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Bile Acid-Induced Lung Injury in Newborn Infants: A Bronchoalveolar Lavage Fluid Study

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ABSTRACT

OBJECTIVES. Neonatal respiratory distress syndrome is associated with intrahepatic cholestasis of pregnancy, and bile acids may play a major role in neonatal bile acid pneumonia. Our aim was to demonstrate the bile acid presence in the bronchoalveolar lavage fluid of neonates affected by respiratory distress syndrome who were born from intrahepatic cholestasis of pregnancy and to investigate bile acid mechanisms of action in acute lung injury.

METHODS. In this prospective study, we enrolled 10 neonates delivered from intrahepatic cholestasis of pregnancy, affected by respiratory distress syndrome requiring mechanical ventilation (intrahepatic cholestasis of pregnancy group) and 2 control groups. The first group consisted of 20 infants with respiratory distress syndrome delivered from pregnancies without any sign of intrahepatic cholestasis of pregnancy (respiratory-distress-syndrome group), and the second group included 20 neonates with no lung disease who were ventilated for extrapulmonary reasons (no-lung-disease group). We measured bile acid and pH in the bronchoalveolar lavage fluid and serum bile acid levels in the first 24 hours of life.

RESULTS. Bile acids were measurable in the bronchoalveolar lavage fluid of all of the infants in the intrahepatic cholestasis of pregnancy group but were absent in the 2 control groups. Bronchoalveolar lavage fluid pH was not different among the 3 groups. Infants in the intrahepatic-cholestasis-of-pregnancy group had significantly higher serum bile acid levels compared with those in both of the control groups.

CONCLUSIONS. Bile acids are detectable in the bronchoalveolar lavage fluid of newborns from intrahepatic cholestasis of pregnancy affected by respiratory distress syndrome. Elevated serum bile acid levels in these infants allow us to hypothesize that bile acid reaches the lung after an uptake from the circulation. These findings strongly support a role for bile acid in causing bile acid pneumonia.

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Key Words
bile acid pneumonia, respiratory distress, pregnancy complications, neonate, cholestasis

Abbreviations
RDS—respiratory distress syndrome
ICP—intrahepatic cholestasis of pregnancy
BA— bile acid
BALF— bronchoalveolar lavage fluid
NLD—no lung disease

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SEVERE RESPIRATORY DISTRESS syndrome (RDS) in neonates from mothers with intrahepatic cholestasis of pregnancy (ICP) was first described in 2004.[1] A causative role of bile acids (BA) was supposed, and the diagnosis of "BA pneumonia" was proposed for this form of acute neonatal lung injury. Two years later, we demonstrated an association between maternal ICP and neonatal RDS in a retrospective cohort study.[2] Reviewing available data concerning BA-induced lung injury,[3] we found that several animal models reflect the picture of BA pneumonia.[4–7] Recent experiences from human adults receiving lung transplant or affected by gastroesophageal reflux showed that patients with BA in the bronchoalveolar lavage fluid (BALF) were more likely to develop surfactant dysfunction and lung inflammation.[8,9] The physiopathology of BA-induced lung injury is still unclear, and BA levels in the BALF of newborn infants have never been studied. We performed this pilot study to demonstrate the presence of BA in the BALF of neonates with BA pneumonia and to better clarify BA mechanisms of action.

PATIENTS AND METHODS

Population

We performed a prospective study from January 1, 2005, to December 31, 2006, enrolling all of the infants delivered from ICP pregnancies who developed RDS requiring intubation and mechanical ventilation (ICP group). Eligible infants were excluded in the case of premature rupture of membranes, maternal diabetes, or eclampsia. Infants with a 5-minute Apgar score of <7 or any evidence of infection, liver disease, esophageal atresia, or tracheoesophageal fistula were also excluded. We established 2 control groups of infants born from pregnancies without any sign of ICP. The first group consisted of infants with neonatal RDS requiring mechanical ventilation (RDS group), and the second included infants with no lung disease (NLD) who were ventilated for extrapulmonary reasons (NLD group). Control neonates were selected as the 2 infants satisfying the inclusion criteria who were born immediately before and after every ICP infant. Gestational age estimate was based on the postmenstrual date and early gestation prenatal sonographic findings. ICP was diagnosed when maternal serum BA levels of ≥10 μmol/L were associated with diffuse pruritus during the second half of gestation without any skin or other medical conditions known to be associated with pruritus.[2] When ICP was diagnosed, the women underwent treatment with *S*-adenosyl-L-methionine (Samyr; Abbot Laboratories, Chicago, IL) as a 600-mg intravenous dose once a day. Ursodeoxycholic acid (Deursil; Sanofi-Synthelabo, Milan, Italy) was given when BA levels were at >20 μmol/L as a 200-mg dose 3 times per day.[10] Antenatal corticosteroids were administered intramuscularly in the form of one 12-mg dose of betamethasone (Bentelan; Biofutura Pharma, Pomezia, Italy) followed by a second dose 24 hours later, whenever delivery was expected to occur before 34 weeks' gestation. Asphyxia at birth was diagnosed for newborn infants who required resuscitation according to the American Heart Association guidelines.[11] Neonatal RDS was defined as clinical signs of respiratory distress needing oxygen and mechanical ventilation with the typical radiograph appearance.[12] Synchronized intermittent mandatory ventilation with a Baby Log 8000 Plus ventilator (Drager Medical, Lubeck, Germany) was used for all of the infants. All of the infants with RDS needing >30% oxygen received 200 mg/kg of surfactant (Curosurf; Chiesi Farmaceutici, Parma, Italy). A blood sample was drawn from all of the infants in the first 24 hours of life to measure BA levels. BA levels at delivery were also measured in mothers affected by ICP. The ethics board of our university approved this study, and informed consent was obtained from all of the parents.

Bronchoalveolar Lavage and Laboratory Methods

A standardized procedure for BALF sampling was performed as soon as possible after the onset of mechanical ventilation and before surfactant administration.[13,14] The neonate was placed supine with the head turned to the right so that the right lung would be predominantly sampled. One milliliter per kilogram of 0.9% sodium chloride was instilled into the endotracheal tube. After 2 ventilator cycles, the suction catheter was gently inserted 0.5 cm beyond the tube tip, and the airway fluid was aspirated into a sterile specimen trap (BALF Trap; Vigon, Ecouen, France) with 50 mm Hg of negative pressure. This procedure was repeated with the head turned to the left, so that the left lung would be predominantly sampled. To rinse the suction catheter, normal saline solution was added up to 2 mL. BALF samples were excluded from further analysis if there was visible blood staining or if the recovered volume was <30% of the instilled volume. After collection, BALF specimens were homogenized and centrifuged at 1000*g* for 5 minutes. Cell-free supernatants were removed, and pH was measured by using a potentiometric micromethod analyzer (ABL 725 Plus; Radiometer Medica A/S, Copenhagen, Denmark). The remaining volume of supernatants was immediately frozen at −80°C for later testing.

Total serum BA was analyzed on an automated analyzer (Olympus AU640; Olympus Life Europe, Hamburg, Germany) with an enzymatic colorimetric assay (Kit Bile Acids TBA; Sentinel Diagnostics, Milano, Italy). BAs were measured in BALF samples using the same kit and analyzer, and the calibration curve was obtained by successive dilutions of the standard (standard Randox [Randox Laboratories Ltd., Crumlin, Co., Antrim, United Kingdom]; 0.6–1.2–2.5–5.0 μmol/L) to increase the method sensitivity at low concentrations, as reported previously.[15,16] A commercial control of 4.3 μmol/L (Sentinel Diagnostics) was diluted in physiologic saline solution up to a final concentration of 0.3 μmol/L to verify the interassay reproducibility (coefficient of variation: <5%). All of the laboratory procedures were performed by the same skilled biochemist who was blind for the specimen origin.

Statistics

Proportions were compared by using the χ^2 test. Continuous variables were contrasted with a 1-way analysis-of-variance procedure with Bonferroni posthoc correction or with the Kruskal-Wallis *H* test, where appropriate. Data were analyzed by using SPSS 13.0 (SPSS Inc, Chicago, IL), and *P* values of <.05 were considered to be statistically significant.

RESULTS

Indications for assisted ventilation in the NLD group were asphyxia ($n = 8$), congenital heart defect ($n = 6$), myelomeningocele ($n = 3$), hydrocephalus ($n = 1$), Galen's vein aneurysm ($n = 1$), and sacrococcygeal teratoma ($n = 1$). The median maternal BA level in the ICP group was 65.5 μmol/L (interquartile range: 45–74).

Table 1 reports baseline data of the study population. Gestational age was significantly different between the study groups. This was because of the difference between the ICP and the NLD groups (Bonferroni correction: $P < .001$). No other significant differences in perinatal data were observed.

Table 2 shows results of the BALF analysis. BAs were absent in controls, whereas measurable BA levels were

detected in all of the ICP infants. Alveolar pH was not statistically different among the 3 groups, and no correlations were found between pH and alveolar BA levels. The timing of BALF samplings was not different among the 3 groups.

Figure 1 shows serum BA levels in the neonates. ICP infants had significantly higher BA (median: 28.9 μmol/L; interquartile range: 19.1–43.4) compared with both control groups (RDS median: 14.8 μmol/L [interquartile range: 10.1–18.7]; NLD median: 15 μmol/L [interquartile range: 9.6–16.4]). No significant correlations were noticed among maternal, neonatal, and BALF BA levels.

DISCUSSION

Animal models and studies on human adults allow hypothesizing that BA can be injurious for the lung. Possible mechanisms of action include surfactant dysfunction, inflammation, and chemical pneumonia.[3] In the adult, lung injury may occur because of bile aspiration after a gastroesophageal reflux.[8,9] ICP is a pregnancy complication in which the fetus is exposed to high BA levels. We know that ICP is associated with the RDS occurrence in the neonate through the raised concentration of serum BA,[2] but mechanisms of BA lung injury are still not clarified in newborn infants. We performed this study to demonstrate the presence of BAs in the BALF and to better understand their causative role in BA pneumonia. We selected the NLD control group because we supposed BA to be undetectable in the BALF of healthy lungs, whereas the RDS control group included infants affected by a lung injury that was unrelated to BA.

Our data clearly show that BAs were present in the BALF of ICP neonates with RDS but were absent in both of the control groups. The 3 study groups had similar basic characteristics, except for gestational age, but preterm delivery is a well-known consequence of maternal ICP.[3] Even if the ICP group had a lower gestational age, the presence of BA only in the BALF of this group supports the concept of damaging effects of BA on the lung epithelium. BA in the lung could enhance secretory phospholipase A_2, which diminishes surfactant levels. Measuring phospholipase A_2 activity in the BALF could contribute to verify the possible clinical significance of our actual findings, and we mean to work on it. We measured the BALF pH, because BA could lower the alveolar pH, damaging type II pneumocyte enzymatic activities.[9,17,18] BA could also disrupt cellular membranes in the alveoli, increasing cationic permeability and intracellular Ca^{++} concentration, leading to apoptosis.[5,19,20] BALF pH was not different among our groups, probably because BAs are weak acid compounds. Moreover, some experimental data suggest that the damage related to BA acidity is conceivable only when BAs are added in extreme pH environments that can hardly be reached in lung tissue.[21] How BAs pass into the newborn lung still remains an open question. Aspiration from the amniotic fluid is unlikely, because BA levels in the amniotic fluid do not correlate with perinatal morbidity.[22] Uptake from circulation is more probable, because the impaired maternal clearance caused by ICP can limit the passage of BA from the fetal to the maternal circulation.[1,23,24] We measured serum BA levels at birth, and our data support this hypothesis. In fact, BA levels measured in the RDS and NLD groups were similar to those reported by Feldmann et al[25] in healthy neonates (19.6 ± 10.4 μmol/L), whereas ICP infants had serum BA levels significantly higher than control subjects.

Our study has some limitations. First, the causative role of BA in BA pneumonia could be definitely confirmed by demonstrating the absence of BA in the BALF of ICP infants without respiratory distress, but ethical reasons discouraged us from considering such a control group. Second, ICP is a rare disease, and enrolling large populations is not easy. Our population is probably too small to be exhaustive about the possible correlations between serum and alveolar BA levels. Finally, exposure time to BA during pregnancy also seems to be important for determining the lung damage,[26] but we have no data about this point, which could be more deeply investigated only in adequate multicenter studies.

TABLE 1 Baseline Characteristics of the Study Population

Characteristic	ICP ($n = 10$)	RDS ($n = 20$)	NLD ($n = 20$)	*P*
Gestational age, mean ± SD, wk	32.4 ± 1.0	34.2 ± 2.7	36.1 ± 2.6	.001
Birth weight, mean ± SD, g	1731 ± 469	2373 ± 709	2527 ± 663	.057
Boys, *n* (%)	5 (50)	9 (45)	10 (50)	.942
Cesarean section, *n* (%)	5 (50)	11 (55)	14 (70)	.161
Antenatal steroids, *n* (%)	4 (40)	5 (25)	4 (20)	.496
5-min Apgar score, mean ± SD	8.1 ± 0.6	8.1 ± 0.7	8.0 ± 0.8	.775

P levels indicate overall differences between groups by analysis of variance. Gestational age was significantly different between the ICP and NLD groups (Bonferroni correction: $P < .001$).

TABLE 2 BALF Analysis

Variable	ICP ($n = 10$)	RDS ($n = 20$)	NLD ($n = 20$)	*P*
Total BAs, median (interquartile range), μmol/L	0.8 (0.3–1.0)	0	0	<.001
pH, median (interquartile range)	6.0 (6.0–7.5)	5.8 (5.0–6.5)	5.5 (5.0–6.0)	.483

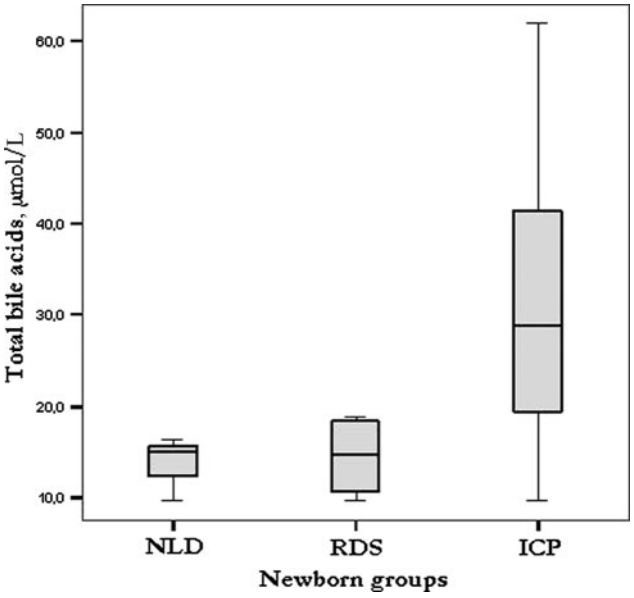

FIGURE 1
Total serum BAs. Median bile acid levels were significantly different within the 3 groups ($P = .037$, Kruskal-Wallis *H* test). The boxes show median and interquartile range.

CONCLUSIONS

Our study shows that BAs are detectable in the BALF of newborns delivered from ICP pregnancies and affected by RDS. Elevated serum BA levels in these infants allow hypothesizing that BAs reach the lung after an uptake from the circulation. These findings strongly support a role for BA in causing BA pneumonia.

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