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Surfactant therapy reduces pulmonary IL-8 in experimental meconium aspiration

Abstract

Background: Interleukin-8 (IL-8) production, as part of the inflammatory response, plays an important role in lung injury associated with meconium aspiration syndrome (MAS). Although surfactant therapy improves pulmonary function, its effects on pulmonary inflammation remain controversial.

Objective: To evaluate the effect of surfactant (beractant) administration on IL-8 levels in a neonatal rabbit model of MAS.

Methods: Anesthetized newborn rabbits underwent tracheotomy, received intratracheal human meconium (6 mL/kg; 65 mg/mL), and were mechanically ventilated using a pneumotachograph-ventilator system. After 5 minutes of ventilation, animals were randomly assigned to three groups: untreated MAS (MEC), MAS treated with bovine surfactant (SURF; 100 mg/kg), and control. After 25 minutes of ventilation, animals were euthanized, and lung tissue was harvested for analysis. IL-8 concentrations were determined in lung tissue homogenates using an enzyme-linked immunosorbent assay (ELISA; UCNLIFE). Statistical analysis was performed using one-way ANOVA, with significance set at $P < 0.05$.

Results: Twenty-five newborn rabbits were studied (MEC, n = 10; SURF, n = 8; control, n = 7). No significant differences were observed among groups regarding body weight or tidal volume during ventilation. IL-8 concentrations (pg/mL.kg) in lung homogenates were significantly lower in the SURF group compared with the MEC group and were comparable to control values: 386.3 ± 102.2* (SURF), 712.9 ± 379.8 (MEC), and 355.8 ± 129.7 (control); *P = 0.006 for SURF vs. MEC.

Conclusion: In this experimental model of MAS, surfactant replacement therapy attenuated pulmonary inflammation, as evidenced by reduced IL-8 levels in lung tissue.

Keywords: newborn, interleukin, meconium aspiration

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Introduction

Meconium aspiration syndrome (MAS) is defined as the presence of respiratory distress in neonates born through meconium-stained amniotic fluid (MSAF) with typical lung radiologic findings.[1] The incidence of MSAF is around 4–22% of all births and 3–12% of the babies born through MSAF develop MAS.[2] The severity of respiratory failure varies, with one-third of newborns requiring mechanical ventilation, and mortality rates ranging from 5-12%.[3]

Pathophysiology of MAS is not well defined and comprises a complex and multifactorial process that includes airway obstruction, surfactant inhibition and inflammation.[4] The inflammatory process found in MAS is one of the major mechanisms of lung injury involved in the pathophysiology of the disease and has been well documented in a number of *in vivo* and *in vitro* studies.[4,5] Meconium contains high levels of proinflammatory cytokines and chemokine, such as interleukins (IL-1, IL-6, IL-8) and tumor necrosis factor, which may be directly involved in the pathogenesis of pneumonitis.[5] Moreover, it has been established that intra-alveolar meconium deposition may stimulate neutrophil chemotaxis via IL-8 production. This process is postulated to occur through meconium's oxidative actions or its inherent phospholipase A2 activity, which facilitates the generation of eicosanoids. A significant finding is that the increase in pulmonary tissue IL-8 levels is directly associated with the subsequent worsening of respiratory function.[6,7]

Clinical studies have shown that the surfactant replacement in MAS decreases the severity of the disease,[4,8–11] however, little is known about the role of exogenous surfactant in the control of meconium-induced lung inflammation.[12–14]

We hypothesize that bovine surfactant administration will ameliorate the inflammatory response by reducing pulmonary IL-8 levels in newborn rabbits with meconium aspiration syndrome. The specific objective was to determine and compare the lung tissue IL-8 concentrations between untreated and bovine surfactant-treated animals.

Material and methods

It is an experimental, randomized study using a well-known model of newborn rabbits with meconium aspiration syndrome.[15,16] Figure 1 demonstrates the design of the study.

Meconium was collected from healthy term human newborns, diluted in distilled water at a concentration of 25 mg/ml, followed by filtration to remove particles with diameters larger than 3 mm, in order to rule out airway's obstruction. Subsequently, the meconium was lyophilized and, prior to endotracheal administration to the animals, diluted in saline solution at a concentration of 65 mg/ml according to techniques described in literature and already applied in previous studies.[16]

The surfactant used in the study was Beractante (Survanta™). The commercial product is obtained from bovine lung macerate, it is composed of phospholipids, neutral lipids, fatty acids and proteins (SP-B e SP-C) and available in the concentration of 25 mg of lipids/ml.[17]

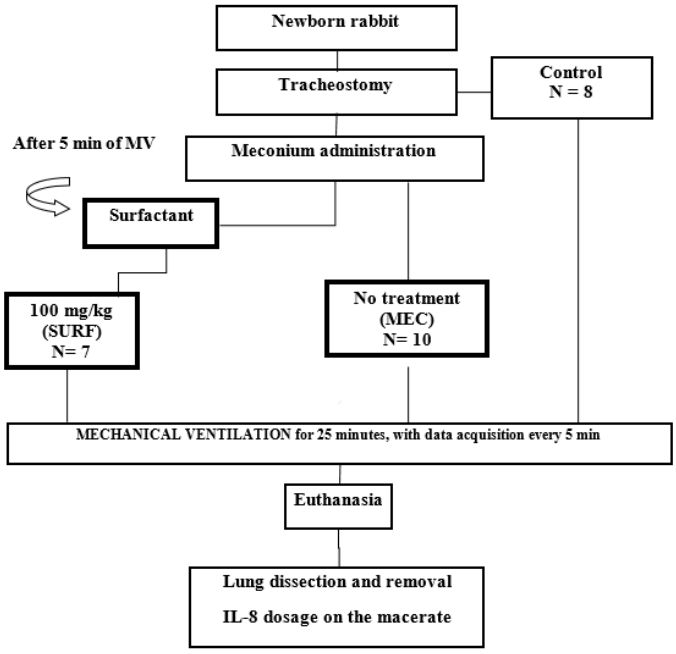

Figure 1 Study design.

Min, minutes; MV, mechanical ventilation; SURF, surfactant group; MEC, meconium group; IL-8, interleukin-8.

Experimental animal model

Birth and ventilation procedure: Term newborn rabbits were obtained through cesarean at 30 days of pregnancy, from pregnant rabbits belonging to the Botucatu genetic group[18] and provided by the Rabbit Production Area of the Veterinary Medicine and Animal Science School, UNESP – Botucatu. Animals with pneumothorax at any time during the study and animals that did not present a 30% reduction in lung compliance (compared to normal complacency values for term newborn rabbits) were excluded. According to previous studies, the reduction in lung compliance values denotes the inactivation of the surfactant by meconium and satisfy the criteria for definition of MAS experimental model.[19] C-section was performed after sedation of the rabbits with intramuscular application of ketamine and acepromazine solution (10mg/kg and 0.1 mg/kg), followed by spinal anesthesia with 2 ml of 1:1 (vol:vol) solution of lidocaine at 2% and bipuvacaine at 0.5%.

After birth, each animal was dried, weighed and anesthetized with ketamine-acepromazine intraperitoneal administration (10 mg/kg – 0.1 mg/kg). A tracheostomy was performed using a metallic cannula (1 mm inner diameter). Before initiating mechanical ventilation, meconium (65 mg/mL) was administered endotracheally at a dosage of 6 mL/kg, followed by 10 seconds of manual ventilation with a self-inflating bag. The animals were given pancuronium via intraperitoneal (20 μg), to prevent spontaneous breathing. Mechanical ventilation was started (INTER 7- Plus ®- Intermed-São Paulo, Brasil), using a respiratory rate (RR) of 60 cycles/min; inspired oxygen fraction (FiO_2) of 1.0; peak inspiratory pressure (PIP) required to achieve 8 ml/kg tidal volume and positive end-expiratory pressure (PEEP) of 3 cmH_2O, in controlled ventilation mode, for a 25 minutes period.

Randomization: Each litter contributed animals to all three experimental groups, and neonatal rabbits were randomly assigned to each group. If there were any reasons for exclusion, another animal from the previously allotted group was picked to replace the excluded animal. Animals were divided in 3 study groups according to the type of treatment administered: **MEC** group – animals with MAS, without treatment; **SURF** group: animals with MAS treated with surfactant -100 mg/kg and **Control** group – animals with no disease and no treatment.

Ventilation and collection of pulmonary mechanics data: During ventilation, data of tidal volume (TV), dynamic compliance (DC) and ventilatory pressure (VP) were collected within 5, 10, 15, 20 and 25 minutes by means of a pneumotacograph (model 3700 series, Hans Rudolph Inc, Kansas City, MO) attached to a signal amplifier transmitting data in real time to a computerized data acquisition system (LabView 5.1, National Instruments), specifically developed for this purpose. Ventilatory pressure was the difference between PIP and PEEP, and the dynamic compliance was calculated by dividing the tidal volume (ml/kg) by VP (cm H_2O). The PIP adjustment was made as necessary, to keep the 8ml/kg target TV, up to 30 seconds prior to each measurement.

Euthanasia of the animals and handling of the lungs: After 25 minutes of ventilation, the animals were deeply sedated with sodium pentobarbital (25 mg/kg) via intraperitoneal, then carrying out the euthanasia by intrathecal injection of 0.5 ml of 2% lidocaine. The lungs were removed by dissection and then macerated for the IL-8 dosage in the homogenate.

IL-8 dosage: The IL-8 dosages were performed in pulmonary tissue homogenate samples of animals from the three evaluated groups using the enzyme-linked immunosorbent assay (ELISA) method, set for rabbits from Kingfisher Biotech, Inc. (St. Paul, MN, USA). The ratio of lung tissue per mL of PBS (Phosphate Buffer Saline) was 10 mg of lung tissue/ 0.25 mL of PBS. The test principle applied was sandwich enzyme immunoassay, which is performed in the following steps: the microtiter plate is coated with an antibody specific to IL-8 and standards or samples are added. After incubation period, the rabbit IL-8 detection antibody (biotin-conjugated antibody specific to IL-8) is added, followed by the addition of Avidin conjugated to Horseradish Peroxidase (HRP) to each microplate well. After addition of tetrametilbenzidina (TMB) substrate solution, those wells that contain IL-8 exhibit a change in color. At this point the enzyme-substrate reaction is stopped by the addition of 0.18M of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of IL-8 in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve. The range of standard curve was 5 to 320 pg/mL, the interassay and intra-assay coefficients of variation were 10% and 7%, respectively.

Statistical analysis: The minimum calculated sample size was 6 animals in each group, based on a difference between the means of the groups in the dimension of 0.17 ml/cmH_2O.kg (for pulmonary compliance), with a standard deviation considered the same for all the groups and ranging around 0.07, with a test power of 0.80 and a significance level of 0.05. The pulmonary compliance values considered were obtained from literature data.[15,16,21]

The comparison of means between groups was made using "ONE-WAY ANOVA". "Student-Newman-Keuls" was used as discriminatory post-test, assuming a level of significance of 5%. For data not showing the normal distribution and same variance prerequisites, the "Kruskal-Wallis One Way Variance Analysis in Ranks" was used as a test option for non-parametric data. The proportions were compared using the chi-square test.

Ethical considerations: The study was approved by the Ethics Committee on Animal Experimentation of Botucatu Medical School.

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The euthanasia of the animals was performed under proper sedation and specific painless procedures were used, with no suffering for the animals, respecting the standards and recommendations in effect at the institution and recognized internationally.[20]

Results

Out of the 29 animals included in the study, two were excluded due to pneumothorax and two because they did not meet the criteria for the MAS model. The birth weight of the rabbit pups ranged from 45 to 53 g, with no statistically significant differences between groups.

Dynamic lung compliance was monitored throughout the 25-minute period of mechanical ventilation to confirm reproduction of the meconium aspiration syndrome model and to assess the effect of surfactant treatment. Figure 2 shows pulmonary mechanics in the three study groups during ventilation. Animals exposed to meconium had lower dynamic lung compliance than controls. After surfactant administration (SURF group), dynamic lung compliance increased and remained stable until the end of the ventilation period. Tidal volume remained stable throughout ventilation (8.0–8.9 mL/kg), with no significant differences between groups.

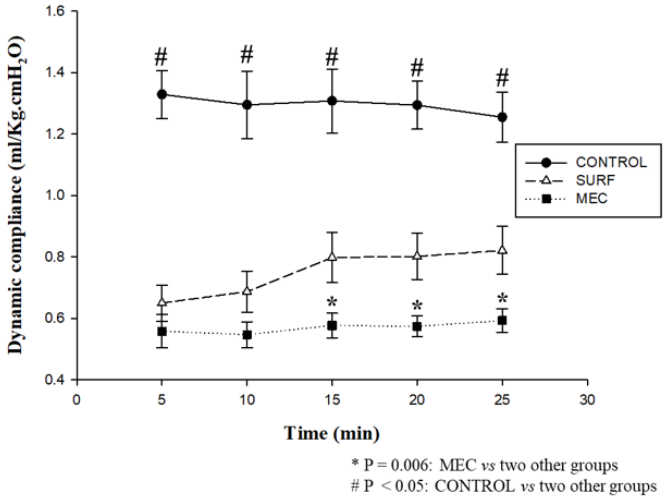

Figure 2 Dynamic lung compliance values during ventilation period (Mean ± se).

IL-8 concentrations in homogenized lung tissue (pg/mL·kg) were 355.8 ± 129.7 in the Control group, 386.3 ± 102.2 in the SURF group, and 712.9 ± 379.8 in the MEC group. Animals with meconium aspiration treated with surfactant had lower IL-8 levels than untreated animals, with values comparable to those observed in the Control group (Figure 3).

Following the administration of meconium via tracheostomy, animals in the MEC and SURF groups showed a reduction in dynamic lung compliance (DLC) greater than 30% compared to the control group, which confirms the establishment of the proposed experimental model. It was observed that after five minutes of ventilation, following surfactant administration, there was a significant increase in DLC in SURF group.

SURF: Group of animals with meconium aspiration, treated with bovine surfactant (100mg/kg); MEC: Group of animals with meconium aspiration, without treatment; min: minutes; se: standard error.

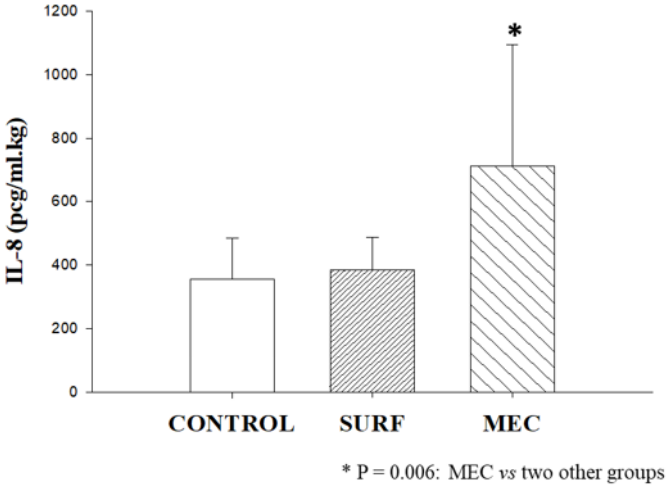

Figure 3 Interleukin 8 (IL-8) values in lung tissue of the study groups (mean ± sd).

SURF: Group of animals with meconium aspiration treated with bovine surfactant (100mg/kg); MEC: Group of animals with meconium aspiration without treatment; IL-8, interleukin 8; sd, standard deviation.

The IL-8 levels in the lung tissue of animals in the group that received surfactant were significantly lower than in the MEC group (without treatment), with values similar to those observed in the control group.

Discussion

Meconium aspiration syndrome may cause severe respiratory failure and is associated to high mortality rates, posing a challenge to neonatal care.[21,22] Within the complex and multifactorial pathophysiology of meconium aspiration syndrome (MAS), inhibition of pulmonary surfactant activity represents a key mechanism, mediated by cholesterol molecules and bile acids present in meconium. In addition, the intense intra-alveolar inflammatory response triggered by meconium plays a central role in disease progression. Elevated levels of IL-8, along with other pro-inflammatory cytokines and mediators, have been consistently demonstrated in experimental and clinical studies.[23–25]

The results of this study confirmed that surfactant administration reverses the observed effects on dynamic lung compliance in animals with meconium aspiration. These results are comparable to previous studies in which surfactant administration at doses of 100 or 200 mg/kg improved lung compliance and reduced lung tissue damage, with less atelectasis and hyperinflation.[16,26]

Our results have also shown that newborn rabbits with meconium aspiration had IL-8 values twice as high in pulmonary tissue when compared to the animals without the disease. The IL-8 values observed were similar to those found in previous studies, performed on samples obtained by bronchoalveolar lavage in rabbits with MAS.[27,28] In our study, animals with MAS treated with exogenous surfactant showed significantly lower levels of IL-8 compared to untreated animals, achieving similar values to the ones found in the control group. This effect may be explained by the modulatory role of surfactant on the intra-alveolar inflammatory response, leading to reduced IL-8 production.[29,30] In addition, we speculate that surfactant replacement, by restoring lung mechanics through a reduction in alveolar surface

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tension, permitted the use of lower inspiratory pressures, thereby decreasing the risk of ventilator-induced lung injury. This, in turn, may have contributed to attenuated local inflammation and, consequently, lower IL-8 levels.

Studies with experimental models of MAS show that anti-inflammatory (glucocorticoids) and antioxidant (N-acetylcysteine) agents can modulate pulmonary inflammatory reaction by the reduction of the edema, the influx of neutrophils and a lower production of oxidative stress markers.[13,31,32] It has also been demonstrated that the administration of inhibitors of cicloxigenase-2, an enzyme involved in the pathogenesis of MAS, also decreases the production of inflammation markers, like IL-8, with less pulmonary tissue injury in rabbits with MAS.[28]

This study has several limitations. The relatively short ventilation period (25 min) may have been insufficient to determine whether the effects of surfactant administration are sustained over time. However, this constraint is inherent to the experimental model, as ventilation periods exceeding 25–30 minutes are associated with high mortality, rendering longer protocols unfeasible. Nevertheless, the model remains valuable, as it is well established that early lung injury occurring within the first minutes of life is closely associated with an intense local inflammatory response, characterized by the production of inflammatory mediators and free radicals.[33,34]Another issue to consider is that the reduced IL-8 levels observed in the treated group could theoretically reflect either the absence of disease or the development of a less severe inflammatory process compared with the untreated MEC group. However, pulmonary mechanics assessment, based on dynamic lung compliance measurements, supports the conclusion that alveolar injury induced by meconium aspiration occurred homogeneously in both the MAS and SURF groups. Furthermore, in both groups, only animals exhibiting a reduction in lung compliance greater than 30% of values considered normal for the species were included, in accordance with established criteria for this experimental model.[19]

We concluded that induced MAS in newborn rabbits increased IL-8 lung tissue production and that the surfactant replacement therapy decreased the local inflammatory response, evaluated by the lower IL-8 values in the pulmonary tissue macerate. In the future, new studies can be conducted to evaluate the effects of surfactants enriched with hydrophilic proteins or combined with anti-inflammatory agents on the alveolar inflammatory response in meconium aspiration syndrome.

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None.

Conflicts of interest

The authors declare that they have no competing interests.

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