 1.43 per 1000 live births.

Characteristics of mothers to infants with MAS are shown in Table 1. Ninety-eight percent of patients were born inside a healthcare facility and only 2% were born at home. The maternal HIV status was positive in 31% of cases, which was similar to the institutional statistics from antenatal clinic. Thirty-one percent were born at ≥ 41 weeks gestation. Electronic monitoring was documented in 49% of cases, of which 78% had abnormal tracing and the common abnormality was late decelerations. Forty-four
Meconium aspiration syndrome and ventilation

S Velaphi and A Van Kwawegen

Figure 2 Number of live births, patients requiring mechanical ventilation and those excluded or included in the study.

Table 1 Maternal characteristics of infants with MAS requiring ventilation

<table>
<thead>
<tr>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
</tr>
<tr>
<td>&lt; 18 years</td>
</tr>
<tr>
<td>18–35 years</td>
</tr>
<tr>
<td>&gt; 35 years</td>
</tr>
<tr>
<td>Not recorded</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1–3</td>
</tr>
<tr>
<td>&gt; 3</td>
</tr>
<tr>
<td>Not recorded</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Antenatal care</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Monitoring with CTG</td>
</tr>
<tr>
<td>Yes, with abnormalities</td>
</tr>
<tr>
<td>Yes, with no abnormalities</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Gestational age by dates</td>
</tr>
<tr>
<td>&lt; 35 weeks</td>
</tr>
<tr>
<td>35–37 weeks</td>
</tr>
<tr>
<td>38–40 weeks</td>
</tr>
<tr>
<td>≥ 41 weeks</td>
</tr>
<tr>
<td>Not recorded</td>
</tr>
<tr>
<td>Place of birth</td>
</tr>
<tr>
<td>Inborn</td>
</tr>
<tr>
<td>Referrals from other hospitals</td>
</tr>
<tr>
<td>Clinic</td>
</tr>
<tr>
<td>Home</td>
</tr>
<tr>
<td>Mode of delivery</td>
</tr>
<tr>
<td>Normal vaginal delivery</td>
</tr>
<tr>
<td>Cesarian section</td>
</tr>
<tr>
<td>Vacuum</td>
</tr>
<tr>
<td>Breech</td>
</tr>
<tr>
<td>Consistency of MSAF</td>
</tr>
<tr>
<td>Thick</td>
</tr>
<tr>
<td>Thin</td>
</tr>
<tr>
<td>Unrecorded</td>
</tr>
</tbody>
</table>

percent were born by cesarian section, and the indication for the cesarian section was fetal distress in 90% of cases. Although consistency of MSAF was not recorded in 44% of patients, the majority of them were born through thick meconium.

Infant details are shown in Table 2. There were 38 (43%) patients who required resuscitation, with almost all of them responding to bag mask ventilation. The number of patients who required resuscitation was greater than those with low Apgar score (score ≤ 5) at 1 min, suggesting over-reading of Apgar scores or reflecting our practice of withholding assisted ventilation from infants with the lowest Apgar scores because of concerns about poor neurodevelopmental outcome. Among those who required resuscitation, only 53% were recorded as having been intubated for suctioning of meconium. Severity of the lung disease was moderate to severe in 64% of patients as defined by an oxygen index of more than 10. Twenty-three patients out of the 28 who did not have C-reactive protein (CRP) results are those who died on day 1 of life before CRP level could be done. Among those who had CRP results, 92% had CRP levels more that 10 mg l⁻¹.

Morbidity and mortality of infants with MAS are shown in Table 3. Common complications among patients with MAS were PPHN (57%) and pneumothorax (24%). Of the 50 patients who
had PPHN, 47 were diagnosed by differential pulse oximetry saturations and only 17 (36%) had echocardiography, which confirmed the diagnosis of PPHN, and the other 30 did not have echocardiography because of limited human resources. Three were diagnosed only on echocardiography. Median duration of stay in NICU was 4 days, with 22% of them staying for more than 7 days. One-third of patients with MAS did not survive. Factors associated with mortality were female gender (<i>P</i> = 0.046), maximum peak inspiratory pressure (<i>P</i> < 0.001), development of pneumothorax (<i>P</i> < 0.001) and PPHN (<i>P</i> = 0.001) (Table 4). Most of the deaths occurred early during their NICU stay.

### Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3080 (2100–4530)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>45 (51)</td>
</tr>
<tr>
<td>Male</td>
<td>43 (49)</td>
</tr>
<tr>
<td>1 min Apgar score</td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>19 (21)</td>
</tr>
<tr>
<td>≥ 5</td>
<td>64 (73)</td>
</tr>
<tr>
<td>Not recorded</td>
<td>5 (6)</td>
</tr>
<tr>
<td>5 min Apgar score</td>
<td></td>
</tr>
<tr>
<td>&lt; 7</td>
<td>21 (24)</td>
</tr>
<tr>
<td>≥ 7</td>
<td>54 (61)</td>
</tr>
<tr>
<td>Not recorded</td>
<td>13 (15)</td>
</tr>
<tr>
<td>Required resuscitation</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50 (57)</td>
</tr>
<tr>
<td>Yes</td>
<td>38 (43)</td>
</tr>
<tr>
<td>Bag mask ventilation</td>
<td>38 (43)</td>
</tr>
<tr>
<td>Bag mask ventilation + chest compression</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Above + adrenalin</td>
<td>0</td>
</tr>
<tr>
<td>Intubated and suctioned for meconium</td>
<td>20/38 (53)</td>
</tr>
<tr>
<td>Median oxygen index (OI) (range)</td>
<td>15.17 (0.97–71.16)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of patients with OI &lt; 10</td>
<td>32 (36)</td>
</tr>
<tr>
<td>Number of patients with OI between 10 and 20</td>
<td>42 (48)</td>
</tr>
<tr>
<td>Number of patients with OI &gt; 20</td>
<td>14 (16)</td>
</tr>
<tr>
<td>C-reactive protein (CRP) (range)</td>
<td>52.9 (1–567)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of patients with CRP &lt; 10 mg l&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Number of patients with CRP between 10 and 20 mg l&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Number of patients with CRP &gt; 20 mg l&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>47 (53)</td>
</tr>
<tr>
<td>Number of patients with unrecorded CRP</td>
<td>28 (32)</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; MAS, meconium aspiration syndrome; OI, oxygen index. *Median (range).

### Table 3

<table>
<thead>
<tr>
<th>Complications</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumothorax</td>
<td>21 (24)</td>
</tr>
<tr>
<td>PPHN</td>
<td>50 (57)</td>
</tr>
<tr>
<td>Median duration of stay in NICU (days)</td>
<td></td>
</tr>
<tr>
<td>≤ 3 days</td>
<td>35 (38)</td>
</tr>
<tr>
<td>4–7 days</td>
<td>35 (40)</td>
</tr>
<tr>
<td>8–14 days</td>
<td>10 (11)</td>
</tr>
<tr>
<td>&gt; 14 days</td>
<td>10 (11)</td>
</tr>
<tr>
<td>Outcome among all patients (n = 88)</td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>29 (33)</td>
</tr>
<tr>
<td>Survived</td>
<td>59 (67)</td>
</tr>
<tr>
<td>Outcome among those without PPHN (n = 38)</td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Survived</td>
<td>33 (87)</td>
</tr>
<tr>
<td>Outcome among those with PPHN (n = 50)</td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>24 (48)</td>
</tr>
<tr>
<td>Survived</td>
<td>26 (52)</td>
</tr>
</tbody>
</table>

Abbreviations: MAS, meconium aspiration syndrome; NICU, neonatal intensive care unit; PPHN, persistent pulmonary hypertension of the newborn. *Median (range).

The use of exogenous surfactant, number of patients who were put on high-frequency oscillatory ventilation and nitric oxide were low during the study period, and it did not change significantly over the years (<i>P</i>-values all more than 0.100) (Table 5). Incidence of pneumothorax, PPHN and mortality rate did not change significantly over the years.

### Discussion

Severe MAS is defined as those cases that require assisted MV.<sup>13</sup> It has been reported that between 40 and 50% of infants with MAS will need MV.<sup>11,14,15</sup> In public hospitals of developing countries, offering MV is often restricted to those who are expected to have favorable outcomes, and among those who qualify, it is often delayed due to limited bed availability in NICUs. Thus, patients who are ventilated in countries with limited resources are more likely to have poor outcome because of delays in instituting treatment, limited adjunct respiratory therapies and high incidence of having associated conditions such as infections. In this study, we sought to determine characteristics and outcome of infants who were ventilated with MAS, in a setting where there are limited resources.
About one-third of patients with severe MAS were born at 41 weeks gestation or more; and 51% of patients were born to mothers who did not have electronic monitoring during labor despite having a history of MSAF. Postmaturity may have contributed to these patients developing MAS. The risk of MAS has been shown to be higher in post-term than term infants with relative risk of 3.1 with 95% confidence interval of 2.6 to 3.7. Inadequate monitoring might have led to delays in expediting delivery of infants who were compromised, and this allowed for ongoing hypoxia resulting in severe disease. The effect of postmaturity and monitoring during pregnancy and labor on MAS is supported by reduction of MAS in institutions where postmaturity has been reduced and monitoring is intensive.

The protocol of intubating and suctioning infants suffering from absent or depressed respiration was not followed consistently, as only 53% of infants who should have been intubated according to the protocol were actually intubated. The possible reasons for this low rate of suctioning could be poor documentation of what happens during resuscitation, or that junior doctors who commonly conduct neonatal resuscitation in our hospital were not...
confident enough to perform intubations and therefore not adhering to protocol. Inadequate suctioning of meconium could have contributed to the development of severe MAS, as it has been reported that meconium in the trachea is one of the major risk factors associated with diagnosis of MAS.\textsuperscript{11,17–20} It is also possible that this did not affect the development of severe MAS, as some studies have suggested that aspiration of meconium occurs \textit{in utero} and that damage in the lungs has already taken place by the time the baby is born.\textsuperscript{1,19,21–23}

The high mortality rate of 33% in this study could be related to associated conditions, such as infections and PPHN, and low usage of adjunct respiratory therapies. Among the patients who had their CRP level assessed, 92% had CRP > 10 mg\textsuperscript{-1} and 78% had CRP > 20 mg\textsuperscript{-1}, suggesting the presence of infection or inflammation. \textit{In vitro} studies have suggested that meconium may activate alveolar macrophages with subsequent release of inflammatory cytokines resulting in an increase in CRP.\textsuperscript{24} Although only one patient had positive culture, it is difficult to exclude infection when there is abnormal CXR and high CRP. Some studies have reported that clinical chorioamnionitis and neonatal sepsis are more common in neonates with severe MAS.\textsuperscript{11,25,26} More than 50% of patients in this study had PPHN. Should one exclude or include infants with PPHN when one is looking at outcome of infants with MAS? The literature suggests that these patients should be excluded. The reason for this is that the cause of PPHN in patients with MAS might have preceded the aspiration of meconium. This is supported by findings in autopsies from cases dying from severe MAS within 48 h of birth, which revealed muscularization of distal pulmonary arterioles, which requires 3 to 8 days to develop, making it unlikely that PPHN was a complication of MAS.\textsuperscript{25,27,28} As PPHN on its own is associated with high mortality rate, one would expect less mortality rate from MAS if infants with PPHN are excluded. In this study, the mortality rate was 73% lower in those with PPHN compared to those without PPHN. The low mortality rate in developed countries has been associated with an increase in the use of exogenous surfactant, high-frequency ventilation and inhaled nitric oxide, with >50% of infants receiving one or more of these therapies.\textsuperscript{5} A postal survey of 227 neonatal units in the United Kingdom revealed that 96, 42 and 29% of units were using exogenous surfactant, high-frequency ventilation and inhaled nitric oxide for MAS, respectively.\textsuperscript{8} In this study, use of exogenous surfactant, high-frequency ventilation and inhaled nitric oxide ranged from 6 to 19, 21 to 45 and 3 to 6%, respectively, over the 3 years.

In conclusion, patients who were ventilated for MAS were more likely to have been born postdates, to have not been monitored electronically during labor, to have been born by cesarean section and to have not been intubated for suctioning of meconium at birth. MAS requiring MV is still associated with significant morbidity and mortality in the setting where there are limited resources. Reducing patients who deliver post-term, intensive monitoring during labor and offering appropriate neonatal care including exogenous surfactant will improve incidence of MAS and those requiring ventilation, and therefore less strain on already limited resources.

**Disclosure**

The authors have declared no financial interests.

**References**


Meconium aspiration syndrome: experiences in Taiwan

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Meconium aspiration syndrome (MAS) is still one of the most challenging diseases for the neonatologists. We reviewed our earlier studies of MAS in an attempt to provide some idea for more understanding of MAS. This study is a retrospective review and summarization of our earlier studies in MAS at two tertiary neonatal centers in Taiwan. Incidence of MAS was decreased sharply in Taiwan. MAS infants who required resuscitation in the birth room being out-born, birth asphyxia and infants who developed persistent pulmonary hypertension (PPHN) and pneumothorax were associated with increasing mortality. In MAS infants who neither required mechanical ventilation nor had a history suggestive of perinatal infection, antibiotic treatments would not affect the outcome of MAS. Dexamethasone did reduce inflammation response and improve cardiopulmonary perfusion. However, steroids did not prevent the development of PPHN. Our review provided the risk factors of mortality for MAS. Antibiotic treatments should not be a routine for every infant with MAS. Although steroids reduce pulmonary inflammation, their role in the prevention of PPHN remains to be further studied.

Incidence of MAS

Table 2 compares the incidence and mortality of infants suffering from MAS between the United States and one of the tertiary centers in Taiwan (China medical university hospital, Taichung, Taiwan).1–5 It is interesting to note that data of the incidences of meconium staining of amniotic fluid, MAS and mortality are almost comparable with those from the United States. However, these data are obtained from one tertiary center with many high-risk maternal transfers; the actual overall incidence in Taiwan could be much lower. The outborn infants have higher mortality rates (7.3%) than inborn infants (0.81%) (Table 3).

Perinatal and postnatal factors and mortality in MAS

A review of 314 cases of MAS between 1995 and 20011,5 indicated that infants who required resuscitation in the birth room, who were first out-born or with birth asphyxia, and infants who developed persistent pulmonary hypertension (PPHN) and pneumothorax are important factors associated with increased mortality in MAS (Table 4).

Management of meconium-stained amniotic fluid in delivery room

Since 1995, the management of meconium-stained amniotic fluid has followed a guideline as shown in Figure 1.6 This guideline is not much different from the recent recommendations of American Academy of Pediatrics.7,8 We do endotracheal suction in infants who have thick meconium staining, who have low Apgar score or who have respiratory distress shortly after birth.9 We perform intrapartum oro- and nasopharyngeal suction in all infants if there is meconium staining of amniotic fluid.5,10 We feel that this procedure is simple and does not carry significant adverse effects. Using this guideline, about 50% of meconium-stained infants required endotracheal suction. We do amnioinfusion only if there is oligohydramnios and thick meconium staining.11–15

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The incidence of meconium aspiration syndrome (MAS) varies from population to population and from countries to countries.1–4 With the recent improvement in socioeconomic status and the advances in prenatal care in Taiwan, the incidence of MAS in Taiwan sharply decreased in the past decade.1 The adoption of National Healthcare Insurance System is probably the most important factor leading to better health access and care. Table 1 shows some of the factors that characterized health care in Taiwan. At present, nearly 100% of the pregnant women have prenatal care and majority of the deliveries are carried out in large hospitals. However, the mean age of the mother at the time of first childbirth is about 26 years, older than that in most of the developing and developed countries.
The role of antibiotics in MAS

Meconium has been shown to enhance bacterial growth in vitro, particularly Gram-negative organisms. It is a common practice to place these infants on antibiotics. We have conducted a study on 259 MAS infants who did not require mechanical ventilation and who did not have a history suggesting perinatal infection. The infants were divided into two groups; one received antibiotics and the other did not. Both groups were comparable in their baseline data. There was no significant difference between the groups in clinical course and mortality (Table 5).

Thus, antibiotic treatments would not affect the clinical course and outcome in MAS without perinatal risk factors for infection and without ventilator use.

The role of pulmonary inflammation in the development of PPHN in MAS

Pulmonary hypertension or PPHN of the newborn occurs in 10 to 15% of infants with MAS. This condition usually presents as persistent hypoxemia occurred at 6 to 24 h after birth. A spontaneous recovery usually occurs within 3 to 4 days if the patient survives, suggesting that a functional vascular constriction is probably involved in the pathogenesis.

Pulmonary hypertension or PPHN in infants with MAS could be due to (1) hypertrophy or neo-muscularization of
postacinar pulmonary capillaries as a result of chronic intrauterine hypoxia, (2) functional pulmonary vasoconstriction as a result of hypoxemia, hypercarbia or acidosis or (3) functional pulmonary vasoconstriction as a result of lung inflammation. We hypothesized that lung inflammation may play an important role in pulmonary vascular constriction and hypertension. We therefore conducted a MAS study on newborn piglet models. The purposes of the study were to investigate the following: (1) Does MAS in newborn piglets produce pulmonary hypertension? (2) Does pulmonary inflammation play a role in the development of pulmonary hypertension. (3) Can anti-inflammatory agents, such as dexamethasone, prevent pulmonary hypertension. Figure 2 shows the method of the study on 35 newborn piglets (aged 1 to 7 days). We also administered dexamethasone early in several infants. The results of piglet and newborn studies are shown in Tables 6 and 7. On the basis of the results of this study, we made the following conclusions: (1) Following meconium aspiration, elevation of pulmonary arterial pressure appears to be biphasic, the early phase starting...
The use of dexamethasone reduced tracheal aspirate TXB2 and 6-keto PGF1α. (4) Dexamethasone increased cardiac stroke volume, increased pulmonary blood flow and improved ventilation/perfusion match. 

The role of pulmonary inflammation in the development of pulmonary hypertension or PPHN in MAS and the possible role of steroids in the prevention of PPHN remain to be further studied (Figures 3 to 7).

Table 7 Respiratory status in MAS infants with and without dexamethasone therapy

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>d-1</th>
<th>d-2</th>
<th>d-3</th>
<th>d-4</th>
<th>d-5</th>
<th>d-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMV C</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>IMV D</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FIO2 C</td>
<td>0.54 ± 0.31</td>
<td>0.35 ± 0.16</td>
<td>0.26 ± 0.16</td>
<td>0.29 ± 0.16</td>
<td>0.29 ± 0.08</td>
<td>0.29 ± 0.09</td>
<td>0.28 ± 0.18</td>
</tr>
<tr>
<td>FIO2 D</td>
<td>0.52 ± 0.24</td>
<td>0.40 ± 0.20</td>
<td>0.30 ± 0.18</td>
<td>0.32 ± 0.29</td>
<td>0.30 ± 0.22</td>
<td>0.31 ± 0.24</td>
<td>0.30 ± 0.21</td>
</tr>
<tr>
<td>P_{O2} (mm Hg) C</td>
<td>86 ± 58</td>
<td>84 ± 53</td>
<td>74 ± 22</td>
<td>75 ± 30</td>
<td>70 ± 26</td>
<td>80 ± 18</td>
<td>75 ± 25</td>
</tr>
<tr>
<td>P_{O2} (mm Hg) D</td>
<td>91 ± 80</td>
<td>86 ± 42</td>
<td>70 ± 42</td>
<td>78 ± 28</td>
<td>67 ± 23</td>
<td>76 ± 14</td>
<td>74 ± 14</td>
</tr>
<tr>
<td>P_{CO2} (mm Hg) C</td>
<td>43 ± 12</td>
<td>36 ± 9</td>
<td>36 ± 8</td>
<td>39 ± 5</td>
<td>43 ± 8</td>
<td>37 ± 8</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>P_{CO2} (mm Hg) D</td>
<td>42 ± 12</td>
<td>34 ± 7</td>
<td>32 ± 5*</td>
<td>33 ± 9*</td>
<td>30 ± 7**</td>
<td>35 ± 7</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>pH C</td>
<td>7.28 ± 0.12</td>
<td>7.41 ± 0.07</td>
<td>7.40 ± 0.05</td>
<td>7.40 ± 0.07</td>
<td>7.39 ± 0.09</td>
<td>7.39 ± 0.06</td>
<td>7.45 ± 0.17</td>
</tr>
<tr>
<td>pH D</td>
<td>7.30 ± 0.70</td>
<td>7.39 ± 0.05</td>
<td>7.40 ± 0.05</td>
<td>7.45 ± 0.03*</td>
<td>7.43 ± 0.04*</td>
<td>7.40 ± 0.06</td>
<td>7.44 ± 0.05</td>
</tr>
</tbody>
</table>

Abbreviations: IMV, intermittent mandatory ventilation; MAS, meconium aspiration syndrome.
*P<0.05, **P<0.01.
C: control infants, n = 23; D: dexamethasone-treated infants, n = 27. Adapted from Yeh et al.17

Figure 3 Mean blood pressure during the study. MAS piglets that received dexamethasone had significantly higher mean blood pressure at 4, 16, 36, 72 and 82 h than saline control piglets. *P<0.05. Adapted from Wu et al.16
Disclosure
The authors have declared no financial interests.

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Continuous positive airway pressure and conventional mechanical ventilation in the treatment of meconium aspiration syndrome

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Meconium aspiration syndrome (MAS) is a complex syndrome that ranges in severity from mild respiratory distress to severe respiratory failure, persistent pulmonary hypertension of the newborn and sometimes death. Understanding of the syndrome’s complicated pathophysiology will help determine the appropriate treatment strategy, including the use of continuous positive airway pressure (CPAP), conventional mechanical ventilation (CMV) and other therapies. Approximately 30 to 50% of infants diagnosed with MAS will require CPAP or mechanical ventilation. The optimum modes of ventilation for MAS are not known. Very few studies have been conducted to determine ‘best’ ventilatory strategies. Despite the introduction, over the last two decades, of innovative ventilatory treatments for this disease (for example, surfactant, high-frequency ventilation, inhaled nitric oxide, extracorporeal membrane oxygenation), the majority of infants can be successfully managed with CPAP or mechanical ventilation alone.

Introduction

Approximately 30 to 50% of infants diagnosed with meconium aspiration syndrome (MAS) will require continuous positive airway pressure (CPAP) or mechanical ventilation. The optimum modes of ventilation for MAS are not known. Very few studies have been conducted to determine ‘best’ ventilatory strategies. Retrospective reviews of the application of conventional mechanical ventilation (CMV) to infants with this malady span three decades, during which time a host of adjunctive therapies have become available.1,2 Despite multiple therapies for this syndrome, there is a disturbingly high-mortality rate (4 to 19%) historically. However, in the most recent of these reviews, only 20% of 40 infants with MAS requiring positive pressure assistance needed ‘innovative ventilatory support’ (defined as the use of high-frequency ventilation (HFV), inhaled nitric oxide or surfactant) and no infants died or required extracorporeal membrane oxygenation.2 It appears that when used optimally, conventional therapies are adequate to treat the majority of infants with this disease. Neonatal MAS challenges the practicing clinician to make the correct diagnosis, understand the changing and complex pathophysiology of the disease, and tailor the respiratory support to the specific alterations in pulmonary mechanics.

Making the diagnosis of MAS

Approximately 8 to 19% of all term deliveries occur through meconium-stained amniotic fluid (MSAF) and MAS develops in 5 to 33% of these infants.3 Respiratory complications of MAS are often the consequence of hypoxia in utero with significant acidosis and depressed respirations at birth. There also appears to be a greater risk of developing MAS as well as all respiratory complications (that is, tachypnea, pneumonia, pulmonary air leaks) if the meconium is ‘thick’ as opposed to ‘thin’.4 However, a recent meta-analysis of the use of amnioinfusion showed no neonatal benefit when meconium was thinned through this process except in settings with limited peripartal surveillance.5 Infants who are vigorous at birth have a very low risk of developing MAS.6 Those who develop MAS are often greater than 41 weeks gestation and have clinical signs of postmaturity (decreased subcutaneous tissue, peeling skin, long nails and so on). The respiratory signs of MAS are similar to other neonatal respiratory diseases. These include tachypnea, retractions, grunting, nasal flaring and cyanosis. If the meconium has been present in utero for greater than 3 h, the infant may have meconium staining of the skin, nails and umbilical cord. The anterior–posterior diameter of the chest may be increased if there is significant air trapping.

The classic radiographic picture of MAS includes diffuse patchy infiltrates with areas of atelectasis mixed with areas of hyperinflation throughout the lung fields. However, initially the chest X-ray may not be very diagnostic, as it may take many hours for the chemical pneumonitis secondary to MAS to develop. Other findings on X-ray include possible air leaks and cardiomegaly, if significant perinatal asphyxia has resulted in cardiomyopathy.
Later in the course of the disease, the MAS picture may be complicated by the radiographic signs of surfactant deficiency (atelectasis, air bronchograms, decreased lung volume) as the surfactant system may be negatively impacted by the primary disease.

Despite the classic clinical and radiological appearance of MAS, it may be difficult in certain cases to distinguish MAS from other neonatal respiratory diseases. Table 1 lists the differential diagnosis. It is important diagnostically and physiologically to determine if persistent pulmonary hypertension of the newborn (PPHN) is present with significant right-to-left shunting of blood through the foramen ovale and/or the ductus arteriosus resulting in exaggerated hypoxemia. PPHN may result secondary to MAS or may be present by itself (‘primary PPHN’) with concurrent MSAF but no true aspiration. As noted by Goetzman in these cases of primary PPHN, ‘when meconium is present, it is a fellow traveler and not the cause of the PHN (PPHN) and hypoxemia, contrary to what is often implied when MAS is used as the primary diagnosis.’ Importantly, the treatment of the baby’s respiratory disease will depend on the presence and severity of pulmonary hypertension.

Thus the diagnosis of MAS may be assumed when three criteria are met: (1) a history of meconium in the trachea; (2) clinical evidence of significant respiratory distress; and (3) X-ray evidence of aspiration pneumonia. The association of transient tachypnea and MSAF without meconium in the airway or X-ray evidence of pneumonitis is often misdiagnosed as MAS and may lead to inappropriate treatment, including early positive pressure, which then may result in inadvertent pulmonary air leaks.

### Pulmonary pathophysiology of MAS

Multiple mechanisms are involved in the pulmonary pathophysiology of MAS. Meconium has deleterious effects on the airways, injures the pulmonary parenchyma and alveoli, inhibits surfactant function and may cause hypoxic pulmonary vasoconstriction resulting in PPHN. The complex pulmonary effects of MAS are seen in Figure 1. Individual infants may manifest any one or more of these effects and the degree of respiratory distress may wax and wane according to the severity of the pathophysiology. Often there is a vicious cycle of air trapping, ventilation-perfusion mismatch, hypoxemia, right-to-left shunting, acidosis and increased pulmonary vascular resistance, which may be difficult to treat successfully with standard therapies.

Meconium may easily obstruct both the large and small airways in the newborn lung. When the obstruction is partial, a ‘ball-valve’ mechanism may lead to air trapping and air leaks (Figure 2). On inspiration the airway dilates allowing gas to enter, but during

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**Table 1** Differential diagnosis of MAS (when MSAF is present at delivery)

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient tachypnea of the newborn</td>
</tr>
<tr>
<td>Aspiration of amniotic fluid or blood</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
</tr>
<tr>
<td>Sepsis with pulmonary edema</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Congenital heart disease</td>
</tr>
<tr>
<td>Intrapartum asphyxia with cardiomyopathy</td>
</tr>
<tr>
<td>Persistent pulmonary hypertension of the newborn</td>
</tr>
<tr>
<td>Delayed transition</td>
</tr>
</tbody>
</table>

**Effects of mediators (cytokines, eicosanoids)**

**Surfactant dysfunction**

**Protein leak into the airways**

**Direct toxicity by meconium constituents**

**Altered lung elastic forces (increased resistance, decreased compliance)**

**Airway obstruction**

**Alveolar and parenchymal inflammation and edema**

**Effects of in utero hypoxemia (pulmonary vascular remodeling, lung parenchymal changes)**

**Altered pulmonary vasoactivity**

**Pulmonary vasoconstriction due to components of meconium**

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**Figure 1** The complex pulmonary pathophysiology of meconium aspiration syndrome. From reference 9 with permission.
expiration the airway constricts around the obstruction, thus trapping air inside the lung. This may result in overexpansion of the lung, ventilation–perfusion mismatch, decreased compliance and possibly air leak. Complete obstruction of the airway by meconium may result in atelectasis and resultant hypoxemia and hypercapnia.

Meconium also causes direct pulmonary parenchymal and alveolar injury resulting in a profound inflammatory response. The release and migration of inflammatory cells and mediators, such as cytokines and phospholipase A₂, to the lung may cause direct pulmonary injury such as epithelial cell necrosis, cell death and apoptosis. These mediators may also result in constriction of pulmonary vessels, alteration and impairment of neutrophil function and cause capillary leakage and edema similar to respiratory distress syndrome.

Multiple investigations in both animals and humans have shown the deleterious effects of meconium on endogenous surfactant. Meconium may inhibit surfactant production, displace surfactant from the alveolar surface and decrease its surface tension lowering function. Surfactant inhibition is concentration dependent and results from a variety of toxic biochemical effects of the meconium constituents. The influx of neutrophils into the airways subsequent to these toxic effects produces proteases, which further degrade surfactant proteins and frequently results in hemorrhagic edema of the lung.

Fox et al. initially reported the association of pulmonary hypertension in association with perinatal aspiration syndromes. The majority of cases of significant PPHN are associated with MAS. The relationship of PPHN with MAS is complex and multifactorial. Vasoconstriction of pulmonary vessels may be associated with normal or increased muscularization of the vessel walls. Prolonged in utero hypoxia may result in vascular remodeling secondary to hypertrophy of the medial musculature. Acute maladaptation secondary to intrapartum asphyxia with normal vessel anatomy is caused by the inhibition of endogenous pulmonary vasodilators and an inflammatory response resulting from MAS, which releases vasoconstrictive substances and causes platelet aggregation thus increasing pulmonary vascular resistance. Hypoxemia and acidosis also result in increased pulmonary artery pressure. Thus in both the acute and chronic hypoxic states, with normal or increased pulmonary vessel muscularization, the inflammatory response to MAS may trigger the vicious cycle of postnatal hypoxia, pulmonary vasoconstriction, right-to-left shunting and further hypoxia. In the presence of chronic vascular remodeling, the combination of MAS and PPHN is much more difficult to treat.

### Altered pulmonary mechanics of MAS

In the theoretical classification of neonatal pulmonary disorders, MAS is considered an obstructive disease, although in reality the condition is both obstructive and atelectatic, and changes with time and treatment. Table 2 shows the physiologic differences between ‘pure’ atelectatic and obstructive lung disease. MAS is characterized in this simplified chart as an obstructive disease with increased lung volume, decreased compliance, increased functional residual capacity and increased airway resistance and time constants. The pathophysiology of MAS discussed in the previous section alters the respiratory mechanics of the lung in four major ways as seen in Table 3. Understanding the principles of normal ventilation and the alteration in respiratory mechanics secondary to MAS pathology is necessary to devise ventilation strategies to treat affected infants with CPAP and/or CMV.

Meconium in the pulmonary airways leads to increased airway resistance and prolonged time constants. The time constant is a measure of how quickly the lung can inflate or deflate, or how long it takes for alveolar and proximal airway pressures to equilibrate. The expiratory time constant ($K_e$) is directly related to both compliance ($C_L$) and airway resistance (Raw) by the equation: $K_e = C_L \times \text{Raw}$. It takes three time constants to discharge 95% of the tidal volume of one breath. If the time constant is prolonged secondary to increased airway resistance (without a concomitant reduction in lung compliance), the patient is at risk.
### Table 3 Ventilation strategies to match pathophysiology of MAS

<table>
<thead>
<tr>
<th>Pathophysiology</th>
<th>Pulmonary dynamics</th>
<th>Ventilation strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway obstruction; ball-valve</td>
<td>↑ Lung volume</td>
<td>Avoid ventilation on ‘C’ portion of compliance curve; Adequate expiratory time; Avoid inverse I:E ratios</td>
</tr>
<tr>
<td>Phenomenon</td>
<td>↑ Airway resistance</td>
<td>Adequate CPAP or PEEP</td>
</tr>
<tr>
<td>Parenchymal/ alveolar injury</td>
<td>↓ Compliance</td>
<td>Adequate CPAP or PEEP; Volume recruitment strategy; Avoid overdistension to ↓ incidence of PAL</td>
</tr>
<tr>
<td>Surfactant dysfunction</td>
<td>↓ Compliance;</td>
<td>Adequate CPAP or PEEP; Volume recruitment strategy; Avoid overdistension to ↓ incidence of PAL</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>Exaggerated</td>
<td>Generous oxygenation</td>
</tr>
<tr>
<td></td>
<td>hypoxia;</td>
<td>(80 to 120 mm Hg);</td>
</tr>
<tr>
<td></td>
<td>Ventilation—</td>
<td>Avoid significant acidosis</td>
</tr>
<tr>
<td></td>
<td>perfusion mismatch</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ↑, increased; ↓, decreased; CPAP, continuous positive airway pressure; I:E, inspiratory:expiratory; MAS, meconium aspiration syndrome; PAL, pulmonary air leak; PEEP, Positive end expiratory pressure.

for incomplete emptying of the previously inspired breath. This condition may be exaggerated when the patient is on assisted ventilation and there is insufficient ventilator time for exhalation because of an abnormally long time constant. The result of this ‘inadvertent PEEP’ will be gas trapping accompanied by an increase in lung volume, a build up of pressure in the alveoli and distal airways, and decreased compliance.18 Significant air trapping may result in hyperinflation and flattening of the diaphragms on chest X-ray, increased anterior–posterior diameter of the chest and even cardiovascular effects such as decreased cardiac output, diminished blood pressure and signs of poor perfusion. Babies with increased functional residual capacity and high lung volumes have decreased compliance as seen in the flattened portion or section ‘C’ of the standard pressure–volume curve (Figure 3).19 Attempting to assist ventilation at the upper end of the compliance curve (that is, at high lung volumes) results in a further decrease in compliance and may lead to pulmonary air leaks.

As noted above, meconium has a direct injurious effect on the pulmonary airways, parenchyma and alveoli. This often results in capillary leak and edema. The additional injury of barotrauma from assisted ventilation through an endotracheal tube should be avoided if possible. Thus the use of oxygen by hood or cannula and nasal CPAP is advocated to avoid intubation and the bellows action of CMV. However, term and near-term infants may not tolerate nasal CPAP despite adequate sedation. Moreover, the use of a ‘permissive hypercapnia’ technique in these infants is problematic because of the sensitivity of the pulmonary vasculature to acidosis.

The deactivation of surfactant from MAS complicates this picture. It makes the use of PEEP mandatory during CMV despite the high functional residual capacity, increased time constant and air trapping that often occurs. Fox et al.20 showed in a small number of infants (n = 14) that moderate levels of PEEP (4 to 7 cm H2O) resulted in the greatest increase in oxygenation in babies ventilated for MAS (Figure 4).

The addition of PPHN to the MAS pathophysiology further complicates the pulmonary mechanics and makes ventilatory strategies very difficult. Increased pulmonary vascular resistance may result from hypoxia, acidosis and reduced cardiac output. Ventilatory strategies must recognize these triggers. Allowing mild hypoxia in these infants may reduce the periods of hypoxemia that occur with interventions or agitation. Pulmonary toilet must be done judiciously with careful attention to the pulse oximeter. Appropriate sedation and limiting interventions while nursing the infant in a dimly lit and quiet area of the neonatal intensive care unit are all helpful adjuncts to care. Although ‘gentle ventilation’ is recommended by some,21 permissive hypercapnia and the resultant respiratory acidosis may worsen the pulmonary hypertension. At the other extreme, very aggressive assisted ventilation with hyperventilation in an attempt to improve oxygenation may contribute to overdistension of the lung, air leaks and decrease cardiac output, further aggravating the PPHN.

### Conventional ventilation strategies

As there is little evidence from clinical trials on ventilator treatment of MAS, conventional management is primarily based on pulmonary pathophysiology. To avoid additional air trapping and
The complicated pulmonary pathophysiology resulting from areas of atelectasis and areas of hyperinflation, in association with ventilation–perfusion mismatch and airway compromise, makes ventilator management of MAS one of neonatology’s greatest challenges. The ventilator strategy used will depend on the stage and severity of the disease, especially the presence or absence of significant pulmonary hypertension. Clinicians vary in their desire to keep blood gas values in various ranges. Prior to the use of inhaled nitric oxide and HFV, some clinicians employed hyperventilation to achieve alkalois by hypocapnia in an attempt to decrease pulmonary vascular resistance. However, follow-up studies on infants managed with this strategy have shown an increased incidence of sensorineural hearing loss, pulmonary barotrauma and adverse neurologic outcomes. Thus hyperventilation has become a less-used strategy, as clinicians feel that the newer modalities such as HFV and/or inhaled nitric oxide are less dangerous than sustained hypocapnia. Contemporary management employs the acceptance of standard blood gas ranges or ‘gentle ventilation,’ a strategy in which higher PaCO 2 levels and lower PaO 2 levels are targeted in the belief that ventilator-induced lung injury will be reduced. Other than the fear of intermittent hypoxemia worsening PPHN, there is probably little reason to keep babies hyperoxic. Moreover, catheterization data in older infants with pulmonary hypertension secondary to bronchopulmonary dysplasia suggest that oxygen tensions that exceed 70 to 80 mm Hg do little to decrease pulmonary artery pressure. No trials have compared any of these strategies in the treatment of infants with MAS.

Infants with MAS without associated PPHN may be managed in a relatively standard fashion with slightly higher oxygen targets than used for premature infants with respiratory distress syndrome. The pH should be maintained above 7.3, PaCO 2 in the 40 to 50 mm Hg range and the PaO 2 targeted at 70 to 80 mm Hg. This may be achieved with a moderate peak inflating pressure preferably not exceeding 25 cm H 2O, a relatively rapid ventilator rate (40 to 60 breaths per minute), a moderate PEEP (4 to 6 cm H 2O) and an adequate expiratory time (0.5 to 0.7 s) to prevent air trapping. This strategy requires a relatively short inspiratory time of 0.3 to 0.4 s. If the diaphragms on chest X-ray are flat and gas trapping is suspected, the expiratory time should be increased to 0.7 to 0.9 s. PEEP decreased to 3 to 4 cm H 2O and the rate decreased to allow at least a 0.25 inspiratory time. Borderline hypoxemia in this situation may be acceptable as increased ventilator settings may cause lung injury or the patient may be converted to HFV to improve oxygenation.

In infants whose MAS is complicated by significant PPHN, the ventilatory strategy is somewhat different. Intermittent hypoxemia can be mitigated by allowing the PaO 2 to be as high as 80 to 100 mm Hg without great concern for oxygen toxicity. As oxygen saturations have never been studied in this context and are surrogates for oxygen tensions, which do not correlate well when
saturations are greater than 95%, monitoring by saturation alone is not recommended. Some clinicians also prefer to target mild hypcapnia (PaCO₂ range of 30 to 35 mm Hg), which results in mild alkalosis as a buffer to acidosis that may trigger a downward PPHN spiral. In both clinical situations of MAS with and without PPHN, a volume-recruitment strategy is preferred to recruit aletectic areas of the lung and treat ventilation-perfusion mismatch. Such an approach resulted in improved oxygenation and less ventilator-induced lung injury in an experimental piglet model.28 This strategy has to be tempered with the knowledge that such an approach may worsen air trapping in the face of mechanical airway obstruction.

All types of conventional methods of ventilation have been used in these cases. Most standard ventilators now allow the operator to choose among intermittent mandatory ventilation, patient-triggered ventilation, pressure support ventilation and/or volume controlled ventilation modes. None of these modalities have been compared with any other in a rigorous way, although each mode of ventilation has its supporters. As non-asphyxiated term infants may vigorously fight assisted ventilation, one approach has been to use a patient-triggered mode (synchronized intermittent mandatory ventilation or assist/control) in conjunction with adequate sedation. Muscle relaxation is rarely necessary and has significant drawbacks when this step is considered to achieve acceptable blood gases and avoid high ventilatory pressures and possible barotrauma, it may be time to move to alternative therapies (that is, inhaled nitric oxide, HFV). Careful attention must be paid to the environment in which these babies are nursed and ventilated. Although it seems appropriate to provide chest physiotherapy and pulmonary toilet, these interventions must be done with caution (or not at all) as they may trigger hypoxemia, agitation and an increase in pulmonary vascular resistance. Other nursing interventions must be considered in the same context with particular attention paid to minimize environmental stimuli such as light and noise.

Conclusions

MAS is a complex syndrome that ranges in severity from mild respiratory distress to severe respiratory failure, PPHN and sometimes death. Understanding of the syndrome’s-complicated pathophysiology will help determine the appropriate treatment strategy, including the use of CPAP, CMV and other therapies. The use of echocardiography, pulmonary function studies and graphics will help guide the clinician in the choice of therapies. As shown by Bhutani et al.2 at Pennsylvania Hospital over a period of 6 years, the use of a systems-based strategy for babies delivered through MSAF can minimize adverse outcomes. This review showed that conventional respiratory therapies such as oxygen, CPAP and CMV were adequate treatment for 80% of MAS-affected infants with no infant requiring extracorporeal membrane oxygenation or dying from the disease. In a time when the newest and most costly therapy seems to find a use in every patient, we can only admire and aspire to the benchmark that Dr Bhutani et al. have set.

Disclosure

JP Goldsmith has received consulting fees from Discovery Labs.

References

The objective of the study was to compare the effectiveness of surfactant treatment either by bolus or surfactant lung lavage followed by inhaled nitric oxide (iNO) therapy in infants with meconium aspiration syndrome (MAS) complicated by persistent pulmonary hypertension (PPHN). In this study, thirteen infants with diagnosis of MAS and PPHN were first treated with conventional respiratory support. Then between 2 and 22 h of life they were randomized either to bolus surfactant treatment (n=6) or surfactant lung lavage (SLL, n=7) treatment. Then all infants were treated with iNO therapy. The groups were compared with regard to their clinical course: changes in PaO₂, FiO₂, MAP, OI, A-a oxygen gradient, duration of iNO therapy, length of ventilation and hospitalization. Complications and mortality were also compared. The results showed that infants treated with SLL had significant improvements in oxygenation, decreases in MAP and A-a gradients. But there were no significant differences in duration of ventilation, iNO treatment, length of hospitalization or complications. In conclusion these data show no advantage of SLL therapy over bolus surfactant treatment in infants with MAS complicated by PPHN.


### Introduction

Improved perinatal management of meconium aspiration has contributed substantially to a decrease in incidence and mortality because of meconium aspiration syndrome (MAS). In infants not responding to conventional treatment of MAS, other methods such as bolus surfactant, surfactant lung lavage (SLL) as well as extracorporeal membrane oxygenation (ECMO) can be used.

Surfactant treatment by bolus may be associated with inactivation by meconium in the airways, which may explain non-responsiveness in earlier studies. It has been suggested that the administration of surfactant followed by SLL may lead to a washout of residual meconium both mechanically and because of the improved mucociliary transport of meconium. This approach of treatment may thus lead to a thorough removal of meconium from the respiratory tracts and minimize the damaging effect of meconium on the endogenous surfactant. Another major complication of MAS is the development of persistent pulmonary hypertension (PPHN). Inhaled nitric oxide (iNO) has been used successfully to manage infants with PPHN. The effectiveness of iNO would depend on unimpeded delivery of the gases to the well-ventilated parts of the lungs. It is possible that iNO does not reach the alveoli, which are covered with meconium. Therefore, we hypothesize that SLL will clear the meconium, stabilize the alveoli and increase the diffusion of iNO resulting in better oxygenation.

**The objective of the trial**

The objective of our trial was to study the effectiveness of the combined therapies of bolus surfactant treatment or SLL followed by iNO in infants with meconium aspiration syndrome complicated by pulmonary hypertension.

**Materials**

This study was conducted in the neonatal intensive care unit (NICU) of the Neonatology Department of the Poznan University of Medical Sciences, Poznan, Poland. We enrolled 13 neonates into the study during the period between 1998 and 2004. The diagnosis of MAS was based on clinical and radiological criteria. PPHN was diagnosed on the basis of echocardiogram results.

**Methodology of the trial**

Randomization is shown in the diagram.

**Group A1**

Group A1 included seven newborns who were treated with a combined therapy (SLL + bolus surfactant + iNO). Besides the conventional treatment, SLL with a surfactant solution Survanta was carried out between the 2nd and 22nd hour of life (average time of treatment initiation 9.7 ± 7.4 h). After the lavage
treatment, one dose of bolus *Survanta* at 100 mg of phospholipids per kg of body weight was given.

Subsequently, echocardiogram assessment was carried out for the diagnosis of PPHN; iNO was administered when PPHN was diagnosed. The initial dose of nitric oxide was 20 p.p.m. (Figure 1).

**Group A2**

Group A2 included six newborns treated conventionally. Between the 2nd and 22nd hour of life (average time of treatment initiation 11 ± 6.4 h), the newborns were given one dose of *Survanta* as a bolus in the amount of 100 mg per kg of body weight. Infants did not undergo SLL. After giving one dose of surfactant as well as conducting an echocardiographic assessment and diagnosing with PPHN, iNO was administered with an initial dose of NO at 20 p.p.m.

Lavage with a surfactant solution. Bronchoalveolar lavage with saline natural surfactant solution was in newborns fulfilling the inclusion criteria, which was carried out after sedation and initial cardiopulmonary stabilization. The steps in surfactant lavage are described below.

(A) Preparation of surfactant solution for lavage

1. A natural surfactant *Survanta* (Ross Abbott Laboratories, Zwolle, The Netherlands) was used.
2. The dose of phospholipids for the lavage solution was 5 mg in 1 ml of physiological salt.
3. The volume of the solution was 15 ml per kg of the patient’s body weight.
4. The calculated volume was divided into four parts.
5. A single lavage volume was 2 ml kg⁻¹.

Example of calculation: If the patient’s body weight was 3000 g, 45 ml of surfactant solution was used as a lavage: 9 ml of Survanta supplemented with normal saline up to 45 ml.

(B) Lavage technique

Installation of a closed circuit system was used for the administration of lavage. This was possible using a Bodai valve in the connector in the endotracheal tube. The surfactant solution was instilled through the connector, whereas the lung fluid was suctioned through the Bodai valve (Figure 2). This closed lavage and suctioning system helped maintain both mechanical ventilation and positive end-expiratory pressure (PEEP) at constant levels. The prepared volume of the solution was divided into four parts. Lavage and suctioning were conducted in four body positions: on the right and left sides, and in the Trendelenburg and anti-Trendelenburg positions, similar to the administration of the *Survanta* dose given as treatment (in line with the producer’s recommendations).

The surfactant solution was administered through the endotracheal tube. After 2 ml of the solution was instilled, the mechanical ventilation was continued. After 3 to 5 respiratory cycles, the secretions were suctioned (Figure 2).

**Treatment with bolus surfactant.** Bolus surfactant was administered through the endotracheal tube after sedation and stabilization.

(A) Surfactant preparation

A natural surfactant *Survanta* (Ross Abbott Laboratories) was used.

(B) The technique of surfactant administration

The surfactant was administered endotracheally with a catheter through an intubation tube and through the Bodai valve placed in the connector by the intubation tube. During the application, the mechanical ventilation was continued. The dose of surfactant was 100 mg per kg of body weight. The surfactant was administered in four positions: on the right and left sides, and in the Trendelenburg and anti-Trendelenburg positions. Bolus surfactant was administered once after SLL (group A1) and also once but without SLL prior to it (group A2).
Safety of the procedures. During the whole procedure, saturation and heart rate were monitored. Saturation was considered safe if, during lavage, it was within the range of 87 to ≥ 93%. If saturation fell below 87%, the parameters of mechanical ventilation were adjusted: peak pressure, fraction of inspired oxygen (FiO₂) and frequency of breaths. Lavage or surfactant was not given until saturation reached a safe level. Once this was achieved, the procedure was continued.

Parameters analyzed in the trial
The following parameters were analyzed in both groups:

1. partial arterial oxygen pressure (PaO₂);
2. Fraction of inspired oxygen (FiO₂);
3. oxygenation index (OI);
4. alveolar–arterial oxygen gradient (AaDO₂).

The parameters listed above were analyzed at six time intervals:
- At 15 min before treatment initiation (0 h);
- after 1 h of combined therapy (1 h);
- after 2 h of combined therapy (2 h);
- after 4 h of combined therapy (4 h);
- after 24 h of combined therapy (24 h);
- after 48 h of combined therapy (48 h).

The following were also analyzed:
1. length of time on mechanical ventilation;
2. duration of iNO treatment;
3. length of hospital stay;
4. complications;
5. number of deaths.

Equipment. Intermittent mandatory ventilation (IMV) was used as treatment for respiratory failure. Echocardiographic examination was conducted by using HDI 3500 ATL and a trans-receiving head with a frequency of 7 to 4 MHz. iNO was administered by using SLE 3600 INOSYS. The initial and, at the same time, maximum dose of NO was 20 p.p.m. Saturation was measured by using pulse oximeters, and maintained in the range between 87 and 95%. Arterial blood PO₂ and PCO₂ were measured.

Biochemical analysis was conducted by using Rapidlab.

Clinical classification and characteristics of recruited newborns. After the diagnosis of MAS, the newborns were divided into two groups: A1 and A2 (Figure 1) by randomization. In group A1, infants received bolus surfactant treatment followed by iNO. Group A2 received SLL followed by iNO therapy. There were no statistically significant differences in the following parameters: birth weight, gestational age as well as assessment of clinical condition in the first minutes of life. No statistically significant differences were observed in the frequency of Cesarean section as well as the time for inclusion in the trial.

In group A1, the maturity of the newborns was 38.1 (± 2.3) weeks of gestation. The average birth weight was 3267 g (± 822.4). The overall condition of the newborns in the 1st and 5th minutes of life was assessed according to the Apgar scale. The median in the first minute of life was 5 points and it was 7 points in the fifth minute of life.

In group A2, the maturity of the newborns was 37.8 (± 2.1) weeks of gestation. The average birth weight was 3128 g (± 1155.4). The overall condition of the newborns in the first and fifth minutes of life was assessed on the basis of Apgar scale. The median in the first minute of life was 4 points and it was 6 points in the fifth minute of life.

Inclusion criteria. The trial was prospective and randomized and included newborns who fulfilled the following criteria:

1. Gestational ages ≥ 35 weeks of gestation;
2. postnatal age ≤ 24 h;
3. MAS was diagnosed on the following basis:
   - presence of meconium-stained amniotic fluid below the vocal cords;
   - clinical symptoms: respiratory failure that required treatment with mechanical ventilation (FiO₂ ≥ 40%) to maintain PaO₂ at 50 to 80 mm Hg and saturation of 87 to 93%;
   - radiological findings consistent with MAS.
4. Parental consent to enter the baby in the study.

Echocardiogram assessment was conducted for the diagnosis of PPHN in all infants diagnosed as MAS. PPHN was diagnosed on the basis of the following echocardiographic parameters: 3–6

- mean pulmonary arterial pressure in the pulmonary trunk ≥ 40 mm Hg;
- ductus arteriosus and foramen ovale;
- tricuspid valve insufficiency (TI);
- out of the wave A in M-mode pulmonary valve presentation.

The blood flow in the pulmonary artery was recorded by the Doppler method using a pulse-wave technique and a trans-receiving head with a high frequency of ultrasonic beams (7 to 4 MHz). The pressure in the pulmonary trunk was calculated on the basis of the time from the initial blood flow wave until it reached the maximum speed (acceleration time (AcT)). The outflow wave was registered on the way from the right ventricle next to the ring of the pulmonary valve or in the pulmonary trunk. The flow acceleration time is shortened in the pulmonary artery. 3

The mean pressure was calculated on the basis of a mathematical
formula: $40 - (0.5AcT)$. Mean pulmonary artery pressure (MPAP) $< 40$ mm Hg was defined as the norm.

**Randomization.** Infants were randomized into groups A1 and A2. Each group of infants was managed by a different group of neonatologists. Throughout the study, the infants stayed in the hospital.

**Statistical analysis.** Parameters such as $\text{PaO}_2$, $\text{FiO}_2$, mean airway pressure (MAP), OI, Aa$\text{DO}_{2}$ as well as duration of hospital stay and the length of time on IMV and iNO were expressed in the interval scale and described with the arithmetic mean value, standard deviation as well as the minimum and maximum values and confidential interval.

The normal distribution of these parameters was checked with Shapiro–Wilk’s test. For the parameters that showed normal distribution, the Student’s $t$-test for independent variables was used for comparison of groups A1 and A2; otherwise, non-parametric Mann–Whitney’s test was used.

For comparisons of values obtained at 0, 1, 2, 4, 24 and 48 h, once the normal distribution was confirmed, the analysis of variances for repeatable variables was carried out using the post hoc Newman–Keuls test; if normal distribution was not confirmed, the non-parametric Friedman test was used with Dunn’s test for repeated comparisons. The Apgar scale as a parameter, expressed in the ordinal number scale, was described with the median as well as the minimum and maximum values. Apgar score between the groups was compared using the non-parametric Mann–Whitney test.

The parameters expressed in the nominal scale, such as the number of deaths and complications, were described with numbers and corresponding percentages. Dependence was tested with Fisher’s exact test. Statistical significance of $\alpha = 0.05$ was assumed for verification of statistical tests.

Statistical calculations were performed using a statistical set StatSoft Inc. (2005), STATISTICA, Tulsa, AZ, USA (data analysis software system), version 7.1.

**Results of the trial**

$\text{PaO}_2$

The mean initial (0 h) $\text{PaO}_2$ before treatment initiation was 48.4 mm Hg ($\pm 15.0$) in group A1. After 1 h of treatment, an increase to 114.4 mm Hg ($\pm 49.8$) was observed. After 2 h, the value was 68.1 mm Hg ($\pm 15.3$), after 4 h 69.6 mm Hg ($\pm 19.0$), after 24 h 78.7 mm Hg ($\pm 18.9$) and after 48 h 67.9 mm Hg ($\pm 12.3$) (Table 1).

In group A2, the initial (0 h) oxygen pressure value before treatment was 45.1 mm Hg ($\pm 14.3$). No increase in this parameter was observed, but after 1 h, its value was 46.7 mm Hg ($\pm 14.9$). An increase up to 51.1 mm Hg ($\pm 19.6$) was observed after 2 h, which further increased to 65.5 mm Hg ($\pm 38.6$) after 4 h and a similar level of 61.9 mm Hg ($\pm 18.9$) after 24 h and 64.0 mm Hg ($\pm 14.0$) after 48 h (Table 2).

**Table 1** Partial arterial oxygen pressure in group A1

<table>
<thead>
<tr>
<th>$\text{PaO}_2$ (mm Hg)</th>
<th>N</th>
<th>Mean value</th>
<th>$-95%$ confidence interval</th>
<th>$+95%$ confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>7</td>
<td>48.4</td>
<td>34.6</td>
<td>62.2</td>
<td>15.0</td>
<td>32.6</td>
<td>70.0</td>
<td>45.0</td>
</tr>
<tr>
<td>1 h</td>
<td>7</td>
<td>114.4</td>
<td>68.3</td>
<td>160.4</td>
<td>49.8</td>
<td>36.4</td>
<td>203.0</td>
<td>116.0</td>
</tr>
<tr>
<td>2 h</td>
<td>7</td>
<td>68.1</td>
<td>54.0</td>
<td>82.3</td>
<td>15.3</td>
<td>53.0</td>
<td>86.0</td>
<td>59.0</td>
</tr>
<tr>
<td>4 h</td>
<td>7</td>
<td>69.6</td>
<td>52.0</td>
<td>87.2</td>
<td>19.0</td>
<td>52.0</td>
<td>108.0</td>
<td>67.0</td>
</tr>
<tr>
<td>24 h</td>
<td>6</td>
<td>78.7</td>
<td>61.2</td>
<td>96.2</td>
<td>18.9</td>
<td>58.0</td>
<td>112.0</td>
<td>74.0</td>
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<tr>
<td>48 h</td>
<td>6</td>
<td>67.9</td>
<td>56.5</td>
<td>79.3</td>
<td>12.3</td>
<td>51.0</td>
<td>85.0</td>
<td>62.5</td>
</tr>
</tbody>
</table>

A statistically significant increase of $\text{PaO}_2$ in group A1 was observed between 0 and 4 h of treatment ($P = 0.0311$).

**Table 2** Partial arterial oxygen pressure in group A2

<table>
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<tr>
<th>$\text{PaO}_2$ (mm Hg)</th>
<th>N</th>
<th>Mean value</th>
<th>$-95%$ confidence interval</th>
<th>$+95%$ confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
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</tr>
</thead>
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<tr>
<td>0 h</td>
<td>6</td>
<td>45.1</td>
<td>30.0</td>
<td>60.1</td>
<td>14.3</td>
<td>36.6</td>
<td>74.0</td>
<td>40.2</td>
</tr>
<tr>
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<td>6</td>
<td>46.7</td>
<td>31.0</td>
<td>62.4</td>
<td>14.9</td>
<td>28.6</td>
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<td>50.4</td>
</tr>
<tr>
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<td>30.7</td>
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<td>80.0</td>
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<td>38.6</td>
<td>23.0</td>
<td>134.0</td>
<td>54.5</td>
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<tr>
<td>24 h</td>
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<td>61.9</td>
<td>38.4</td>
<td>85.5</td>
<td>18.9</td>
<td>41.9</td>
<td>88.0</td>
<td>52.9</td>
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<tr>
<td>48 h</td>
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<td>64.0</td>
<td>46.6</td>
<td>81.4</td>
<td>14.0</td>
<td>51.1</td>
<td>88.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>

Increase of $\text{PaO}_2$ in group A2, during the 48 h treatment was not statistically significant ($P > 0.05$).
The initial values of PaO₂ in groups A1 and A2 did not differ statistically. A comparative analysis of both groups demonstrated that in group A1 the increase in PaO₂ was statistically significantly higher in comparison with group A2 after 1 h of treatment (\( P = 0.0105 \)). After several hours, the difference was still noted; however, the value was not statistically significant. After 2, 4, 24 and 48 h, there was a smaller difference in the value of PaO₂ in groups A1 and A2 (Figure 3).

**Fraction of inspired oxygen**
The mean value of the initial (0 h) fraction of inspired oxygen before treatment initiation was 95.7% ± 11.3% in group A1 and 95.0% ± 12.2% in group A2. In group A1, a drop in this parameter to 79.4% ± 23.2% was achieved after 1 h, to 67.1% ± 20.0% after 2 h, to 57.1% ± 16.6% after 4 h, and after 24 h this parameter was maintained at a similar level of 53.7% ± 23.2%. After 48 h, a drop of FiO₂ to 40.1% ± 19.6% was observed (Table 3).

In group A2, before treatment initiation (0 h) the value of FiO₂ was 95.0% ± 12.2%, and after 1 h of treatment a drop in this parameter was achieved (to 93.3% ± 10.3%), and after 2 h the level was 96.8% ± 8.3%. A drop to 89.2% ± 15.6% was observed after 4 h, and to 64.0% ± 22.2% after 24 h and to 46.0% ± 32.3% after 48 h (Table 4).

The difference in the initial values of FiO₂ in groups A1 and A2 was not statistically significant. When comparing both groups, it was observed that after the first hour of treatment, the drop of FiO₂ in group A1 was faster than it was in group A2. After several hours, a difference that was statistically insignificant was still noted. In group A1, a drop of FiO₂ was statistically significantly higher than it was in comparison with group A2 after 2 h (\( P = 0.0130 \)) and

### Table 3 Fraction of inspired oxygen in group A1

<table>
<thead>
<tr>
<th>FiO₂ (%)</th>
<th>N</th>
<th>Mean value</th>
<th>95% confidence interval</th>
<th>+95% confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
</tr>
</thead>
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<td>95.7</td>
<td>85.2</td>
<td>106.2</td>
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<td>100.0</td>
<td>100.0</td>
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<tr>
<td>1 h</td>
<td>7</td>
<td>79.4</td>
<td>57.9</td>
<td>100.7</td>
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<td>50.0</td>
<td>100.0</td>
<td>90.0</td>
</tr>
<tr>
<td>2 h</td>
<td>7</td>
<td>67.1</td>
<td>48.7</td>
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<td>40.0</td>
<td>100.0</td>
<td>60.0</td>
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<tr>
<td>4 h</td>
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<td>41.8</td>
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<td>21.0</td>
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<td>19.6</td>
<td>21.0</td>
<td>70.0</td>
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</table>

A statistically significant drop of FiO₂ in group A1 was observed between zero and first hour of treatment (\( P = 0.0323 \)).

### Table 4 Fraction of inspired oxygen in group A2

<table>
<thead>
<tr>
<th>FiO₂ (%)</th>
<th>N</th>
<th>Mean value</th>
<th>95% confidence interval</th>
<th>+95% confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
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<tr>
<td>0 h</td>
<td>6</td>
<td>95.0</td>
<td>82.1</td>
<td>107.9</td>
<td>12.2</td>
<td>70.0</td>
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<td>100.0</td>
</tr>
<tr>
<td>1 h</td>
<td>6</td>
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<td>82.5</td>
<td>104.2</td>
<td>10.3</td>
<td>80.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
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<td>6</td>
<td>96.8</td>
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<td>80.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>4 h</td>
<td>6</td>
<td>89.2</td>
<td>72.8</td>
<td>105.6</td>
<td>15.6</td>
<td>60.0</td>
<td>100.0</td>
<td>95.0</td>
</tr>
<tr>
<td>24 h</td>
<td>6</td>
<td>64.0</td>
<td>36.5</td>
<td>91.6</td>
<td>22.2</td>
<td>40.0</td>
<td>100.0</td>
<td>60.0</td>
</tr>
<tr>
<td>48 h</td>
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<td>46.0</td>
<td>5.9</td>
<td>86.1</td>
<td>32.3</td>
<td>21.0</td>
<td>100.0</td>
<td>35.0</td>
</tr>
</tbody>
</table>

After 48 h of treatment, the drop in FiO₂ that was observed was not statistically significant (\( P > 0.05 \)).

The initial values of PaO₂ in groups A1 and A2 did not differ statistically. A comparative analysis of both groups demonstrated that in group A1 the increase in PaO₂ was statistically significantly higher in comparison with group A2 after 1 h of treatment (\( P = 0.0105 \)). After several hours, the difference was still noted; however, the value was not statistically significant. After 2, 4, 24 and 48 h, there was a smaller difference in the value of PaO₂ in groups A1 and A2 (Figure 3).

### Figure 3 Partial arterial oxygen pressure in groups A1 and A2.
after 4 h of treatment ($P = 0.0113$). After 24 and 48 h, a smaller difference in the value of FiO$_2$ was observed in groups A1 and A2 (Figure 4).

**MAP**

The initial (0 h) MAP before treatment initiation was 14.3 cm H$_2$O (± 4.2) in group A1, and 13.8 cm H$_2$O (± 8.3) in group A2.

A drop in this parameter was observed after 1 h of treatment in group A1 to 11.5 cm H$_2$O (± 3.7) with a further drop in this group after 2 h to 9.8 cm H$_2$O (± 4.0) and after 4 h to 6.6 cm H$_2$O (± 5.2). A further drop was observed after 24 h to 4.1 cm H$_2$O (± 3.6). After 48 h, a further but small increase to 5.0 cm H$_2$O (± 6.1) was observed (Table 5).

In group A2, before treatment initiation (0 h), the value of MAP was 13.8 cm H$_2$O (± 8.28). After 1 h of treatment, no drop in this parameter was observed and it was 13.2 cm H$_2$O (± 5.0). After 2 h of treatment, a drop to 12.0 cm H$_2$O (± 5.2) was observed. A further drop was observed after 4 h to 10.9 cm H$_2$O (± 3.9) and after 24 h to 8.0 cm H$_2$O (± 3.7). This value decreased to 4.2 cm H$_2$O (± 2.8) after 48 h (Table 6).

The difference between the initial value of MAP in groups A1 and A2 was not statistically significant. When comparing both groups, it was noticed that after the first hour of treatment, the drop in MAP was more rapid in group A1 than in group A2. After 1 and 2 h, the difference was still noted; however, it was statistically insignificant. In group A1, a drop in MAP was statistically significantly higher in comparison with group A2 after 24 h of treatment ($P = 0.0423$) (Figure 5). After 48 h, the values of MAP in groups A1 and A2 were similar.

---

**Table 5** Mean airway pressure in group A1

<table>
<thead>
<tr>
<th>MAP (cm H$_2$O)</th>
<th>N</th>
<th>Mean value</th>
<th>−95% confidence interval</th>
<th>+95% confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>7</td>
<td>14.3</td>
<td>10.5</td>
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<td>4.2</td>
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<td>13.6</td>
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<td>14.9</td>
<td>3.7</td>
<td>8.2</td>
<td>17.4</td>
<td>9.2</td>
</tr>
<tr>
<td>2 h</td>
<td>7</td>
<td>9.8</td>
<td>6.0</td>
<td>13.4</td>
<td>4.0</td>
<td>4.6</td>
<td>15.6</td>
<td>10.8</td>
</tr>
<tr>
<td>4 h</td>
<td>7</td>
<td>6.6</td>
<td>1.8</td>
<td>11.5</td>
<td>5.2</td>
<td>1.4</td>
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<td>4.8</td>
</tr>
<tr>
<td>24 h</td>
<td>7</td>
<td>4.1</td>
<td>0.8</td>
<td>7.4</td>
<td>3.6</td>
<td>1.4</td>
<td>11.7</td>
<td>3.1</td>
</tr>
<tr>
<td>48 h</td>
<td>7</td>
<td>5.0</td>
<td>−0.7</td>
<td>10.7</td>
<td>6.1</td>
<td>1.4</td>
<td>18.6</td>
<td>2.6</td>
</tr>
</tbody>
</table>

In this group of patients, a drop in MAP that was statistically significant took place between 0 and 24 h of treatment ($P = 0.0114$).

**Table 6** Mean airway pressure in group A2

<table>
<thead>
<tr>
<th>MAP (cm H$_2$O)</th>
<th>N</th>
<th>Mean value</th>
<th>−95% confidence interval</th>
<th>+95% confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
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<tr>
<td>0 h</td>
<td>6</td>
<td>13.8</td>
<td>5.1</td>
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<tr>
<td>1 h</td>
<td>6</td>
<td>13.2</td>
<td>8.0</td>
<td>18.4</td>
<td>5.0</td>
<td>7.8</td>
<td>21.7</td>
<td>12.5</td>
</tr>
<tr>
<td>2 h</td>
<td>6</td>
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<td>17.4</td>
<td>5.2</td>
<td>6.2</td>
<td>20.7</td>
<td>12.2</td>
</tr>
<tr>
<td>4 h</td>
<td>6</td>
<td>10.9</td>
<td>6.8</td>
<td>14.9</td>
<td>3.9</td>
<td>7.2</td>
<td>17.3</td>
<td>10.5</td>
</tr>
<tr>
<td>24 h</td>
<td>6</td>
<td>8.0</td>
<td>3.4</td>
<td>12.6</td>
<td>3.7</td>
<td>4.3</td>
<td>13.9</td>
<td>7.2</td>
</tr>
<tr>
<td>48 h</td>
<td>6</td>
<td>4.2</td>
<td>0.8</td>
<td>7.6</td>
<td>2.8</td>
<td>2.2</td>
<td>8.9</td>
<td>3.5</td>
</tr>
</tbody>
</table>

In this group, the drop in MAP during 48 h of treatment was statistically significant between the 1st and 48th hour of treatment ($P = 0.0008$).
The mean initial (0 h) value of the oxygenation index before treatment initiation was 29.8 (±12.5) in group A1 and 32.4 (±25.0) in group A2. In group A1, this value decreased after several hours and was 11.2 (±12.5) after 1 h, 10.0 (±5.5) after 2 h and 6.3 (±6.4) after 4 h. After 24 h, this value decreased to 2.7 (±2.2), and after 48 h to 5.0 (±9.1) (Table 7).

In group A2, a drop in OI between 0 and 48 h of treatment was statistically significant (P=0.0011).

### Table 7 Oxygenation index in group A1

<table>
<thead>
<tr>
<th>OI</th>
<th>N</th>
<th>Mean value</th>
<th>−95% confidence interval</th>
<th>+95% confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
</tr>
</thead>
<tbody>
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<td>29.8</td>
<td>18.3</td>
<td>41.4</td>
<td>12.5</td>
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<td>11.2</td>
<td>−0.3</td>
<td>22.7</td>
<td>12.5</td>
<td>3.8</td>
<td>39.0</td>
<td>6.9</td>
</tr>
<tr>
<td>2 h</td>
<td>7</td>
<td>10.0</td>
<td>4.9</td>
<td>15.1</td>
<td>5.5</td>
<td>3.8</td>
<td>18.1</td>
<td>10.0</td>
</tr>
<tr>
<td>4 h</td>
<td>7</td>
<td>6.3</td>
<td>0.4</td>
<td>12.3</td>
<td>6.4</td>
<td>1.2</td>
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<td>3.9</td>
</tr>
<tr>
<td>24 h</td>
<td>7</td>
<td>2.7</td>
<td>0.7</td>
<td>4.8</td>
<td>2.2</td>
<td>0.4</td>
<td>6.7</td>
<td>2.8</td>
</tr>
<tr>
<td>48 h</td>
<td>7</td>
<td>5.0</td>
<td>−3.4</td>
<td>13.5</td>
<td>9.1</td>
<td>0.4</td>
<td>25.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

A statistically significant drop took place between 0 and 1 h (P=0.0003), 0 and 4 h (P=0.0398) and 0 and 48 h of treatment (P=0.0001).

### Table 8 Oxygenation index in group A2

<table>
<thead>
<tr>
<th>OI</th>
<th>N</th>
<th>Mean value</th>
<th>−95% confidence interval</th>
<th>+95% confidence interval</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Median</th>
</tr>
</thead>
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<td>6.1</td>
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<td>80.0</td>
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<td>28.8</td>
<td>16.1</td>
<td>41.4</td>
<td>12.0</td>
<td>11.0</td>
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<td>35.9</td>
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<tr>
<td>2 h</td>
<td>6</td>
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<td>19.0</td>
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<tr>
<td>4 h</td>
<td>6</td>
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<td>18.4</td>
<td>4.8</td>
<td>53.0</td>
<td>18.2</td>
</tr>
<tr>
<td>24 h</td>
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<td>10.4</td>
<td>0.4</td>
<td>20.5</td>
<td>8.1</td>
<td>1.9</td>
<td>22.1</td>
<td>9.0</td>
</tr>
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<td>−5.6</td>
<td>17.0</td>
<td>9.1</td>
<td>0.7</td>
<td>22.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

In group A2, a drop in OI between 0 and 48 h of treatment was statistically significant (P=0.0011).

### Oxygenation index

The mean initial (0 h) value of the oxygenation index before treatment initiation was 29.8 (±12.5) in group A1 and 32.4 (±25.0) in group A2. In group A1, this value decreased after several hours and was 11.2 (±12.5) after 1 h, 10.0 (±5.5) after 2 h and 6.3 (±6.4) after 4 h. After 24 h, this value decreased to 2.7 (±2.2), and after 48 h to 5.0 (±9.1) (Table 7).

In group A2, a drop in this parameter was observed after 1 h to 28.8 (±12.1), after 2 h to 26.8 (±19.0), after 4 h to 22.2 (±18.4), after 24 h to 10.4 (±8.1), and after 48 h a drop to 5.7 (±9.1) (Table 8).

The difference in the initial value of OI in groups A1 and A2 was not statistically significant. When comparing both groups, it was noted that after 1 h of treatment a drop in OI in group A1 was statistically significantly faster than it was in group A2 (P=0.0222). In group A1, a drop in OI was also statistically significantly higher when compared with group A2 after 2 h (P=0.0455), 4 h (P=0.0455) and 24 h of treatment (P=0.0423). After 48 h, the values of OI in groups A1 and A2 were similar (Figure 6).

### Alveolar–arterial oxygen gradient

The initial values of AaDO2 before treatment initiation (0 h) were 575.6 mm Hg (±91.0) for group A1 and 589.5 mm Hg (±95.8) for group A2. In group A1, a drop in these values was observed after 1 h of treatment to 423.7 mm Hg (±184.3), after 2 h to...
364.2 mm Hg (± 128.8), after 4 h to 305.3 mm Hg (± 122.9), after 24 h to 261.0 mm Hg (± 160.9), and after 48 h to 178.3 (± 144.0).

A drop in AaDO₂ during treatment was statistically significant between 0 and 24 h (P = 0.0024) and between 0 and 48 h of treatment (P = 0.0021).

In group A2, after 1 h the value was 575.3 mm Hg (± 88.2). After 2 h, an increase to 581.2 mm Hg (± 71.2) in this parameter was observed. A drop was noted after 12 h to 506 mm Hg (± 112.6), with a further drop after 24 h to 352.3 mm Hg (± 177.5) and after 48 h to 226.0 mm Hg (± 239.8).

A drop in AaDO₂ during the time of the treatment was statistically significant between 0 and the 48th hour of treatment (P = 0.0017).

The difference between the initial values of AaDO₂ in groups A1 and A2 was not statistically significant. When comparing both groups, it was noted that after 1 h of treatment a drop in AaDO₂ was more rapid in group A1 than in group A2. Subsequently, the difference was statistically significant (P = 0.0015). After 4 h, a drop of AaDO₂ in group A1 was also statistically significantly higher in comparison with group A2 (P = 0.0106). After 24 h and 48 h, a smaller difference was observed between the value of AaDO₂ in groups A1 and A2. These differences were statistically insignificant (Figure 7).

**Duration of mechanical ventilation**
In group A1, the mean time on mechanical ventilation was 6.6 days (± 2.6), and in group A2 it was 7.3 days (± 1.7). This difference was not statistically significant (P > 0.05).

**Duration of iNO treatment**
The mean duration of iNO treatment was 2.9 days (± 1.5) in group A1. In group A2, this value was higher and the duration was 4 days (± 1). This difference was not statistically significant (P > 0.05).

**Length of hospitalization**
In group A1, the length of hospitalization was 16.4 days (± 5.4) and in group A2 it was 19.8 days (± 2.9). This difference was not statistically significant (P > 0.05).

**Complications**

**Air leaks.** The incidence of pneumothorax was analyzed. In group A1, no cases of pneumothorax were noted. This complication was observed in two newborns from group A2. This value was not statistically significant (P > 0.05).

**Deaths.** There were no deaths in group A1. There were two cases of deaths in group A2. This difference was not statistically significant (P > 0.05).

**Discussion**
Meconium in the airways and alveoli leads to inactivation of endogenously and exogenously replaced surfactant. Therefore, we hypothesized that SLL would improve the clearance of meconium from the airways while replacing the surfactant. Earlier studies on
the use of SLL in newborn piglets were published by Paranka et al.\textsuperscript{8} A solution of "Survanta" was used for lung lavage. Following this treatment, a rapid improvement in oxygenation was observed. Similar experimental studies on the use of SLL in the treatment of MAS were conducted at different centers by Dargaville et al.\textsuperscript{2} and Ogawa and co-workers.\textsuperscript{10–12}

The first clinical studies on SLL were conducted by Ogawa\textsuperscript{10} and Lam and Yeung.\textsuperscript{15} Ogawa administered as a lavage a surfactant solution with a concentration of 12 mg of phospholipids in 1 ml of physiological salt. Earlier, Ohama et al.\textsuperscript{11} attempted to prove that a higher concentration is more effective. The volume of the solution was 5 ml per kg of body weight. A significant drop in OI and AaDO\textsubscript{2} was achieved after 4 h of treatment.

Another attempt to define the most optimal dose of surfactant for lavage was undertaken by Chang et al.\textsuperscript{14} In group I, the lungs were washed with a surfactant solution with a concentration of 5 mg ml\textsuperscript{–1} of physiological salt. In group II, lavage was conducted with a surfactant solution with a concentration of 10 mg ml\textsuperscript{–1}. A significant increase in PaO\textsubscript{2} was observed within 24 h. It was noted that both the first and the second doses of surfactant were effective.

The next phase of study was a multi-center study by Ogawa\textsuperscript{10} conducted in 11 centers. A comparative analysis of the group with SLL against the group without SLL demonstrated that a drop in the oxygenation parameter and an increase in the oxygen pressure after 180 min were significantly higher in the SLL group than in the control group.

Interesting results of clinical trials were presented by Lam and Yeung.\textsuperscript{13,15} "Survanta", a natural surfactant solution with a concentration of 5 mg phospholipids per ml of saline and a quantity of 15 ml per kg of body weight, was administered as a lavage. The results of the trial demonstrated that the average values of OI, MAP, AaDO\textsubscript{2} and FiO\textsubscript{2} were significantly and statistically lower after 48 h of treatment.

Similar trial results were published in 2003 by Maroszyńska et al.\textsuperscript{16,17} The inclusion criteria used in our study did not differ much from the ones proposed by other researchers. Some of the researchers quoted above considered the level of OI ≥ 20 as the only inclusion criterion. In our study, the criteria used were similar to those used by Lam and Yeung.\textsuperscript{13,15} The only difference was in the time for enrollment in the trial, which was extended to 24 h (average time to include newborns transferred from a level II unit). We did not use OI and MAP as the inclusion criteria; only the value of FiO\textsubscript{2} ≥ 40% was used.

A more extended time for the inclusion in the study—up to 120 h was proposed only by Maroszyńska et al.\textsuperscript{16,17} The majority of the quoted experimental and clinical trials as well as our own study studied the use of natural surfactant solutions in SLL. Earlier, Meister et al.\textsuperscript{19} compared the natural and the synthetic surfactants. They proved that there are no statistically significant differences in the results achieved after administrations of either type of surfactant in SLL. Studies on these subjects were also published by Wiswell et al.\textsuperscript{20} Their trial was focused on the use of "Survanta", a new generation synthetic surfactant solution. The trial group comprised 22 newborns and the control group included 15 newborns. The inclusion criteria were similar to the ones quoted above. A statistically significantly faster drop of OI after SLL was observed in comparison with the control group.

The dose of surfactant used for lavage is still a subject for discussion. The smallest dose (8 ml per kg of body weight) of lavage solution was proposed by Wiswell et al.\textsuperscript{21} Others have proposed a dose of 10 ml solution per kg of body weight.\textsuperscript{9,14} The most frequently suggested dose is a higher dose—from 15 to 20 ml kg\textsuperscript{–1}.\textsuperscript{10,17} In his earlier experimental trials, Ogawa\textsuperscript{10} attempted to define the most optimal dose of the lavage solution and he came to the conclusion that a dose of 10 ml kg\textsuperscript{–1} is most effective.

Similarly, the concentration used in the solution is also a subject of trials. Wiswell et al.\textsuperscript{21} proposed the lowest concentration of the drug—2.5 mg ml\textsuperscript{–1} of physiological salt. The majority of researchers propose a dose from 4 to 5 mg ml\textsuperscript{–1}.\textsuperscript{12} Only Ogawa in their subsequent clinical studies proposed a higher dose of 12 mg ml\textsuperscript{–1}. On the other hand, Chang et al.\textsuperscript{14} proved that a dose of 5 mg ml\textsuperscript{–1} is as effective as a dose of 10 mg ml\textsuperscript{–1}. In our trial, a concentration of 5 mg ml\textsuperscript{–1} at a dose of 15 ml per kg of body weight was used.

All of the aforementioned clinical trials on the use of SLL in MAS used saline as control groups. None compared bolus treatments with SLL. Meister et al.\textsuperscript{10} conducted experimental trials on a rabbit model in which they compared the results of surfactant with bolus surfactant treatment. They also studied the differences in natural and synthetic surfactant treatments. A significant increase in PaO\textsubscript{2} was observed in both natural and synthetic surfactants, SLL, in comparison with groups treated with bolus surfactant and a control group. The increase was observed after 4 h of treatment.

The group of patients with the most severe clinical course of MAS includes cases complicated by PPHN. In the majority of cases, studies on the use of iNO in the treatment of PPHN in term neonates with MAS confirm its effectiveness in oxygenation improvement.\textsuperscript{2,24} It was observed that, 30 min after the administration of NO, there was a visible oxygenation improvement manifested by an increase in PaO\textsubscript{2} and a drop in OI and AaDO\textsubscript{2}.

When conducting independent randomized trials, Chen and Kao observed a significant drop in OI after iNO treatment of PPHN in MAS. Chen et al.\textsuperscript{1} noted improvement in 30 min of iNO, whereas Kao et al. observed a statistically significant drop in OI after 60 min of iNO.\textsuperscript{35} The effectiveness of iNO in the treatment of PPHN in MAS was also proved by Gupta et al.\textsuperscript{30} A clinical trial on the use of iNO in PPHN in MAS was also conducted by Hwang et al.\textsuperscript{27} After 1 h of iNO treatment, they observed a statistically significant drop of OI and MAP.
The neonates presented in our study who were diagnosed by an echocardiographic examination with PPHN in the course of a severe MAS, were treated with surfactant prior to the administration of iNO. In this group of patients, the intention was to assess the combined therapy as well as to answer the question whether it was more effective to use SLL followed by bolus surfactant or surfactant only prior to the administration of iNO. The initial dose of NO was 20 p.p.m. The treatment was conducted in the first 24 h of the newborn’s life. The average time of treatment initiation was 9.7 ± 7.4 h in group A1 and 11 ± 6.4 h in group A2. Oxygenation, ventilation and other selected clinical parameters were assessed.

Rais-Bachrami et al.28 attained a faster improvement in oxygenation after surfactant administration followed by iNO. After 20 min, they lowered the dose of NO and after 15 min they stopped the dose of iNO.

The administration of bolus surfactant followed by iNO (group A2) had a positive influence on the improvement in oxygenation. However, in a majority of cases, these results were not statistically significant. An increase in PaO2 was observed only after 2 h of treatment, and a further increase was noted within 48 h; however, this was not statistically significant. A similar tendency was observed in the values of FiO2 and OI. Within 48 h, a gradual decrease in these parameters was observed; however, this drop was not statistically significant either. AaDO2 declined as well. A statistically significant drop was noted after 24 h and then after 48 h of treatment as well as in the time range between the 12th and 24th hour of treatment. A drop in MAP was observed only after 4 h of treatment and a further drop was noted until the 48th hour of treatment; however, this drop was not statistically significant. Substantially better results were observed in cases when, prior to iNO, SLL was used followed by one dose of bolus surfactant.

There is a lack of information regarding the effectiveness of this therapy. Investigators have used solely iNO treatment in severe cases of MAS complicated by PPHN. A drop in OI and an increase in PaO2 were observed after 30 min,29 after 60 min8 and after 24 h of iNO treatment29 in different studies. In our studies, a significant increase in PaO2 was observed in the group with SLL after 1 h. In the SLL group, a drop in O1 and FiO2 was statistically significant after 24 and 48 h. When we compared SLL with the group without SLL, we noticed statistically significant differences much sooner, that is, after the first, second and fourth hours.

The drop in AaDO2 in the SLL group was substantial after 2 and 4 h, with a further drop after 24 and 48 h. MAP also declined significantly in the SLL group after 24 h. However, when comparing the two groups we observed a significant difference only after 48 h. These results are similar to others who did not use SLL but were treated with only iNO. Therefore, we cannot conclude with certainty whether the use of SLL increases the effectiveness of iNO.

Similar to our findings, others did not find statistically significant differences in the length of time on IMV, number of complications and deaths when in comparison with the group that was not treated with iNO. Similarly, in our trial no statistically significant differences were observed in the length of mechanical ventilation and hospitalization. In the group with SLL, bolus surfactant and iNO, as well as no air leaks or deaths, were encountered. In the group with bolus surfactant and iNO, there were 2/6 cases of pneumothorax and 2/6 deaths. However, this difference was not statistically significant. In the first case, pneumothorax was one of the causes of death. In the second case which was complicated by pneumothorax, the patient survived. The other death was due to a sudden stop in heart activity in the course of a severe circulatory failure.

During our study, there was no attempt to answer the important question whether the use of bolus surfactant along with SLL will prevent the development of PPHN and whether it may decrease the need for iNO use. Chang et al.14 attempted to address this question. They conducted clinical studies on neonates with MAS in whom OI was ≥ 20. Prior to iNO use, the airways were washed with a surfactant solution with a concentration of 5 mg ml⁻¹ or 10 mg ml⁻¹ of physiological salt. A statistically significant increase in PaO2 was observed within 24 h; 5 of 12 neonates required iNO treatment.

Therefore, the results of the trials conducted by researchers quoted in this paper as well as by our own trial indicate that, in severe cases of MAS complicated by PPHN, the administration of bolus surfactant or SLL followed by bolus surfactant prior to iNO treatment improves oxygenation of the patient and may lead to a decrease both in the severity of PPHN and in the duration of iNO treatment. However, no significant decrease in the duration of IMV, hospitalization or in the number of complications and deaths was observed.

Conclusions

(1) The combined therapy: SLL followed by bolus surfactant, in combination with iNO in the treatment of meconium aspiration syndrome accompanied by PPHN, showed significant improvement in oxygenation as well as a decrease in mechanical ventilation parameters.

(2) However, SLL followed by bolus surfactant as well as combined therapy: SLL followed by bolus surfactant combined with iNO did not exert significant influence over the duration of mechanical ventilation, iNO treatment as well as the length of hospitalization and the number of complications.

(3) On account of small number of the patients, these studies do not have enough power to choose a particular combination of therapies. There is a need for larger series to answer these questions.

Disclosure

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Nitric oxide and beyond: new insights and therapies for pulmonary hypertension

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Persistent pulmonary hypertension of the newborn (PPHN) contributes significantly to the morbidity and mortality associated with meconium aspiration syndrome. This review article discusses new insights into the vascular abnormalities that are associated with PPHN, including the recent recognition of the importance of oxidant stress in its pathogenesis. Recent data are presented showing that treatment with high oxygen concentrations may increase production of oxygen free radicals. The rationale for the use of inhaled nitric oxide, and strategies for enhancing nitric oxide signaling are discussed. Finally, the rationale for new treatment approaches is reviewed, including inhibition of cyclic guanosine monophosphate-specific phosphodiesterases and scavengers of reactive oxygen species.

Introduction

Neonatal respiratory failure affects 2% of all live births and is responsible for a substantial proportion of neonatal mortality. Although preterm infants are at higher risk of respiratory failure, term and near-term infants account for one-third of the cases. A better understanding of the pathophysiology of hypoxic respiratory failure is needed to develop more specific and effective therapies. This review will focus on recent progress in our understanding of pulmonary hypertension, which is commonly associated with the severe respiratory failure that accompanies meconium aspiration syndrome (MAS).

Pathophysiology of PPHN

Shortly after birth, the fetus normally undergoes a rapid cardiopulmonary transition to meet the new demands of extrauterine life. However, if pulmonary vascular resistance does not fall, pulmonary blood flow cannot increase, and the result is hypoxic respiratory failure or persistent pulmonary hypertension of the newborn (PPHN). The incidence of severe PPHN is estimated at 0.2% of live-born term infants. MAS is the most common cause of PPHN, and when present, it can contribute significantly to its morbidity and mortality. Newborns with hypoxemic respiratory failure and/or PPHN are at risk for numerous complications including death, neurological injury and other morbidities.

Persistent pulmonary hypertension of the newborn can generally be characterized as one of three types: (1) the abnormally constricted pulmonary vasculature due to lung parenchymal diseases, such as MAS, respiratory distress syndrome or pneumonia; (2) the lung with normal parenchyma and remodeled pulmonary vasculature, also known as idiopathic PPHN; or (3) the hypoplastic vasculature as seen in congenital diaphragmatic hernia. The most common cause of PPHN is MAS. Infants with MAS will typically fall into the first or second categories, and the most severe cases are probably affected by both parenchymal and vascular disease.

Understanding the pathophysiology of the abnormally remodeled pulmonary vasculature is of utmost importance in directing therapy. As it is not feasible to study the remodeling process in the human infant, much of our current understanding is derived from animal models. One cause of idiopathic PPHN is constriction of the fetal ductus arteriosus in utero because of exposure to nonsteroidal anti-inflammatory drugs during the third trimester. Ductal constriction or ligation can be surgically performed in utero in lambs, leading to rapid antenatal remodeling of the pulmonary vasculature. Findings in PPHN lambs are similar to those observed in human infants, including increased fetal pulmonary artery pressure, pulmonary vascular remodeling and profound hypoxemia after birth.

On the basis of work from animal models, there is strong evidence that disruptions of the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP), prostacyclin-cyclic adenosine monophosphate (cAMP) and endothelin signaling pathways play an important role in the vascular abnormalities associated with PPHN. The NO-cGMP pathway has been a topic of particularly intense investigation over the past decade. Decreased expression and activity of endothelial NO synthase have been documented in the PPHN lamb model, and decreased endothelial NO synthase expression has also been reported in umbilical venous endothelial cell cultures from human infants with meconium staining who develop PPHN. These important findings were rapidly followed by
clinical testing of inhaled NO (iNO) as a therapy for hypoxemic respiratory failure and PPHN.

Inhaled nitric oxide

The primary aim of PPHN therapy is selective pulmonary vasodilatation. Inhaled NO appears to be well suited for this effect: it is a rapid and potent vasodilator, and because NO is a small gas molecule, it can be delivered through a ventilator directly to airspaces approximating the pulmonary vascular bed. Once in the blood stream, NO binds avidly to hemoglobin, limiting its systemic vascular activity and increasing its selectivity for the pulmonary circulation. Large placebo-controlled trials provided clear evidence that iNO significantly decreases the need for extracorporeal membrane oxygenation (ECMO) support in newborns with PPHN. However, it is important to note that up to 40% of infants will not improve oxygenation or maintain a response to iNO, and iNO did not reduce mortality or length of hospitalization. In addition, follow-up studies for 12 to 24 months indicate that iNO does not significantly alter the incidence of chronic lung disease or neurodevelopmental impairment. This is an interesting and important observation that may indicate that the underlying disease is associated with early neurological injury. Finally, Konduri et al. determined that starting iNO earlier in the disease course (for an oxygenation index of 15 to 25) did not decrease the incidence of ECMO and/or death or improve other patient outcomes, including the incidence of neurodevelopmental impairment.

Following the introduction of high-frequency ventilation (HFV), surfactant and iNO in the early 1990s, the patient demographic of neonatal support with ECMO has changed. Data from the large registry maintained by the Extracorporeal Life Support Organization indicate that the use of these therapies has increased steadily over the past 10 years, accompanied by a greater than 40% reduction in the number of neonates cannulated for ECMO. However, as overall ECMO survival has diminished over the same time period, some physicians have speculated that these new treatment modalities may delay ECMO cannulation and have a negative effect on mortality and morbidity in those infants that continue to require extracorporeal support. Therefore, we recently examined data from the Extracorporeal Life Support Organization registry between 1996 and 2003. We found that NO, HFV and surfactant use were not associated with any adverse outcomes during ECMO, including increased hours on ECMO or increased time to extubation. Furthermore, both surfactant and NO use were associated with lower ECMO mortality, and NO use was associated with a decreased risk of cardiac arrest before cannulation. As ECMO is a proven therapy for severe respiratory failure, it is reassuring that these new therapies have not had a negative impact on the most severely affected infants.

New insights into PPHN pathophysiology

As NO is not universally effective, there has been considerable interest in better understanding the biochemical pathways that regulate pulmonary vasoconstriction and remodeling in PPHN. For instance, NO mediates vasodilatation by stimulating soluble guanylate cyclase in vascular smooth muscle cells, which then converts guanosine triphosphate to cGMP (Figure 1). cGMP is the central and critical second messenger that regulates contractility of the smooth muscle cell by modulating the activity of cGMP-dependent kinases, phosphodiesterases and ion channels. The cGMP-dependent or type 5 phosphodiesterase is potentially important, as it can downregulate cGMP concentrations by degrading cGMP to the inactive 5′-GMP. Therefore, there are critical points in the pathway downstream of NO production that serve as attractive targets for manipulating cellular cGMP concentrations. For example, expression and activity of soluble guanylate cyclase are decreased in the abnormally remodeled pulmonary vessels of the PPHN lamb model, which could potentially diminish responses to both endogenous and exogenous NO. This finding would indicate that new compounds that directly stimulate sGC at an NO-independent but heme-dependent site may be helpful, a hypothesis that appears to be promising in preclinical testing. Another potential cause for low cGMP concentrations would be increased expression and/or activity of cGMP-specific phosphodiesterases, which could then be manipulated through use of specific inhibitors.

Several new lines of evidence now indicate that oxidant stress is important in the pathogenesis of PPHN. An increase in reactive...
oxygen species (ROS) such as superoxide and hydrogen peroxide in the smooth muscle and adventitia of pulmonary arteries has been demonstrated in the PPHN lamb model. Possible sources of elevated concentrations of ROS include increased expression and activity of NADPH oxidase and a reduction in superoxide dismutase (SOD) activity. PPHN lambs also demonstrate diminished binding of the chaperone protein, heat shock protein 90, to endothelial NO synthase. Decreased heat shock protein 90–endothelial NO synthase interactions lead to an ‘uncoupling’ of NOS activity, which results in decreased synthesis of NO and increased superoxide production. Once present in the lung, elevated concentrations of ROS are believed to play a role in vascular smooth muscle cell proliferation in PPHN, as well as abnormal vascular reactivity.

Finally, current therapeutic practices may have an effect on pulmonary vascular reactivity and remodeling. In particular, the use of oxygen has recently become controversial in numerous settings. While oxygen is a pulmonary vasodilator, the extreme hyperoxia routinely used in PPHN management may be toxic to the developing lung by the formation of ROS. Superoxide anions combine with nitric oxide (NO) to form peroxynitrite, a potent pulmonary vasoconstrictor. Superoxide dismutase (SOD) enzyme converts superoxide anions to hydrogen peroxide (H₂O₂), also a pulmonary vasoconstrictor. When membrane lipids (arachidonic acid and polyunsaturated fatty acids (PUFA)) are exposed to ROS, such as superoxide anions and hydrogen peroxide or peroxynitrite, a variety of isoprostanes are formed. Isoprostanes are known constrictors of pulmonary arteries. Adapted from Lakshminrusimha et al. with permission.

Figure 2 Hypothesized role of reactive oxygen species (ROS) and their metabolites in mediating increased pulmonary arterial contractility following exposure to 100% oxygen. Exposure to high oxygen concentrations results in the formation of superoxide anions (O₂⁻). Superoxide anions combine with nitric oxide (NO) to form peroxynitrite, a potent pulmonary vasoconstrictor. Superoxide dismutase (SOD) enzyme converts superoxide anions to hydrogen peroxide (H₂O₂), also a pulmonary vasoconstrictor. When membrane lipids (arachidonic acid and polyunsaturated fatty acids (PUFA)) are exposed to ROS, such as superoxide anions and hydrogen peroxide or peroxynitrite, a variety of isoprostanes are formed. Isoprostanes are known constrictors of pulmonary arteries. Adapted from Lakshminrusimha et al. with permission.

Alternative and emerging pulmonary vasodilators
An improved knowledge of the biochemical abnormalities responsible for refractory PPHN is leading to a growing list of promising new therapeutic strategies. Many investigators are seeking to enhance cGMP-mediated vasodilation through the use of cGMP-specific phosphodiesterase inhibitors, direct soluble guanylate cyclase activators, scavengers of ROS as well as manipulation of the cAMP pathway.

Similar to cGMP, cAMP also stimulates vasodilatation (Figure 1). One potential approach that might take advantage of this mechanism is using milrinone to inhibit PDE3, the phosphodiesterase that metabolizes cAMP. Milrinone has been shown to decrease pulmonary artery pressure and resistance and to act additively with iNO in animal studies. Recent reports indicate that it may decrease rebound pulmonary hypertension after discontinuation of iNO and may enhance pulmonary vasodilation of infants refractory to iNO. Prostacyclin (PGI₂) stimulates...
membrane bound adenylate cyclase, increases cAMP and inhibits pulmonary artery smooth muscle cell proliferation in vitro. Although the use of systemic infusions of PGI₂ may be limited by systemic hypotension, inhaled PGI₂ has been shown to have vasodilator effects limited to the pulmonary circulation. Reports in children have been positive, but to date there have been few reports of inhaled PGI₂ use in neonates with PPHN. ¹⁹ It is most likely that further investigations will focus on preparations specifically designed for inhalation, such as iloprost.

There has been particular interest in the inhibition of cGMP-metabolizing phosphodiesterase activity, which would theoretically increase cGMP concentrations and result in pulmonary vasodilation and/or increased efficacy of iNO (Figure 1). On the basis of clinical trials, sildenafil, a potent and highly specific phosphodiesterase inhibitor, has recently been approved by the Food and Drugs Administration for the treatment of pulmonary hypertension in adults. In lambs with experimental pulmonary hypertension, both enteric and aerosolized sildenafil dilate the pulmonary vasculature and augment the pulmonary vascular response to iNO. In lambs with experimental pulmonary hypertension, both systemic and inhalational sildenafil induce vasodilation and augment the pulmonary vascular response to iNO. Intravenous sildenafil was found to be a selective pulmonary vasodilator with efficacy equivalent to inhaled NO in a piglet model of meconium aspiration, although hypotension and worsening oxygenation resulted when it was used in combination with iNO.²⁰,²¹ Sildenafil may attenuate rebound pulmonary hypertension after withdrawal of iNO in newborn and pediatric patients.²² Use of sildenafil in PPHN has been limited by its availability only as an enteric form, although a recent report indicates that it improved oxygenation and survival in human infants with PPHN compared with placebo.²³ A pilot trial of intravenous sildenafil was recently conducted in newborns with pulmonary hypertension, and data analysis is nearing completion.

New laboratory studies indicate that scavengers of ROS such as SOD may augment responsiveness to iNO. As described above, increased production of superoxide is noted in experimental models of PPHN. As iNO is usually delivered with high concentrations of oxygen, there is the potential for enhanced production of additional oxidants such as peroxynitrite. Superoxide dismutase scavengers and converts superoxide radical to hydrogen peroxide, which is subsequently converted to water by the enzyme catalase. Administration of recombinant human SOD (rhSOD) has been tested in preterm infants without adverse effects and with trends toward decreased pulmonary morbidity. In lambs with pulmonary hypertension, rhSOD was found to dilate the pulmonary circulation and enhance responsiveness to inhaled NO.²⁴ A recent study examined the effects of rhSOD on oxygenation over a 24-h period in ventilated PPHN lambs.²⁵ The results showed that a single dose of rhSOD by itself improved oxygenation to a degree that was similar to iNO (Figure 3). Furthermore, rhSOD treatment appeared to block formation of oxidants such as peroxynitrite and isoprostanes. Thus, an antioxidant therapeutic approach may have multiple beneficial effects: scavenging superoxide may make both endogenous and inhaled NO more available to stimulate vasodilation and may also reduce oxidative stress and limit lung injury. It is hoped that human trials will begin soon.

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References


REVIEW
Pharmacotherapy for meconium aspiration

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In this article we have attempted to review the current pharmacological treatment options for infants with meconium aspiration syndrome with or without persistent pulmonary hypertension. These treatments include ventilatory support, surfactant treatment and inhaled nitric oxide (INO), in addition to older and newer pharmacological treatments. These include sedatives, muscle relaxants, alkali infusion, antibiotics and the newer vasodilators. Many aspects of treatment, including ventilatory care, surfactant treatment and the use of INO, are reviewed in great detail in this issue. On the other hand, many newer pharmacological modalities of treatment described here have not been evaluated with randomized control trials. We have given an overview of these emerging therapies.


Introduction
Meconium aspiration syndrome (MAS) is defined as the presence of respiratory distress and hypoxemia associated with the presence of meconium during or just before delivery. Meconium-stained amniotic fluid is a relatively common occurrence seen in approximately 10 to 15% of deliveries and approximately 5% of these develop true MAS.1 The most severe cases require assisted ventilation for greater than 48 h and are often associated with persistent pulmonary hypertension. Several recent publications and other reviews in this issue have described in detail the pathophysiology and different aspects of the management. Our goal is to review the pharmacological management of MAS.

Muscle relaxants
Prior to 2000, depolarizing muscle relaxants were widely used along with opioids to decrease agitation and subsequent hypoxic episodes in ventilated infants. Pancuronium and Vecuronium (0.1 mg kg\(^{-1}\) h\(^{-1}\)) are the two most commonly used muscle relaxants in neonatal intensive care unit. The benefits of neuromuscular blockade include improved oxygenation, decreased oxygen consumption and decreased accidental extubations. However, a prospective multicenter observational study by Walsh-Sukys et al.5 showed considerable variations in muscle relaxant use between centers (33 to 98%). Use of muscle relaxants was also associated with increased mortality (Odds ratio: 1.95, confidence interval: 0.74, 5.18). In their series, 41% (total \(N = 385\)) of the cases had MAS as the primary diagnosis. The side
effects of nondepolarizing agents include cardiovascular effects from histamine release, though this is less common in neonates and also tachycardia (vagolytic effect). Neuromuscular blockade can also mask seizures especially in infants depressed at birth. No randomized control studies are available at this time. Neonatologists are urged to consider the risks and benefits of neuromuscular blockade prior to their use.

Alkalinization
Increasing the pH from 7.45 to 7.5 either by hyperventilation or by sodium bicarbonate infusion has been shown to produce pulmonary vasodilatation both in animal models and human newborns. However, both hyperventilation and sodium bicarbonate infusion should be used with caution in newborns due to their adverse effects on cerebral and coronary circulation. Sodium bicarbonate infusion can increase intracellular acidosis and worsen myocardial perfusion and cardiac output. Hyperventilation may also increase barotrauma and volutrauma, and increase the risk for chronic lung disease. Hypocarbia below 25 mm Hg can also result in hearing loss secondary to injury to hair cells. Walsh-Sukys et al. reported an increased need for extracorporeal membrane oxygenation (ECMO) and oxygen at 28 days in patients treated with sodium bicarbonate. Neither hyperventilation nor alkali infusion have been rigorously tested in a RCT. Fortunately their use seems to be on the decline since the availability of INO.

Initial management has also traditionally included inotropic support to assist cardiac function against suprasystemic pulmonary pressures. Once again, there is no randomized study to support this practice. Drummond et al. were able to show in a case series that dopamine infusion along with tolazoline and hyperventilation helped reduce pulmonary pressure. The combination of tolazoline and dopamine had a variable and unpredictable effect on oxygenation. It is noted that these drugs were not studied independently of each other. Hypotension is frequently secondary to heavy sedation and the use of high mean airway pressure. Dopamine and dobutamine are the two most frequently used inotropes in neonates. High doses of dopamine (>10 μg kg⁻¹ min⁻¹) on its own can also contribute to pulmonary vasoconstriction.

Pulmonary vasodilators
Regulation of pulmonary vascular tone is a dynamic process and depends on the balance between various endogenous constrictors (endothelin and thromboxane) and dilators (nitric oxide (NO) and prostaglandins). Majority of these substances are produced by the pulmonary endothelium. In addition, arterial oxygen and carbon dioxide tension, and lung volume play a major role in the regulation of pulmonary vascular tone. Pulmonary hypertension in patients with MAS is secondary to hypoxia, acidosis, release of the inflammatory mediators and alveolar atelectasis. Thus the pulmonary hypertension of MAS should ideally be treated with adequate ventilation and pulmonary vasodilators. The problem until recently was the inability to find such a truly selective pulmonary vasodilator. We will briefly review the past and present pulmonary vasodilators used in MAS. Table 1 shows dosages of various pulmonary vasodilators used in pulmonary hypertension.

Tolazoline
Tolazoline is a nonspecific vasodilator, which had been used for the treatment of pulmonary hypertension at least two decades before the introduction of INO. Lee and Hox showed that tolazoline led to pulmonary vasodilatation even in the absence of endothelium. Whereas Curtis et al. showed in the animal model that the vasodilatation produced was independent of NO production. Its mechanism of action is directly on the vascular...
Table 1 Pulmonary vasodilators

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dose</th>
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<tbody>
<tr>
<td>Tolazoline</td>
<td>IV</td>
<td>0.5–2 mg kg⁻¹</td>
</tr>
<tr>
<td></td>
<td>(NEB)</td>
<td>1–2.5 mg kg⁻¹</td>
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<tr>
<td>Magnesium sulfate</td>
<td>IV</td>
<td>200 mg kg⁻¹ bolus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–150 mg kg⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>IV</td>
<td>1–2 q12 to q6 h¹⁷</td>
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**PDE inhibitors**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipyridamole</td>
<td>IV</td>
<td>0.3–0.6 mg kg⁻¹</td>
</tr>
<tr>
<td>Esprostenol/prostacyclin</td>
<td>IV</td>
<td>2–5 ng kg⁻¹ min⁻¹</td>
</tr>
<tr>
<td>Milrinone</td>
<td>IV</td>
<td>Loading 75 mcg kg⁻¹ × 60 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maintenance 0.5–0.75 mcg kg⁻¹ min⁻¹ &lt; 30 GA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loading 0.75 mcg kg⁻¹ min⁻¹ over 3 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maintenance 0.2 mcg kg⁻¹ min⁻¹ &lt; 30%</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PO</td>
<td>0.25–1 mg kg⁻¹</td>
</tr>
<tr>
<td>Tezosentan</td>
<td>IV</td>
<td>5 mg h⁻¹ for 30 min, 1 mg h⁻¹ for 24–72 h²⁹</td>
</tr>
<tr>
<td>Adenosine</td>
<td>UVC</td>
<td>25–50 µg kg⁻¹ min⁻¹</td>
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**Abbreviations:** ET, endotracheal; IV, intravenous; GA, gestational age; NEB, nebulized; PO, per oral; UVC, through umbilical vein catheter.

Several of these agents are being used in adults with severe pulmonary arterial hypertension. Milrinone, dipyridamole, zaprinast and sildenafil (Viagra) have all been used in newborns but the number of patients treated so far are few and no large control studies are available to date.

**Milrinone.** Milrinone is a PDE-3-specific inhibitor and is one of the earliest drugs studied in newborns. It acts synergistically with INO inducing pulmonary vasodilatation, but in addition it is also a systemic vasodilator and has positive myocardial inotropic activity. It is frequently used in newborns, pediatric and adult patients following cardiac surgery. Inhaled milrinone has been tried in adults with pulmonary hypertension undergoing cardiac surgery. In a recent case report, milrinone was successfully used in four infants who failed to respond to INO.³⁵ Pharmacokinetics of milrinone have not been studied in newborns. Milrinone may have limited role with the advent of more specific PDE-5 inhibitors, for example, sildenafil.

**Dipyridamole.** Dipyridamole is a selective PDE-5 inhibitor. In experimental models it has been shown to augment and prolong the response of NO in post-cardiac surgery patients.³⁶ However, augmentation response is not consistent and in some pediatric studies decrease in pulmonary vascular resistance of >20% is noted in only half the treated cases. Dipyridamole has been shown to help in attenuating the rebound phenomenon seen after discontinuation of INO therapy in pediatric patients.¹⁸,¹⁹ Dosage of dipyridamole ranged from 0.3 to 0.6 mg kg⁻¹.

**Zaprinast.** Zaprinast is a more selective inhibitor of PDE-5 and was one of the original inhibitors studied along with dipyridamole. Intravenous administration showed, in a fetal lamb model, to decrease pulmonary arterial resistance as well as a potentiation of inhaled NO-mediated pulmonary vasodilatation.³⁶–³⁸ The concern with intravenous administration was that at moderate-to-high doses systemic vasodilatation was noted. Aerosolized drug therapy has been shown to have less systemic side effects, preferential delivery to better ventilated areas of the lungs and a potentiation of the INO effect.³⁹,⁴⁰

**Sildenafil.** The most specific PDE-5 inhibitor currently available is sildenafil. It is becoming increasingly popular in the treatment of pulmonary hypertension, especially in post-cardiac surgery cases.⁴¹ Initial success has been noted in some pediatric cases in attenuation of INO-rebound pulmonary hypertension.⁴² Baquero et al.,²⁷ in a proof-of-concept, randomized masked study showed an improvement in oxygenation index and pulse oxygen saturation within 6 to 30 h, and significant increase in survival rate (85%, 6/7 infants) in those randomized to receive oral sildenafil. In the placebo group only 1/6 (16%) survived. The dose of sildenafil was 1 mg kg⁻¹ every 6 h till oxygenation index decreased.
Pharmacotherapy for MAS
A Asad and R Bhat

Pharmacotherapy for MAS

Recent studies have reported elevated ET-1 levels in neonatal pulmonary vasculature following MAS. ET-1 is a potent vasoconstrictor and smooth muscle cell proliferation through ET-A receptors. ET-A receptors have been found on smooth muscle cells, leading to vasoconstriction and smooth muscle cell proliferation through ET-1 signaling. Of the two major types of endothelins, ET-1 and ET-2, ET-1 is more prevalent in the pulmonary vasculature following MAS. ET-1 has been shown to be a significant mediator of vasoconstriction in the pulmonary vasculature.

Prostaglandins and prostacyclins
It has been known that both the fetal and neonatal pulmonary vascular beds are sensitive to the arachidonic acid metabolites. Prostaglandin E1 (PGE1) has been used in clinical practice for more than three decades for maintaining ducital patency, but it is a weak pulmonary vasodilator when compared with prostacyclin (PGI2). PGI2 has been in use to treat adult pulmonary hypertension for several years and is the first drug to be approved for clinical use. It is a potent pulmonary and systemic vasodilator, and its action is mediated through increased cyclic adenosine monophosphate. It has antiproliferative and antiplatelet adhesion effects. The major drawback of PGI2 is the need for long-term intravenous infusion and its very short half-life. Various synthetic analogs of PGI2, namely treprostinil, iloprost and beraprost, are in clinical use in pulmonary hypertension but is yet to show any clinical benefit.

Endothelin antagonists
Endothelins are vasoconstrictors derived from the endothelial cells. Of the two major types of endothelins, ET-1 mediates vasoconstriction and smooth muscle cell proliferation through ET-A receptors. ET-A receptors have been found on smooth muscle cells in pulmonary arteries. Endothelins are involved in maintaining the pulmonary vascular tone in the neonatal period. Recent studies have reported elevated ET-1 levels and an upregulation of ET-1 gene expression in the pulmonary vasculature following MAS. In adults with pulmonary hypertension, the combined ET-A and ET-B receptor antagonist bosentan has been used successfully. In pediatric and neonatal populations information is limited to case reports. The drug is currently available only in oral formulation, which may hinder its use in critically ill newborns. Bosentan is approved by the FDA for pulmonary hypertension in adults. Side effects of the drug include abnormal liver enzymes. Second-generation ET-A blockers such as azemetan and satexitan are being studied both in animal models and in adults with pulmonary hypertension and/or heart failure at this time.

Adenosine
ATP is a purine nucleotide and well-known dilator of systemic and pulmonary vasculature in both fetal and neonatal vessels. ATP causes vasodilatation by acting on endothelial A2 adenosine receptors and subsequent release of NO. Its use in a meconium lung was studied by Kappa et al. in the porcine model and he showed that at low doses it was able to cause an amelioration of the pulmonary hypertension. However, withdrawal of ATP led to significant rebound and even an overshoot phenomenon. From a randomized placebo-controlled trial of 18 infants, Konduri et al. showed an improvement in oxygenation only in 4/9 (45%) term infants with persistent pulmonary hypertension. The dosage used was 25 to 50 µg kg⁻¹ min⁻¹ and no systemic side effects were observed. It did not decrease the need for ECMO, bronchopulmonary dysplasia or mortality. Adenosine does have a short half-life and rapid metabolism, therefore it should be given through the umbilical venous line directly to right atrium to reach the pulmonary artery.

Magnesium sulfate
Magnesium at high doses is a smooth muscle relaxant and vasodilator. It acts by antagonizing calcium entry into smooth muscle cells. Wu et al. have shown that it also suppresses catecholamine release and alters metabolism of prostaglandins and responsiveness of smooth muscles to vasoconstrictors. Owing to its varying modes and site of action, it is a relatively nonspecific vasodilator with a significant risk for systemic hypotension. However, in clinical trials in children who have failed conventional therapies, significant improvement has been shown in both oxygenation and oxygenation index without significant hypotension. Magnesium sulfate also has sedative and antithrombotic activities. In third world countries where INO is not easily available, this multifaceted drug can be used to alleviate hypoxia. RCTs have not been done so far.

Future pulmonary vasodilators
Table 2 shows some of the newer pulmonary vasodilators currently undergoing investigation.

<table>
<thead>
<tr>
<th>Table 2 Emerging pulmonary vasodilators</th>
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<tbody>
<tr>
<td>1. Superoxide dismutase</td>
</tr>
<tr>
<td>2. Vasoactive intestinal peptide</td>
</tr>
<tr>
<td>3. Adrenomedulline</td>
</tr>
<tr>
<td>4. Arginine</td>
</tr>
</tbody>
</table>
Arginine

L-arginine infusion is being studied as an adjunct for NO as well as for its potential use in the weaning process. L-arginine is a required substrate for NO synthesis and under oxidative stress conditions it increases the activity of nitric oxide synthase. It also helps to preserve endogenous nitric oxide synthase activity thus has potential for helping in the weaning of NO. RCTs of L-arginine in persistent pulmonary hypertension are still lacking.

Superoxide dismutase

Superoxide dismutase is an endogenous scavenger and potent anti-inflammatory and anti-oxidative enzyme. There is now a recombinant version (rhSOD) that is commercially available. The rhSOD is being studied as an adjunct with INO therapy. Steinhorn et al.58 initially showed how single dose administration of rhSOD in a lamb model with and without INO led to selective pulmonary vasodilatation. In view of these anti-inflammatory and pulmonary vasodilator effects, rhSOD appears to be an exciting new addition to treat pulmonary hypertension in MAS.

Other drugs

Surfactant. Meconium contents are highly viscous and consist of desquamated epithelial cells and inspissated intestinal secretions. The large glycoproteins in meconium cause increased adhesiveness.59 Rubin et al.60 in 1996 reported that the mucociliary transportability of meconium was lower than that of sputum clearance in cystic fibrosis patients. Meconium constituents especially the fatty acids and other soluble proteins and bilirubin are direct inhibitors of surfactant.61,62 Two small randomized control studies have reported significant improvement in oxygenation and decrease in barotrauma with surfactant treatment.63,64 Readers are referred to excellent reviews on surfactant therapy and lavage in meconium aspiration in this issue.

Antibiotics

The use of antibiotics in MAS has been controversial. Initial use was advocated as it was believed that stress caused the passage of meconium and the most likely reason for perinatal stress was an infection, hence the need to treat with antibiotics. This was substantiated in 1967 when Bryan65 showed that sterile meconium was never fatal on its own, but when given intratracheally along with Escherichia coli it reduced the number of organisms needed to cause death. A more recent in vitro study by Eidelman et al.66 showed once again that clear amniotic fluid was inhibitory for bacterial growth but in the presence of meconium β-Streptococci grew at much faster rate. It is still controversial whether every infant with meconium aspiration needs antibiotic therapy. Few prospective RCTs are available from outside the United States for comparison of antibiotic use. In a prospective RCT of non-ventilated infants Lin et al.67 showed that antibiotic treatment did not alter duration of tachypnea, oxygen support and the need for nasal continuous positive airway pressure between the treated and untreated infants. Of the 306 infants, 2-month follow-up data were available only for 259 infants. None of these infants required ventilator support and none had perinatal risk factors. The use of antibiotics gave no advantage in terms of oxygen supplementation, severity of respiratory distress or mortality. Two other prospective studies from Shankar et al.68 and Krishnan et al.69 from India also concluded that routine post-natal antibiotic therapy is of no benefit. These studies do support the current view that routine antibiotic therapy in MAS does not alter clinical course. Currently in our unit only infants admitted with MAS requiring ventilatory support or infants admitted with risk factors (prolonged rupture of membranes, chorioamnionitis or positive antenatal group B beta streptococcus screen) receive antibiotics after obtaining initial cultures.

Anti-inflammatory agents

Steroids. Although the initial phase of deterioration in the lung mechanics is because of the mechanical obstruction and surfactant inactivation, the persistence of the dysfunction is thought to be because of activation of the inflammatory cascade. Chemical pneumonitis is the second cardinal finding in MAS. Khan et al.70 in 2002 showed histological and biochemical evidence of meconium-induced airway dysfunction in a murine model. Our own animal studies in newborn rabbits following tracheal instillation of meconium showed significant increase in several cytokines namely TNFα, IL-8 and IL-1β in tracheal aspirate. This was associated with significant apoptosis.72 As inflammation is one of the major findings in MAS; several investigators have attempted to treat MAS with steroids. Steroids are potent anti-inflammatory medications. Glucocorticoids, particularly dexamethasone, has been shown to improve oxygenation and lung function in animal models of MAS73 and in at least one uncontrolled study of human newborns with MAS.74 A recent Cochrane review75 found two small RCTs (a 3rd study was unpublished) and the meta-analysis of the data showed no differences in mortality, chronic lung disease and length of stay. The major weaknesses in these studies were small sample size and lack of long-term follow-up. Owing to the lack of consistent short-term benefit as well as recent concerns about its long-term outcome, dexamethasone cannot be recommended at this time as an anti-inflammatory agent in MAS.

In summary the recent advances in pharmacological treatment namely INO and surfactant has certainly decreased the need for ECMO in MAS infants. However there is a definite need for further multicenter studies to evaluate some of the specific and nonspecific therapies discussed in this review. As MAS is a major cause of mortality and morbidity in the developing countries, studies focusing on prevention and early treatment should be continued to alleviate this tragedy.
Acknowledgments

We thank Dr Bharmapuri Vidyasagar for his many contributions on the pathophysiology and treatment of MAS during the last 30 years.

Disclosure

R Bh at has lectured while on a speakers bureau. A Asad has declared no financial interests.

References


Pharmacotherapy for MAS

A Asad and R Bh at
Pharmacotherapy for MAS

A Asal and R Bhat

Journal of Perinatology


Extracorporeal membrane oxygenation (ECMO) has been successful as a rescue therapy for infants with respiratory failure with some diagnoses such as meconium aspiration syndrome (MAS) having a survival rate of more than 94%. New therapies have allowed many infants who would have required ECMO to be kept off ECMO, but at what cost. The survival rate for the neonatal ECMO patient has dropped over the years, whereas the time of ECMO has increased, indicating that the new therapies are keeping the less ill infants off ECMO. The major cause of non-survival in this population remains intraventricular hemorrhage. The primary risk factors related to this are thought to be pre-ECMO events, such as hypoxia and/or ischemia either prenatally or post-delivery. ECMO events that may complicate this are heparinization that is required while on ECMO and concern for the effect of shear stress and blood flow pattern changes created by the ECMO pump with venoarterial ECMO, although these changes are not seen in venovenous ECMO, the more common form of ECMO. Newer low-resistant microporous artificial lungs and miniaturized pumping systems may allow ECMO to be performed using less blood and safer equipment. The smaller low-resistant artificial lungs provide the ability to consider giving extracorporeal life support using only this membrane with flow provided by an arterial–venous shunt, thus eliminating the pumping system all together. Trials are ongoing in adults and, if effective, may direct further research into using this technique in newborns where the umbilical artery and vein could be used as the arterial–venous shunt.

Introduction

Extracorporeal membrane oxygenation (ECMO) is the use of a modified cardiopulmonary bypass circuit to supply respiratory and/or cardiac support for patients in cardiorespiratory failure. The ECMO circuit supplies an artificial lung and an artificial pump, which allow the patient to be supported for both respiratory and cardiac needs. On account of this, many describe ECMO therapy as a ‘time-buying’ procedure. If you give extracorporeally respiratory and cardiac support to the level, the patient can be placed on minimal ventilatory and pressor therapy over time, and a percentage of the patients will have recovery of lung and/or cardiac function. As all patients have to be systemically heparinized to be on an ECMO circuit, the known vasodilatory effect of heparin on the pulmonary vessels may assist in the reduction of pulmonary vascular resistance in patients placed on ECMO.

In the mid-1960s, ECMO use was directed at the premature infant with the hopes that ECMO would provide the ‘artificial’ placenta for these infants. Although the procedure provided sufficient cardiorespiratory support for these infants, all infants died secondary to the high intracranial hemorrhage (ICH) rate in the premature population. This was thought to be related to the immature brain of these infants with an already high rate of ICH and to the heparinization required for the procedure. The first newborn survivor in 1976 was an infant with meconium aspiration syndrome (MAS), turning the focus of treatment to the term infant. Why would MAS be a good disease to treat with a therapy such as ECMO? Figure 1 depicts the complex pathophysiology associated with MAS, with a combination of obstructive lung disease, parenchymal disease caused by inflammation and persistent pulmonary hypertension. Ventilation alone, either conventional or high-frequency ventilation, of the severe cases may not be able to break the cycle associated with severe hypoxia and persistent pulmonary hypertension, thus making ECMO an ideal therapy for this complex disease. While on ECMO, the ventilator can be placed at lung rest, allowing gentle ventilation, time for the body to absorb the meconium and time for the pulmonary hypertension to resolve. Figure 2 from the Extracorporeal Life Support Organization database shows the changing patterns of use in ECMO, with the MAS infant ranging from 45 to now 35% of the neonatal patients treated with ECMO. The survival rate for MAS infants compared with infants with other disease states is shown in Figure 3. Infants with MAS, who do not survive ECMO, do not do so because of a major intracranial complication, not because of irreversibility of the lung disease. These findings indicate that infants with severe MAS need to be referred to an ECMO center.
prior to meeting conventional ECMO criteria to reduce the risk for pre-ECMO cardiovascular instability making transfer difficult. Most intracranial bleeds are thought to be secondary to hypoxic/ischemic insults prior to ECMO that would, in a non-heparinized patient, result in an infarction, but in the heparinized ECMO population results in a hemorrhage and/or hemorrhagic infarction.\textsuperscript{6,7} Studies are now indicating that ECMO conducted using venoarterial (VA) ECMO, in which non-pulsatile flow is created with the ECMO pump, may also place the brain at risk.\textsuperscript{7–11} This risk is minimal in venovenous (VV) ECMO, the more common form of ECMO for the infant with MAS.

As new therapies have become available, such as the use of high-frequency ventilation, surfactant use in term infants, and inhaled nitric oxide, the need for ECMO has reduced.\textsuperscript{1,12–15} Figure 4 shows the reduction in the number of infants being placed on ECMO over time. Also depicted in this figure is the reduction in the survival rate of the ECMO patient over time. Although the cause of this reduction is not clear, it may be assumed that as increasing numbers are rescued by other therapies, the severity and acuity of the patients going on to ECMO has increased. This finding differs from those of Fliman et al.,\textsuperscript{15} who only looked at cases from 1996 to 2003. This difference in survival data is probably related to the time period studied, with the late 1980s, as depicted in Figure 4, representing the highest survival rate prior to most new interventions. Figure 5 may support the theory of sicker patients now being placed on ECMO. This figure shows that as the number of ECMO patients has decreased, the time on ECMO has increased, indicating that the severity of lung disease in infants going on ECMO is greater, that is, the less severe are being rescued by other therapies. Table 1 compares the pre-ECMO ventilator and blood gas

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Mechanical obstruction and chemical inflammation are the hallmark for meconium aspiration syndrome (MAS), leading to respiratory failure and the development of pulmonary hypertension in the MAS patient.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{This figure shows the major diagnoses treated with extracorporeal membrane oxygenation (ECMO). Although the numbers are reduced, the meconium aspiration syndrome (MAS) population continues to be the largest group of patients treated with ECMO (data from the Extracorporeal Life Support Organization database from 1987 to 2005).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Survival data by diagnoses for extracorporeal membrane oxygenation (ECMO) patients (data from the Extracorporeal Life Support (ECLS) database, January 2007 report, n = 21,258 patients).}
\end{figure}
data for the top three diagnoses going on to ECMO. It is interesting that the MAS infant has an average mean airway pressure of 16 compared with the congenital diaphragmatic infant with a mean airway pressure of 15 mm Hg, indicating the severity of the lung disease in these infants.

The risk for cerebral injury in the ECMO population ranges from 5 to 12%, with severe hypoxia, birth weight and sepsis representing the highest risk factors. Although the pre-ECMO events are thought to be the primary factors leading to ICH, data from animal models simulating clinical ECMO have shown an altered cerebral autoregulation pattern in animals exposed to VA ECMO both with and without prior exposure to hypoxia. Figure 6 depicts the changes in cerebral blood flow with lower cerebral perfusion pressure (lower end of the autoregulatory curve). In the newborn lamb model, 2 h of exposure to severe hypoxia altered cerebral autoregulation, but when the animals were recovered by placing them on ECMO, the alteration in autoregulation was markedly worse. Further studies using isolated
arterial vascular chamber techniques have shown that cerebral vessels taken from animals exposed to VA ECMO have a marked alteration in reactivity and that this reactivity is associated with an altered production of nitric oxide (see Figure 7). This alteration is thought to be secondary to the shear stress changes caused by the non-pulsatile VA ECMO circuit, because this alteration is seen only in the VA ECMO-exposed animals and not to animals exposed to VV ECMO, in which the pumping chamber is the native heart with normal pulsatile blood flow patterns (see Figure 7). These findings indicate that patients placed on VA ECMO may have an alteration of cerebral vascular reactivity resulting in altered autoregulation of the cerebral circulation. The cerebral circulation becomes pressure-passive under these conditions, making hypertension and hypotension risks for either cerebral ischemia (infarction) or cerebral overperfusion (cerebral hemorrhage). There has been no randomized comparison of VA versus VV ECMO patients to determine whether the incidence of ICH is higher in the VA ECMO population. Many centers still use VA ECMO primarily, but when they use VV ECMO, it is in the less severe infants, making a comparison of the rate of ICH in these populations difficult. A recent study by Radhakrishnan et al. reviewing data from the Extracorporeal Life Support Organization’s registry on the outcome and complication rate in the MAS population treated with ECMO, found that infants with MAS were treated with VV ECMO 55% of the time compared with 29% for the non-MAS patient. They also found that the MAS population had a significantly higher survival rate and lower number of complications per patient versus the non-MAS patient. They concluded that, with the lower mortality and morbidity, it was time to consider relaxing the criteria for the MAS patient and ECMO use. This may be a consideration when using VV ECMO, in which potential cerebral alterations do not exist and ligation of the carotid artery is not a concern. It may be time to consider a randomized trial looking at morbidity instead of mortality in this population.

Table 1 Pre-ECMO ventilator and blood gas data (data from the ECLS organization neonatal database, summary from 1997–2007)

<table>
<thead>
<tr>
<th></th>
<th>MAS</th>
<th>PPHN</th>
<th>CDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age on ECMO (days)</td>
<td>2.00</td>
<td>2.86</td>
<td>2.27</td>
</tr>
<tr>
<td>Time on ECMO (hours)</td>
<td>138</td>
<td>159</td>
<td>266</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>3.0</td>
<td>3.15</td>
<td>2.89</td>
</tr>
<tr>
<td>GA</td>
<td>38</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Apgar (1/5 min)</td>
<td>4/6</td>
<td>6/7</td>
<td>4/6</td>
</tr>
<tr>
<td>FIO2</td>
<td>0.96</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>PIP/PEEP</td>
<td>34/3</td>
<td>32/3</td>
<td>32/3</td>
</tr>
<tr>
<td>MAP</td>
<td>16</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Pre-pH</td>
<td>7.00</td>
<td>7.18</td>
<td>7.08</td>
</tr>
<tr>
<td>Pre-pCO₂</td>
<td>44</td>
<td>44</td>
<td>58</td>
</tr>
<tr>
<td>Pre-pO₂</td>
<td>45</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>Pre-sats (%)</td>
<td>65</td>
<td>61</td>
<td>56</td>
</tr>
</tbody>
</table>

Abbreviations: BW, birth weight; CDH, congenital diaphragmatic hernia; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; FIO2, fractional inspired oxygenation; GA, gestational age; MAP, mean airway pressure; MAS, meconium aspiration syndrome; PEEP, peak end expiratory pressure; PIP, peak inspiratory pressure; PPHN, persistent pulmonary hypertension; Pre-sat, pre-oxygen saturation.

VA ECMO may have an alteration of cerebral vascular reactivity resulting in altered autoregulation of the cerebral circulation. The cerebral circulation becomes pressure-passive under these conditions, making hypertension and hypotension risks for either cerebral ischemia (infarction) or cerebral overperfusion (cerebral hemorrhage). There has been no randomized comparison of VA versus VV ECMO patients to determine whether the incidence of ICH is higher in the VA ECMO population. Many centers still use VA ECMO primarily, but when they use VV ECMO, it is in the less severe infants, making a comparison of the rate of ICH in these populations difficult. A recent study by Radhakrishnan et al. reviewing data from the Extracorporeal Life Support Organization’s registry on the outcome and complication rate in the MAS population treated with ECMO, found that infants with MAS were treated with VV ECMO 55% of the time compared with 29% for the non-MAS patient. They also found that the MAS population had a significantly higher survival rate and lower number of complications per patient versus the non-MAS patient. They concluded that, with the lower mortality and morbidity, it was time to consider relaxing the criteria for the MAS patient and ECMO use. This may be a consideration when using VV ECMO, in which potential cerebral alterations do not exist and ligation of the carotid artery is not a concern. It may be time to consider a randomized trial looking at morbidity instead of mortality in this population.

Although MAS is the most common neonatal respiratory failure diagnoses going on in ECMO, the increasing use of ECMO is now in neonatal and pediatric patients with heart failure post-surgery for congenital heart disease. With this increase, there has been the introduction of newer devices for cardiovascular support.
including various types of ventricular assist devices. Artificial heart support for bridge to transplant available for the adult population is now available for the newborn and pediatric population in Europe. Research and development of similar devices is ongoing in the United States. The field of conventional ECMO is also changing, with newer equipment, including low-resistant microporous oxygenators and centrifugal pumps that will allow the procedure to be performed with smaller and safer systems. On account of low-resistant microporous membrane lungs have the ability of allowing ECMO to be conducted without a pump. Newer low-resistant microporous membrane lungs have the ability of allowing ECMO to be conducted with smaller and safer systems. Newer non-pump-driven circuit with potentially less complications and lower blood prime may allow for the consideration of studies using extracorporeal support for early intervention instead as a rescue therapy. These trials would be designed to look at reduction in the lung injury, time of ventilation and reduction in hospital length of stay. To date, this technique has not been used in newborns, but the umbilical vessels make this an attractive approach. The MAS population would be perfect candidates to consider for such a study.

Disclosure

The author has declared no financial interests.

References

Inhaled nitric oxide (iNO) has quickly become a standard therapy for term and near-term infants with hypoxic respiratory failure and persistent pulmonary hypertension. Its effect on the lung is believed to be through the stimulation of soluble guanylyl cyclase and the increased production of cyclic guanosine 3',5'-monophosphate (cGMP). However, in addition to pulmonary vasodilation and a decrease in pulmonary vascular resistance, nitric oxide (NO) shows several additional potential beneficial effects on the lung. This article reviews NO mechanisms of action, early clinical trial of iNO and clinical aspects for the use of iNO in acute respiratory failure of the term and near-term neonates.

**Introduction: nitric oxide**

Nitric oxide (NO) is a colorless, odorless gas. Earlier, NO was considered only as the toxic product of internal combustion engines, burning cigarettes and lightening storms. The atmospheric concentration of NO is 10 to 500 p.p.b. (parts per billion) in general, 1.5 p.p.m. (parts per million) in heavy traffic and 1000 p.p.m. in tobacco smoke. Human airways produce NO; within the nasal mucosa, NO concentrations reach 25 to 64 p.p.b. NO concentrations are considerably lower more distal, reaching 1 to 6 p.p.b. in the mouth, trachea and distal airways.

In spite of its Food and Drug Administration (FDA) approval in December 1999, NO continues to be confused with other compounds. Some are listed in Table 1 along with their chemical formula and bioactivity. The ability of NO to exist and exert biological activity in several states strengthens its position as a perfect second messenger (Table 2).

**Historical perspective**

In 1980, Furchgott and Zawadzki described how intact blood vessels, possessing intact endothelium and smooth muscle, dilated to stimuli that induced vasoconstriction when the endothelium was disturbed. He termed this substance, produced in vascular endothelial cells that relaxed blood vessels, endothelium-derived relaxing factor. Ignarro et al., in 1987, help solve the riddle with compelling evidence supporting NO as the active signaling component of endothelium-derived relaxing factor. With Moncada’s work in 1987, also showing that NO is the active component of endothelium-derived relaxing factor, NO has also become appreciated as an important neurotransmitter. In 1996, by allowing NO gas to bubble through a tissue preparation containing guanylylcyclase, Ferid Murad showed an increase in the production of cyclic guanosine 3',5'-monophosphate (cGMP), thus identifying the mechanism of action of this important molecule. For their pioneering work, Drs Furchgott, Ignarro and Murad received the 1998 Nobel Prize for Medicine.

**Pulmonary vasodilatation: mechanism of action**

Nitric oxide activates soluble guanylyl cyclase, leading to the activation of cGMP-dependent protein kinase. In turn, cGMP-dependent protein kinase decreases the sensitivity of myosin to calcium-induced contraction and lowers the intracellular calcium concentration by activating calcium-sensitive potassium channels and inhibiting the release of calcium from the sarcoplasmatic reticulum. The degradation of cGMP back to guanosine triphosphate is regulated under the control of a specific

<table>
<thead>
<tr>
<th><strong>Table 1</strong> NO impersonators</th>
</tr>
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<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td>Nitrous oxide</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>Nitrate</td>
</tr>
</tbody>
</table>

**Abbreviation: NO, nitric oxide.**

<table>
<thead>
<tr>
<th><strong>Table 2</strong> NO equivalents</th>
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<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Nitroxyl anion</td>
</tr>
<tr>
<td>Nitrosonium</td>
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</tbody>
</table>

**Abbreviation: NO, nitric oxide.**
Inhaled nitric oxide (iNO) is the first virtually selective pulmonary vasodilator in contrast to other medications. Tolazoline, nitroprusside or prostaglandins E₁ and D₂, which also decrease pulmonary vascular resistance, but as non-selective vasodilating compounds, often has profound systemic effects (Figures 1 and 2).

The biochemical fates of iNO at the alveolar-capillary membrane: small amounts of nitrogen dioxide (NO₂) may be formed if iNO mixes with high concentrations of oxygen (O₂) in the air space. Depending on the milieu of the lung parenchyma, NO may react with reactive oxygen species to form reactive nitrogen species such as peroxynitrite. In the vascular space, dissolved NO is scavenged by oxyhemoglobin (forming methemoglobin and nitrate) and, to a lesser extent, plasma proteins (for example, forming nitrosothiols, which are stable intravascular sources of NO activity).³

The fate of intravascular NO is dose-dependent: Under normal conditions, NO produced by endothelial NO synthase with arginine as a substrate diffuses into smooth muscle to activate soluble guanylyl cyclase (sGC) to form cGMP and thus maintain basal vascular tone. Intravascular destruction of NO by intraerythrocytic hemoglobin is limited by diffusional barriers, including the red-cell-free layer adjacent to the endothelium and factors in and around the erythrocyte membrane that slow NO transit (Figure 3). However, sufficient NO is consumed to limit the activity of NO to the local environment. When NO is administered by inhalation, high intravascular NO concentrations promote NO reactions, with erythrocyte hemoglobin and plasma proteins that can either protect it, thereby promoting systemic vasodilatation, or destroy it. During hemolysis or the infusion of hemoglobin-based blood substitutes, cell-free ferrous hemoglobin in the plasma rapidly destroys NO by being oxidized to methemoglobin and nitrate ions. Cell-free hemoglobin may diffuse into extravascular spaces. Limited NO bioavailability under these conditions promotes systemic vasoconstriction and organ dysfunctions.⁶
Early clinical trials

In 1991, Frostell et al. showed in a lamb model that inhaled nitric oxide (iNO) (5 to 80 p.p.m.) can reverse pulmonary hypertension, without systemic vasodilation. Pulmonary hypertension resumed within minutes of ceasing NO inhalation. While inhaling NO, there was an improved oxygenation and cardiac output and decreased pulmonary artery pressure and pulmonary vascular resistance.
The first human study with iNO was conducted in eight adults with pulmonary hypertension. Pulmonary vascular resistance fell with iNO in all patients. This was not associated with a decrease in systemic vascular resistance, in contrast to treatment with intravenous prostacyclin.

Roberts et al. and Kinsella et al. described in 1992 the first use of iNO for the treatment of persistent pulmonary hypertension of the newborn (PPHN). Roberts treated six newborn infants with PPHN with up to 80 p.p.m. of iNO. In all six patients, preductal oxygen saturation increased; in five infants, postductal oxygen saturation and oxygen tensions also increased. Inhalation of NO did not cause systemic hypotension.

Kinsella used a lower dose (10 to 20 p.p.m.) in nine newborn infants with PPHN. All showed a rapid improvement in oxygenation without a reduction in systemic blood pressure. Inhalation of NO did not cause systemic hypotension.

Larger randomized controlled studies examining different strategies of treatment with iNO in acute severe PPHN soon followed these pilot studies. The NINOS trial enrolled 235 term and near-term newborn infants with hypoxic respiratory failure, randomized to receive either iNO (20 p.p.m.) or a standard medical treatment (FiO2 100%). Infants receiving iNO were significantly less likely to be treated with extracorporeal membrane oxygenation (ECMO).

In another study, Roberts randomized 58 newborn infants with PPHN to either iNO (80 p.p.m.) or placebo: in 53% of infants treated with iNO, oxygenation improved as compared with 7% in the control group. The use of ECMO was again significantly decreased in the iNO group (40 vs 71%). In the final randomized, controlled trial, which led to the FDA approval, Clark et al. studied a low-dose iNO therapy (20 p.p.m. in the first 24 h and then 5 p.p.m. for up to 4 days) in 248 infants who were randomized to iNO or placebo: ECMO treatment was again significantly lower in the iNO group (38 vs 64%). In addition, this was the first study to show in term infants a reduction in the risk for chronic lung disease (7 vs 20%). Because ECMO was used as a rescue therapy, none of these studies showed a change in mortality.

Endogenously released and exogenously iNO may influence many facets of the developing perinatal lung, including the lung parenchyma, bronchi and vascular structures. In a fetal or preterm lung during the transition from a saccular to an alveolar stage (25 to 28 weeks of gestation), endogenous NO is released primarily from epithelial and endothelial cells that contain NO synthase, and it is implicated in the structural and functional aspects of the development of pulmonary vasculature and airway smooth muscle. NO also contributes to growth of the lung parenchyma and to extracellular matrix deposition and may modulate surfactant and inflammation in the developing lung. The implications of supplementation with iNO in preterm infants are beyond the scope of this review.

Potential beneficial effects of iNO

Thus, given the multiple mechanisms of action exerted by NO on the lung, its benefit may be mediated in several ways. Several are listed here:

1. **Improved V/Q matching**: Hypoxic pulmonary vasoconstriction minimizes ventilation—perfusion mismatching in the presence of abnormal ventilation. iNO improves oxygenation by increasing blood flow to ventilated lung units.
2. **Improved PVR/SVR ratio**: This results in a decrease in R to L shunting at the ductal and foramen level and an improvement in oxygenation.
3. **Enhancement of pulmonary surfactant activity**: Neonatal animal models showed that low-dose iNO decreased or prevented the O2-induced detrimental effects on alveolar surfactant.
surfactant, improved the apparent ability of hydrophobic surfactant proteins to promote low-surface tension and improved lung volume by a process unrelated to surfactant function.\textsuperscript{15}

(4) \textit{Prevention of neomuscularization in patients with PPHN}: In a model of injured pup lungs, iNO protected against pulmonary remodeling induced by lung injury by mechanisms that are independent of pulmonary tone, inflammation or thrombosis.\textsuperscript{16}

(5) \textit{Decreased reperfusion injury}: A neonatal animal model of lung ischemia—reperfusion showed that iNO can prevent microvascular injury, endothelial dysfunction and pulmonary neutrophil accumulation.\textsuperscript{16}

(6) \textit{NO replacement}: NO, produced by endothelial NO synthase, is critically involved in the cardiopulmonary transition from fetal to neonatal life. Endothelial NO synthase protein expression and mRNA were decreased by a half and two-thirds, respectively, in an animal model of pulmonary hypertension (Figure 6).\textsuperscript{17}

\textbf{Clinical considerations}

Hypoxic respiratory failure of the term near-term infant: Hypoxemia of the near-term infant is caused by complex pathophysiological pathways that have a common end point of reduced perfusion of the lungs. In some patients, one of these mechanisms dominates but in others several mechanisms play along, contributing to the severe clinical picture.

- Patent ductus arteriosus and/or patent foramen ovale with right-to-left extrapulmonary shunting.
- V/Q mismatching in the lungs: as in meconium aspiration syndrome. In some segments of the lungs, there is reduced ventilation and right-to-left intrapulmonary shunting, and other segments are hyperinflated, increasing the dead space. In a heterogeneous, patchy, a form of lung disease, iNO may improve the V/Q matching by dilating the vasculature bed around the ventilated segments. In a homogenous parenchymal lung disease, the response to iNO may be low; treatment of the underlying lung disease and better recruitment of the atelectatic lung may improve the response to iNO.
- Left ventricular dysfunction.

It is important to evaluate and treat each of these factors when treating the hypoxicemic infants.

\textbf{Echocardiography}. Echocardiography is crucial in the evaluation and management of the hypoxicemic (blue) baby. The study will measure evidence for PPHN: existence of patent ductus arteriosus and patent foramen ovale in the direction of the shunt, and the pressure at the right ventricle. PPHN can be also shown by the evidence of right-to left-extrapulmonary shunting, by a difference between the preductal and the postductal O\textsubscript{2} saturation.

Currently, the best way to distinguish between a congenital heart lesion, which is dependent on right-to-left shunting (for example, interrupted aortic arch, total anomalous pulmonary venous return and hypoplastic left heart syndrome) in which treatment with iNO may not be indicated, other anatomical variations or a normal heart with maladaptation to the extraterine circulation is with echocardiography. Echocardiography is also important to evaluate the cardiac performance and assess ventricular function.

\textbf{Timing of treatment}. Large-scale randomized studies support the efficacy of iNO in term and near-term infants (>34 weeks of gestation) in the first 2 weeks of life.\textsuperscript{18} iNO has also been used post-ECMO. iNO has also been used in the treatment of late pulmonary hypertension complicating chronic lung disease with some reported success,\textsuperscript{19,20} although much of the data are anecdotal. The treatment of the premature infant is still under investigation and is beyond the scope of this review.

\textbf{Indication for treatment}. Most of the studies that tested the efficacy of iNO in acute respiratory failure used enrolled infants with oxygenation index (OI) of 25 or above.\textsuperscript{18} Most evidence supports the use of iNO to reduce the need for ECMO and/or death. Some studies that used less-stringent entry criteria showed improved oxygenation but did not reduce ECMO/death when compared with the more ‘traditional’ criteria.\textsuperscript{21,22}

\textbf{Dose}. The first clinical trials used higher initial doses of 80 p.p.m. Subsequent studies changed the practice to an initial dose of 20 p.p.m. A review of the iNO treatment of 476 neonates revealed that starting at higher doses does not improve the major outcomes (ECMO and death), and there was no evidence that using higher dose leads to a more rapid improvement of the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Pulmonary endothelial NO synthase gene expression is decreased in fetal lambs with pulmonary hypertension.\textsuperscript{17} *P<0.05.}
\end{figure}
hypoxemia. Using higher doses of iNO (>22 p.p.m.) led to an increase in methemoglobin levels. Using lower doses of iNO (<18 p.p.m.) achieved the same response of PaO₂ as the higher doses, and there were no differences in the adverse events. 

Risks and complications

Methemoglobinemia. The incidence of methemoglobinemia has been low in clinical trials, occurring more commonly in neonates and with high inhaled doses, but usually is tolerated well.

Left ventricular dysfunction

Inhaled NO may have adverse hemodynamic effects in patients with significant preexisting left ventricular dysfunction. Studies with iNO in adults with preexisting, severe left ventricular dysfunction showed an increase in the left ventricular end-diastolic pressure. This increase in end-diastolic volume resulting from an iNO-induced increased pulmonary perfusion may not be tolerated by the poorly compliant left ventricle resulting in worsened, rather than improved, oxygenation.

Withdrawal. A randomized control study of iNO also examined the safety of its withdrawal in 155 term infants with pulmonary hypertension. For infants responding to the treatment, a dose–response relationship between the iNO dose and decrease in PaO₂ after discontinuing iNO was found (Figure 7). A reduction in iNO to 1 p.p.m. before discontinuation of the drug seems to minimize the observed decrease in PaO₂. For infants failing treatment, discontinuation of iNO could pose a life-threatening reduction in oxygenation (Figure 8).

A possible explanation for this phenomenon might be through an increase in the concentration of superoxide and peroxynitrite when treating with iNO. In an animal model, reactive oxygen species scavenging ameliorated alterations in endogenous NO signaling during iNO therapy (Figure 9).

Direct toxicity. At high inspired levels, NO is directly toxic to tissues and can cause methemoglobinemia, formation of NO₂, pulmonary edema, alveolar hemorrhage and hypoxemia. At low inspired levels of iNO (20 p.p.m.) NO₂ formation is minimal.

Bleeding tendency. iNO is associated with increased bleeding time and inhibition of platelet aggregation in adults. A Cochrane review of randomized studies of iNO in the near-term infants did not find an association between the use of iNO and bleeding events.

New information

The new clinical data emerging in the literature concentrate on the safety of iNO: A study in premature infants dropped the concerns that iNO might have a deleterious effect on surfactant proteins and function. This study showed a transient improvement of surfactant function after iNO treatment, and that administration of iNO does not increase inflammatory markers in the alveolar fluid.

Neurodevelopmental outcome. Concerns raised regarding the long-term complications of long-term use of iNO. In a study that examined the effects of iNO in premature infants, the infants in the study group had improved neurodevelopmental outcomes at 2 years of age as compared with the placebo group. A follow-up study at 18 to 24 months of age of the infants participating in the NINOS study showed that early iNO therapy (OI: 15 to 25) as compared...
with late therapy (O1>25) for respiratory failure in term and near-term infants was not associated with an increase in neurodevelopmental impairment or hearing loss. This new data suggest that iNO might be beneficial for the developing brain.

Congenital diaphragmatic hernia. The pulmonary artery hypertension in congenital diaphragmatic hernia is, in part, secondary to underdevelopment and anatomical changes of the pulmonary vasculature (that is, maladaptation). In addition, left ventricular dysfunction and surfactant dysfunction may contribute to hypoxemia. These may lead to a different approach in treating the hypoxemic respiratory failure in these patients. Two studies that randomized infants with congenital diaphragmatic hernia to iNO or placebo failed to show improved outcome in terms of reduced use of ECMO or death.

Non-invasive low-dose iNO, delivered via nasal canula at the time of tracheal extubation, may benefit a subset of infants with congenital diaphragmatic hernia who cannot be weaned from iNO after surgical correction. In this subset of patients, pulmonary vascular hyperactivity persisted in spite of parenchymal improvement. The role of iNO is likely far more complicated than simply vasodilation alone, as the NO/cGMP pathway is also involved in alveolar growth and lung angiogenesis.

Future directions and research consideration
Unfortunately, not all infants with PPHN improve after the initiation of iNO therapy and refractory PPHN is common. Other pharmacologic modalities are important. On the basis of the regulation pathways that control the production of the NO–cGMP cascade, and other physiologic mechanisms that induce pulmonary vasoconstriction, the following pharmacological strategies might be useful in the prevention or amelioration of PPHN. Some of these have been studied in humans (adults or pediatric population) and some are still experimental, shown to be effective in animal studies.

cGMP-specific phosphodiesterase inhibitors (sildenafil)
The secondary messenger of NO, cGMP, is inactivated predominantly by PDE5 (phosphodiesterase type 5). Sildenafil, an inhibitor of PDE5, is a selective pulmonary vasodilator, partially because PDE5 is highly expressed in the lung.

Amelioration effects of iNO withdrawal. Rebound pulmonary hypertension caused by withdrawal of iNO can be markedly attenuated by increasing cGMP with sildenafil.

Therapy for pulmonary hypertension. A randomized placebo-controlled study in 278 adult patients with symptomatic pulmonary arterial hypertension, treatment with oral sildenafil showed an improvement in exercise capacity, WHO functional class and hemodynamics (Figure 10).

A model for meconium aspiration in piglets tested the efficacy of the intravenous form of sildenafil. Sildenafil completely reversed the increase in pulmonary vascular resistance, and was shown to...
be a highly effective pulmonary vasodilator, which is at least as effective as iNO.59

The effectiveness of the aerosolized form of sildenafil was tested in an animal model of PPHN. Sildenafil selectively decreased the pulmonary artery pressure. When sildenafil was inhaled while simultaneously breathing NO, the pulmonary artery pressure decreased even further.40

Human studies followed the animal studies for PPHN. Six patients with PPHN were randomized to enteral sildenafil and compared with seven patients who received placebo. Oral sildenafil improved OI in infants with severe PPHN, which suggests that oral sildenafil may be effective in the treatment of PPHN.41

Soluble guanylate cyclase activators
BAY 41-2272 is a novel direct activator of soluble guanylate cyclase. This compound was tested in an animal model for PPHN. The effectiveness of BAY 41-2272-induced pulmonary vasodilation increased during the development of pulmonary hypertension in a response that was greater than sildenafil. It also augmented the pulmonary vasodilation induced by iNO.42

PGI2 analogs
Iloprost is a stable analog of prostacyclin. Its mechanism of action is through a different signaling pathway, involving cAMP. The use of the inhaled formulation has been approved by the FDA for the use in adults. When given as the inhaled formulation, iloprost is a selective pulmonary vasodilator. A first study of the use of inhaled iloprost for children with pulmonary artery hypertension has been recently published, reporting sustained functional improvement in some children, although occasionally induced bronchoconstriction.43 There are several anecdotal reports for the use of inhaled iloprost for refractory cases of PPHN, most reporting improved oxygenation after the initiation of therapy. No adverse events were reported.

Endothelin-1 receptor antagonists (bosentan, sitaxsentan)
Endothelin-1 is a potent vasoconstrictor, and has been shown to inactivate iNO. In an animal model, intratracheal recombinant human superoxide dismutase, with or without iNO, increased oxygenation and reduced pulmonary vasoconstriction.48

Expanding the pharmacologic options and the combination of several of these might improve the response to iNO and the clinical outcome of this devastating disease.

Summary
Since its discovery two decades ago, NO found its way to the arsenal of therapeutic approaches available to the neonatologist in the treatment of PPHN, a disease with high morbidity and mortality. Vast research, both in animal models and human, increased our understanding of the features of this gas and its benefits and downsides. iNO was found to be the first potent 'fairly' selective pulmonary vasodilator. NO has multiple mechanisms of actions that may benefit newborn infants with diseases associated with meconium aspiration and persistent pulmonary hypertension. Understanding the mechanism of action of iNO through the cGMP pathway led to the introduction of other medications in this pathway; in addition to increasing cGMP concentration through NO-induced stimulation, inhibiting cGMP metabolism through phosphodiesterase inhibition, by sildenafil, for example, may provide additional benefit. The combination of drugs affecting this pathway and other pathways might have a synergistic effect, increasing the rate of success in the treatment of PPHN.

Disclosure
MD Schreiber has received lecture fees, consulting fees, and grant support from Ikaria. A Bin-Nun has declared no financial interests.

Duality of interest
Dr Schreiber has received grant support and honoraria from iNO Therapeutics/Ikaria.

References


1650–1657.


REVIEW

Neurodevelopmental outcome of infants with meconium aspiration syndrome: report of a study and literature review

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There is a paucity of information on long-term outcome of infants who have suffered from meconium aspiration syndrome (MAS) in the neonatal period. We analyzed long-term developmental outcome data of 35 infants who were admitted to the neonatal intensive care unit (NICU) at the University of Illinois Hospital at Chicago (UICMC) with a diagnosis of MAS, and we reviewed the literature pertinent to the subject. The objective of the study was to assess the neurodevelopmental status of MAS infants and compare the possible effects of different variables that are known to affect the later developmental outcome. The variables included mode of delivery, APGAR score, cord pH, mode of treatment, and neurological findings during the course of NICU. The infants were enrolled in the developmental follow-up program (DFUP) after discharge from the nursery for assessment of long-term developmental status and neurodevelopmental outcome. In order to assess the impact of the treatment on long-term outcome and compare our findings with previously published reports, we also reviewed the previously published literature on neurodevelopment outcome of infants treated for MAS (with different modalities) during the last three decades. Total of 35 infants with a diagnosis of MAS admitted to the NICU at UICMC were followed in the DFUP clinic for 3 years during January 1999 to September 2001. The medical records of these infants were reviewed for the mode of delivery, APGAR score, birth weight (BW), gestational age, mode of treatment during the neonatal period, and neurodevelopmental status. 19/35 (54%) infants were delivered vaginally, 16/35 (46%) by cesarean section (C-section). All were treated in the delivery room using the standard resuscitation protocol. Following initial resuscitation, all except three required intubation and ventilation for varying duration. One infant required inhaled nitric oxide therapy, and two required extracorporeal membrane oxygenation treatment. Subsequent to discharge, the infants were evaluated in the clinic at 2 months of age, and then every 4 months up to 3 years. The developmental assessment of mental development index (MDI), psychomotor development index (PDI), and behavior rating scale (BRS) were obtained using the Bayley II infant motor scale, and neurodevelopmental evaluation was performed using the Amiel-Tison technique. Speech evaluation was performed in infants >18 months using the Rossetti Infant–Toddler language scale. Infants were considered normal when MDI and PDI scores were >85 to 110; mildly delayed when scores were >70 to 84; and severely delayed if the scores were <69. In addition, neurological evaluation also confirmed the disability. The report is based on the final analysis of 29 infants. Data of six infants were not included in the final analysis because of incomplete information. The mean BW of the infants was 3269 ± 671 g; mean gestational age was 39.5 ± 3.1 weeks. The median APGAR score at 1' was 4, and at 5' was 6. Out of 29, 11 (38%) infants were normal. Out of 29, 2 infants (7%) had cerebral palsy (CP) and 4 (14%) had severe delay at 12 months of age. Out of 29, 2 who were neurologically disabled had PDI <69. Out of 29, 12 (41%) had mild delay in speech. No statistical difference in neurodevelopment was found in infants born vaginally or by C-section. Our findings show poor outcome (CP and global delay) in 21% of infants who suffered MAS, even though the majority of the infants (26/29) responded to conventional ventilator support alone. No difference was found in the outcome of infants between NSVD vs C-section delivery. These findings suggest that infants with the diagnosis of MAS manifest later neurodevelopmental delays, even if they respond well to conventional treatment. This abstract was presented at the Society for Pediatric Research Annual Meeting, 2000. Journal of Perinatology (2008) 28, S93–S101; doi:10.1038/jp.2008.154

Introduction

Meconium aspiration syndrome (MAS) is a clinical entity that contributes to the high incidence of mortality and morbidity among term pregnancies.1,2 The prevalence and mortality in MAS is noted to be steadily decreasing with newer and more efficient modalities of treatment.1,2 With steady improvement in survival, the amount of morbidity among the survivors has not changed. There is a great need for follow-up data on the neurodevelopmental outcome of this group of infants. The purpose of this study was to report our findings of neurodevelopmental outcome of infants with the diagnosis of MAS in the neonatal period and review the literature on this supplement.

Methods

We reviewed the medical records of 35 infants with the primary diagnosis of MAS followed in the developmental follow-up program

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During the period of 1999 to 2001, 75 infants were diagnosed as having meconium-stained amniotic fluid at the time of delivery. Of these, only 40 infants who developed respiratory distress and met the criteria for MAS were admitted to the NICU. Parents of all infants who were admitted NICU with a diagnosis of MAS were counseled by the clinic nurse and enrolled for developmental follow-up clinic during January 1999 to September 2001 at the University of Illinois Hospital at Chicago (UICMC). Information was collected using the specific diagnostic code for MAS ICD-907 criteria recorded in the obstetric and neonatal intensive care units (NICUs). The criteria for the diagnosis of MAS (ICD-907) include: history of meconium-stained amniotic fluid before delivery, the presence of meconium below the vocal cords at the time of birth in infants >37 weeks of gestation, evidence of respiratory distress, and radiological evidence of aspiration pneumonia. Many of these infants also developed persistent pulmonary hypertension (PPHN). The medical records of the infants who met the above criteria were selected for the retrospective analysis of perinatal and neonatal events. The study was approved by the internal review board at the University of Illinois at Chicago.

Obstetrical and NICU registry and medical records were reviewed for maternal complications of pregnancy, such as diabetes, hypertension, toxemia, and chorioamnionitis. The presence of meconium-stained amniotic fluid, consistency of meconium, and presence of amnioinfusion were also ascertained. The presence of fetal distress and the mode of delivery were also noted. The infant’s condition in the delivery room and any interventions were reviewed. The following data were retrieved from the infants chart: APGAR score at 1 and 5 min, presence of meconium below the vocal cords, method of resuscitation, presence of respiratory distress at birth, the requirement of the use of supplemental oxygen, and ventilator support. The data of cord blood gases and blood gas analysis at the time of admission to the NICU were also noted. The gestational age was determined using fetal ultrasound assessment or Ballard score. Birth weight (BW), length, and head circumference on admission to the NICU were recorded.

**Developmental assessment**

During the period of 1999 to 2001, 75 infants were diagnosed as having meconium-stained amniotic fluid at the time of delivery. Of these, only 40 infants who developed respiratory distress and met the criteria for MAS were admitted to the NICU. Parents of all infants who were admitted to NICU with a diagnosis of MAS were counseled by the clinic nurse and enrolled for developmental follow-up clinic during January 1999 to September 2001 at the University of Illinois Hospital at Chicago (UICMC). Information was collected using the specific diagnostic code for MAS ICD-907 criteria recorded in the obstetric and neonatal intensive care units (NICUs). The criteria for the diagnosis of MAS (ICD-907) include: history of meconium-stained amniotic fluid before delivery, the presence of meconium below the vocal cords at the time of birth in infants >37 weeks of gestation, evidence of respiratory distress, and radiological evidence of aspiration pneumonia. Many of these infants also developed persistent pulmonary hypertension (PPHN). The medical records of the infants who met the above criteria were selected for the retrospective analysis of perinatal and neonatal events. The study was approved by the internal review board at the University of Illinois at Chicago.

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**Analysis and results**

**Maternal data**

Complete maternal data were available in all 29 infants who had complete follow-up data. Total of 16 of these infants were delivered by C-section for fetal distress. 13 infants were delivered by normal spontaneous vaginal delivery. Pregnancy complications included diabetes in 4 patients who were on insulin. Pregnancy induced hypertension in 3, fetal deceleration in 4, chorioamnionitis in 3, premature rupture of membranes in 3 maternal drug use in 6, sickle-cell disease in 4, fever of unknown origin in 3, and presence of prolapsed cord in 3. Umbilical cord pH was available in 16/18 inborn infants and in 2/11 outborn infants. It was noted 8/18 infants had cord pH>7.2 and in two infants cord pH was 6.75 and 7.10.

**Neonatal data**

Table 1 shows the perinatal data of infants in the study. Total of 11 outborn infants were transferred to UICMC within 12 h after birth. Method of resuscitation was not available. Mean admission age was 6 h after birth APGAR score ranged from 0 to 7 at 1 min with mean of 4, and at 5 min APGAR score ranged from 4 to 9 with mean of 6.
APGAR score ± in accordance with the standard NRP protocol. All infants were below the vocal cord in four infants. Mean gestational age was at the time of delivery in 14 infants and meconium was present infants therefore not included in our data. Thick meconium fluid We were unable to get the fetal heart rate pattern in many of the delivered by NSVD: 6/16 (37.5%) were normal, 8/16 (50%) had mild delay, and 4/16 (12.5%) had severe delay. 16 infants were admitted to the NICU, all required assisted ventilation. Duration of the ventilatory support varied between 2 and 12 days with a mean of 6.4 days. Only one infant was treated with inhaled nitric oxide (INO) and high frequency ventilator who had severe metabolic acidosis and pulmonary hypertension. Two infants required extracorporeal membrane oxygenation (ECMO) for 12 to 24 days who did not respond to mechanical ventilation.

The neurological findings in the neonatal period showed: fourteen infants developed seizure activity during the first week of life. All had electroencephalography (EEG) recordings. Six of the EEGs demonstrated abnormal foci in the temporal and parietal areas. Thirteen infants had magnetic resonance imaging (MRI) scans during the neonatal course. Eleven infants had normal MRI, and two infants demonstrated cerebral hemorrhage.

**Developmental outcome data**

Table 2 shows developmental outcome at 36 months. In 38% (11/29) of infants, the developmental status at 3 years of age was normal. 41% (12/29) had mild deficits, which consisted of mild hypotonia or mild speech delay. 7% (2/29) had CP. 14% (4/29) had severe global delay. None required oral or gastric tube feeding. All had normal nutritional status. 19/29 infants (65%) were enrolled in the EIP. Other 10 required anticipatory development counseling.

The growth and development of these infants at 3 years were appropriate for age, with the exception of one infant who had suffered from birth asphyxia resulting in CP. Two infants required phenobarbital for control of seizure even at 2 years of age.

Of the 13 infants delivered by C-section, only 5 were normal, 4 had mild delay, and 4 had severe delay. 16 infants were delivered by NSVD: 6/16 (37.5%) were normal, 8/16 (50%) had mild delay, and 2/16 (12.5%) had severe delay.

We were unable to get the fetal heart rate pattern in many of the infants therefore not included in our data. Thick meconium fluid at the time of delivery in 14 infants and meconium was present below the vocal cord in four infants. Mean gestational age was 39.5 ± 3.1 weeks. All infants were resuscitated in the delivery room, in accordance with the standard NRP protocol. All infants were admitted to the NICU, all required assisted ventilation. Duration of the ventilatory support varied between 2 and 12 days with a mean of 6.4 days. Only one infant was treated with inhaled nitric oxide (INO) and high frequency ventilator who had severe metabolic acidosis and pulmonary hypertension. Two infants required extracorporeal membrane oxygenation (ECMO) for 12 to 24 days who did not respond to mechanical ventilation.

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Neurodevelopmental outcome did not differ whether they were born vaginally or by C-section.

**Discussion**

MAS is a distinct clinical syndrome that occurs in term infants. Severe meconium aspiration occurs in one in 500 deliveries: that is, in only 0.2% of all births. Intraperitoneal fetal distress and fetal asphyxia in utero before birth have been recognized as stimulating and enhancing the intestinal peristalsis of the infant, resulting in relaxation of the anal sphincter and passage of meconium in utero. Previous reports have described the pathophysiology of MAS. The hypoxic distress of the fetus and gasping in utero have been speculated as the cause of the meconium aspiration during labor and at birth. Aspiration of thick meconium may occur during the respiratory effort of the first breath. This leads to obstruction of airways, resulting in profound hypoxia. In addition, meconium may lead to chemical pneumonitis, parenchymal lung damage, and inactivation of surfactant. Other complications such as pulmonary hypertension or pulmonary atelectasis with or without pneumothorax have been reported. Severe hypoxia may cause brain injury and hypoxic ischemic encephalopathy.

Since the recognition of these issues, both obstetricians and neonatologists have intensified their efforts to decrease the incidence of MAS using new interventions. The obstetric preventive interventions include amnioinfusion in the presence of thick or thin meconium-stained amniotic fluid before birth of the infant, and suctioning of the oropharynx at the perineum before the delivery of the shoulders. The neonatal interventions include tracheal suctioning at birth in the presence of meconium-stained amniotic fluid to prevent meconium aspiration.

Although the survival rate of MAS has greatly improved over the last three decades, the long-term morbidities among survivors have been a major concern. We undertook a study to analyze the long-term outcome of infants treated for MAS in our NICU and compare the same with published reports. However, comparisons of our findings with the published studies are constrained for several reasons. First, the treatment modalities differed. Second, the primary objective of most reports was to study ‘the treatment effect’ rather than the ‘effect of MAS’ on long-term outcome. The third...
issue was that the population included in follow-up studies was the sum total of varied diagnoses (MAS, CDH, PPHN, sepsis, and pneumonia), making it difficult to assess the independent effect of MAS alone on the outcome. Finally, the methods of developmental assessment were not uniform and comparable. We attempt here to summarize the uniformity of evaluation and follow-up methods and findings of the published reports of long-term studies. We try to provide some perspective on our follow-up study, its importance, and its relation to the current literature. Review of the literature during the past 30 years has shown that there were three major strategies of treatment and outcome reports. They included the use of mechanical ventilation in the 1970s, additional ECMO treatment in the 1980s, and INO treatment in the 1990s. The outcome results of the infants treated with these different modalities are summarized in Table 3 and discussed below.

Studies in which the primary treatment of MAS was conventional ventilation alone

In the 1970s, the treatment of MAS infants who developed acute respiratory failure consisted of providing early ventilatory support. The survival was low. The clinical course during the immediate neonatal period and complications of MAS included hypoxic injury of brain, pulmonary atelectasis, air leak syndrome, and other systemic insults. During 1978 to 2000, there were 10 other studies published in this category, and they reported long-term outcome of infants with the primary diagnosis of MAS. In two of these studies, MAS infants were treated with conventional ventilation alone. Marshall et al. in a retrospective study in 1978 identified 13 of the 17 infants (78%) who survived following conventional ventilator therapy. Follow-up of 11 of the survivors showed that 2/11 had developmental delay (18%). Both had seizures. Brett et al. in 1981 followed 9 of their 11 survivors of hyperventilation using conventional mechanical ventilation therapy. Seven of the survivors had MAS. The range of MDI was 89 to 130. They concluded that hyperventilation was not associated with adverse outcome. Here, the emphasis was on the efficacy of treatment rather than the MAS. Walsh and Suky compared the infants treated with conventional ventilation with the ECMO survivors. 90% of the survivors of the ECMO-treated group had primary diagnosis of MAS. The reported morbidity in the ECMO-treated group was twice that of conventional ventilation treated group (55 vs 28%). The reported high morbidity among the long-time survivors, Macrocephaly developed in 24% of the ECMO group. They also found 4/17 (28%) of the conventionally treated group had severe neurodevelopmental disabilities, compared with the 20/38 (26%) of the ECMO-treated group.

Outcome of infants following ECMO treatment in the 1980s

Bartlett et al. used ECMO in the management of infants with MAS who failed to respond to conventional therapy. The rest of the eight studies reported following outcome with ECMO treatment. Four of them showed adverse outcome. Three studies showed that, although MAS infants had higher disabilities compared with the normal infants, they were better than infants with diaphragmatic hernia (CDH) and sepsis. Idle et al. showed in their study no significant difference in the intelligence quotient (IQ) of children at school age, in the ECMO-treated group, except the children with diagnosis of MAS had lower IQ. These studies indicate that infants with the primary diagnosis of MAS have poor neurodevelopmental outcome. Within the ECMO-treated group, the MAS group had a lower incidence of disability than the CDH and sepsis group, suggesting that the presence of sepsis will increase the neurological insult in comparison to MAS. The ECMO trials reported long-term outcome results. These studies included both MAS and PPHN infants. However, separation of the MAS group was not possible. Overall, the studies showed improved survival following ECMO without concomitant increase in morbidities. There were a few differences among the studies. Vaucher et al. on the other hand reported higher morbidity in the conventional group compared with the ECMO group (25 vs 6% of CP). They also reported that abnormalities found in neuroimaging predicted later clinical neurodevelopmental abnormalities. The UK Collaborative study showed no concomitant increase in morbidities in the ECMO group. Rais-Bahrami et al. similarly showed that the neurodevelopmental outcome in the ‘near-miss ECMO’ group (who were sick enough to be on ECMO but were not) had higher handicaps (25 vs 16%) at follow-up than those treated with ECMO.

Glass et al. is the only report that compared the long-term outcome of MAS infants treated with ECMO with healthy controls. These data showed that MAS infants had significantly higher neurodevelopmental delays compared with controls. Most infants undergoing ECMO were in the MAS group. It follows that MAS is associated with higher adverse neurodevelopmental sequelae than the normal population and that these disabilities did not decrease with different treatment modalities, as demonstrated in most of the studies discussed above.

Blackwell et al. showed that there were no differences in the occurrence of seizures between infants with MAS and cord pH<7.20 and those with cord pH>7.20, suggesting that there was a pre-existing injury or that a nonhypoxic mechanism is involved in infants with severe MAS. Casey et al. showed that pH change from birth to immediate neonatal period predicted neonatal morbidity. Infants whose both cord pH and subsequent 2 h arterial pH were <7.20 had a higher incidence of seizures. Total of 55% of infants in this group had associated meconium aspiration, compared with only 36% in infants who had cord pH>7.20. Blackwell et al. showed that pH change from birth to immediate neonatal period predicted neonatal morbidity. Infants whose both cord pH and subsequent 2 h arterial pH were <7.20 had a higher incidence of seizures. Total of 55% of infants in this group had associated meconium aspiration, compared with only 36% in infants who had cord pH>7.20. In our study, only 2/29 (6.9%) had severe neurodevelopmental impairment; neither required ECMO treatment. These infants experienced severe intrauterine hypoxia, as noted by their cord pH.
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<tr>
<th>Reference no.</th>
<th>Author(s)</th>
<th>Number of cases</th>
<th>Outcome</th>
<th>Comments</th>
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<tbody>
<tr>
<td>14</td>
<td>Marshall R <em>et al.</em>, 1978</td>
<td>Study period, 1974; records of 1 year Follow-up MAS: 11 infants</td>
<td>Outcome at 1 year: four (23.5%) died of respiratory failure Two had PFC by cardiac catheterization, two had significant psychomotor retardation at 8 and 13 months. CP 11.9%, both had seizures and one had microcephaly &lt;5%</td>
<td>Significant psychomotor retardation and cognitive delay among the survivors</td>
</tr>
<tr>
<td>15</td>
<td>Brett C <em>et al.</em>, 1981</td>
<td>Study period March 1977–1979, number of cases followed 9, MAS: 7, RDS: 1, sepsis: 1, age at follow-up 16 months Hyper ventilated: 64.4 ± 18.6</td>
<td>Outcome at 16 months: seven infants were normal neurodevelopment, one had severe delay, six had normal neurological exam, one infant had growth retardation</td>
<td>Although short-term follow-up reported, preliminary observation assures normal development outcome with prolonged hyperventilation</td>
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**Outcome of ECMO trials**

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<th>Reference no.</th>
<th>Author(s)</th>
<th>Number of cases</th>
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<tbody>
<tr>
<td>16</td>
<td>Walsh-Sukys MC <em>et al.</em>, 1994</td>
<td>Study period 1987–1998 Cases followed: 61 For 20 months of both CV group, and ECMO group</td>
<td>Outcome at 20 months ECMO: 2 CP, 2 seures, disability 20 and 1 deaf. CV: 2 disability, 1 CP for blind</td>
<td>Disability among CV was 4 (24%) and in ECMO 20 (26%) No difference between the two groups followed. 24% ECMO group had macrocephaly at 20 months</td>
</tr>
<tr>
<td>18</td>
<td>Towne BH <em>et al.</em>, 1985</td>
<td>Study period 1973–1980 Cases followed: 18 MAS: 14/18 11 of MAS cases follow-up</td>
<td>Outcome at 12 months: 9/11 MAS infants were normal; 1 had developmental delay, seizures; and 1 had hemipertias</td>
<td>ECMO—life saving without increasing morbidity</td>
</tr>
<tr>
<td>19</td>
<td>Andrews AF <em>et al.</em>, 1986</td>
<td>Study period March 1981–September 1983 Cases followed: 14 with PPHN MAS: 5, PPHN: 13, CDH: 1, RDS: 3, others: 4</td>
<td>Outcome mental function: normal EEG in 71%, abnormal in 14, one long-time treatment Normal outcome in seven (50%), five with delay and failure to thrive, three with CP, three had microcephaly, and dilated ventricles</td>
<td>Majority of survivors of neonates with high risk of respiratory failure can be expected to have near normal growth and development</td>
</tr>
<tr>
<td>20</td>
<td>Hofkosh D <em>et al.</em>, 1991</td>
<td>Study period 1990–1993 in 67 cases from 6 months to 10 years, 6–30 months: 47 cases; 2.5–4.11 years: 10 cases; 5–10 years—school age: 10 cases Diagnosis MAS: 33, CDH: 13, RDS: 8, PPHN: 10, others: 3</td>
<td>At 6–30 months outcome by MDI/PDI: normal: 27, abnormal: 4, suspect: 16. 2.5–4.11 years, IQ test normal: 7, abnormal: 2, CP: 2. School age 5–10 years. IQ normal: 9, abnormal CP: 1 (10%), sensory neural hearing loss: 21%, seizures: 4, with abnormal outcome</td>
<td>Out of entire cohort, normal in 43 (64%), abnormal 7 (11%), suspect 17 (25%). ECMO did not increase the morbidity; comparison of control conventional ventilation group is absent</td>
</tr>
<tr>
<td>21</td>
<td>Wilden SR <em>et al.</em>, 1994</td>
<td>Study period January 1990–1992 Cases followed: 22 Diagnosis MAS: 10, RDS: 7, PPHN: 3, sepsis: 6, CDH: 8</td>
<td>At 24 months ECMO: GP MDI &lt;85, PDI &lt;73, abnormal expressive and receptive language in 48%. Hearing loss in four (15%), visual impairment in one, and abnormal MRI in nine infants</td>
<td>Overall ECMO group 23% significant impairment</td>
</tr>
<tr>
<td>22</td>
<td>Bernbaum J <em>et al.</em>, 1995</td>
<td>Study period 1990–1993 Cases followed: 60 MAS: 21, CDH: 19, sepsis: 7</td>
<td>Follow-up 6–12 months Neurologic morbidity in four MAS, and in five CDH. Hearing loss in two MAS, hypotonicity in 8% of MAS, and low cognitive scores in CDH. Total of 68% of CDH had the worst outcome, with BPD and feeding difficulty at 12 months</td>
<td>BPD and feeding difficulties, hypotonicity were greater in CDH, compared to MAS</td>
</tr>
</tbody>
</table>
### Table 3 Continued

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Author(s)</th>
<th>Number of cases</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Robertson C et al., 1995</td>
<td>Study period 1989–1992</td>
<td>Outcome at 2 years of age</td>
<td>In ECMO: GP disability: 6, hearing loss: 3, severe cognitive disability: 2, seizures: 1, visual loss: 3, expressive language delay: 7, moderate cognitive delay: 2. MDI were lower in sepsis group, with mean MDI 91.8 (9.5)</td>
</tr>
<tr>
<td>24</td>
<td>Ikle L et al., 1999</td>
<td>Study period 1988–1991</td>
<td>Cases followed: 17 for 5–8 years; studied IQ at school age</td>
<td>Outcome of 17 MAS: full-scale IQ varied from 90 to 115, performance IQ, 81–121, verbal IQ, 89–118; 15 children in MAS with low functioning had low average IQ</td>
</tr>
<tr>
<td>25</td>
<td>Nield TA et al., 2000</td>
<td>Study period 1987–January 1993</td>
<td>Cases followed: 130 for 3.5 years</td>
<td>Outcome MAS: major neurologic sequelae in 20%, including MR and CP, functioning disability in 17%, 24% at risk for disability, normal function in 59%</td>
</tr>
<tr>
<td>26</td>
<td>Vaucher Y et al., 1996</td>
<td>Study period 1987–1993</td>
<td>Comparative study, conventional HFV ventilation to ECMO, CV: 53, ECMO: 138, MAS: 97, PPHN: 19, RDS: 46, sepsis: 28, studied CLD, and MRI</td>
<td>Neuromotor outcome at age CLD had lower MDI, PDI scores &lt;84, 27% had CP, neuroimaging abnormalities. Severe in 11%, moderate in 9%, mild in 24%</td>
</tr>
<tr>
<td>27</td>
<td>Benett CE et al., 2001</td>
<td>Neurodevelopmental outcome at 1 year of age in ECMO</td>
<td>Infants compared with those treated with conventional therapy (99 survivors)</td>
<td>1. Death 2. Severe disability DQ &lt;50 using Griffiths mental developmental scale</td>
</tr>
<tr>
<td>28</td>
<td>Rais-Bahrami K et al., 2000</td>
<td>Study period for 11 years</td>
<td>Cases followed: ECMO: 76, near miss ECMO: 20 ECMO GP; MAS: 47, PPHN: 16, RDS: 13 Near miss ECMO group; MAS: 11, PPHN: 6, RDS: 3</td>
<td>Outcome tested for IQ at 5 years of age. FSIQ &lt;0.70 as cognitive disability found in 12% of ECMO group, 11% in near miss ECMO, half of them had MR CP. Sensory neural hearing loss academic achievement test, using V IQ, PIQ, FSIQ, academic school failure, ECMO (38%), near miss ECMO (37%)</td>
</tr>
<tr>
<td>29</td>
<td>Glass P et al., 1995</td>
<td>Study period, 1984–1988: 103 post-ECMO compared with 37 healthy controls</td>
<td>Diagnosis</td>
<td>Parent interview at 5 years, major disability in 17%, 13 had MR, 4 seizures, 5 severe motor delay, 3 with SNHL FSIQ (96 vs 115), VIQ (96 vs 115), PIQ (96 vs 110)</td>
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**Outcome of INO trials**

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<tr>
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<tbody>
<tr>
<td>35</td>
<td>Rosenberg A et al., 1997</td>
<td>Study period 1992–1995</td>
<td>Cases followed: 51 with PPHN, 61% followed up to 1 year, 33% followed to 2 years, MAS: 23, CDH: 13, PPHN: all sepsis: 22, RDS: 8, other: 2</td>
<td>At 1 year, 6 (11.8%) had major handicap, MDI, PDI &lt;69, mild disability in 7 (13.7%). At 2 years, 4 (12.1%) had neurodevelopmental disability, MDI, PDI &lt;69, 4 infants had MDI, PDI &lt;84, 3 had sensory neural hearing loss</td>
</tr>
</tbody>
</table>
Outcome of infants treated with INO

In 1990, INO was introduced to treat severe PPHN associated with MAS. Recently, Rosenberg critically reviewed the long-term outcomes of MAS and PPHN infants treated with INO therapy. He concluded that, overall, INO was effective in treating PPHN in MAS. He observed that all the four studies under review reported significant medical and neurodevelopmental sequelae. Ichiba et al. later reported that the outcome of INO-treated infants depended on their response to treatment. Infants who responded early had zero sequelae, compared with those who responded late, who had 11% disabilities. Rosenberg et al. reported one-year survival of INO-treated infants with severe morbidities. He also reported the outcome of infants who survived the randomized trial of INO treatment with confirmed diagnosis of PPHN. These infants were followed for 1 to 2 years. At 1 year of age, 6/51 (11.8%) were found to have severe neurological disability as measured by a Bayley II MDI or PDI score of <69, abnormal neurological findings, and poor weight gain. He concluded that, regardless of the various postnatal treatment strategies, the predisposing factors and underlying factors leading to MAS are fundamental to the pathogenesis of neurodevelopmental handicap among the survivors. The NINOS study reported no difference in the outcome between INO-treated infants of the larger group and the control group. The NICHD collaborative reports on infants treated with INO who were followed up at 24 months showed no difference in disabilities between the control and the INO-treated groups.

Table 3

<table>
<thead>
<tr>
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<th>Number of cases</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>NINOS, 2000</td>
<td>Study period</td>
<td>Cases followed: 173 for 18–24 months</td>
<td>Outcome at 24 months, cerebral palsy in 30%, with MDI, PDI &lt;70, bilateral blindness in 12%, 6 had CP in INO group, SNHL: 10 (12%) in INO 34.5% of the INO group had at least one disability</td>
</tr>
<tr>
<td>37</td>
<td>Ellington M et al., 2001</td>
<td>Study period September 1992–1997</td>
<td>Satisfaction survey of families of children treated with INO between 1 and 4 years. Cases followed: 60, INO treated: 42, CV: 40</td>
<td>Outcome INO and CV group: any morbidity: 34% (35%) disability CP: 9% (20%), hearing, speech: 11% (28%), visual impaired: 12% (3%), DQ: 70 0% (16%)</td>
</tr>
<tr>
<td>38</td>
<td>Lipkin PH et al., 2002</td>
<td>Study period 1994–1996</td>
<td>Cases followed: 129, INO treated: 94, control: 35, diagnosis: PPHN only</td>
<td>INO group: 13% had major disability, hearing loss in 14%. Cognitive delay: 30%, rehospitalization: 22%. INO had 8% CP, and CD in 8%, s.d. in 10%, compared with control had 6% CP, and 6% CD, 6% s.d.</td>
</tr>
<tr>
<td>39</td>
<td>Ichiba H et al., 2003</td>
<td>Study period 1996–1998 total 15 of INO treated follow-up to 3 years</td>
<td>MAS: 11, CDH: 1, pneumonia: 1 RDS: 1, hypoplastic lung: 1</td>
<td>Only diagnosis PPHN, response to INO treatment classified as early, late and poor</td>
</tr>
</tbody>
</table>

Abbreviations: BPB, broncho pulmonary dysplasia; CDL, chronic lung disease; CP, cerebral palsy; CV, conventional ventilation; ECMO, extracorporeal membrane oxygenation; FSIQ, full scale intelligent quotient; HMD, hyaline membrane disease; INO, inhaled nitric oxide; IQ, intelligence quotient; MAS, meconium aspiration syndrome; MDI, mental development index; PFC, persistant fetal circulation; PIQ, performant intelligent quotient; PPHN, persistent pulmonary hypertension; RDS, respiratory distress syndrome; VIQ, verbal intelligent quotient.
Summary

We report follow-up of 29 MAS infants, almost all of whom were treated only with conventional ventilation. Only one infant was treated with INO, and two other infants required ECMO. The infants were followed up to 3 years of age. The findings of this study showed that, in spite of a milder clinical course of illness, severe delay was noted in 21% of the infants. Our study shows that infants with MAS, in spite of adequate respiratory support, have the potential to develop later neurodevelopmental deficits. In our study, we analyzed the importance of cord pH to determine the factors that predispose to eventual morbidity. We were able to identify cord pH<6.9 in two infants who also developed CP. There were no differences in the outcome by mode of delivery. As reviewed above, previous follow-up studies show that although different treatment modalities, improved the survival, did not improve the outcome among MAS infants.

Conclusion

Although various degrees of neurologic abnormalities are found in infants who survived MAS in the neonatal period, establishing the causation of neurological insult has been a difficult problem. The above findings suggest poor outcome in MAS, regardless of the mode of delivery (NSVD vs C-section) or mode of treatment (ECMO vs INO). Most infants are asymptomatic at the time of discharge from the nursery. However, on later follow-up, 10–20% of infants demonstrated developmental disabilities. The findings above suggest that there are yet other unidentified events that occur antecedent to delivery of the infant. The presence of meconium at birth may simply be an associated event. The retrospective descriptive study has limitations because of the limited number of patients available. A multicenter trial involving a large number of cohorts of infants with meconium-stained amniotic fluid as well as MAS is very much needed to better understand the mechanisms of development of neurodevelopmental disabilities in these infants.

Acknowledgments

This study was partially supported by Perinatal Program Grant, IDHS #53789004.

Disclosure

The authors have declared no financial interests.

References


To review current literature related to cellular mechanisms of meconium-induced lung injury (MILI). Review of published experimental in vitro and in vivo MAS studies using human and animal lung cells. We found that meconium induces expression of cytokines and angiotensin II (ANG II)-induced apoptotic process in the lung cells. We postulate that inflammatory cytokines induce ANG II expression, which causes apoptotic cell death after binding to its AT1 receptors. We also demonstrated expression of serpins associated with meconium instillation into the lungs. Serpins are proteins that inhibit cellular proteases and elastases. Expression of serpins may be an attempt to recover lung from these injurious effects. In summary our studies show that whereas meconium induces inflammatory cytokines and subsequent cell apoptosis, the lung cells also try to protect themselves by inducing serpins. The balance of these interactions will determine the residual damage. We believe these new findings are very important in understanding of MILI.

Introduction

Meconium aspiration syndrome (MAS) is one of the major clinical problems encountered in the neonatal period. It is an inflammatory newborn lung disease that frequently leads to severe respiratory failure with a potential fatal outcome in term and post-term infants. Aspiration of meconium is known to cause airway obstruction, damage of airways and surfactant inactivation. Meconium also causes chemical pneumonitis. In the newborn, the major clinical manifestations include problems of oxygenation and respiratory failure associated with increased pulmonary vascular reactivity. It has been shown that persistent pulmonary hypotension is a hallmark of severe MAS. Number of investigators has extensively studied these aspects of MAS.

In addition to the above-described derangements meconium also induces intense inflammatory reaction in the airways and alveoli. However not much has been explored in this field. There is emerging evidence from different animal models that intense inflammatory reaction may be central to the initial progression of MAS and associated severe pulmonary hypertension.

Investigators using animal models have shown the presence of inflammatory-induced cell death through apoptotic process in meconium-instilled lungs. There is increased interest to better understand complex mechanisms of inducing meconium-induced lung cell apoptosis. The purpose of this paper is to review the literature and better understands the mechanisms of meconium-induced lung injury (MILI).

The evidence for meconium-induced inflammation and apoptosis comes from different investigators. These studies can be classified into two categories: one from human studies and the other from experimental animal models. Table 1 shows selected papers in this regard. In general, these findings showed that meconium in both human and animal models produces inflammatory reaction, which is followed by cascade of events leading to lung epithelial cell apoptosis. Findings of these studies are summarized below.

Human studies

There are very few human studies (Table 1). De Beaufort et al. were the earliest investigators who studied in vitro response of human neutrophils to MAS. They showed that there was overexpression of interleukin 8 (IL-8) in neutrophils exposed to meconium. They also showed anti-IL-8 antibodies inhibited neutrophils migration. These studies suggested that MAS in infants may induce neutrophils migration and overexpression of IL-8 lead to inflammation.

Uotila et al. studied meconium exposure to monocytes in culture and showed increased expression of cycloxygenase 2 expression. Jones et al. demonstrated increased production of inflammatory cytokines in preterm infants with MAS. Castelheim showed compliment activation in human monocytes culture of MAS model.

Animal studies

Investigators have studied meconium-induced inflammation using different animal models (Table 1). Davey et al., in piglets, showed...
acute decrease in gas exchange and dynamic lung compliance, increase of total lung protein and inhibition of surfactant. Holopainen\textsuperscript{12} made extensive observations in piglets exposed to meconium. They demonstrated severe inflammation and airway epithelial damage, necrotic changes associated with increased phospholipase 2 activities. Mollnes also showed an increased compliment activation following experimental MAS in piglets.\textsuperscript{13}

Khan\textsuperscript{14} showed that MAS increases IL-13 expression along with lymphocytic and eosinophilic inflammation. Tyler\textsuperscript{3} in animal model showed that MAS leads to surfactant inactivation and pneumonitis. In our own laboratory, using rabbit pups,\textsuperscript{6,15,16} we have shown that lung exposure to meconium leads to intense inflammatory reaction with increased expression of lymphocytes, macrophages and neutrophils. Experimental studies showed that inflammatory response and apoptotic epithelial cell death is important feature of meconium-induced newborn lung injury. Our studies differ from previously published studies because of the methodology used. In our studies we used rabbit pups with no previous hypoxia. Another major distinction from other studies is that we did not use whole meconium, because whole meconium contains many components that can cause lung injury. Instead, we used debris-free meconium to avoid mechanical obstruction of airways. One gram of fresh newborn meconium (1 g) was homogenized on ice with 9 ml of 0.9% NaCl (pH 7.2) to a final concentration of 10% (weight/volume) and was spun down at 1000 g for 20 min (at 4 °C) to separate supernatant and meconium debris. Supernatant was filtered using 0.45 mm filter paper (Millipore Co., Bedford, MA, USA). The cell-free filtered solution instilled into the lungs. Following instillation, rabbits were allowed to breathe spontaneously in room air with no additional oxygen. No respiratory support was provided to pups following instillation of meconium. Animals were sacrificed at 0, 2, 4, 8 and 24 h after meconium instillation. The findings of other studies including ours are described below.

### Inflammatory responses to meconium

The first response to meconium injury is the release of active inflammatory cells, the most important of which are neutrophils and macrophages.\textsuperscript{8,9,16} Especially, neutrophils are dramatically increased in meconium-instilled lungs compared to saline-instilled lungs. Macrophages are also increased, but not as dramatically as neutrophils. Tessler recently suggested that meconium also induced free oxygen radicals.\textsuperscript{17} Now we describe the pathways of induction of inflammatory response following cell influx.

### Table 1 Human and animals models of MAS

<table>
<thead>
<tr>
<th>Human models of MAS</th>
<th>Animal models of MAS</th>
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</thead>
<tbody>
<tr>
<td>Srinivasan and Vidyasagar\textsuperscript{2}</td>
<td>Tyler et al.\textsuperscript{3}</td>
</tr>
<tr>
<td>Jones et al.\textsuperscript{4}</td>
<td>Animal models</td>
</tr>
<tr>
<td>de Beaufort et al.\textsuperscript{8}</td>
<td>Rabbit pups</td>
</tr>
<tr>
<td>Uotila and Kaapa\textsuperscript{9}</td>
<td>Piglets</td>
</tr>
<tr>
<td>Castelhaim et al.\textsuperscript{10}</td>
<td>Piglets</td>
</tr>
<tr>
<td>Holopainen et al.\textsuperscript{12}</td>
<td>Holopainen et al.\textsuperscript{12}</td>
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<tr>
<td>Molines et al.\textsuperscript{15}</td>
<td>Molines et al.\textsuperscript{15}</td>
</tr>
<tr>
<td>Khan et al.\textsuperscript{14}</td>
<td>Rabbit pups</td>
</tr>
<tr>
<td>Zagariya et al.\textsuperscript{15}</td>
<td>Rabbit pups</td>
</tr>
<tr>
<td>Zagariya et al.\textsuperscript{16}</td>
<td>Rabbit pups</td>
</tr>
<tr>
<td>Tessler et al\textsuperscript{17}</td>
<td>Newborn infants</td>
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</table>

Abbreviations: IL, interleukin; MAS, meconium aspiration syndrome; TNF, tumor-necrosis factor.
Neutrophil influx

Aspirated meconium produces a vigorous but transient leukocytic inflammatory reaction in the newborn lungs, but the mechanisms of the neutrophil influx are still unclear. Meconium itself may stimulate neutrophil chemotaxis and it supposedly induces the lung inflammatory cells to produce proinflammatory chemotactic cytokines. Ex vivo studies using a piglet model show that moderate and high concentrations of aspirated meconium rapidly activate circulating neutrophils. Experimental study in piglet model suggests that meconium-induced inflammation is localized in the meconium-contaminated areas of the lungs with no generalized inflammatory injury. Correspondingly, local accumulation of neutrophils and macrophages and production of IL-1 and tumor necrosis factor-α (TNFα) cytokines have been observed. Current experimental data suggests that intrapulmonary accumulation of activated neutrophils, similar to various forms of acute injury in adult lungs could aggravate the hypoxic respiratory failure. Myeloperoxidase activity is usually used as an important indicator of neutrophil accumulation. The chemotaxis is related to the presence of powerful chemotactic role of IL-8.

In a study by Ruiz et al., addition of polyclonal immunoglobulin G (IgG) activated the mononuclear cells to express IL-8 cytokine production, which could promote intrapulmonary accumulation and infiltration of neutrophils. Other studies have shown that intravenous IgG may increase of neutrophil migration to the sites of inflammation via unknown mechanisms. Sterile meconium increased migration of neutrophils obtained from neonates in comparison with random migration in vitro studies. These studies show that meconium induces chemotaxis of inflammatory cells especially neutrophils. These above studies showed the neutrophil response to meconium.

Cytokine release and their effect

In experimental models of MAS, an intense pulmonary inflammatory reaction with influx of polymorphonuclear leukocytes, T-lymphocytes, monocytes and macrophages is demonstrated within a few hours. It is further apparent that intrapulmonary meconium stimulates the lung inflammatory cells to express inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, and may thereby propagate development of parenchyma cell injury in the exposed animal and human lungs. A novel signaling pathway involved in MAS is the role of IL-13. This molecule has been shown to be markedly elevated in a murine model of MAS and could be a mediator of persistent airway dysfunction and remodeling in survivors of MAS. A dramatic initiation of the pulmonary inflammatory response to meconium aspiration is highlighted by immediate appearance of the lung pro-inflammatory cytokine expression in rabbit models, with a maximum at 8 to 14 h after the meconium insult. As low levels of anti-inflammatory IL-10 is simultaneously found in these lungs, the resulting cytokine imbalance may regulate the susceptibility of the newborn lung to meconium-induced injury.

Complement activation

Various investigators using animal and human cells showed that human meconium is a potent activator of complement, an important mediator of inflammatory tissue damage. Meconium activated the alternate complement pathway by increase of alternative convertase C3bBbp. Meconium can also induce systemic complement activation, paralleling the increase in lung dysfunctions.

Phospholipase activation

Local release of phospholipid-degrading enzyme, phospholipase A2, may further play an exacerbating role in the pathogenesis of acute inflammatory lung injury after meconium aspiration in a human model. As showed by Kaapa, experimental studies in animal models indicate that this proinflammatory activity is also contained in human meconium itself. The existing data on human and animal models thus indicate that constituents of aspirated meconium, including cytokines, phospholipases, bile acids, lipids and polysaccharides, may trigger a local inflammatory reaction supposedly through stimulation of lung inflammatory cells to produce proinflammatory cytokines and prostaglandins. Moreover, recent data demonstrate that activation of lung tissue phospholipase A2, in meconium injury, may mediate cellular apoptosis, as a noninflammatory mechanism of programmed cell death, in porcine meconium aspiration.

Meconium-induced apoptosis

Apoptosis of cells exposed to meconium was one of the interesting findings of our experimental studies. Apoptosis is a programmed cell death, which does not result in inflammatory tissue injury, but rather is a cell clearance mechanism promoting resolution of inflammation. Normal rate of apoptosis is beneficial to the host organ. However, increased apoptosis may clearly cause harm for the tissue. Recent literature including data from our laboratory demonstrate that apoptosis may play an important role in acute lung injury of newborn. As was demonstrated by several techniques, meconium-induced apoptosis is localized preferably in lung and its airway epithelial cells. These data demonstrate that meconium-induced lung cell apoptosis leads to damage and detachment of lung airway or epithelial cells. It is a very important process as airway epithelium plays a very important role in maintenance of cell viability and normal lung functions. In spite a several existing theories, exact mechanism of meconium-induced lung cell apoptosis is presently unknown.

Usually apoptosis is seen immediately after instillation of meconium into the lungs. Apoptosis occurs under the influence of different substances present in meconium. Apoptotic cells are
smaller in size; they appear shrunk, their cytoplasm and nuclei are condensed. Examination of the lung lavage cells demonstrated that apoptosis usually is accompanied with inflammatory reactions, extensive cell death. The apoptotic cells are identified by measuring caspase 3 expression. Maximal expression of caspase 3 was also seen at 8 h after meconium instillation (Figure 1).

ISEL labeling is another method of identifying the apoptotic cells. Using ISEL labeling, we found a fragmented DNA in numerous airways and alveolar epithelial nuclei. ISEL-positive cells were seen specifically in meconium-instilled lungs but not in saline-instilled lungs. ISEL-positive cells surround the bronchioles and gradually expanded to the entire lung field.

Apoptosis destroys many important cells in the lung airway or alveolar epithelium. In worst cases, such destruction may reach up to 50% of airway epithelial cells. The mechanism of meconium-induced epithelial cell apoptosis and their detachment presently remains unknown. Also unknown are mediating mechanisms of apoptotic cell death, which we will describe below.

Oxygen radicals in apoptosis

Increased release of reactive oxygen species from the animal pulmonary inflammatory cells may be associated with apoptotic cell death in the lung. Neutrophil influx following meconium exposure may also activate alveolar macrophages to produce oxygen radicals, which may, at least in part, explain the proapoptotic effect of meconium in animal and human lungs.

Nitric oxide and apoptosis

Although the bulk of evidence indicates that nitric oxide (NO) may induce apoptosis in different cell lines, including macrophages, it may have a dual effect. It is interesting to note that inhaled smaller quantities of NO may also inhibit apoptosis. Inhalation of NO (10 p.p.m.), in addition to having a beneficial effect on hemodynamics and oxygenation in newborn lungs, also significantly inhibited cell death by apoptosis. The effect of a smaller amount (1 p.p.m.) of NO inhalation was similar but did not reach statistical significance. Apoptotic cell death was induced by exogenous NO in rat type II pneumocytes in vitro but not in cultured human cells of pulmonary epithelial origin. Although the inhibitory effect of NO is still unclear, interaction of NO with oxygen-reactive species may eliminate the adverse affects of these toxic radicals or, alternatively, upregulate protective genes that attenuate apoptosis. Furthermore, increased cyclooxygenase-2 expression, shown to be stimulated in monocytes by human meconium in vitro, may modulate NO-mediated apoptosis in macrophages.

Role of ANG II and its receptors in apoptosis

Pulmonary angiotensin II (ANG II) has been recently proposed to contribute to receptor-mediated lung epithelial apoptosis in vivo and in vitro, but in neonatal lung injuries in vivo this mechanism is still unclear. We demonstrated using a rabbit model of MAS that apoptosis occurs via cytokine-induced angiotensinogen gene expression, conversion of ANG I to ANG II, and binding of ANG II to its AT1 receptors. This conversion requires the presence of ANG-converting enzyme, ACE. In animal models captopril may also decrease the apoptosis process in alveolar epithelium induced by FAS, bleomycin, plant alkaloid monocrotaline, or γ-irradiation. Consequently, it was hypothesized that meconium-induced apoptosis could be prevented by suppression of ANG I conversion to ANG II by captopril pretreatment or by blocking of its AT1 receptors. Recent data from our laboratory indicate that captopril and inhibition of AT receptor action may diminish pulmonary epithelial apoptosis in meconium lungs. Additional data from our laboratory show that cytokine-treated rabbit lung and human lung airway epithelial cell line A549 demonstrate a similar rate of lung apoptosis, compared to meconium-instilled lungs. From the above discussion it may be postulated that intrapulmonary meconium induces influx of inflammatory cells in the lungs. These cells express inflammatory cytokines, involved in expression of ANG I, which is immediately processed to ANG II by ACE. Lung cell damage and apoptosis occur when ANG II binds to its AT1 but not AT2 receptors (Figure 1). It is, however, not clear how the above-discussed mechanisms are initiated.

Protective role of serpin in apoptosis

During our studies to explain the mechanisms of apoptosis, we isolated a protein from tracheal aspirates. We found that this protein to be a novel serine proteinase inhibitor, with an apparent molecular mass of 50 kDa, which was identified to be e-1-antitripsin. Serpins are known inhibitors of proteases and apoptosis. We hypothesize that serpin may attenuate pulmonary inflammation and improve surfactant function after meconium instillation into 2-week-old rabbit lungs.
aspiration. α1-antitripsin is a member of the proteinase inhibitor (serpin) superfamily and inhibitor of neutrophil elastase. α1-Antitripsin is synthesized by epithelial cells, macrophages, monocytes and neutrophils. Deficiency in α1-antitripsin leads to exposure of lungs to uncontrolled proteolytic attack from neutrophil elastase or other damaging factors culminating in lung destruction and cell apoptosis. We hypothesize that accumulation of α1-antitripsin in the lungs serves as a predisposed protection against MILI.

Unified concept of MILI

Taking into consideration of all the above findings we propose a unified concept of the mechanism of MILI. The construct of the hypothesis is shown in Figure 2. It is proposed that meconium aspiration by the newborn besides causing airway obstruction causes local inflammation. The inflammatory reaction leads to cellular influx of neutrophils and macrophages. Both release inflammatory cytokines, which in turn cause inflammation and set the process of apoptosis. The end result depends on the extent of inflammation and degree of apoptosis. Moderate apoptosis may leave no permanent damage. Severe apoptosis initiates fibrotic reaction. These results may become perceptible on histological examination of the tissues.

The sequence of events in the epithelial cells is different. Epithelial cells express ANG II and their AT1 and AT2 receptors. Both participate in the process of cell apoptotic cell death.

It is also to be noted that meconium can induce serpin expression, which plays a protective role against injury and apoptosis which outcomes depends from the ration of proteases and serpins. If this ration is large a large damage is observed; if small, it is minimal.

Figure 2 Meconium-induced lung injury.

In summary, meconium aspiration induces a rapid and intensive inflammatory reaction and lung injury within the contaminated areas of the newborn lungs and produces simultaneously lung cell death by apoptosis. In addition, based on our work, we believe that meconium-induced lung cell apoptosis is also induced by accumulation of ANG II and binding to its AT1 receptors. TNFα or IL-8 cytokines may be involved in processing ANG II and thus is also involved in increasing meconium-induced cell apoptosis.

We also believe that degree of apoptosis is dependent on the production of proteases and serpins in the cells. Serpins inactivate meconium-induced proteases thus prevent lung damage. In summary, we propose that meconium induces cytokine expression and lung cell apoptosis. At the same time expression of serpins in response to meconium may negate the effect of proteases induced by meconium (Figure 3).

The significance and contribution of these lung injury processes to the clinical manifestation of MAS, however, needs to be further investigated. Better understanding of meconium-induced inflammatory injury and apoptosis may lead to a development of new therapeutic interventions of MAS.

Figure 3 Cytokines and serpin in meconium-induced apoptosis.

Summary

In summary, meconium aspiration induces a rapid and intensive inflammatory reaction and lung injury within the contaminated areas of the newborn lungs and produces simultaneously lung cell death by apoptosis. In addition, based on our work, we believe that meconium-induced lung cell apoptosis is also induced by accumulation of ANG II and binding to its AT1 receptors. TNFα or IL-8 cytokines may be involved in processing ANG II and thus is also involved in increasing meconium-induced cell apoptosis.

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Disclosure

The authors have declared no financial interests.
Inflammatory cytokine expression and apoptosis

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References


REVIEW

Angiotensin II in apoptotic lung injury: potential role in meconium aspiration syndrome

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Meconium aspiration injures a number of cell types in the lung, most notably airway and alveolar epithelial lining cells. Recent data show that at least some of the cell death induced by meconium occurs by apoptosis, and therefore has the potential for pharmacologic inhibition through the use of apoptosis blockers or other strategies. Related work in adult animal models of lung injury has shown that apoptosis of lung epithelial cells induces a local (that is, entirely lung tissue specific) renin-angiotensin system (RASL). Furthermore, this inducible RASL is required for the apoptotic response and affects other adjacent cell types through the release of angiotensin II and related peptides. This manuscript reviews the published data supporting this viewpoint as well as more recent works that suggest the involvement of a RASL in the perinatal lung damage associated with meconium aspiration syndrome (MAS). The implications of these findings regarding their potential for the clinical management of MAS are also discussed.


General mechanisms in apoptosis

Apoptosis, sometimes referred to as programmed cell death (PCD) or ‘cell suicide’, is a metabolically active process of cellular dismantling. Apoptosis avoids the inflammation often associated with necrotic cell death because cellular contents are often not released from apoptotic cells as they are from necrotic cells. A variety of cellular signaling pathways regulates apoptosis, which is characterized morphologically by nuclear fragmentation, cleavage of chromosomal DNA into internucleosomal fragments, packaging of cellular debris into ‘apoptotic bodies’ without plasma membrane breakdown and exposure of surface molecules targeting the cell remnants for phagocytosis.1,2

Major signaling pathways leading to apoptosis involve the activation of cystein-dependent, aspartate-directed proteases termed caspases. Depending on the type of stimuli and/or cell type, the apoptotic cascade can follow either an intrinsic pathway, involving apoptogenic mitochondrial proteins such as cytochrome c, or an extrinsic pathway that follows activation of a death receptor. The receptor is usually activated by its extracellular ligand, such as the binding of Fas ligand (FasL) to Fas (APO-1 or CD95) or tumor necrosis factor-α (TNF-α) binding to TNF-α receptor. On activation, initiator caspases (caspase-2, -8, -9, and -10) propagate death signals by activating downstream effector caspases (caspase-3, -6, and -7) in a cascade-like manner. Activated effector caspases mediate the release of caspase-activated DNase (CAD) from the complex with its endogenous inhibitor (ICAD) through a proteolytic cleavage. The activated CAD then cleaves genomic DNA between nucleosomes to generate DNA fragments, which are multiples of 180 to 200 bp in length. These fragments can form the ‘DNA ladder’ pattern seen on electrophoresis, a frequently used marker of apoptosis.

Caspase-independent mechanisms of apoptosis are mediated by mitochondrial effectors; in this pathway, poly(ADP-ribose) polymerase-1-mediated DNA damage is signaled to mitochondria. This leads to release and translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus. AIF enhances endonuclease activity through its cooperation with endonuclease G or cyclophilin A, which leads to large-scale DNA fragmentation and chromatin condensation.

In contrast to apoptosis, which requires energy in the form of ATP, necrosis is the end result of a bioenergetic catastrophe resulting from ATP depletion to a level incompatible with cell survival. Cells that die by necrosis frequently exhibit changes in plasma membrane and nuclear morphology but not the organized chromatin condensation or DNA laddering that are characteristic of apoptosis.1 Necrosis-like PCD, or autophagy, is also considered as alternative PCD that is caspase independent. Autophagy features degradation of cellular components within the intact dying cell in autophagic vacuoles that undergoes phagocytosis. Autophagic cells exhibit vacuolization, degradation of cytoplasmic contents and slight chromatin condensation; the molecular mechanisms regulating autophagy are now being elucidated.2,3

Mechanisms of apoptosis specific to the lungs

The topic of apoptosis and its regulation in the lungs has been reviewed recently,4 this section will briefly summarize apoptosis...
mechanisms specific to acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis (PF) that may be relevant to meconium aspiration syndrome (MAS).

Apoptosis of epithelial cells, neutrophils and endothelial cells are all thought to play roles in ARDS. The Fas-FasL system is thought to play a role in epithelial apoptosis in ARDS on the basis that bronchoalveolar lavage (BAL) fluids obtained from ARDS patients contain soluble Fas and FasL and induce apoptosis in lung epithelial cell line.3–8 Involvement of BAX, a homolog of Bcl-2 and its likely proapoptotic effect on type II pneumocytes was suggested in studies of diffuse alveolar damage.9,10 Neutrophils were found to accumulate at inflammatory sites in the lung, in pulmonary edema fluid and BAL fluid from patients with ARDS.11 This accumulation is due in part to delayed neutrophil apoptosis; in addition, apoptosis of epithelial cells is enhanced at the same site.12–14 BAL fluids from patients with ARDS had elevated levels of granulocyte/macrophage colony-stimulating factor that inhibits neutrophil apoptosis through phosphoinositide 3-kinase and ERK pathway15,16 and stabilizes the factor Mcl-1 that is crucial for the delay of apoptosis initiated by antiapoptotic factors.17,18 BAL fluids from patients with ARDS was also cytotoxic to lung microvascular endothelial cells due to the presence of TNF-α and angiotatin, an inhibitor of angiogenesis in vivo.19,20 Furthermore, apoptosis signal-regulating kinase (ASK)-1 was involved in apoptosis of pulmonary vascular endothelial cells challenged with H2O2.21 Given the known roles of H2O2 generated by activated neutrophils and other phagocytes, this suggests the possibility that inhibitors of ASK-1 and its signaling pathway might have potential benefit in the management of ARDS.

Cell death by apoptosis is likely an important contributor to the pathogenesis of COPD. Apoptosis of both inflammatory and alveolar wall cells in emphysema was reported by several research groups.22–25 Induction of apoptosis by active caspase-3 instillation in mice caused architectural changes in the lungs similar to those observed in human emphysema.26 Oxidant stress, such as that resulting from cigarette smoke, results in the killing of lung cells by apoptosis and necrosis. Glutathione S-transferase was shown to have a protective effect on tobacco smoke-induced apoptosis.27,28

Other studies have implicated endogenous growth factors and cytokines in COPD-associated apoptosis. One example is vascular endothelial growth factor (VEGF); decreased VEGF expression or blockade of VGEF receptors caused emphysematous changes in lung architecture.29,30 Another group of endogenous mediators believed to play role in apoptosis of parenchymal cells of the lung in COPD is TNF-α, FasL and Fas. However, it is unclear at present whether these molecules are elevated or contribute to the severity of COPD.31–33 and more work is needed to clarify these issues.

Recent studies support the notion that apoptosis contributes to the pathogenesis of lung fibrosis as well as to its resolution. Many studies strongly suggest a role of epithelial apoptosis as a key profibrotic event in lung fibrogenesis. Apoptosis of alveolar epithelial cells (AECs) is found in patients with idiopathic PF (IPF) and in animal models of the disease.34–36 The epithelial apoptosis colocalizes with myofibroblasts where collagen deposition is severe.37 Blockade of apoptosis by either the angiogenxin-converting enzyme (ACE) inhibitor captopril, caspase inhibition or the forced expression of p21 in lung epithelial cells exerted antifibrotic effects in animal models.38,39,40 Together, these data suggested that the fibrotic response is secondary to the apoptotic death of epithelial cells in the lung. The death receptor Fas was found to be expressed in AECs within the lungs of IPF patients and circulating levels of its ligand FasL correlated with disease activity.40–42 Similar observations were observed in animal models.43,44

Activation of Fas-induced apoptosis of bronchial and AECs is sufficient to initiate a fibrotic response in animal models.45 However, development of bronchiolar and alveolar epithelial apoptosis and fibrosis in the lungs of Fas-null mice was similar to wild-type mice,45,46 which suggests that other pathways different from Fas can initiate epithelial apoptosis and facilitate fibrogenesis. Other works have suggested the involvement of c-Jun N-terminal kinase and p38, members of the mitogen-activated protein kinases family pathways in the signaling of epithelial apoptosis.46,47 Finally, several lines of evidence have shown that the angiotesin system plays a critical role in AEC apoptosis and in lung fibrogenesis, and these will be discussed next.

The endocrine RAS versus local tissue RASes

In the classical or ‘endocrine’ RAS described many years ago, the 58 kDa precursor protein angiotensinogen (AGT) is synthesized primarily by the liver and is secreted into the circulation, where it is proteolytically cleaved by the kidney-derived aspartyl protease renin to yield the 10 amino-acid peptide angiotensin 1 (ANGI). ANGI travels through the vasculature and on entry into the pulmonary vascular bed, is cleaved by the dipeptidyl carboxypeptidase ACE to yield the eight amino-acid peptide angiotensin II (ANGII). ANGII then travels through the blood and elicits a number of endocrine functions including the release of aldosterone from the adrenals and contraction of vascular smooth muscle throughout a variety of vascular beds.48

More recently described local RASes, which occur in virtually every organ and tissue in which their presence has been evaluated, are classified as ‘extrinsic’ or ‘intrinsic’ depending on the origin of RAS system components. In extrinsic local RASes (RAS L[ext]), such as is believed to occur in the heart,49 one or more of the RAS components are synthesized locally but others are derived from the endocrine RAS. In intrinsic local RASes (RAS L[int]), all of the components of a functional RAS are synthesized locally. In many local organ systems the term ‘RAS’ is a misnomer because often renin is not expressed locally but other aspartyl proteases such as
cathepsin D can accomplish the same proteolytic conversion of AGT to ANGI; moreover, other dipeptidyl carboxypeptidases such as chymase can cleave ANGI to ANGII. For this reason, many tissues have ‘ACE-independent’ pathways of ANGII formation that may not be affected by specific ACE inhibitors. Regardless, most tissues exhibit some degree of expression of the major ANGII receptor subtypes AT1 and AT2, either at baseline or after injury, which often upregulates one or both of these receptors.50

**The role of RASes in lung injury and fibrogenesis**

A wealth of literature supports the ability of ACE inhibitors or ANG receptor blockers to attenuate experimental lung cell death and fibrogenesis. The prototype ACE inhibitors captopril or lisinopril attenuated experimental lung fibrosis induced by monocrotaline.51 Bleomycin52 or paraquat53 and also blocked apoptosis of lung alveolar epithelial cells in response to bleomycin57 or the antiarrhythmic agent amiodarone.59 Given the central role of epithelial cells death in lung injury in a variety of disease states, attention as been focused on defining the role of ANGII in apoptosis of lung alveolar epithelials (AECs).

In cell culture studies, ANGII itself was found to be a potent inducer of apoptosis in both the human AEC cell line A549 and in primary AECs isolated from adult rats.60 The EC50s for ANGII-induced apoptosis in these cells (50 and 10 nm, respectively) are far above the concentrations of ANGII found in the circulation.48 which provides an explanation of why circulating ANGII does not kill AECs as it passes through the lungs. Although the cultured AECs expressed both the AT1 and AT2 ANG receptor subtypes, only AT1-selective antagonists could prevent ANGII-induced apoptosis.61

Subsequent studies found that when cultured AECs are exposed to other inducers of apoptosis, they begin to synthesize their own ANGII de nova, that is, from the precursor AGT. The apoptosis inducers Fas ligand,62 TNF-α,55 bleomycin54 and amiodarone63 all induce expression of the gene for ANGII in AECs at the level of AGT transcription, and cause a significant increase in the steady-state level of AGT mRNA. Moreover, AECs express all the enzymes necessary for conversion of AGT to the mature peptide ANGII, including ACE62 and the aspartyl protease cathepsin D.54 Thus, the alveolar epithelial cell expresses its own intrinsic local RAS in response to a variety of apoptosis inducers.

Further work showed that the expression of this local RAS and the generation of ANGII by AECs are required for AEC apoptosis in response to FAS ligand, TNF-α, bleomycin or amiodarone. In all those cases, AEC apoptosis in response to the inducer could be blocked by either ANG receptor blockers,55,62 ACE inhibitors54,55 or by antisense oligonucleotides against AGT mRNA.54,55,62 Therefore, not only do AECs produce ANGII in response to apoptosis inducers, but the ANGII production is an essential component of the apoptotic response and blockade of ANGII production or receptor interaction can block the cell death. Recent studies of human lung biopsies from patients with IPF have found double labeling of ANG peptides and markers of apoptosis in AECs,65 these data support the notion that ANG peptide synthesis occurs in apoptotic AECs in the intact injured human lung as well as in animal models and cultured cells.

**The role of RAS in MAS and potential for pharmacologic control**

Apoptotic AECs have been observed in several models of experimental MAS,66,67 Interest therefore was turned to the possibility that AECs dying in response to meconium might also require the local intrinsic RAS for their death and might be prevented from dying by ACE inhibitors of ANG receptor blockers. Several lines of investigation support the theory that meconium does indeed induce expression of the local RAS that might be amenable to pharmacologic blockade. Pretreatment of 2-week-old rabbit pups with the ACE inhibitor captopril before intratracheal instillation of human meconium prevented both meconium-induced upregulation of interleukin (IL)-6 and IL-8 and also reduced lung epithelial cell apoptosis detected by DNA fragmentation assay.68 The captopril also appeared to reduced meconium-induced upregulation of AGT mRNA.

In a similar study of rats, Lukkarinen et al.69 showed that pretreatment of rats with the nonselective ANG receptor blocker saralasin, given intraperitoneally before meconium instillation, prevented AEC apoptosis detected by both DNA fragmentation assay and electron microscopy. The meconium increase AGT mRNA in the lungs, suggesting apoptosis-induced upregulation of AGT in AECs. The saralasin also reduced meconium-induced upregulation of endothelial monocyte-activating polypeptide (EMAP-II) and total lung neutrophil accumulation, but had little effect on the increased wet/dry weight ratio induced by the meconium. The saralasin also had a small but statistically insignificant ability to reverse altered arterial blood gases.

Although not statistically significant, this latter result suggests that further refinement of dosing or route of administration might offer improvement of lung function, especially if more potent and selective ANGII antagonists (AT1 receptor-selective) or intratracheal administrations are explored. Regardless, all the data taken together strongly suggest that meconium, like other inducers of AEC apoptosis and lung injury, elicits the expression of the local RAS in AECs and that the RAS is intimately involved in their demise. Given the many agents already in clinical use for the manipulation of the RAS, it is proposed that further exploration of antagonists of the RAS and creative approaches to their application...
(for example, aerosols) may offer clinically useful adjuncts to the current pharmacotherapy of MAS in the future.

Disclosure

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Apoptosis and angiotensin II in meconium aspiration
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To review and summarize experimental data examining the effects of different fractions of meconium, and to test the effect of albumin on meconium aspiration both as prophylactic and rescue treatment. Newborn piglets 2 to 5 days of age were made hypoxic and then instilled meconium or fractions of meconium intratracheally. Meconium-added albumin and albumin instilled after meconium were also tested. Lung function and inflammatory cytokines were measured. Both the lipid- and water-soluble fractions induce inflammation in the lungs with elevation of inflammatory cytokines. When meconium was mixed with albumin, the inflammatory effects of meconium were significantly ameliorated. Rescue therapy with intratracheal albumin 5 min after the meconium aspiration syndrome was induced also improved lung function. These results indicate that at least part of the symptoms seen in the meconium aspiration syndrome could be prevented by blocking the active substances of meconium such as bile acids and free fatty acids.

**Introduction**

Aspiration of meconium in the airways of the newborn infant leads to plugging and subsequently to inflammatory changes, surfactant inhibition, respiratory failure and pulmonary hypertension. There have been three approaches to moderate these effects: (1) dilute meconium (amnionfusion, lavage), (2) improve/strengthen surfactant by adding for instance polymers and (3) inhibit harmful effects of meconium per se.

Meconium is a complex mixture containing 75 to 80% water. The other chemical components can be divided into lipid- and water-soluble material. The lipid includes free fatty acids (FFA), triglycerides and cholesterol. The FFA include mainly palmitic, stearic and oleic acids. The water-soluble components include bile acids, bilirubin, dietary fibers and inorganic molecules. The main bile acids of meconium are cholic, hydroxycholic and chenodeoxycholic acids. 1–3 These different subfractions of meconium are leading to reduced activity of surfactant and inflammation therefore promoting atelectasis and impaired gas exchange. Both hydrophilic and hydrophobic components of meconium reduce surfactant function. Furthermore, the lipid fraction has a stronger inhibitory effect on surfactant function than the water-soluble subfraction. 4–7 It is well known that bile salts may cause inflammation 8,9 and that FFA, such as oleic acid, induce severe lung failure when administered intravenously. 10

We have tested the effects of different meconium subfractions on pulmonary function in an animal model of newborn piglets. Because albumin is able to bind FFA, we also examined whether albumin added to meconium alleviates the clinical symptoms of meconium aspiration. In case such an inhibitory effect was found, perhaps this also could be used as rescue therapy. We therefore developed a rescue model instilling albumin intratracheally following meconium aspiration. In this article we summarize and review some of our previous results.

**Methods**

We used newborn piglets undergoing hypoxia and meconium aspiration followed by resuscitation. 11 Human meconium was collected from healthy term newborns and diluted in distilled water, and rotavaporated dry. It was sterilized by γ-irradiation and diluted with sterile water to 110 mg dry weight per ml. Hypoxia with FI02 of 0.08 was induced in newborn piglets 2 to 5 days of age until they were close to collapse. The piglets were then hand-ventilated for 30 s and put back on ventilator with rate increased from 30 to 60 per min, and peak inspiratory pressure increased to 5 Cm H2O. Tidal volume was kept constant at 10 to 15 ml kg⁻¹ and paCO2 4.5 to 6 kPa (34 to 45 mm Hg).

In the first study 3 ml kg⁻¹ body weight of meconium was instilled intratracheally giving an oxygenation index (OI) of 6 to 7 indicating a mild meconium aspiration syndrome (MAS). In this
study meconium was also mixed with albumin (30% albumin in stoichiometric relation to FFA estimated to be 1.4 ml kg\(^{-1}\)) before intratracheal in administration of the mixture.\textsuperscript{12,13}

In the second study 3 ml kg\(^{-1}\) body weight of either meconium, lipid- or water-soluble meconium fractions was instilled intratracheally in separate groups.\textsuperscript{14}

In the third study a moderate and severe model was developed. A moderate MAS was developed by instilling meconium of 4 ml kg\(^{-1}\) body weight, giving an OI of approximately 12. A severe MAS was induced by instilling 5 ml meconium mixture per kg leading to a typical OI of 25. Five min after meconium had been instilled, 3 ml of 30% albumin was instilled intratracheally.\textsuperscript{15}

The animals in all studies were resuscitated for 8 h with room air or supplemental \(\text{O}_2\) to achieve a \(\text{SaO}_2\) of 85%.

**Results**

**Effect of albumin**

Animals receiving albumin mixed with meconium compared with meconium alone had a significantly reduced OI as well as ventilation index. OI was approximately 50% in animals given meconium mixed with albumin compared with those given meconium. The need for oxygen and mean airway pressure on the ventilator was also significantly reduced and compliance increased in animals given the combination of meconium and albumin.\textsuperscript{12}

The inflammatory cytokine interleukin (IL)-8 in tracheal aspirate, 8 h after reoxygenation was started, was in mean fivefold higher (\(P<0.05\)) in animals given meconium alone (93 pg ml\(^{-1}\)) as compared to those given meconium mixed with albumin (18 pg ml\(^{-1}\)) before intratracheal instillation.\textsuperscript{13}

**Effect of meconium fractions**

Both the lipid- and water-soluble fractions of meconium induced inflammatory changes, however, more in the lipid extract than the water extract group. At 8 h after instillation of meconium, IL-8 in tracheal aspirate was threefold higher in the lipid extract group than in the animals given the water-soluble fractions of meconium. Intact meconium had more severe effects on the lungs than the lipid- and water-soluble fractions of meconium separately.\textsuperscript{14}

**Effect of rescue therapy with albumin**

Rescue therapy with a low-dose intratracheal albumin (stoichiometric binding of FFA) did not affect OI or IL-8 in tracheal aspirate when measured at 8 h. However, a significantly attenuated decrease in lung compliance was found.\textsuperscript{15}

**Discussion**

In these studies we found that albumin inhibits clinical effects of meconium and that the lipid fraction is more harmful than the water fraction. Rescue therapy with albumin increases compliance but does not seem to have significant clinical effects in the doses and administration tested out.

Both the lipid- and water-soluble fractions of meconium induce pulmonary inflammation and injury, however, less than meconium alone. The lipid fraction was responsible for most of the cytokine activation assessed by IL-8 in tracheal aspirate. However, the water-soluble fraction also had some inflammatory properties.

We chose albumin as a protein with high capacity to bind FFA in spite of the fact it is known that albumin also inhibits surfactant function. However, in our animal model, and probably in patients with MAS as well, surfactant function is already abolished.

Albumin added to meconium before intratracheal instillation, almost completely blocked the negative effects of meconium. Albumin seems to prevent development of MAS when added to meconium. However, when albumin is given as rescue therapy the effect is less clear. In another of our studies rescue therapy by lavaging the lungs with excess albumin gave worse outcome than meconium alone (submitted). Rescue therapy with albumin in low doses and low volume therefore had a small but significant and positive effect on compliance, however, larger doses and volumes seem to have a detrimental effect on pulmonary function.

The optimal albumin dose, timing and mode of administration therefore are unknown. Other inhibitors of both the lipid- and water-soluble fractions of meconium should be searched for and tested aiming at developing an efficient rescue therapy. Several such inhibitors as polymers and anti-inflammatory agents have been described already. Furthermore, meconium-induced apoptosis may be inhibited by angiotensin-converting enzyme inhibitors, which may reduce injury.\textsuperscript{18,19}

Meconium is a potent complement activator and complement inhibition may represent a future therapeutic principle in MAS.\textsuperscript{20}

In the future all the principles discussed above may be combined to develop a powerful therapy for MAS. Given the available presented data, there does not appear to be a robust argument for the use of albumin in the human infant with meconium aspiration, let alone suggest that there may be ‘an optimum dose’, timing and mode of administration.

**References**

The role of complement in meconium aspiration syndrome

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The complement system is part of the host defense with a number of biological effects, most of which contribute to the inflammatory reaction by activation of cells like leukocytes and endothelial cells. An intact complement system is required for protection against infection and for maintaining internal inflammatory homeostasis. However, the system is a double-edged sword as improperly or uncontrolled activation is disadvantageous and potentially harmful for the host. Meconium aspiration syndrome (MAS) is associated with a local inflammatory reaction in the lungs, frequently described as a chemical pneumonitis. Cytokines, arachidonic acid metabolites and reactive oxygen species are involved in this reaction. We have recently documented that meconium is a potent activator of complement in vitro and in an experimental piglet model of MAS, the latter presenting with an inflammatory profile closely resembling systemic inflammatory response syndrome. We postulate that complement activation may contribute to the pathogenesis of MAS.

Inflammatory mediators in MAS

The pathophysiology of meconium aspiration syndrome (MAS) is complex. Several mechanisms may be operative in the development of lung injury in MAS, including mechanical obstruction, inactivation of surfactant, pulmonary hypertension and lung inflammation. Although the controversies still remain in the literature as to what extent meconium per se leads to MAS, increasing evidence indicates that the inflammation induced by meconium is an essential part of the pathophysiology. The assumed chemical pneumonitis caused by meconium was first documented in rabbits by infiltration of polymorphonuclear leukocytes in alveolar septa within 6 h after instillation of meconium, and a later study documented increased counts and chemotactic activity of neutrophils in lung lavage fluid from pigs. Leukocytes are important sources for three main inflammatory branches that are induced or activated by meconium: cytokines, arachidonic acid metabolites and reactive oxygen species.

In vitro meconium has been shown to trigger peritoneal macrophages to produce a proinflammatory tumor necrosis factor-α (TNF-α) response and in animals instilled with human meconium interleukin (IL)-6, IL-8, TNF-α and IL-1β have been detected in cells harvested from lung lavage within 8 h after meconium instillation. In another animal studies with the same observation time, TNF-α and IL-8 have been detected in bronchoalveolar lavage fluid whereas IL-5 and IL-13 were elevated at day 7 in mice. Furthermore, meconium may stimulate chemotaxis, the latter effect claimed to be because of IL-8 present in meconium. Finally, the levels of IL-1β, IL-6 and TNF-α have been compared in sterile meconium vs meconium contaminated with bacteria and in spite of variation among samples the median concentrations did not differ. The transcription factor NF-κB, known to induce a number of proinflammatory cytokines, was shown to be activated by meconium in rat alveolar macrophages.

Human meconium is shown to contain phospholipase A2, thereby capable of directly damaging alveolar cells. Meconium was found to increase the production of arachidonic acid metabolites by phospholipase A2 and elevated catalytic activity of phospholipase A2 was found both in tissue and lavage fluid. In addition, meconium has been shown capable of upregulating cyclooxygenase-2 mRNA expression in rat lungs, a process that is inhibited by dexamethasone, but not indomethacin and enhance the release of thromboxane-A2 in human airway epithelial cells. Finally, apoptosis associated with meconium could be inhibited by angiotensin II receptor blockade.

The effect of meconium on oxidative burst (release of reactive oxygen species) has been difficult to interpret. Although one study showed that meconium stimulated alveolar macrophages to produce superoxide anion, another study showed that light and very light meconium inhibited neutrophil oxidative burst. Recently, it has been showed that meconium has an inhibitory effect on neutrophils in low concentrations (0.2 mg ml⁻¹) but in higher concentrations (1 and 2 mg ml⁻¹) stimulates neutrophil oxygen radical production progressively. A possible role for...
Complement in meconium-induced oxidative burst has been recently postulated (see below).

**The complement system**

Complement is one of the plasma cascade systems and represents a main part of fluid-phase innate immunity. It consists of more than 30 proteins acting together in a specific manner with the principal function to protect the host against invading organisms. A simplified cartoon of the complement system is shown in Figure 1. There are three initial pathways of activation.

The classical pathway (upper left) is activated either by natural or elicited antibodies binding to antigen, or by direct, antibody-independent activation, for example, by C-reactive protein. C1q triggers the serine proteases C1r and C1s, the latter cleaving C4 to C4b, which exposes a specific binding site for C2. C1s then cleaves C2 and the resulting C3 convertase C4b2a cleaves C3 to C3b to form the C5 convertase C4b2a3b. Splitting of C5 to the highly potent anaphylatoxin C5a and the C6-binding fragment C5b is the last enzymatic step in the cascade. Activation of the lectin pathway (upper middle) is initiated by mannose-binding lectin (MBL) recognizing mannose on bacteria, by immunoglobulin A and probably by structures exposed by damaged endothelium. MBL is homologous to C1q and triggers the MBL-associated serine proteases (MASPs), of which three forms (MASP1, MASP2 and MASP3) have been described. Further lectin pathway activation is virtually identical to classical pathway activation forming the same C3 and C5 convertases. The alternative pathway (upper right) activation differs from the classical and lectin pathways by a physiological, low-graded spontaneous hydrolysis of the internal thiol ester of the C3 molecule thereby binding factor B, which is then cleaved by factor D. The C3 convertase then formed cleaves C3 to C3a and C3b, the latter binds factor B forming C3bB, which is cleaved by factor D and the alternative pathway C3 convertase, C3bBb, is formed leading to further cleavage of C3. C5 is then activated in the same manner as for the classical and lectin pathway C5 convertase.

Thus, all three pathways converge at C3 and the terminal pathway (lower part of Figure 1) proceeds in the same way irrespective of the initial pathway activation, by assembly of C7 to C5b-6, forming an amphiphilic complex able to insert into a lipid membrane. One C5b-7 moiety binds one C8 and one or several C9 molecules, creating a physical pore penetrating the membrane (C5b-9(m)) or membrane attack complex, leading to transmembrane leakage and subsequent cell activation, or more infrequently to lysis (lower right). If the activation occurs in the fluid phase and there is no membrane present, the C5b-7 complex binds to vitronectin and clusterin (fluid-phase regulators of the terminal pathway) and thus retains hydrophilic properties. Final assembly of a soluble C5b-9 (SC5b-9), the second form of the terminal complement complex (TCC), occurs by binding of C8 and C9. Soluble TCC is a particularly useful indicator of complement activation and an assay for use in humans, baboons and pigs has been developed.

Complement activation is strictly regulated by inhibitory proteins of which some are illustrated in Figure 1 (boxes with broken lines). The biological potency of this system is substantial and when activated out of control it may break down essential homeostatic mechanisms, for example, as seen in irreversible septic shock. Thus, it is crucially important to keep the system under control by the regulatory proteins. C1 inhibitor regulates the initial classical and lectin pathways. Carboxypeptidase N inactivates the anaphylatoxins C5a, C3a and C4a, of which C5a is particularly potent. Factor I cleaves and inactivates C4b and C3b and uses C4b-binding protein (C4BP) as cofactor in the classical/lectin pathway and factor H in the alternative pathway. The membrane regulators complement receptor 1 (CR1; CD35), membrane cofactor protein (MCP; CD46) and decay-accelerating factor (DAF; CD55) regulate complement activation by either acting as cofactors for factor I-mediated cleavage of C4b and C3b (CR1 and MCP), or accelerating the decay of the biomolecular C3 and C5 convertases (CR1 and DAF). CD59, also a membrane regulator, prevents the binding of C9 to the C5b-8 complex in the terminal pathway.

The main biological effect of complement activation is induction of an inflammatory reaction. Many of the activation products are involved in this process, but C5a is particularly important. Innumerable inflammatory mediators are induced by C5a, when it reacts with its receptor (C5aR). Most of the inflammatory branches can be triggered by C5a (Figure 2), illustrating that complement has a key function as an upstream mediator of a broad spectrum of inflammatory reactions. Thus, activated complement is a double-edged sword with undesired effects in many conditions. Various reagents with potential
Inflammatory effects mediated by C5a

<table>
<thead>
<tr>
<th>Chemotaxis</th>
<th>Histamin release</th>
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<td>Neutrophil migration</td>
<td>Smooth muscle contraction</td>
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<td>Neutrophil aggregation</td>
<td>Increased permeability</td>
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**Figure 2** Biological effects of C5a. The potent anaphylatoxin C5a released during complement activation induces a number of inflammatory mediators of which several are released to the fluid phase whereas others are expressed on the surface of cells. The fluid-phase mediators may then act directly as inflammatory agents or may trigger secondary effects through receptor binding. The surface proteins induced by C5a include adhesion molecules, leading to trafficking of cells through interaction between endothelial cells and leukocytes.

Therapeutic applications have been recently developed to target complement activation and function. 35

**MAS and the complement system**

In the first study of a possible interaction of complement and MAS it was documented that meconium *in vitro* is a highly potent activator of the complement system. 36 Adding meconium to human umbilical serum induced a substantial increase in TCC formation, which was found to be mediated mainly through the alternative activation pathway. As judged by their relative amounts in whole meconium, the lipid and water fractions contributed equally to this activation. However, corrected for the lower amount of the lipid fractions compared with the water fractions in normal meconium, the lipid fraction per weight was more potent.

In a whole-blood model established for studying the interaction of the complement system with the inflammatory network, 37 we studied the role of complement in meconium-induced leukocyte activation as measured by oxidative burst and expression of the surface activation markers CD11b and L-selectin. 38 Meconium induced a marked oxidative burst that was reduced by 60 to 70% when adding the alternative complement pathway inhibitor anti-factor D. Furthermore, meconium induced a substantial increase in CD11b and decrease in L-selectin, typical for leukocyte activation, which was virtually abolished (95 to 100%) by adding anti-factor D. Importantly, inhibition of complement using a specific C5aR antagonist gave the same inhibition of both oxidative burst and expression of CD11b and L-selectin as anti-factor D, implying that C5a was solely responsible for the meconium-induced complement-dependent leukocyte activation.

On the basis of these *in vitro* experiments we proceeded with *in vivo* studies using a well-established piglet model of MAS. 39 Here we demonstrated that complement is activated systemically, as detected by a substantial increase in plasma TCC. The increase in TCC correlated positively with oxygenation and ventilatory indexes and negatively with lung compliance. Finally, the mortality of the piglets correlated with the increase in TCC, indicating that the breakdown in homeostatic mechanisms may be associated with uncontrolled complement activation. These observations led us to further investigate the role of complement in experimental MAS. 40

In this study we found that experimental MAS piglets reflected an inflammatory response, measured by cytokines and complement, closely resembling the systemic inflammatory response syndrome (SIRS). We therefore propose that MAS is associated with a general whole-body SIRS-like inflammatory reaction. It remains, however, to be shown whether complement activation actually contributes to the pathophysiology of clinical MAS and if inhibition of complement may attenuate inflammation and severity in MAS.

Lately, we have focused our research on the two main branches of innate immunity: the fluid-phase complement system and the cellular Toll-like receptor (TLR) system, the latter with focus on the CD14/MD2/TLR4 pathway. Thus, we studied the effect of meconium on these two branches as well as the effect of specific inhibition of both complement and CD14. Interestingly, we found that inhibition of both pathways efficiently attenuated the inflammatory reaction induced by meconium (B. Salvesen et al., manuscript submitted).

**Addendum**

To the best of our knowledge we are the first and so far the only group that has studied the interaction between complement and MAS. How comes that these studies were initiated? The answer is simple: an incidental meeting in a PhD dissertation a few years ago by two scientists (first and last authors of this paper), one an expert on complement and the other on MAS—both of them at that time virtually devoid of knowledge on the other’s research field—led to an interesting discussion. First author asked: ‘what is at present your main research?’ After getting the answer he replied ‘what is really MAS?’ Having got a brief introduction to MAS, the last author asked: ‘but what is your main focus?’ After answering, last author replied: ‘but what is actually complement?’ Two completely different scientific worlds met and recognized that we had to proceed on a novel track—does meconium as such activate the complement system and is complement activated in experimental MAS? As there were no reports of this topic in the literature we initiated studies by mutually utilizing well-established models to study complement activation and MAS, respectively. Lessons to learn are that scientific groups to a greater extent should discuss possible interacting mechanisms of pathophysiology when they meet, and we should all accept that homeostasis in human biology is more complex than seen just from our own window, consequently leading to collaboration to obtain scientific progress.
Disclosure
The authors have declared no financial interests.

References
Although the triggering mechanisms of tissue inflammation and injury in meconium-contaminated lungs are still unclear, there is increasing evidence to suggest a central role for phospholipase A2's (PLA2). In fact, elevated PLA2 activities together with high enzyme concentrations, especially the amount of pancreatic (group I) secretory PLA2 (PLA2-I), have been detected in human meconium and in meconium-contaminated lungs. Recent data from our laboratory further indicate that human pancreatic PLA2, introduced in high amounts within aspirated particulate meconium, is a potent inducer of lung tissue inflammatory injury. Our finding of elevated human PLA2-I concentrations in plasma during the first hours after intratracheal meconium administration in newborn piglets further suggests that intrapulmonary aspiration of meconium could also have systemic inflammatory and injurious effects. This, however, remains to be studied in further detail.

Aspiration of meconium is known to initiate a complex cascade of pulmonary processes, resulting in progressively increasing pulmonary vascular tone and a rapidly developing inflammatory tissue injury with concomitant surfactant dysfunction in the affected lungs. Although the critical role of pulmonary inflammation in the course of neonatal meconium aspiration syndrome (MAS) has been emphasized, the triggering mechanisms and the manifold interplay of the injurious mediators in MAS are still poorly identified. There is yet evidence that human meconium may induce production of proinflammatory agents within the lungs, and that some proinflammatory components of meconium itself, such as cytokines and phospholipase A2 (PLA2), may additionally contribute to intense inflammation and injury in the meconium-contaminated lung tissue. PLA2 represents a family of ubiquitous enzymes that through cleavage of membrane phospholipids release arachidonic acid, the precursor of proinflammatory eicosanoids. Although PLA2 may occur as cytosolic (cPLA2) and secretory (sPLA2) types, increasing attention has been paid to the role of secretory PLA2 as an acute phase protein in inflammatory conditions.

In this paper, the present understanding of the role of secretory PLA2 in acute lung injury, specifically when induced by meconium aspiration, is delineated. A new line of basic and clinical research of MAS, namely the possible contribution of meconium-introduced secretory PLA2 to systemic inflammation, is also highlighted.

Aspiration of meconium is known to initiate a complex cascade of pulmonary processes, resulting in progressively increasing pulmonary vascular tone and a rapidly developing inflammatory tissue injury with concomitant surfactant dysfunction in the affected lungs. Although the critical role of pulmonary inflammation in the course of neonatal meconium aspiration syndrome (MAS) has been emphasized, the triggering mechanisms and the manifold interplay of the injurious mediators in MAS are still poorly identified. There is yet evidence that human meconium may induce production of proinflammatory agents within the lungs, and that some proinflammatory components of meconium itself, such as cytokines and phospholipase A2 (PLA2), may additionally contribute to intense inflammation and injury in the meconium-contaminated lung tissue. PLA2 represents a family of ubiquitous enzymes that through cleavage of membrane phospholipids release arachidonic acid, the precursor of proinflammatory eicosanoids. Although PLA2 activity associated with upregulation of PLA2-I may be important in the pathogenesis of acute inflammatory lung injury. Still, variations in the tissue expressions of different PLA2 enzyme types in the insulted lungs may also have consequences in the clinical course and therapeutic approaches (for example, use of specific PLA2 inhibitors) of acute lung injuries from various origin.
The role of PLA₂ in meconium-induced lung injury

Earlier studies in our laboratory have demonstrated that human meconium has very high catalytic PLA₂ activity, mainly (>90%) due to high concentration of human pancreatic PLA₂-I.⁴ In line with this finding, tissue PLA₂ activity and concentration of human pancreatic PLA₂ in the meconium-contaminated lungs are concentration-dependently elevated and correlate directly with the severity of the meconium-induced lung injury.⁴,¹⁶,¹⁷ In these studies, promotion of lung edema formation and neutrophil influx through insufflated human PLA₂-I, either in soluble form or within meconium, further substantiates the central role of pulmonary PLA₂-I in the development of lung injury after meconium aspiration.¹⁶,¹⁷ High PLA₂ activity in meconium, mainly due to secretory PLA₂-I, is additionally shown to be associated with decreased surfactant biophysical activity in the affected lungs.¹⁸

This inactivation could be explained by enzymatic hydrolysis of dipalmitoyl-phosphatidylcholine, the major lipid constituent of surfactant, but also the produced lyso-phosphatidylcholine may have an inhibitory role.¹⁹ Although some experimental data suggest that type I secretory PLA₂ may induce type II PLA₂ expression,²⁰ challenge of the lungs with high pancreatic PLA₂ activity within aspirated meconium does not seem to influence pulmonary PLA₂-II production.¹⁶,¹⁷ It is thus conceivable that exposure of the lungs meconium with high pancreatic PLA₂ activity, specifically through aspiration of thick particulate meconium, may markedly participate in propagation of the ensuing pulmonary failure.¹⁷ Yet, the possible involvement of other phospholipases in the development of meconium-induced lung injury remains to be investigated.

Although the above considerations may potentially offer new ways to treat infants with MAS, most of the current therapies of MAS are symptomatic, and their clinical effects have been unsatisfactory and often conflicting.¹ Accordingly, our earlier data indicate that early administration of dexamethasone, known to inhibit stimulated PLA₂ synthesis,²¹ does not reduce lung PLA₂ activity or inflammation in experimental meconium aspiration.²² Alike, some of our preliminary data show that mepacrine, an unspecific PLA₂ inhibitor, does not decrease PLA₂ activity or prevent inflammatory injury, but rather tends to elevate tissue enzyme activity in the meconium-contaminated lungs (P. Kääpä, unpublished observation). Clearly, more investigations are needed before the pathophysiological relevance of PLA₂ in neonatal MAS is revealed.

The role of PLA₂ in systemic inflammatory reaction after meconium aspiration

Recent investigations have suggested that the inflammatory injury processes found in MAS may not be restricted to the lungs, but may also result in systemic inflammatory reaction and possible sequelae in extrapulmonary organs.²³ Intrapulmonary meconium exposure is indeed demonstrated to result in systemic complement activation, cytokine release, and activation of circulating neutrophils with production of a variety of biologically active mediators, including reactive oxygen radicals.¹,²³ In line with these findings, our recent studies indicate that pancreatic PLA₂, contained in high amount in meconium and thereby introduced into the lungs, may be absorbed, at least to some extent, into the pulmonary circulation.⁴,¹⁷ In fact, we were able to demonstrate that human pancreatic PLA₂ concentrations in plasma are elevated during the first hours after intratracheal human meconium administration in newborn piglets (Figure 1). Similar PLA₂ activation and circulatory release have been recognized in clinical disorders that promote systemic inflammation.²⁴ Alike, markedly elevated lung and blood PLA₂ activity, correlating with the disease severity, has been found in adults with acute respiratory distress.¹⁰

The systemic inflammatory reaction with systemic release of mediators, such as PLA₂ and cytokines, is supposed to ultimately lead to extrapulmonary organ dysfunction and injury.²⁴ Recent experimental data from our laboratory in fact indicate that severe meconium aspiration itself, without any complicating incidents, may result in brain tissue injury, specifically in the hippocampus.²⁵ Nevertheless, it still remains undetermined how often and at what intensity systemic inflammation occurs in connection with meconium aspiration and what is its significance for the outcome of infants with severe MAS.

Conclusions

Taken together, there is a bulk of evidence indicating that intrapulmonary aspirated meconium, specifically in thick particulate form, challenges the lungs with high human pancreatic PLA₂ concentration and activity, and may thereby contribute to pulmonary inflammatory perturbations, systemic inflammatory reactions and eventually distant organ damage. These findings...
may be amenable to development of new modes of more specific therapeutic approaches.

Acknowledgments

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Disclosure

The authors have declared no financial interests.

References

Meconium-induced release of nitric oxide in rabbit alveolar cells

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Previous studies have shown meconium-induced lung injury occurs throughout release of inflammatory cytokines. The exact mechanism of cytokine-induced apoptosis is not known. In this study we hypothesized that meconium-induced apoptosis in the lungs is mediated through the production of inducible nitric oxide (NO). We studied two groups of newborn rabbit pups: one group was instilled with meconium and other with normal saline. We measured precursors of NO in lung lavage from both groups of rabbits and NO levels were calculated accordingly. The levels of NO and NO-derivatives increased significantly in both groups. However NO expression in meconium group 2 h after meconium instillation was significantly higher than in saline-instilled group suggesting NO production plays a role in meconium-induced inflammation.

Introduction

Meconium aspiration syndrome (MAS) is a major cause of morbidity and mortality in neonates. It is characterized by initial obstruction of the airway resulting in ventilation-perfusion mismatch, hypoxemia and increased vascular resistance. MAS effects 1 to 3% of both term and post-term infants. Earlier studies have shown that MAS includes accumulation of inflammatory cells, release of inflammatory cytokines and massive cell death in experimental models. The pathogenesis is still unclear, although the role of bioactive mediators such as nitric oxide (NO), pro-inflammatory cytokines, prostaglandins, thromboxane, reactive oxygen species has been proposed in sepsis-induced lung injury.

Nitric oxide is a biological signaling and effector molecule involved in many biologic functions including maintaining blood pressure, modulating neural transmissions and host defense mechanisms. It is catalyzed by enzymes of the NO synthase (NOS) family. It is generated through the reaction of L-arginine, nicotinamide adenine dinucleotide phosphate oxidase and O₂ to NO and citrulline. Reactions of NO with oxygen in aqueous solution produce a relatively unreactive nitrate and nitrite ion as an end product. Two of the three isoforms are constitutive and the third is inducible, Ca-independent NO (iNOS). It has been shown in the lungs of septic murine to have high levels of NO produced by iNOS. NO is also a known regulator of apoptosis and has been shown to inhibit the activity of many caspases both in vitro and in vivo conditions in endotoxin-induced lung injury.

High levels of NO can react with superoxide to produce a highly oxidative peroxynitrate. We have reported in an earlier study apoptosis and necrosis occurring after meconium instillation in rabbit lungs using DNA staining and detection of DNA fragments by in situ end labeling.

Nitric oxide is an important inflammatory mediator. In MAS, an intense inflammatory reaction occurs and Li et al. (2001) reported an increase in rat alveolar macrophage cell line (ATCC8383) when exposed to meconium. They also found iNOS expression leading to increased production of NO. The purpose of the study is to measure the NO production in meconium-induced injury in rabbit alveolar cells.

Hypothesis

Meconium-induced apoptosis in the lungs is mediated through the production of inducible NO. NO production is a major intermediary step in meconium-induced lung cell apoptosis.

Methodology

Animal model (in vivo)

The care and handling of the animals were in accordance with National Institutes of Health guidelines. The Animal Care and USE Committee of Michael Reese Hospital (Chicago, IL, USA) approved the experimental protocol. Pregnant rabbits were housed for 3 days before the experiments with the mother in stainless steel rabbit cages. Mothers were given regular Purina rabbit chow (Scientific Animal Feed Co., Arlington Heights, IL, USA). After delivery, pups were used in the study.

Two groups, 2-week-old New Zealand white rabbit pups, were used in the study. Ten rabbits per group: group 1, saline-instilled rabbits and group 2, meconium-instilled rabbits. Tracheotomy was performed under sedation with intraperitoneal ketamine (10 mg kg⁻¹) and xylazine (1 mg kg⁻¹). Tracheal instillation of...
Meconium-induced release of NO in alveolar cells
R Fontanilla et al

10% meconium solution (1.2 ml kg$^{-1}$) or 0.9% NaCl (1.2 ml kg$^{-1}$) was made. Skin incision was closed with a 4-0 nylon suture, and rabbits were allowed to breathe spontaneously in the room air without assisted ventilation or supplemental oxygen. At set point (0, 2, 4 and 8 h), the animals were euthanized by intraperitoneal injection of Nembutal (100 mg kg$^{-1}$) for studies.

**Meconium preparation**
A total of 15 first-pass human meconium samples were obtained from term, healthy neonates. Fresh newborn infant meconium of 1 g was homogenized on ice in a blender with 9 ml of 0.9% NaCl to a 10% (w/v) final concentration and was spun down at 5000 r.p.m. for 20 min (4 °C) to separate the supernatant and pellet. The supernatant was filtered by a glass filter followed by sterilization by 0.2 µm filter (both filters from Millipore Co., Bedford, MA, USA) and was used for instillation in the lungs of 2-week-old newborn rabbit pups.

**Reagents**
ELISA kits were obtained from R&D System Co. (Minneapolis, MI, USA). All other materials are obtained from different sources and were of reagent grade.

**In vivo animal model experiments**
The lungs were isolated and homogenized in saline and centrifuged at 5000 r.p.m. for 5 min. The supernatant was filtered before measuring NO. Filtration eliminates nucleophiles, antioxidants and compounds containing sulphydryl groups such as cysteine and glutathione, which may interfere with color formation in Griess reaction.

**Studies of NO using ELISA**
NO has a very short half-life (<10 s), which makes it difficult to measure directly. However, NO is metabolized to nitrate and nitrite. Quantification of these anions can be used indirectly to determine the amount of NO originally present. There is a direct correlation between the nitrate and nitrite levels and NO. Nitrates and nitrites were measured using a commercially available ELISA kit (R&D System Co.). The assay is based on colorimetric detection of nitrite as an azo dye product of Griess reaction. The Griess reaction is a two-step diazotization reaction in which acidified NO$_2$ produces a nitrosating agent, which reacts with sulfanilic acid to produce a diazonium ion. This ion is then coupled to N-1-naphthylethylenediamine to form the chromophoric azo-derivative, which absorbs light between 540 and 570 nm and is quantitated by spectrophotometer.

**Data analysis**
Data from the experiments were reported as the mean ± s.d. Data were analyzed using analysis of variance and unpaired Student’s $t$-test. A $P<0.05$ was considered to be significant.

**Result and analysis**
NO derivative (nitrite) production in meconium-instilled lungs was significantly high ($P<0.05$) at 2 h after meconium instillation compared with saline instillation. The levels of nitrite also increased at 4 and 8 h after meconium instillation, but these values were not significantly different compared with saline-instilled lungs. Nitrites in saline-instilled lungs show low levels at 2 h and increased levels at 4 and 8 h comparable with meconium-instilled pups. This shows that meconium initiates lung injury faster than saline and that both meconium and saline induced NO production. The NO is released in response to the external injury factors such as meconium or saline (Table 1 and Figure 1).

NO is a biological signaling and effector molecule involved in many biologic functions including maintaining blood pressure, modulating neural transmissions and host defense mechanisms. It is a free radical, which makes it react very readily with superoxide to form peroxynitrite. Peroxynitrite is a highly oxidative species that is capable of nitrating tyrosine residues of numerous proteins lead to the production of nitrotyrosine.$^{11}$ High levels of nitrotyrosine formation have been shown to be involved in acute lung injury by way of increasing vascular permeability of endothelial cells, inhibiting leukocyte adhesion, degrading carbohydrates, inhibiting lipid peroxidation and cleaving DNA through nitration and oxidation.$^{11,12}$ The excess amount of NO required to form peroxynitrite to produce apoptosis is not known. What has been shown in this research is that within 2 h after instillation, meconium or saline enhances the production of NO. Khan et al.$^{10}$

**Table 1 NO production 2, 4 and 8 h after meconium instillation.**

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<th>2h*</th>
<th>4h</th>
<th>8h</th>
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<tr>
<td>Meconium</td>
<td>43.5 ± 12.3 µmol l$^{-1}$</td>
<td>52.5 ± 5 µmol l$^{-1}$</td>
<td>55.59 ± 26.3 µmol l$^{-1}$</td>
</tr>
<tr>
<td>Saline</td>
<td>28.24 ± 9.8 µmol l$^{-1}$</td>
<td>52.28 ± 26.3 µmol l$^{-1}$</td>
<td>48.9 ± 12.7 µmol l$^{-1}$</td>
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*$P<0.05$.

**Figure 1** NO production at 2, 4, 8 h after meconium and saline instillation in rabbit lungs. Measured using ELISA technique.
showed the production of NO from monolayers of A549 cells immediately after incubating the cells with meconium.

The instillation of meconium or saline itself does not possess any significant neutrophil chemotaxis but could stimulate the production of the pro-inflammatory cytokine and upregulate the production of the inducible form of iNOS, which produces large amount of NO. InOS is produced in many cells including alveolar cells in response to infection, inflammation and other circumstances when the immune system is activated. The other form is neuronal NOS from nerve cells and endothelial cells. The inflammatory damage in this research marked by increased levels of NO lasted for 8 h. A study carried out Holopainen et al., in the ultrastructural analysis of porcine lungs, 6 h after instillation of meconium, showed a significant amount of alveolar exudates and edematous alveolar epithelium and endothelium. The increase in the production of NO at 4 and 8 h could have been due to the worsening inflammatory process and increasing apoptosis in the alveolar cells. This inflammatory reaction causes an increased pulmonary vascular permeability producing the exudates and eventually inactivation of the surfactant and decreased pulmonary compliance. Earlier studies also showed in vitro, those neutrophils and plasma proteins in the alveoli inactivate surfactant. This may also explain why meconium can inactivate a surfactant. Meconium enhancing the production of NO and the inflammatory reactions may play an important part in the pathogenesis of MAS.

Meconium aspiration may also cause apoptosis in alveolar cells. Apoptosis is characterized by loss of cell function and decrease in cell size and its morphology. Zagaria et al. showed apoptosis in the airway epithelium 8 h after meconium instillation using ISEL-DNA end labeling. The apoptosis reported from an earlier study coincide with an increase in the production of NO in this study up to 8 h. This might play a crucial role in meconium-induced apoptosis. Apoptosis is induced by two main pathway: (1) mitochondrial pathway, which involves cytochrome c release from the mitochondria, and the activation of caspase-9, which then cleaves and activates caspases 3, 6 and 7; and (2) the death domain receptor pathway that involves the activation of Fas or tumor necrosis factor receptors followed by the activation of caspase-8 and subsequent activation of other caspases. The long-lasting overproduction of NO acts as a modulator of apoptosis, activating the caspase family through the release of cytochrome c into the cytosol. Once in the cytosol, cytochrome c interacts with Apaf-1 (apoptotic protease-activating factor 1), leading to the activation of caspase-9 and subsequent caspases, thereby leading to apoptosis. The activated caspases lead to cleavage of the nuclear lamin and breakdown of the nucleus through caspase-3. In our study, the meconium-induced NO production may represent a key mechanism for the apoptosis associated with meconium aspiration syndrome.

Despite the significant advances in management and diagnosis, MAS still causes an important morbidity and mortality in neonates. Earlier studies by Holopainen et al. showed that NO inhalation controls the rise in the pulmonary artery pressure, improves arterial oxygenation and prevents further increase in epithelial apoptosis, but does not protect against early inflammatory damage caused by meconium. Steroids have been used to inhibit the inflammatory response and can downregulate the pro-inflammatory cytokine production in vitro. Li et al. have shown that steroids also inhibits meconium-stimulated NO production and suggested that steroids could be used in the treatment of MAS.

In conclusion, our findings show that meconium is a potent inducer of NO production in the alveolar and airway cells (Figure 2), resulting in inflammation and apoptosis, which could result in MAS.
Significance of the research

This study might be essential in understanding the role of endogenous NO in the pathophysiology of MAS and might represent a key point in future therapeutic application.

Disclosure

The authors have declared no financial interests.

References

REVIEW

Intracellular and extracellular serpins modulate lung disease

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An imbalance between peptidases and their inhibitors leads to pulmonary disease. Imbalances occur in the adult and the neonate at risk for a specific set of lung pathologies. Serpins (serine peptidase inhibitors) make up the major source of antipeptidase activity in the lung. The purpose of this review is to describe the serpin mechanism of inhibition, their roles in the normal and pathological lung and their potential as therapeutic agents.

Introduction

The lung functions in the face of many physical challenges: exposure to oxygen and environmental toxins, airborn pathogens, continuous expansion and compression while breathing and maintenance of a delicate interface enabling gas exchange with the body’s vascular system. As a result, tissue damage, inflammation, repair and remodeling are constant. These processes, from the induction of apoptosis and necrosis in acute injury to the defense mechanisms of inflammatory cells, coagulation and fibrinolysis, to extracellular matrix degradation and cell migration, are all peptidase driven. Peptidase inhibitors are required to regulate these processes and neutralize peptidases upon completion of their intended roles. Most pulmonary diseases are associated with an imbalance between peptidase and peptidase inhibitor activity (Table 1). This holds true in the neonate at risk for a specific set of dysfunctional lung pathologies, including meconium aspiration syndrome (MAS), respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD). The balance between peptidase and antipeptidase activities appears critical in the development and progression of these diseases of premature and full-term newborns. Serpins (serine peptidase inhibitors) make up the major source of peptidase inhibitors in the lung. Others peptidase inhibitors include Kunitz, Kazal and Bowman-Birk protein families. Unlike the other peptidase inhibitors, serpin inhibition occurs at a 1:1 stoichiometry. Importantly, peptidase inhibition by serpins is irreversible. The purpose of this review is to describe the general mechanism and regulation of serpin function in the lung. Critical roles in the normal and pathological lung, and serpins as potential therapeutic agents are examined.

Serpins are found in animals, plants, prokaryotes and viruses. They are distinguished from other peptidase inhibitors because their unique suicide substrate-like mechanism of inhibition (reviewed in Gettins1,2). A total of 37 serpin genes in humans are distributed within 9 clades (A–I). Clade A has 13 members including SERPINA1 (α1-antitrypsin, α1AT) and SERPINAS1 (protein C inhibitor, PCI) that are secreted into the circulation. Clade B is made up of 13 serpins that are primarily intracellular. The remaining eleven serpins are dispersed across clades C–I and are secreted into the fluid phase. The tertiary structure of serpins is highly conserved and consists of three β-sheets (A–B), 7 to 9 α-helices (A–I) and extended reactive site loop (RSL) that acts as the bait for peptidase targets. The structure is critical to the unique suicide-substrate-like mechanism of peptidase inhibition.3 Inhibitory serpins exist in a metastable state resembling a loaded mousetrap. Upon peptidase binding to the RSL, hydrolysis of the P1–P1’ peptide bond releases the RSL and allows the serpin to undergo a rapid conformational transition. With the peptidase covalently bound to the P1 residue, the RSL is inserted into β-sheet A as strand 4 and the peptidase is trapped in an inactive complex with the serpin.

This mechanism of inhibition has a side effect resulting in clinical diseases collectively referred to as serpinopathies.4,5 Mutations that cause structural instability, specifically the opening of β-sheet A, can lead to the formation of serpin polymers. Under conditions of high serpin concentration, such as the endoplasmic reticulum of cells in the liver, the RSL of one molecule is inserted into β-sheet A of another. The prototype of this disease mechanism is the genetic deficiency of α1AT.6 The two most common alleles, α1AT*S and α1AT*Z, result in formation of α1AT polymers in the liver and significantly diminished α1AT plasma levels. The consequence of this is twofold for the individual: hepatocyte cell death leading to cirrhosis,7 and predisposition to emphysema, asthma and additional respiratory diseases.8 Other serpins with
naturally occurring alleles shown to develop polymers and subsequent serpinopathies are C1 inhibitor, antithrombin, α1-antichymotrypsin (ACT), heparin cofactor II and neuroserpin.

**α1AT is the major source of protection against proteolytic damage in the lung**

α1AT (SERPINA1) is the major peptidase inhibitor in plasma and provides the main source of antipeptidase activity in lung. α1AT is a 52 kDa glycoprotein produced in and secreted from the liver, as well as bronchial epithelial cells (BECs). Amino-acid residues Met-Ser at the P1–P1′ position within the RSL make α1AT a potent inhibitor of neutrophil elastase (NE), cathepsin G (catG) and proteinase-3 (Table 1). In the lung, NE is capable of causing extensive damage because of its proteolytic activity against structural components collagen and elastin. During inflammation large amounts of this peptidase are delivered to the lung. Infiltrating neutrophils express catG on the cell surface and release peptidases including NE into the airway space. The primary role of α1AT is to maintain a local balance between peptidase activities required for inflammatory cell function and to protect the lung against peptidase-mediated tissue damage.

α1AT deficiency is one of the most common inherited defects in Caucasians. Mutant alleles α1AT*S (Glu264Val) and α1AT*Z (Glu342Lys) account for most α1AT deficiencies, with α1AT*Z the more deleterious. Approximately 4% of northern Europeans carry the Z allele and approximately 1 in 2000 is homozygous (α1AT*ZZ) whereas 1 in 1000 is heterozygous for the two mutant alleles (α1AT*SZ). Individuals homozygous for the more common S allele (α1AT*SS) exhibit an ∼40% decrease in α1AT plasma levels. The α1AT*ZZ homozygous genotype results in a deficit of ∼85%. Individuals with this phenotype, or heterozygous for the two mutant alleles, are at risk for developing diseases associated with excess elastase activity such as emphysema.

**Table 1 Serpins and their proposed functions in the lung**

<table>
<thead>
<tr>
<th>Serpin</th>
<th>Peptidase targets</th>
<th>Function</th>
<th>Pulmonary diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERPINA1</td>
<td>Neutrophil elastase, cathepsin G, proteinase-3</td>
<td>Protect the lung against elastase activity</td>
<td>Empysema</td>
</tr>
<tr>
<td>α1-antitrypsin</td>
<td></td>
<td></td>
<td>Chronic pulmonary obstructive disease</td>
</tr>
<tr>
<td>SERPIN5</td>
<td>Activated protein C, thrombin-thrombomodulin</td>
<td>Activate coagulation</td>
<td></td>
</tr>
<tr>
<td>Peptidase C inhibitor</td>
<td>Thrombin, factors Xa, XIa, Urokinase-type plasminogen activator, tissue-type plasminogen activator</td>
<td>Suppress coagulation</td>
<td></td>
</tr>
<tr>
<td>SERPINC1</td>
<td>Thrombin, factors IXa, Xa, XIa, kallikrein</td>
<td>Suppress coagulation</td>
<td>Sepsis/ALI</td>
</tr>
<tr>
<td>SERPINE1</td>
<td>Urokinase-type plasminogen activator, tissue-type plasminogen activator</td>
<td>Suppress fibrinolysis</td>
<td>ALI</td>
</tr>
<tr>
<td>SERPINB1</td>
<td>Neutrophil elastase, cathepsin G, proteinase-3</td>
<td>Protect the lung against elastase activity</td>
<td>Idiopathic pulmonary fibrosis</td>
</tr>
<tr>
<td>SERPINB2</td>
<td>Urokinase-type plasminogen activator, tissue-type plasminogen activator</td>
<td>Protect against cell death</td>
<td>ARDS</td>
</tr>
<tr>
<td>SERPINB3</td>
<td>Cathepsins K, L, S, V</td>
<td>Protection of cells against cytosolic lysosomal peptidases, inhibit cell death</td>
<td></td>
</tr>
<tr>
<td>SERPINB4</td>
<td>Cathepsin G, mast cell proteinase</td>
<td>Protect against cell death</td>
<td></td>
</tr>
<tr>
<td>SERPINB6</td>
<td>Cathepsin G</td>
<td>Protect cells from granule peptidases</td>
<td></td>
</tr>
<tr>
<td>SERPINB9</td>
<td>Granzyme B</td>
<td>Protect cytotoxic lymphocytes/maintain granzyme B granules</td>
<td></td>
</tr>
<tr>
<td>SERPINB10</td>
<td>Trypsin, thrombin</td>
<td>Protect against cell death</td>
<td></td>
</tr>
<tr>
<td>SERPINB12</td>
<td>Trypsin</td>
<td>Protect against cell death</td>
<td></td>
</tr>
<tr>
<td>SERPINB13</td>
<td>Cathepsin K, L</td>
<td>Protect against cell death</td>
<td></td>
</tr>
</tbody>
</table>
\(\alpha\)1AT deficiency: COPD and emphysema

The proposed mechanism in the development of chronic obstructive pulmonary disease (COPD), including emphysema, is an imbalance of elastase and anti-elastase activity in the lung (reviewed in Elias et al.\(^{11}\)). \(\alpha\)1AT*ZZ and \(\alpha\)1AT*SZ individuals are at a greater risk of developing COPD. For \(\alpha\)1AT*ZZ individuals, plasma \(\alpha\)1AT*\(Z\) levels of 15% translate into elastase-inhibitory activities in the lung far below this. First, protein encoded by the \(\alpha\)1AT*Z allele exhibits an association rate with NE approximately fivefold slower than normal \(\alpha\)1AT. Second, \(\alpha\)1AT*Z protein maintains its inherent instability and continues to form polymers as it diffuses into the lung, leading to the detection of polymers in lungs of \(\alpha\)1AT*ZZ individuals, and further loss of anti-elastase activity.\(^{12,13}\) Finally, it was observed that \(\alpha\)1AT*Z polymers served as a chemoattractant for human neutrophils.\(^{14}\) This translates into increased inflammation and NE peptidase in conjunction with decreased \(\alpha\)1AT function.

COPD development is largely influenced by environmental factors, of which the most common is tobacco smoke. It has been proposed that the P1-Met residue in the RSL renders \(\alpha\)1AT sensitive to inactivation by oxidation from tobacco smoke exposure and the reactive oxygen burst released by neutrophils (reviewed in Carrell\(^{10}\)). Accordingly, \(\alpha\)1AT*ZZ individuals who smoke exhibit a rapid onset of emphysema, often by 30 years of age, and death by the age of 50.\(^{15}\) However, most \(\alpha\)1AT*ZZ individuals who avoid smoking live normal length lives with minimal complications. Replacement therapy for \(\alpha\)1AT deficiency, with intravenous \(\alpha\)1AT from pooled human plasma, results in longer survival. Data compiled by the National Heart, Lung and Blood Institute indicate that \(\alpha\)1AT serum levels and lung function are improved with \(\alpha\)1AT replacement therapy.\(^{16}\) However, plasma is limited and therapy expensive. Gene therapy is an alternative toward which much progress has been made in animal models.\(^{17,18}\) Synthetic small molecule peptidase inhibitors are also being developed as a tool to counteract imbalances in peptidase activity (reviewed in Chughtai and O’Riordan\(^{19}\)). On the basis of nature of \(\alpha\)1AT deficit caused by polymerization, another therapeutic strategy is to prevent polymerization and facilitate \(\alpha\)1AT secretion from the liver. This may be accomplished using small molecules interacting with \(\alpha\)1AT directly or by enhanced chaperone function.\(^{20–22}\)

Cystic fibrosis and \(\alpha\)1AT

Cystic fibrosis (CF) presents another scenario where lung pathology and disease progression is associated with increased elastase activity. However, unlike \(\alpha\)1AT deficiency, CF patients express normal amounts of \(\alpha\)1AT. Decreased fluidity of mucus in the CF airway impairs mucociliary clearance, obstructs normal diffusion of innate immune components, as well as \(\alpha\)1AT, and creates localized environments bacteria may colonize. Chronic infection recruits an excess of migrating and activated neutrophils that release their serine peptidases to the cell surface or directly into the airway space. The elevated peptidase levels overwhelm the available neutralizing activity of \(\alpha\)1AT. CF progression is associated with exacerbations, frequently caused by increased bacterial load or viral infection (reviewed in Goss and Burns\(^{23}\)). Inflammation is central to this event, including increases in interleukin (IL)-8, IL-6, IL-1\(\beta\), tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)), leukotriene B\(_4\) (LKB\(_4\)) and free NE. Exacerbations lead to airway remodeling and decreased lung function.

\(\alpha\)1AT replacement therapy to combat NE activity in CF lungs has been available for over two decades, however, there is little statistical data derived from clinical trials relating to its effectiveness.\(^{24,25}\) Two recent papers described promising results with inhaled \(\alpha\)1AT in CF patients. Both studies found decreased inflammation associated with lowered cytokines and neutrophil cell numbers.\(^{26,27}\) Griese et al. compared peripheral lung versus the bronchial deposition of aerosolized prolastin (\(\alpha\)1AT purified from plasma; Bayer Corporation, Clayton, NC, USA). Although no difference was observed between the two sites of treatment deposition, both groups receiving 4 weeks of daily prolastin exhibited significant decreases in IL-8, TNF\(\alpha\), IL-1\(\beta\) protein and LKB\(_4\) concentrations in sputum. In a second study, 4 weeks of treatment with recombinant \(\alpha\)1AT (\(\alpha\)1AT) made in sheep produced significant decreases in neutrophil infiltration, and reduced complexes between NE and endogenous \(\alpha\)1AT, suggesting that \(\alpha\)1AT was effective in neutralizing endogenous NE.\(^{27}\) Favorable results have also been collected from nebulized \(\alpha\)1AT therapy studies in animal models.\(^{28}\) Together these results suggested that \(\alpha\)1AT aerosol treatment would benefit CF patients. However, it will be important to examine in detail the mechanism by which \(\alpha\)1AT inhibited inflammation and cytokine production.

\(\alpha\)1AT and lung disease in the newborn

The premature infants lacking surfactant synthesis develop RDS. A direct result of undervdeveloped lungs and respiratory system, therapy includes oxygen and mechanical ventilation, and surfactant replacement. Even with improved oxygen saturation monitoring and ventilation, unavoidable acute lung injury (ALI) adds to the severity of RDS. Infants born prematurely frequently go on to develop BPD. Prematurity is the primary risk factor for BPD, followed by oxygen toxicity and ventilation induced lung damage (reviewed in Jobe and Ikegami\(^{29}\) and Chess et al.\(^{30}\)). The ‘new’ BPD pathology consists of arrested alveolar development, resulting in decreased total alveoli, and abnormal vasculature localization observed in the lung periphery. Inflammatory cytokines have been associated with accelerated lung maturation.\(^{30}\) Serine peptidases, released from inflammatory cells, caused epithelial cell injury and lung remodeling that is associated with RDS and BPD.\(^{31}\) In the neonate the balance between elastase and elastase inhibitor activity
has been associated with lung injury and progression to BPD. In addition to α1AT, low molecular mass inhibitor secretory leukocyte peptidase inhibitor (SLPI) was found to be important in neutralizing NE in tracheal aspirates of infants born prematurely. Watterberg et al. found that SLPI increases in the tracheal lavage of RDS, whereas neonates going on to develop BPD had a significantly higher elastase to elastase-inhibitor ratio. Sweger et al. also reported significant correlations with BPD development and low levels of inhibitors α1AT and ACT (SERPINA3) in tracheobronchial aspirate of preterm infants at 3 to 4 days of age, and low SLPI at 7 to 8 days of age. Two trials assessed the effect of α1AT therapy in preterm infants on recovery from RDS and development of BPD. A meta-analysis of the two trials found that although a trend for reduced risk of oxygen dependency after 28 days existed, no significant difference was observed between groups receiving α1AT therapy and placebo for risk of BPD or long-term neurodevelopmental abnormalities.

Similar to endotoxin exposure, meconium aspiration leads to a rapid induction of cytokines, inflammation, decreased surfactant function, hypoxemia, pulmonary hypertension and excessive cell death in the airway of the newborn. In animal models, all of these events take place within 2 to 4 h of exposure to dilute human meconium. Following the initial inflammatory response, MAS patients frequently undergo further pulmonary distress associated with oxygen toxicity upon intubation and mechanical ventilation. Zagariya et al. hypothesized that α1AT in the lungs of neonates may attenuate meconium aspiration-induced lung injury. As has been observed in α1AT deficiency and CF patients, the absence of adequate anti-elastase activity associated with α1AT resulted in extensive pulmonary damage. Treatment with supplemental α1AT activity would seem to be a promising approach to arrest elastase-dependent lung damage following meconium aspiration.

**Plasminogen activator inhibitor-1**

Plasminogen activator inhibitor-1 (PAI-1, SERPINE1) is an inhibitor of the two plasminogen activators (PAs), urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). uPA is expressed in specific tissues, whereas tPA functions as a soluble protein in the vascular system. The balance between PA and PAI-1 activities determines local fibrinolysis (Table 1). Diseases of acute inflammation, such as ALI and acute respiratory distress (ARDS) (reviewed in Ware and Matthay), and fibrotic diseases like idiopathic pulmonary fibrosis (IPF) share a common pathology of fibrin deposition in the alveolar compartment. To determine the best approach for treatment and prevention of these related diseases, it is important to identify the source of imbalanced fibrinolysis resulting in fibrin deposition. As the main inhibitor of fibrinolysis, PAI-1 is expressed by several cell types found in the lung and is modulated by inflammatory cytokines and tissue damage. Elevated levels of PAI-1 were observed in alveolar macrophages from patients with ARDS and IPF. An investigation of PAI-1, uPA and tPA levels in preterm infants with RDS also identified elevated ratios of PAI-1 to uPA protein in tracheal aspirate fluid. Elevated PAI-1 in BAL fluid occurred in bacterial pneumonia. Excessive PAI-1 levels were observed in ARDS and severe pneumonia cases requiring mechanical ventilation. These findings supported the hypothesis that PAI-1 is a critical regulator of fibrin deposition in the alveolar space in response to ALI. Further support for this theory was gained from animal models of lung injury, including bacterial endotoxin, and bleomycin models of ALI. These studies established PAI-1, its activity and its regulation, as a critical target for therapies treating the family of diseases displaying poor lung function associated with fibrin deposition including ALI, ARDS and COPD.

PAI-1 has been identified as a potential mediator of host defense. In mouse lung infection models, PAI-1 deficiency did not affect the outcome of infection by Gram-positive *Streptococcus pneumoniae*, but was found to be critical in defense against Gram-negative *Klebsiella pneumoniae*. PAI-1-deficient animals exhibited increased mortality at 24 and 48 h, coinciding with increased bacterial dissemination, and elevated fibrinolysis. These results may be attributed to the ascribed function of PAI-1 in regulating fibrinolysis or a potential role of PAI-1 in mediating neutrophil recruitment signals, as was demonstrated in vitro. In either case, these results suggest that PAI-1 function may prove to be a useful alternative target in the battle against bacterial pathogens.

**Antithrombin III**

Antithrombin III (SERPINC1, ATIII) regulates coagulation by inhibiting activated serine peptidases. ATIII can inhibit serine peptidases thrombin, factors IXa, Xa, XIIa and kalikrein (Table 1). The peptidase inhibitor activity of ATIII is positively regulated by its cofactor heparin binding to the D helix. This induces a conformational switch putting the RSL in a more favorable position for serine peptidase binding. Heparin molecules longer than 26 residues further accelerate peptidase inhibition through interactions with the peptidase. Defects in ATIII function primarily result in complications relating to thrombosis. However, a protective role of ATIII has been experimentally demonstrated in animal models of sepsis and ischemia reperfusion of grafted lungs. Recombinant ATIII (rATIII) at elevated levels resulted in reduced to complete inhibition of pulmonary vascular permeability associated with endotoxin treatment. In a lung transplant model, lungs were stored for 28 h with normal saline, then transplanted into dogs receiving ATIII or vehicle alone. Transplant animals treated with ATIII exhibited no change in O2 partial pressure, alveolar–arterial O2 difference or pulmonary vascular resistance for up to 3 h. These encouraging...
results suggested that ATIII might be used to treat sepsis-related lung dysfunction, ARDS and ALI resulting from ischemia.

In humans, plasma ATIII levels normally decline with sepsis severity, and correlate with high mortality rate.66 Small clinical trials suggested improved survival rates in patients receiving rATIII.61–64 Eisele et al.62 reported that rATIII therapy was associated with decreased lung dysfunction. However, Waydas et al.65 found no difference in the duration of organ failure. A single large trial of 2314 patients with severe sepsis produced inconsistent results.65 One group in this trial, receiving ATIII but not heparin, exhibited an increased 90-day survival rate compared with placebo. It was also observed that new pulmonary dysfunction was decreased in the group receiving rATIII therapy. Unfortunately, no critical trials have been performed to date specifically with ALI or ARDS patients to test ATIII or heparin therapy. Overall, improvement in fibrin deposition and lung function observed with ATIII in animal models of sepsis and ALI is promising but is unconfirmed in humans.

**Peptidase C inhibitor**

Peptidase C inhibitor (PCI, SERPINA5) has a broad peptidase inhibitor profile through which it modulates both the coagulation and fibrinolyis systems (Table 1) (reviewed in Church et al66). PCI in plasma is the major inhibitor of the anticoagulant peptidase activated protein C (APC). PCI also inhibits the thrombin–thrombomodulin complex,67 kallikrein, factors Xa, XIa and thrombin. PCI may downregulate fibrinolysis by inhibiting plasmin activator peptidases uPA and tPA. Like ATIII, the interaction between PCI and many of its target peptidases is modulated by heparin and other glycosaminoglycans.68–70 The interaction between PCI and target peptidase kallikrein is inhibited by glycosaminoglycans. In humans, PCI is a plasma protein, present at a concentration of ~100 nM and a half-life of ~23 h.71 PCI is found in many other body fluids and secretions, and in a wide range of tissues. The broad peptidase-inhibitory profile and widely distributed expression has made it difficult to assign specific biological functions to this serpin.

To determine the role of this serpin, PCI function was studied in the mouse. Expression of human PCI was limited to the male and female reproductive systems.72 Homozygous-null PCI-knockout mouse appeared normal except male sterility was observed.72 Limited endogenous PCI in the mouse allowed a unique approach to model human PCI pulmonary function. Hayashi et al.73 expressed hPCI in mice from a transgene consisting of the human PCI gene contained within 25 kb of human genomic DNA. hPCI protein in these transgenic (Tg) animals was found to be an active inhibitor of APC, and expressed in a pattern similar to humans. This hPCI Tg animal system may be used as a tool to further explore PCI function in the lung under physiological and pathological conditions, as well as to test the therapeutic effect of human APC in vivo. Nishi et al.74 used the hPCI Tg mouse to identify a role for PCI in pulmonary hypertension. Monocrotaline treatment was used to specifically induce pulmonary hypertension. A significant increase in right ventricular pressure was observed in treated wild-type (WT) control mice compared to hPCI Tg animals. BAL fluid levels of thrombin–antithrombin complex, monocyte chemoattractant protein-1, platelet-derived growth factor and IL-13, and the plasma level of TNFα were significantly increased in treated WT mice compared to hPCI Tg animals.74 This study established that PCI in the lung is protective against monocrotaline-induce hypertension. Furthermore, it suggested that PCI fulfills both anti-inflammatory and anticoagulant activities in the lung.74,75 To take advantage of PCI therapeutically it will be important to determine which activities as a serine peptidase inhibitor are protective in specific physiological conditions.

Clinical studies on the role of PCI are limited. Examination of 58 patients with interstitial lung disease (ILD) associated with diverse underlying pathologies discovered elevated PCI in the BAL fluid of each of them.76 Groups with cryptogenic-organizing pneumonia, collagen vascular disease (CVD-ILD) and sarcoidosis exhibited elevated levels of PCI and thrombin-activatable fibrinolysis inhibitor (TAFI), supporting the hypothesis that PCI inhibition of APC results in elevated TAFI levels.77

**The clade B serpins protect cells with an intracellular antipeptidase shield**

In humans there are 13 clade B genes that encode serine and cysteine peptidase inhibitors. The intracellular serpins are expressed in a wide range of tissues including lung, and target a wide range of peptidases (Table 1). In the face of environmental insults such as bacterial and viral infection, and excessive peptidase levels associated with inflammation, the induction of cell death is a common yet critical step in ALI. There is increasing evidence that intracellular serpins function as a cytoprotective antipeptidase shield, limiting damage by the misdirected peptidases as well as the induction of necrosis and apoptosis (reviewed in Silverman et al.78 and Scott79). SERPINB1 inhibits elastases expressed by the neutrophil. SERPINB2, B3, B4, B10 and B13 have been implicated in blocking proapoptotic signals.80,81 SERPINB6 and B9 inhibit peptidases stored in the cytolytic granules including catG and granzyme B (GzmB), respectively.82,83 SERPINB12 inhibits trypsin and is expressed in the lung.84 Together, the clade B serpin genes encode proteins with diverse antipeptidase activity, with critical intracellular roles in protecting cells from damage, and maintaining normal function in the lung.

**SERPINB1 inhibits elastases in CF and BPD**

SERPINB1 (monocyte/neutrophil elastase inhibitor) is an inhibitor of NE, catG and neutrophil peptidase-3 (pr-3) (Table 1).
Serpins in pulmonary disease

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Serpins are promising candidates for the development of therapeutic agents, from simple replacement by inhalation, to recombinant gene transfer under the regulation of serpins. The biological function and regulation of serpins will be critical in the development of therapies against pulmonary disease.

Summary and future directions

Lung function is dependent on several peptidase driven systems including the innate and adaptive immune systems, coagulation, fibrinolysis and tissue remodeling. As a result, pulmonary diseases are commonly linked to excessive peptidase activity. Serpins are the major regulators of peptidase activity in the lung. It follows that understanding the biological function and regulation of serpins will be critical in the development of therapies against pulmonary disease.

Serpins occupy two niches in the lung, regulation of extracellular peptidases and intracellular peptidases. The blood plasma serpins α1AT, PAI-1, ATIII and PCI, function extracellularly. Their localization within the lung makes them primary targets for the development of therapeutic agents, from simple replacement by inhalation, to recombinant gene transfer and development of small molecule inhibitors that mimic their activity. In the case of α1AT, this serpin inhibits a limited number of elastolytic peptidases. In diseases associated with excess elastase activity (emphysema, CF), replacement therapy appears very promising. In contrast, ATIII and PCI are multi-peptidase inhibitors and their local active concentration in vivo is likely critical to properly balancing the function of specific peptidase.

Plasminogen activator inhibitor 2 blocks apoptosis

Plasminogen activator inhibitor 2 (SERPINB2, PAI-2) inhibits uPA and tPA, and is expressed in macrophages and monocytes (Table 1). Its primarily intracellular and nuclear localization suggests, however, that PAI-2 has functions in addition to regulating fibrinolysis (reviewed in Medcalf and Stanisopoulos95). Several studies have demonstrated the ability of intracellular PAI-2 to inhibit apoptosis. TNFα-induced apoptosis was inhibited by ectopic PAI-2 expression in HeLa and fibrosarcoma cells.94,95 Recently, PAI-2 was found to be required for macrophage survival following pathogen activation of the toll-like receptor-4 apoptosis pathway.96 On the basis of these studies, PAI-2 appears to be a critical regulator of cell survival in cells of the host defense system where it is expressed.

SERPINB3 and SERPINB4 are serine and cysteine peptidase inhibitors

SERPINB3 and B4 are co-expressed in lung epithelium.97 SERPINB3 inhibits the lysosomal cysteine peptidases, cathepsin L (catL), catK and catS.98 SERPINB4 inhibits the serine peptidases catG and mast cell chymase (Table 1).99 Ectopic SERPINB3 expression was cytoprotective against TNFα and natural killer (NK) cell-induced apoptosis,83 and both SERPINB3 and B4 have been shown independently to protect cells against radiation.82 The peptidase inhibitor activity of SERPINB3 and B4 may provide cellular protection in the environment of the lung during ALI and inflammation. SERPINB3 and B4 expression was induced in BECs in asthmatics and by IL-4 and IL-13.100 SERPINB3 and B4 may provide protection against neutrophil and mast cell peptidases as well as localized bursts of reactive oxygen generated by neutrophils used to destroy pathogens. Cells exposed to reactive oxygen succumb to necrosis or apoptosis following lysosomal damage and release of its cysteine peptidases into the cytosol (reviewed in Lockshin and Zakeri101 and Guicciardi et al.102). Cysteine peptidases are likely to be involved in development of BPD, as catK, catL, and catS were shown to be elevated in a baboon model of BPD.103 SERPINB3 is predicted to provide protection to cells against cysteine peptidases. Maintenance of BEC by SERPINB3 and B4 would limit ALI and prevent epithelial permeability during infection.

SERPINB9 inhibits granzyme B

SERPINB9 is the only known inhibitor of GzmB in humans (Table 1).84 SERPINB9 is expressed in cytotoxic lymphocytes (CTLs), dendritic cells (DCs) and NK cells of the monocyte and lymphocyte lineages.104,105 Using a Serpinb9-deficient mouse model, Zhang et al.106 demonstrated that Serpinb9 is required to protect CTLs from ‘accidental death’ induced by residual GzmB in the cytosol. Serpinb9 was found to be required for the maintenance of GzmB-containing granule integrity in CTLs. Finally, in the absence of Serpinb9, animals exhibited impaired clearance of lymphocytic choriomeningitis virus.106 On the basis of these findings and the localization of SERPINB9-positive monocytes and DCs in lung tissue,107 it is proposed that this serpin is required for normal host defense in the lung, particularly against bacterial and viral pathogens requiring cell contact-mediated killing by the immune system.

SERPINE1 protein was shown to exist at elevated levels and in a complex with NE in the lavage of CF patients compared to normal individuals.87 Recombinant human SERPINB1 (rSERPINB1) was able to protect rat lungs against injury, including hemorrhage and epithelial permeability, from the instillation of NE or CF patient sputum preparations.88 rSERPINB1 was shown to inhibit Surfactant-A degradation by the peptidase(s) in BAL fluid from CF patients.89 Yasumatsu et al. probed SERPINB1 function in the established baboon BPD model.90,91 As in humans, SERPINB1 was localized to bronchial and glandular epithelial cells, as well as mast cells, neutrophils and macrophages in the baboon lung.92 SERPINB1 protein in baboon lung tissue was found in high molecular weight complexes with both NE and catG in BPD models but not gestational controls.90 These results suggested that SERPINB1 function in the newborn lung is critical as an antipeptidase shield against elastases associated with inflammation and responsible for lung injury.
targets. To modulate the activity of these serpins therapeutically, we may take advantage of the endogenous mechanisms regulating their activity, interactions with glycosaminoglycans and conformational changes in structure. Alternatively, extensive success has been met using recombinant APC, a PCI target peptidase, in the treatment of sepsis and ALI.

The clade B serpins are mainly intracellular. They are predicted to form an anti-peptidase shield, protecting cells against exogenous and endogenous peptidase activity. This hypothesis is based upon their intracellular localization and broad tissue distribution, combined with the varied peptidase-inhibitory specificities of the clade B members. In their absence, cellular injury would lead to cellular stress and death. SERPINB9, the only inhibitor of GzmB in humans, is required for CTL survival, and therefore host defense against some viral infections. SERPINB3 and SERPINB4, expressed in BEC, are poised to protect the bronchial airways against both exogenous and endogenous serine and lysosomal cysteine peptidases. In lung epithelium, this anti-peptidase shield is very important due to repeated exposure to neutrophil-derived serine peptidases and oxidative stress accompanying inflammation in response to pulmonary injury or infection. On the basis of the ability to block cellular damage, including that caused by lysosomal cysteine peptidases, and cell death, the intracellular clade B serpins are powerful agents to target in the development of therapeutics.

Disclosure
The authors have declared no financial interests.

References
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