The Pathophysiologic Changes Following Bile Aspiration in a Porcine Lung Model*


Aspiration of bile is an underpublicized aspiration syndrome. Using a porcine lung model, the physiologic response and the histopathology of lung tissue were evaluated after the intratracheal instillation of sublethal doses of bile. Twenty-one domestic swine (11 to 19 kg) were the studied population. Three groups of five swine were evaluated: a control group received intratracheal physiologic saline (pH 7.45); study group 1 received strained gastric contents (pH 2.74); and study group 2 received strained bile (pH 7.19). All animals received the solutions at 0.5 ml/kg intratracheally. Lungs of six additional animals were studied (two gastric, two bile, and two physiologic saline) after aspiration by scanning electron microscopy (SEM). A seventh untreated animal was used as the SEM control. The physiologic data were analyzed using analysis of variance for repeated measures. The SEM and histopathologic results were graded by an observer blinded to the groups and were analyzed using the analysis of variance (ANOVA) and Scheffe tests. The group with bile aspiration was consistently characterized by significant deterioration of PaO2, the alveolar-arterial (A-a) gradient, shunt fraction, and static compliance (p<0.01); and the light histopathologic and SEM findings demonstrated pathologic changes in the bile-exposed lung (p<0.05) greater than the gastric- or saline-exposed lungs. It is concluded that bile aspiration produces a severe chemical pneumonia leading to noncardiac pulmonary edema.

(Chest 1993; 104:919-24)

A-a = alveolar-arterial; ANOVA = analysis of variance; SEM = scanning electron microscopy

Aspiration of gastric contents, blood, meconium, petrochemicals, water (salt or fresh), and the like occurs in a variety of clinical settings.1-6 Historically, Mendelson1-4 took his clinical observation from obstetric patients and investigated his theory in his laboratory. His laboratory investigation in rabbits demonstrated pulmonary parenchymal damage from acid aspiration and an obstruction phenomenon from large particulate matter. These studies have been replicated by others to define significant characteristics of aspirates, such as pH, volume, particulate and nonparticulate matter, osmolarity, infectious agents and toxic inhalational agents. From these studies came the appreciation and the identification of "high-risk" situations.

Bile aspiration was seen by us in a patient with a paralytic ileus who clinically aspirated some "gastric contents." The immediate clinical course was characterized by a rapid deterioration of arterial oxygenation requiring artificial ventilation with rapidly increasing levels of positive end-expiratory pressure. Typically, this patient remained hypotensive, similar to patients with septic shock. Further histopathologic examinations revealed that the aspirate was of a bilious nature. While no septic foci or bacteremia could be identified, the clinical course was rapid onset of adult acute respiratory distress syndrome leading to the patient's death in approximately 48 h.1-6 Few reports have described the effects of aspiration of bile in humans or animals. Henderson et al10 studied the effects of bile, bile salts, and HCl aspiration on the lungs in rabbits. The extent of pulmonary damage that occurs in aspiration syndromes has been attributed to the composition of the aspirate.1-2,5,6,9,10 The identification of bile in this patient's gastric aspirate by thin-layer chromatography stimulated this laboratory study of bile aspiration.

Materials and Methods

Twenty-one healthy domestic swine (11 to 19 kg in weight) were anesthetized with ketamine (20 mg/kg) administered intramuscularly. Following the establishment of peripheral intravenous access, pentobarbital sodium (25 mg/kg) was administered to facilitate endotracheal intubation by direct observation. Anesthesia was maintained by intermittent injection of intravenous pentobarbital (75 to 150 mg) every 15 to 30 min as needed. Then 14-gauge arterial and venous catheters were inserted into the femoral artery and vein, respectively. Throughout the experiment the animal's body temperature was maintained at 38°C using a heating pad, increased ambient temperature, overhead heat lamps, and enclosure of the animal in cellophane. A pediatric 5F pulmonary artery catheter (Baxter-Edwards) was introduced through an external jugular vein. Ventilation was controlled using a ventilator (Siemens-Elema Servo 900B) to maintain normal oxygenation and normocapnia (tidal volume remained constant at 12 to 15 ml/kg). Positive end-expiratory pressure was not used. Pharmacologic support with inotropic or vasopressor agents was avoided. Fluid status was maintained with 5 percent dextrose/lactated Ringer's solution at 3 ml/kg/h. Samples of arterial and mixed-venous blood were drawn for gas analysis using a blood gas analyzer (Radiometer ABL-30). Cardiac output...
and other cardiac parameters were obtained by the thermodilution technique (3.0 ml of cold saline), using a cardiac output computer (Barter-Edwards COM-1). Each determination of cardiac output was the average of three sequential measurements with less than 10 percent variance. Previously obtained swine bile and gastric juice had been collected, strained, and analyzed by thin-layer chromatography and then divided into aliquots and frozen for future use. The absence of bile or bile salts was confirmed in the gastric juice. After the pulmonary and hemodynamic parameters were stable on a fractional concentration of oxygen in the inspired gas (FIo2) of 1.0 for 30 min, the studied fluids at 0.5 ml/kg (physiologic saline, preservative-free [pH 7.45]; gastric juice [pH 2.24]); or strained bile [pH 7.19] were instilled into the animal's trachea distal to the cuffed endotracheal tube via the distal port of a triple-lumen catheter. After the solution was injected intratracheally, four manual breaths were given to assist in the intrapulmonary distribution of the studied solution.

Measurements were taken every 15 min for the first hour and then every 30 min for the next 3 h. The physiologic parameters recorded were heart rate, systemic blood pressure, central venous pressure, pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output, minute ventilation, hematocrit, arterial and mixed-venous blood gas levels (pH, Pco2, PaO2, HCO3, saturation, and base excess [BE]). Calculations were made for left and right ventricular stroke work, oxygen delivery and consumption, oxygen extraction, alveolar-arterial (A-a) gradient, shunt fraction, and static compliance. Four hours after aspiration of the solution, the animal was killed with an intravenous overdose of sodium pentobarbital. The lungs were immediately removed and placed in a formaldehyde solution (10 percent Formalin) for later sectioning and analysis by light microscopy.

Six additional animals were similarly prepared: two bile, two gastric, and two normal saline. A seventh, untreated animal was similarly prepared (without any aspirate) for the scanning electron microscope (SEM) histopathologic control. Following an identical study period, the lungs were placed in glutaraldehyde fixative. Five sections per lung were submitted for analysis by SEM.

The hemodynamic and pulmonary parameters were analyzed using an analysis of variance (ANOVA) of repeated measures. The histopathologic findings, both light microscopy and SEM, were evaluated by a histopathologist blinded to the treatment of the animal and analyzed by ANOVA and the Scheffe test. The following grading scales for light microscopy and SEM were used:

**Light microscopy**

1. No significant lesions
2. Minimal focal bronchial and alveolar leukocyte accumulations, principally neutrophilia
3. Slight focal acute bronchiar and alveolar supplicative inflammation with increased numbers of neutrophils and macrophages in alveoli, bronchiolar lumina, and within alveolar walls and around bronchioles
4. Focal peribronchiolar inflammation; increased thickness of alveolar walls with neutrophils and macrophages; slight to moderate bronchiolar epithelial cell suffusion, mild edema between lobules, and perivascular and peribronchiolar mild

**FIGURE 1. Comparative pulmonary effects after aspiration. NS, Normal saline; and Qs, Qt, shunt fraction. Asterisk indicates p<0.01 for bile versus control or gastric after injection at all times; solid bullet indicates not significant for gastric versus control after injection at all times (control is 0.9 percent preservative-free normal saline).**

Pathophysiologic Changes Following Bile Aspiration (Pulemba et al)
focal edema, slight focal hemorrhage.
4. Extensive focal to lobular acute suppurative inflammation with moderate bronchiolar epithelial stuff ing; generalized increased numbers of inflammatory cells in and around bronchi oles, vessels, and alveolar walls; slight to moderate focal hemorrhage or focal vascular congestion (or both).
5. Extensive lobular acute suppurative inflammation with extensive bronchiolar epithelial stuff ing; lobular inflammation, extensive large areas of hemorrhage, or vascular congestion.

**Scanning electron microscopy**

0. Cilia present in more than 90 percent of cells; few macrophages or other leukocytes seen.
1. Cilia present in 50 to 90 percent of cells; small denuded areas devoid of bronchiolar epithelial cells; moderate numbers of macrophages and neutrophils, some with pseudopods and ruffles.
2. Cilia present in less than 50 percent of cells; large denuded areas of bronchiolar epithelial cells; moderate to large numbers of macrophages and neutrophils, most with pseudopods and ruffles.

**RESULTS**

While physiologic changes were noted in the groups,

**Table 1—Histologic Quantification With Light Microscopy**

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**Discussion**

Aspiration of gastric contents has been shown by many studies to result in a significant morbidity and mortality. In our study, animals receiving gastric juice and saline solution, these changes did not statistically differentiate between these two groups; however, the group receiving bile differed from the gastric juice and saline solution groups (p < 0.01) in PaO2, A-a gradient, shunt fraction, and static compliance (Fig 1). The bile group differed histopathologically (both by light microscopy and SEM) from the gastric juice and control groups in the severity of lung damage as judged by a pathologist blinded to the treatment (p < 0.05) (Tables 1 and 2).

Analysis of hemodynamic parameters for cardiac output, central venous pressure, mean arterial pressure, pulmonary artery occlusion pressure, and heart rate revealed no statistical differences among the three groups. All of these groups had a decreasing trend for cardiac output, heart rate, and left and right ventricular stroke work which was attributed to the depth of anesthesia. In the bile group, there was a trend for the hematocrit reading to increase and pulmonary artery occlusion pressure to fall at the end of the study period. Five of seven animals in the bile group developed clinically evident noncardiac pulmonary edema compared to zero of seven animals in the gastric and zero of seven animals in the saline group, which is statistically significant.

Two swine were not used initially because of rapid cardiovascular collapse and subsequent cardiac arrest after instilling an aliquot of bile of 1.0 ml/kg. Two animals early on in the study developed a tracheal disruption during a traumatic intubation that resulted in some hemorrhage and subcutaneous emphysema, and the experiment was discontinued.

**Table 2—Scanning Electron Microscopy**

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*0, Cilia present in more than 90 percent of cells, and few macrophages or other leukocytes seen; 1, cilia present in 50 to 90 percent of cells, small denuded areas devoid of bronchiolar epithelial cells, moderate numbers of macrophages and neutrophils, some with pseudopods and ruffles; 2, cilia present in less than 50 percent of cells, large denuded areas of bronchiolar epithelial cells, moderate to large numbers of macrophages and neutrophils, most with pseudopods and ruffles.
†B, bile; G, gastric; and NS, normal saline.
‡p < 0.05 vs gastric vs control.
§Not significant for gastric vs control.
The incidence of aspiration of gastric contents has been variously reported to occur between 0.008 to 0.2 times per 1,000 anesthetics. Mortality has been suggested to occur in 5 percent of proven cases of aspiration. Prior studies by Mendelson, Teabeaut, James et al., Schwartz et al., Gibbs et al., and others have concentrated on the pH, volume, and character of the aspirate. Our initial appreciation of aspiration pneumonitis came from the literature on obstetric anesthesia, when Mendelson first described it in the clinical environment and subsequently studied aspiration in the rabbit; however, Teabeaut quantified the effects of acid aspiration by varying the pH of hydrochloric acid solutions and described the histologic findings. He found that a pH of less than 2.5 resulted in significant pulmonary parenchymal damage and that food particles exacerbated the damage. When reviewing prior laboratory investigations of acid aspiration, the instillation of contents of hydrochloric acid that had a pH of less than 2.5 with a pH greater than 2.5 appeared to cause significant lung injury. In addition, Schwartz et al. studied the intratracheal injection of human bile in rabbits and reported 100 percent mortality at 24 h. The findings from necropsy of these animals were reported as severe pulmonary hemorrhage. Henderson et al. reported that synthetic bile intratracheally in rabbits induced pulmonary hemorrhage and edema. Brown studied bile from another animal species, while Henderson et al. used a concentration of bile in excess of 3 percent. Neither study correlated hemodynamic and pulmonary changes in the animal models; however, what is consistent from these studies is that lung parenchymal damage was similar in our study when sublethal doses of aspirate were instilled.

In previous studies, amounts of aspirate from 2.0 to 4.0 mL/kg were used, resulting in greater variations. This study is significant for the use of a high pH (7.24) and low volume (0.5 mL/kg) of aspirate to produce severe pulmonary and hemodynamic changes associated with bile aspiration. The PaO₂ is a sensitive indicator of physiologic injury during aspiration. In this study, the immediately postaspirational PaO₂ of 50 mm Hg in the bile group characterizes the severe pulmonary injury. In the bile group, PaO₂ values remained significantly lower than the other two groups throughout the study period. The PaO₂ rose only marginally, possibly from increasing the alveolar minute ventilation (to maintain normocapnia) and from alveolar recruitment. Activation of hypoxic pulmonary vasoconstriction could possibly contribute to the slow increase in PaO₂. This value correlated well with the A-a gradient, shunt fraction, and static compliance measurement in the bile group (Fig 1).

Histopathologically (light and SEM), the bile group demonstrated marked parenchymal damage consisting of hemorrhage, edema, atelectasis, and polymorphonuclear leukocyte infiltration (Fig 2 and 3) (Table 1).
The rise in hematocrit reading in the bile group correlated with the appearance of clinically significant noncardiac pulmonary edema. This compares to the study of Kennedy et al demonstrating the permeability index after acid aspiration. The increase in the permeability index is believed to reflect the amount of lung injury. Histologically, the changes seen in gastric aspiration were not different from prior investigations when considering low volume of aspirate.

In addition, the resultant injury from gastric aspiration was less severe and extensive when compared to the bile group (Fig 2 and 3, Table 1 and 2).

Recent attention has been given to the use of surfactant replacement in acute lung injury. Lamm and Albert showed that surfactant replacement improved lung recoil in rabbit lungs after aspiration. Kaneko et al studied intratracheal instillation of taurocholic acid (1 ml/kg) in rabbits. These investigators reported a progressively significant decrease in PaO\(_2\) in all animals, leading to death at a mean time of 3.3 h. Kaneko et al then instilled exogenous surfactant in another group of animals after bile aspiration, reporting survival and increasing PaO\(_2\) values in animals receiving doses of surfactant. The pathologic findings in the group not receiving surfactant were similar to this study. What is apparent from this study is that there was acute damage very similar to the chemical pneumonitis seen from acid aspiration (Mendelson's syndrome), but of greater magnitude. In that population (Mendelson's syndrome), prophylactic therapy with antibiotics is usually not given, and a resultant pneumonia is seen in approximately 40 percent of the patients; however, in patients who aspirate gastric contents that contain bile, even if the pH is greater than 2.5, the question arises as to whether a more prevalent pneumonic process would arise from the resultant pneumonitis or destruction. The prevalence of bile in the gastric contents in our intensive care population was not uncommon (40 percent of the patients). Recent studies evaluated the need to selectively decontaminate the gut from bacterial pathogens to prevent the complication of esophageal reflux leading to a disease process of the lower airway (pneumonia). Physicians presently do not evaluate if there is any bile in the gastric contents, nor what adjustment of the antibiotic therapy is needed. Also, no prior investigations have evaluated the response of aspiration of bile with the use of the pulmonary artery catheter for analysis of shunt fraction, static compliance, and A-a gradient and correlated with histopathologic findings by light microscopy and SEM.

In conclusion, this study demonstrates the potential for significant lung injury and physiologic deterioration of PaO\(_2\) associated with bile aspiration. This study describes an underappreciated aspiration syndrome. The development of noncardiac pulmonary edema after gastric aspiration should alert the clinician to suspect bile aspiration. The advantage of surfactant instillation in this clinical setting remains to be identified. This study demonstrates that bile aspiration (as one of the aspiration syndromes presently not characterized) will produce significant pulmonary parenchymal damage.

ACKNOWLEDGMENTS: We thank Boleslaw Lirincz, Ph.D., for the preparation of the light microscopic sections; Thomas Joyce III, M.D., for a critical review of this manuscript; and Ms. Terri Emerson for assistance in preparation of this manuscript. We dedicate this article to the memory of Roger Stuebing, Ph.D.

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