Acute Lung Injury after Instillation of Human Breast Milk or Infant Formula into Rabbits’ Lungs


Background: Recent interest in shortening the fasting interval after ingestion of milk products demonstrated large volumes of breast milk in the stomach 2 h after breastfeeding. Although aspiration is a rare event, if it were to occur with human breast milk, it is important to understand the extent of the lung injury that might occur. Therefore, the response to instillation of acidified breast milk and infant formula in the lungs of adult rabbits was studied.

Methods: In 18 anesthetized adult rabbits, 1 of 3 fluids (in a volume of 0.8 ml·kg⁻¹ and pH level of 1.8, acidified with hydrochloric acid); saline, breast milk, or infant formula (SMA, Wyeth, Windsor, Ontario), was instilled into the lungs via a tracheotomy. The lungs were ventilated for 4 h after instillation. Alveolar-to-arterial oxygen gradient and dynamic compliance were measured before and at hourly intervals after instillation. After 4 h, the rabbits were killed and the lungs were excised. Neutrophil infiltration was quantitated by a pathologist blinded to the instilled fluid. A histologic control group of four rabbits was ventilated under study conditions without any intratracheal fluid instillation.

Results: Alveolar-to-arterial oxygen gradient increased and dynamic compliance decreased significantly during the 4 h after instillation of both breast milk and infant formula compared with baseline measurements and with saline controls (P < 0.05). The neutrophil counts in the lungs from the saline, breast milk, and formula rabbits were significantly greater than those in the control group.

Conclusions: Instillation of acidified breast milk or infant formula (in a volume of 0.8 ml·kg⁻¹ and pH level of 1.8) into rabbits’ lungs induces acute lung injury of similar intensity that lasts at least 4 h. (Key words: Acidification; hydrochloric acid. Compliance: dynamic. Lungs, aspiration: breast milk; infant formula. Lungs: injury.)

RECENT attention has focused on the rationale for preoperative fasting guidelines for infants and children after ingestion of clear fluids and milk products. In the case of clear fluids, published data indicate that the risk of pneumonitis after aspiration is no greater after a fasting interval of 1⁻8 h than it is after 2 h before elective surgery. In the case of milk products, there is surprisingly little evidence on which to base preoperative fasting guidelines.

To develop meaningful fasting guidelines after ingestion of milk products, three issues must be addressed: first, a definition of milk products; second, the rate of gastric fluid emptying; and third, the severity of lung injury after aspiration of milk products. The common milk products used in infancy are breast milk and infant formulas (such as SMA, Wyeth, Windsor, Ontario). Few studies have addressed the rate of gastric emptying of milk products. The rate of gastric emptying of breast milk is approximately one fifth that of water but twice as fast as infant formula. These data are supported by a report of large residual volumes of breast milk in the stomachs of children who were fasted only 2 h before elective surgery. On the basis of these data, the fasting interval after breast milk is distinct from and should be greater than that after clear fluids if we intend for the stomach to be emptied of these products by the time anesthesia is induced. The third issue and the least understood of the three, is the severity of lung injury that can be expected if milk products were aspirated.

Previous studies of the pulmonary effects of the intratracheal instillation of milk products in rabbits identified several factors that predisposed to severe lung injury and/or death. Moran reported that large volumes of milk products and hypertonic milk products at their native pH levels were often acutely fatal when instilled into the tracheas of rabbits. He also noted, however,
that while smaller volumes of both human breast milk and cow's milk (3 or 4 ml) were not fatal, they did induce acute and delayed reactions in the lungs as evidenced by the presence of red cells, granulocytes, and macrophages. More recently, Olson concluded that Similac (3 or 4 ml at its native pH level) injured rabbits' lungs to a greater extent than did distilled water. When we instilled 0.4 ml·kg⁻¹ breast milk at its native pH level (6.6–7.4) into rabbits' lungs, we found no evidence of lung injury. However, these studies may not reflect the extent of lung injury after milk product aspiration in the clinical setting because most fluids that are aspirated have been acidified after mixing with gastric fluid secretions. To quantitate the pulmonary effects of acidified milk products within the lungs, we instilled acidified human breast milk and infant formula into the lungs of rabbits and quantitated the physiologic and histologic changes.

Materials and Methods

Surgical Preparation

With approval of the institutional animal care committee 18 pathogen-free adult New Zealand white rabbits (Charles River, Canada) weighing 3.4 ± 0.3 kg were studied. The rabbits were fasted overnight for solids and allowed free access to clear fluids up to 4 h before induction of anesthesia. Each rabbit was premedicated with 14.5 mg·kg⁻¹ intraperitoneal ketamine, 0.25 mg·kg⁻¹ atropine, and 0.3 mg·kg⁻¹ acepromazine maleate. After application of electrocardiographic monitoring electrodes, 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·h⁻¹ through a 24-G cannula in a marginal vein in the ear. Systemic blood pressure was monitored continuously through a 22-G catheter inserted percutaneously into an artery in the ear. The internal jugular vein was cannulated with a 5-French umbilical catheter that was advanced into the right atrium. After subcutaneous infiltration with bupivacaine (1 mg·kg⁻¹) with epinephrine in the anterior neck caudal to the cricoid cartilage, a surgical tracheostomy was fashioned between the third and fourth tracheal rings. A 3.5-mm ID uncuffed tracheal tube was inserted through the stoma to a depth of 3 cm and sealed with a ligature. Ventilation was controlled after paralysis with intravenous 0.5 mg·kg⁻¹ pancuronium bromide. Neuromuscular blockade was maintained throughout the study with a continuous infusion of 0.4 mg·kg⁻¹·h⁻¹ pancuronium. Bilateral inflation of the lungs was confirmed by observation of symmetrical chest expansion and auscultation of the thorax. Intermittent positive pressure ventilation was delivered with a pneumatically operated, time cycled, and volume controlled ventilator (Airshield's Ventimeter, Hatboro, PA). All rabbits were ventilated with 1% halothane in humidified oxygen. Rectal temperature was maintained at 38 ± 1°C by using a warming blanket and an overhead infrared radiant heater. Hemodynamic stability was maintained by optimizing the preload with lactated Ringer's solution based on filling pressures and monitoring the base deficit.

Experimental Protocol

Tidal volume was adjusted to deliver between 10 and 12 ml·kg⁻¹. Respiratory rate was adjusted to produce normocapnia (Pa₂CO₃ 35–45 mmHg) and was not altered thereafter. Deliberate positive end expiratory pressure and cardiotoxic and vasoactive drugs were avoided during the study. Baseline (time zero) arterial blood gas measurements and dynamic pulmonary compliance were recorded 10 min after stable cardiorespiratory variables. The rabbits were then randomly assigned to 1 of 3 groups: 0.9% saline, human breast milk, or infant formula (SMA 2800). Each study solution was titrated to a pH level of 1.8 by the addition of 6 N, 1 N, or 0.1 N hydrochloric acid. The pH level was measured using a Radiometer Copenhagen PHM62b (Copenhagen, Denmark), that was calibrated before each measurement with two commercial buffer solutions (pH 1 and 4; Fisher Scientific, Nepean, Ontario). The accuracy range of this instrument was ±0.01 pH units. Four additional rabbits (control group) were anesthetized for 4 h but received no fluids intratracheally. These rabbits composed a control group for neutrophil infiltration in ventilated normal lungs.

After baseline measurements were completed, the tracheotomy tube was disconnected from the ventilator and 0.8 ml·kg⁻¹ of the assigned fluid was instilled into the trachea. Throughout the study, all rabbits were supine. Blood gas analysis, peak inspiratory pressure, and lung volume measurements were recorded at hourly intervals for 4 h 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 5–10 ml·kg⁻¹. After the 4-h measurements, the rabbits were killed by rapid intravenous administration of 35
mg·kg⁻¹ pentobarbital into the central venous catheter. The lungs were then removed en bloc and fixed by immersion in 10% buffered formalin for histopathologic assessment. Representative sections for histopathologic assessment from the lungs of each rabbit were stained with hematoxylin and eosin.

Methods of Measurement
Arterial blood gas tensions (carbon dioxide and oxygen) and pH were measured using a Radiometer Copenhagen ABL320 analyzer. The alveolar to arterial oxygen tension difference (Aa\textsubscript{DO₂}) was calculated as follows: Aa\textsubscript{DO₂} = Fi\textsubscript{O₂} [Pb - pH\textsubscript{2}O] - Pa\textsubscript{CO₂}/0.8 - Pa\textsubscript{O₂}. The arterial and central venous pressures were measured with a transducer via a heparinized saline-filled tubing and displayed on a monitor (Hewlett Packard 783 i+2A, Mississauga, Ontario). The transducers were zeroed to the mid thoracic level. To determine compliance, both the peak inspiratory pressure and expiratory tidal volume were measured. Peak inspiratory pressure was measured in the distal inspiratory limb of the ventilator tubing using an airway pressure manometer. Expiratory tidal volume (averaged over 5 sequential breaths) was measured at the endotracheal tube connector (Neonatal Volume Monitor 1900, Riverside, CA) using a precalibrated pneumotachograph.

A qualitative histopathologic assessment of each lung section was performed at low magnification (×10) by a pathologist (EC) blinded to the treatment administered. Alveolar edema and congestion, atelectasis, the presence or absence of particulate matter in the alveolar ducts, and lumen and neutrophil migration were recorded. In addition, a semiquantitative assessment of the neutrophil infiltration was performed using a 25× objective lens incorporated into a projecting microscope (Leitz, Wetzlar, Germany). This provided sufficient resolution to identify intraalveolar and septal neutrophils. For this assessment, each lung section (2 per rabbit) was divided into ten approximately equal areas and within each area, a uniformly sized section devoid of major vascular or bronchial structures was examined. The examined area was projected onto a blank sheet and the neutrophils were sequentially marked to avoid duplication and then counted.

Data Analysis
Demographic data (such as weight), Aa\textsubscript{DO₂}, and dynamic compliance are expressed as means ± standard deviation (SD), and were analyzed using one-way analysis of variance for intergroup analysis and repeated measure analysis of variance for within-group analysis. Post hoc testing was performed using the Student-Newman-Keuls test. Numbers of neutrophils were analyzed using the Kruskal Wallis test. Post hoc testing was performed using Dunn’s multiple comparison test. A P level < 0.05 was accepted as statistically significant.

Results
The baseline Aa\textsubscript{DO₂} in the breast milk group was greater than in the infant formula and saline groups (P < 0.05; fig. 1). The Aa\textsubscript{DO₂} in the breast milk group 1 h postinstillation was greater than that in the infant formula group (P < 0.05), although there were no significant differences between these two groups at subsequent measurements. The Aa\textsubscript{DO₂} in both the breast milk and infant formula groups was greater than both their respective baseline values and the Aa\textsubscript{DO₂} in the saline group at all measurements postinstillation (P < 0.05). In addition, the Aa\textsubscript{DO₂} in the saline group did not change significantly at any time postinstillation compared with the baseline measurement.

Baseline dynamic compliance was similar in all three groups (fig. 2). Compliance in the breast milk group was similar to that in the infant formula group at all
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four postinstillation measurements, although the measurements in both groups at all four times were significantly less than their respective baseline values ($P < 0.05$). In comparison with the saline group, compliance in the breast milk group was significantly less at all postinstillation measurements, whereas compliance in the infant formula group was less at the 3- and 4-h measurements only ($P < 0.05$). Dynamic compliance in the saline control group did not change significantly postinstillation compared with the baseline measurement.

Qualitative histologic assessment revealed alveolar edema, vascular engorgement, and septal and intraalveolar neutrophil margination with focal areas of atelectasis and hemorrhage. According to the pathologist, these qualitative features were most evident in the two milk product groups. The quantitative assessment indicated that the neutrophil count in the saline, breast milk, and infant formula groups were all significantly greater than that in the control group ($P < 0.05$ for the saline group, $P < 0.01$ for the breast milk and infant formula groups), but were not different from one another (fig. 3).

**Discussion**

This study was designed to compare the pulmonary effects of acidified breast milk or infant formula with saline in adult rabbits. We found that both breast milk and infant formula (at a $pH$ level of 1.8 and a volume of 0.8 mL·kg$^{-1}$) produced an acute lung injury within 1 h of its instillation into the trachea compared with saline at the same $pH$ level and volume. This was confirmed physiologically by an increase in $A_{dO_2}$ and a decrease in dynamic compliance in the two milk product groups. The magnitude of the injury appeared to be similar. These physiologic changes persisted for the 4 h of the study, without evidence of resolution or deterioration in the measurements. At post mortem examination, the pulmonary sequestration of neutrophils in all three study groups confirmed the histologic injury.

We acidified all of the fluids to a $pH$ level of 1.8 immediately before instillation into the rabbits’ lungs for three reasons. First, although a $pH$ level of 2.4 is widely considered the threshold for severe lung injury after aspiration, some data suggest that the threshold $pH$ level for lung injury may be closer to 1.8. Second, we targeted a $pH$ level of 1.8 because it was approximately 1 SD below the mean $pH$ of residual fluid in the stomachs of infants who had been breastfed 2 h earlier, 2.6 ± 1. Third, this targeted $pH$ level was also consistent with the mean $pH$ after 2–8 h of fasting after clear fluids in children. However, a $pH$ level of 1.8 may not accurately represent the gastric fluid $pH$ level in all infants, particularly those 1–2 days old. Gastric acid hyposecretion has been reported in full-term neonates during the first 6 h after birth and in preterm neonates during the first 49 h after birth. After the first two postnatal days, gastric acid secretion increases reaching normal adult values. Hence, a $pH$ level of 1.8 reflects the gastric fluid of neonates beyond the first 36–49 h postnatal age, and of older infants.

The baseline $A_{dO_2}$ in the breast milk group was significantly greater than that in both the infant formula and saline groups (fig. 1). The magnitude of the difference in $A_{dO_2}$ between the breast milk group and the group with the smallest baseline $A_{dO_2}$, saline, was 65 mmHg ($P < 0.05$). The reason for this difference is not apparent. To ensure that there was no systematic bias in this study, all rabbits were purchased from the same pathogen-free source, all were assigned to their treatments using random number tables and were free of clinical evidence of pulmonary dysfunction. In ad-
diation, compliance in the breast milk group was similar to that in the other two groups. The $Aa_{\text{DO}}_2$ in the breast milk group was similar to that in the infant group at all measurements postinflation, except at the 1 h measurement. If the difference in the baseline $Aa_{\text{DO}}_2$ between the breast milk and other groups also held true for the postinflation measurements then the $Aa_{\text{DO}}_2$ in the breast milk group at 1 h postinflation may not have differed from that in the infant formula group. The difference in baseline $Aa_{\text{DO}}_2$ between the breast milk group and the other two groups, although statistically significant, remains of questionable relevance in this study.

Our results are distinct from published studies of the pulmonary effects of milk product instillation for several reasons. First, in this study, the $pH$ level was uniform for all fluids administered, whereas in previous studies the $pH$ level of the fluids was the native $pH$ level. Because the native $pH$ level differed, the extent of the lung injury that occurred also differed. By maintaining a uniform $pH$ level for all fluids, we eliminated this variable. Second, the volume of fluid instilled into the lungs was smaller than that reported previously. This together with the high inspired fraction of oxygen meant that the severity of the injury could not be attributed to hypoxemia. Third, the anesthetic, ventilation, and position of the rabbits in this study were uniform throughout the study period. In previous studies, anesthesia was used only to facilitate introduction of the milk products into the trachea, the mode of ventilation was not clearly described, and the position of the rabbits was varied from supine under anesthesia to prone during awakening.

The results of this study may not be directly applicable to the pulmonary effects of aspiration in humans for several reasons. First, we acidified all fluids with hydrochloric acid instead of gastric fluid. In these experiments, our goal was to study the pulmonary effects of acidified fluids, not of gastric juice aspiration. In the absence of the proteolytic enzymes and other substances present in the gastric fluid, the true clinical effects of milk product aspiration may have been underestimated. Further studies are warranted to address this issue. Second, we ventilated the lungs with 100% oxygen without positive end expiratory pressure for the entire study. Atelectasis is known to occur in this setting, although we assumed that the extent of the atelectasis would be minimal after 4 h. Indeed, the absence of histologic evidence of atelectasis, even in the control (no fluid instilled) group, supports this assumption. Third, our model consisted of mature adult rabbits, which may differ in their responses to milk product instillation.
when compared with human infants. Although previous studies suggest that the pulmonary response to milk product aspiration in rabbits is consistent with autopsy findings after similar aspiration in human infants, the time course of the responses and the extent of the lung injury in rabbits may differ from that in human infants. Thus, caution must be exercised when considering the clinical implications of these results.

Histologically, it is possible that the changes in the lungs after instillation of milk products could be explained in part by changes attributable to terminal cardiac failure (i.e., congestion and edema of the lungs at the time of death). However, we killed the rabbits with a rapid intravenous bolus injection of barbiturate. This should have minimized the risk of histologic changes in the lungs during cardiac failure. In addition, we quantitated the neutrophil migration, a relatively specific marker that heralds the onset of an inflammatory cascade of mediator release and possesses a latency of onset. Therefore, by using a quick-acting barbiturate together with a marker of inflammation in the lungs, we believe that the histologic changes that we documented closely reflect the acute lung injury associated with milk product instillation.

In summary, physiologic and histologic evidence of acute lung injury occurs after instillation of 0.8 ml kg⁻¹ acidified (pH 1.8) breast milk and infant formula into rabbits’ lungs. The extent of the injury is similar to the two milk products and lasts at least 4 h.

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