Laboratory Investigation

Acute lung injury after tracheal instillation of acidified soya-based or Enfalac® formula or human breast milk in rabbits

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Purpose: The aim of this study was to compare the severity of the acute lung injury after tracheal instillation of acidified soya-based or Enfalac® infant formula, or human breast milk (HBM) in anesthetized rabbits.

Methods: Alveolar-arterial oxygen tension gradient ($A-aDO_2$) and dynamic compliance were measured before (baseline) and hourly for four hours after tracheal instillation of $0.8 \text{ ml}\cdot\text{kg}^{-1}$ soya-based or Enfalac® infant formula or HBM (all acidified to pH 1.8 with hydrochloric acid) or no fluid (control) in 24 anesthetized and tracheotomized adult rabbits. The $A-aDO_2$, the difference between alveolar and arterial oxygen tensions, was corrected for barometric pressure and carbon dioxide tension. Dynamic compliance was the ratio of the expired tidal volume to the peak inspiratory airway pressure, normalized to body weight.

Results: Baseline $A-aDO_2$ and dynamic compliance were similar among the four groups. In the control rabbits, $A-aDO_2$ remained unchanged throughout the four hours, whereas mean $A-aDO_2$ increased 180 mm Hg in the soya-based group ($P < 0.0025$) and 350 and 275 mm Hg in the Enfalac® and HBM groups respectively ($P < 0.0002$). The order of the $A-aDO_2$ post-instillation was $\text{Enfalac}^\circ \approx \text{HBM} > \text{soya} > \text{control}$ ($P < 0.0002$). Dynamic compliance decreased 10-12% in the control rabbits during the four post-instillation measurements compared with baseline ($P < 0.033$), decreased 20% in the soya-based group ($P < 0.0002$) and 40-50% in the Enfalac® and HBM groups ($P < 0.0002$).

Conclusion: The severity of the acute lung injury after intratracheal instillation of infant feeds in a volume of $0.8 \text{ ml}\cdot\text{kg}^{-1}$ and at pH 1.8 in rabbits depends in part, on the type of feed: $\text{Enfalac}^\circ \approx \text{HBM} > \text{soya} > \text{control}$.

Objectif : Comparer la sévérité de la lésion pulmonaire aiguë qui suit une instillation de lait pour bébé à base de soya ou d'Enfalac ou de lait maternel humain (LMH), tous acidifiés, chez des lapins sous anesthésie.

Méthode : Le gradient alvéolo-artériel de pression d'oxygène ($A-aDO_2$) et la compliance dynamique ont été mesurés avant (mesures de base) et après (à chaque heure pendant 4 h) l'instillation trachéale de $0,8 \text{ ml}\cdot\text{kg}^{-1}$ de lait pour bébé à base de soya ou d'Enfalac® ou de LMH (tous acidifiés à un pH de 1,8 avec de l'acide chlorhydrique) ou sans liquide (témoin) chez 24 lapins adultes sous anesthésie et trachéotomie. Le $A-aDO_2$, différence entre les pressions d'oxygène alvéolaire et artérielle, corrige les déviations entre la pression barométrique et celle du gaz carbonique. La compliance dynamique est le rapport du volume courant expiré sur la pression inspiratoire maximale des voies aériennes, normalisé selon le poids corporel.

Résultats : L' $A-aDO_2$ et la compliance dynamique étaient similaires au départ dans les quatre groupes. Chez les témoins, l' $A-aDO_2$ est demeuré le même pendant les quatre heures, alors qu'il a augmenté jusqu'à 175 mmHg dans le groupe soya, ($P < 0,0025$) et jusqu'à 300 mmHg dans les groupes Enfalac® et LMH ($P < 0,0002$). Le $A-aDO_2$ post-instillation était, dans l'ordre : $\text{Enfalac}^\circ \approx \text{LMH} > \text{soya} > \text{témoin}$ ($P < 0,0002$). La compliance dynamique post-instillation, comparée à celle de base, a diminué de 10-12 % chez les témoins ($P < 0,033$), de 20 % dans le groupe soya ($P < 0,0002$) et de 40-50 % dans les groupes Enfalac® et LMH ($P < 0,0002$).

Conclusion: La sévérité de la lésion aiguë du poumon, après une instillation intratrachéale de lait pour bébé chez des lapins, dépend en partie du type d'aliment : $\text{Enfalac}^\circ \approx \text{LMH} > \text{soya} > \text{témoin}$.

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THE factors that contribute to severe lung injury after tracheal instillation of milk products and infant feeds are poorly understood. In 1952, Moran demonstrated that neither human breast milk (HBM) nor cow's milk products was injurious to the lungs, unless they had been fortified with carbohydrates.¹ In the following year, Teabeaut reported that the pathological changes after pulmonary aspiration of a vegetable-based feed (pH 2.7) were less injurious to the lungs of rabbits than aspiration of a dairy-based feed (pH 3.8).² Following these two studies, there was little research in milk product aspiration until recently, when interest to reduce the fasting interval after breast-feeding began to mount. To address this issue, Litman *et al.* fasted infants only two hours after breast-feeding. They found that the residual gastric volume was ≥ 1 ml·kg⁻¹ in 29% of the infants who were breast-fed, compared with none of the infants who were fed dextrose solution.³ Although Litman *et al.* demonstrated that a substantial proportion of infants retained a large volume of gastric fluid two hours after breast-feeding, they did not determine whether this fluid was injurious to the lungs if it were aspirated. To address this issue, we investigated the severity of the lung injury after tracheal instillation of infant feeds in rabbits. We found that human breast milk and SMA® feeds injured the lungs to similar extents,⁴ not an unexpected finding since these feeds are both dairy products and similar in composition.^{5,a} Subsequently, we demonstrated that the severity of the lung injury after tracheal instillation of 0.8 ml·kg⁻¹ HBM was independent of the pH of the feed between pH values of 1.8 and 7.0.⁶ These results, together with the earlier results, prompted us to speculate that the putative cause of the acute lung injury may be the infant feeds themselves and that soya-based feed, being vegetarian-based, may be less injurious to the lungs than is SMA®, a dairy-based feed. Accordingly, we designed the following study to determine whether the severity of the lung injury depends on the type of infant feed that is instilled into the trachea.

Materials and methods

Surgical preparation

With approval by the institutional animal care committee, 24 pathogen-free adult New Zealand white rabbits (Charles River, Canada) weighing 3.2 ± 0.3 kg were

studied. The rabbits were fasted overnight for solids and allowed free access to clear fluids up to four hours before induction of anesthesia. Each rabbit was premedicated with 10 mg·kg⁻¹ ketamine, 0.16 mg·kg⁻¹ atropine and 0.2 mg·kg⁻¹ acepromazine *im*. After application of ECG monitoring, anesthesia was induced with halothane in a nitrous oxide/oxygen gas mixture. Lactated Ringer's solution was infused at a rate of 10 ml·kg⁻¹·hr⁻¹ through a 24G cannula in a marginal ear vein. Systemic blood pressure was monitored continuously via a 24G catheter that was inserted into the common carotid artery under direct vision. The internal jugular vein was cannulated with a 5 Fr. umbilical catheter that was subsequently advanced into the right atrium. After injecting 1 mg·kg⁻¹ bupivacaine with epinephrine *sc* into the anterior neck caudal to the cricoid cartilage, a surgical tracheostomy was fashioned between the third and fourth tracheal rings. A 3.5 mm Portex uncuffed tracheal tube was inserted through the tracheostomy to a depth of 3 cm and sealed with a ligature. Ventilation was controlled using intermittent positive pressure ventilation delivered via a pneumatically operated, time cycled and volume controlled ventilator (Airshield's Ventimeter Ventilator®, Hatboro, Penn) after the rabbits were paralyzed with 0.5 mg·kg⁻¹ pancuronium. Neuromuscular blockade was maintained throughout the experiment by a continuous infusion of pancuronium (0.4 mg·kg⁻¹·hr⁻¹). Bilateral inflation of the lungs was confirmed by observation of symmetrical chest expansion and by auscultation of the thorax bilaterally. Anesthesia was maintained with halothane 1%, inspired, in humidified oxygen. Rectal body temperature was maintained at $38 \pm 1^\circ\text{C}$ using a warming blanket and an overhead infrared radiant heater. Hemodynamic stability was maintained by optimizing preload with lactated Ringer's solution based on filling pressures and the base deficit. All rabbits were supine throughout the study.

Experimental protocol

After induction of anesthesia, tidal volume was adjusted to deliver 10 - 12 ml·kg⁻¹. Respiratory rate was adjusted before instillation of the milk to maintain normocapnia (PaCO₂ 35 - 45 mm Hg) and was not adjusted thereafter. Positive end expiratory pressure and cardiotonic or vasoactive drugs were not used in this study. Baseline (time zero) arterial blood/gas measurements and dynamic compliance were recorded 10 min after stable cardiorespiratory variables. The rabbits were then assigned by random selection to one of four groups: Enfalac® formula (n = 6), soya-based formula (Isomil®) (n = 6), HBM (n = 6) and control (n = 6). Control rabbits received no fluids intratracheally. The infant formu-

^a Formulary of Infant and Enteral Formulas, Clinical Dieticians, The Hospital for Sick Children, Toronto, November 1997.

las were acidified to pH 1.8 with HCl immediately before the study. The pH of the study fluid was titrated to the assigned pH using a Radiometer Copenhagen PHM62b pH meter (Copenhagen, Denmark) that had been calibrated with commercial buffer solutions (pH 1, pH 4, and pH 7 Fisher Scientific, Nepean, Ontario) before each measurement. The accuracy range of this instrument is ± 0.01 pH units.

After baseline measurements were recorded, either $0.8 \text{ ml} \cdot \text{kg}^{-1}$ of the study fluid or no fluid was instilled into the trachea through the tracheotomy. Blood gas analysis, peak inspiratory pressure and lung volumes were recorded at hourly intervals for four hours after instillation. Mean arterial pressure > 40 mmHg and right atrial pressures of 2 - 5 mmHg were maintained throughout the study period with intermittent fluid boluses of lactated Ringer's solution of $5\text{--}10 \text{ ml} \cdot \text{kg}^{-1}$ as necessary. When the final measurement was completed, the rabbits were killed rapidly by intravenous administration of $35 \text{ mg} \cdot \text{kg}^{-1}$ pentobarbital followed by $6 \text{ mmol} \cdot \text{kg}^{-1}$ KCl into the central venous catheter.

Methods of measurement

Arterial blood gas tensions (carbon dioxide and oxygen) and pH were measured using a Radiometer Copenhagen

FIGURE 1 The A-aDO₂ (mean $\pm$ SD) before and hourly for four hours after tracheal instillation of soya-based formula, Enfalac®, HBM or no fluid (control). Baseline A-aDO₂ was similar in all four groups. In the control rabbits, A-aDO₂ did not change throughout the study period but was less than that in the three infant feed group at all times ($P < 0.001$). The A-aDO₂ in the three infant feed groups increased after instillation of the feed compared with their respective baseline values at all times post-instillation ($P < 0.0025$). The A-aDO₂ measurements in the Enfalac® and HBM groups were similar at all times post-installation and both were significantly greater than that in the soya-based group at all measurements post-instillation ($P < 0.0002$).

ABL330 analyzer (Copenhagen, Denmark). The alveolar to arterial oxygen tension gradient (A-aDO₂) was calculated as follows: $A\text{-}a\text{DO}_2 = \{F_i\text{O}_2[P_B - P_{\text{H}_2\text{O}}] - \text{PaCO}_2/0.8 - \text{PaO}_2\}$. The arterial and central venous pressures were measured via a heparinized saline filled pressure transducer (Hewlett Packard 78342A, Mississauga, Ontario). The transducers were zeroed at mid-thoracic level. Dynamic compliance was the ratio of the expired tidal volume and the peak inspiratory pressure, normalized to the body weight of the rabbit. Expired tidal volume was measured (Monitor 1900, Riverside, Ca.) using a calibrated pneumotachograph that averaged the tidal volumes of five sequential breaths. Peak inspiratory pressure was measured at the distal inspiratory limb of the ventilator tubing using a saline filled pressure transducer (Hewlett Packard 78342A, Mississauga, Ontario).

Dynamic Compliance

FIGURE 2 Dynamic compliance (mean $\pm$ SD) before and hourly for four hours after tracheal instillation of soya-based formula, Enfalac®, HBM or no fluid (control). Baseline dynamic compliance was similar in all four groups. Dynamic compliance in the control group decreased at 1, 3, and 4 hours post-instillation compared with baseline ($P < 0.033$) and compliance in the soya-based Enfalac®, and HBM groups decreased at all four measurements post-instillation compared with their respective baselines ($P < 0.0002$). Compliance in the soya-based group was similar to that in the control group at all times post-instillation except at the two hour measurement ($P < 0.035$) but greater than that in the Enfalac® and HBM groups at all times post-instillation ($P < 0.0027$). Compliance in the Enfalac® and HBM groups was similar at all post-instillation measurements, but less than that in the control group at all four measurements ($P < 0.0002$).

Data analysis

All data are presented as means $\pm$ standard deviation (SD). Demographic, A-aDO₂ and dynamic compliance data were analyzed by two-way ANOVA with repeated-measures (Statistica® for the Macintosh. Statsoft, Inc, Tulsa 1994). Post-hoc testing was performed for main and interaction effects using the Student Newman Keuls test. $P < 0.05$ was accepted as statistically significant.

Results

Baseline A-aDO₂ measurements in the four groups were similar (Figure 1). The A-aDO₂ measurements did not change throughout the study period in the control rabbits. In contrast, the mean A-aDO₂ measurements increased 180 mm Hg above baseline ($P < 0.0025$) in the soya-based group and 350 to 275 mm Hg ($P < 0.0002$) in the Enfalac® and HBM groups at all four measurements post-instillation respectively. The A-aDO₂ measurements in the Enfalac® and HBM groups were similar at all times post-installation and both were greater than that in the soya-based group at all measurements post-instillation ($P < 0.0002$). The measurements for all three infant feeds were greater than the corresponding control measurements at all times post-instillation ($P < 0.001$).

Baseline dynamic compliance measurements in all four groups were similar (Figure 2). Dynamic compliance in the control group decreased 10-12% at one, three and four hours post-instillation compared with baseline ($P < 0.033$), compliance in the soya-based group decreased 20% at all four measurements post-instillation ($P < 0.0002$) and compliance in the Enfalac® and HBM groups decreased 40-50% at all four measurements ($P < 0.0002$). Compliance in the soya-based group was similar than that in the control group at all times post-instillation except two hours ($P < 0.035$) but greater than that in the Enfalac® and HBM groups at all times post-instillation ($P < 0.0027$). Compliance in the Enfalac® and HBM groups was similar at all post-instillation measurements, but less than that in the control group at all four measurements ($P < 0.0002$).

Discussion

The present study was designed to determine whether the type of infant feed instilled into the trachea affected the severity of the acute lung injury. We found that the severity of the lung injury does depend, in part, on the type of feed according to the order: Enfalac® $\approx$ HBM $>$ soya $>$ no fluid. We also confirmed previous findings that instillation of acidified infant feed into the trachea induces an acute lung injury as evidenced

by an increase in the A-aDO₂ and decrease in compliance in rabbits that persisted for four hours.⁴

The variables that are known to affect the severity of the acute lung injury after aspiration may be classified as either those that can be adjusted under experimental conditions, such as the pH and volume of the aspirate, or those that cannot, such as the composition, type and particle size of the feed.^{2,7-9} In the present study, we acidified all feeds and fluids to a single pH value, 1.8, and we instilled a known volume, 0.8 ml·kg⁻¹, into the trachea of each rabbit. Given these controlled conditions, all observed differences in the severity of the lung injury should be attributed to the particular properties of the feed. Enfalac® and HBM, both dairy-based feeds with similar protein, fat and carbohydrate compositions,^{5b*} should evoke a similar lung injury, which they did in this study. In contrast, the soya-based formula Isomil®, a vegetable-based feed composed of vegetable protein and vegetable oil, contains neither casein nor whey protein. According to Teabeaut,² such a feed should evoke a less severe lung injury than a dairy diet, which it did. Our findings support the notion that the type of infant feed is an important determinant of the severity of the lung injury after instillation of feeds into the trachea of rabbits.

It is also possible that differences in the particle size of the three feeds could explain, in part, the observed differences in the lung injury. Knight *et al.* reported that tracheal instillation of 1.5 ml·kg⁻¹ of a fluid at pH 5.3 that contained gastric juice particles 2-30 microns in diameter in rats produced a similar capillary leak as did a clear solution at pH 1.25.⁹ Thus, particles 2-30 microns in diameter may be injurious to the lungs even in the presence of a minimally acidic pH. Although we did not quantitate the particle size distribution in the three feeds used in this study, the diameter of fat globules in HBM ranges from 1 to 12 microns and the concentration of fat in HBM is 35-45 mg·ml⁻¹.¹⁰ These values, which are comparable with those of the fluids studied by Knight *et al.*,⁹ support the notion that the particles in HBM contribute to the acute lung injury after tracheal instillation of HBM. The explanation for the attenuated effect of the soya-based feed on the lung injury in rabbits remains unclear. Whether the composition or particle size of this feed is less injurious to the lungs or leukoactivation after tracheal instillation of soya is not as severe as that after HBM, remains to be determined.

^b Formulary of Infant and Enteral Formulas, Clinical Dieticians, The Hospital for Sick Children, Toronto, November, 1997

We designed this study to investigate the lung injury after tracheal instillation of feeds that had been acidified to a pH 1.8 and administered in a volume of 0.8 ml·kg⁻¹. In previous studies of acid aspiration, the instillates had been acidified to pH values between 1.0 and 1.25.⁷⁻⁹ Although the latter pH range is more likely to injure the lungs severely than a pH $\geq$ 1.8 and has been used to study acute lung injury after aspiration, this low pH range does not represent the pH of gastric fluid in this population of infants. The mean pH of the gastric fluid two hours after breast-feeding is 2.7 ± 1.0 .³ Using the normal deviate to estimate the population distribution of residual gastric fluid pH,¹¹ less than 7.5% of the population of breast-fed infants will have a gastric fluid pH $\leq$ 1.25. By adjusting the pH of all of the feeds in our studies to 1.8, we estimated that 68% of this population should have a residual gastric fluid pH $\geq$ 1.8. We believe that by using a pH of 1.8, it provides a sound clinical context for interpreting the results of this study.

Although we instilled 0.8 ml·kg⁻¹ of each feed or fluid, the minimum volume of tracheal instillate that is purported to injure the lungs severely is 0.4 ml·kg⁻¹. This volume is based on a single report by Roberts and Shirley in which the fluid was instilled into one bronchus, not the trachea, of a monkey.¹² Thus, the minimum volume of fluid that we should instill into the trachea to replicate this injury is at least double the volume reported or $\geq$ 0.8 ml·kg⁻¹. Several studies have confirmed that volumes of tracheal instillate larger than 0.4 ml·kg⁻¹ are associated with a severe lung injury after acid aspiration.^{7,8} It remains possible that the relative severity of the lung injury associated with these feeds may differ at lower pH values or greater gastric fluid volumes than those reported in this study. Nonetheless, based on the data in this study, soya-based feeds appear to be less injurious to the lungs of rabbits than HBM and Enfalac®.

Conclusion

The results of this study emphasize the need to fully characterize the constituents of instilled or aspirated fluids in models of infant feed aspiration. The severity of the lung injury after tracheal instillation of an infant feed in rabbits follows the order: Enfalac® $\approx$ HBM > soya-based > no fluid.

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