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ANIMAL MODELS

Resolution of Lung Injury after a Single Event of Aspiration

A Model of Bilateral Instillation of Whole Gastric Fluid

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Gastric aspiration is a high-risk condition for lung injury. Consequences range from subclinical pneumonitis to respiratory failure, with fibrosis development in some patients. Little is known about how the lung repairs aspiration-induced injury. By using a rat model of single orotracheal instillation of whole gastric contents, we studied the time course of morphological and biochemical changes during injury and resolution, and evaluated whether repair involved long-term fibrosis. Anesthetized rats received one gastric fluid instillation. At 4, 12, and 24 hours and 4 and 7 days, we performed lung histological studies and biochemical measurements in bronchoalveolar lavage fluid and lung tissue. Physiological measurements were performed at 12 to 24 hours. Long-term outcome was studied histologically at day 60. During the first 24 hours, severe peribronchiolar injury involving edema, intra-alveolar proteinaceous debris, hemorrhage, increased neutrophils and cytokines, and physiological dysfunction were observed. At days 4 and 7, an organizing pneumonia (OP) pattern developed, with foreign-body giant cells and granulomas. Lung matrix metalloproteinase 9 and 2 activities increased, with metalloproteinase-9 linked to early inflammation and metalloproteinase-2 to OP. At day 60, lung architecture was normal. In conclusion, a continuum of alterations starting with severe injury, evolving toward OP and later resolving, characterizes the rat single aspiration event. In addition to identifying markers of staging and severity, this model reveals that OP participates in the repair of aspiration-induced injury. *(Am J Pathol 2015, 185: 2698–2708; http://dx.doi.org/10.1016/j.ajpath.2015.07.001)*

Aspiration of gastric contents into the lungs is common among patients with an impaired gag reflex, a depressed level of consciousness, drug use, and gastrointestinal disorders.[1,2] Consequences of aspiration are variable, ranging from subclinical pneumonitis to severe respiratory failure.[3]

Several approaches have been used in animal models to gain insights into the pathogenesis of aspiration-induced lung injury. Instillation of individual components of gastric fluid has contributed to the understanding of their relative roles in lung injury.[1,4–13] Thus, derangement of the alveolar-capillary barrier with edema and an intense inflammatory reaction occur with acid instillation,[4–7] whereas a delayed inflammatory reaction, followed by granuloma formation without significant edema, results when acid-free gastric food particles are instilled.[8,10,11] Synergistic effects are obtained when acid and gastric food particles are instilled in combination.[1,8] Interestingly, few studies have used the whole gastric fluid to study the pathogenesis of aspiration. Those studies have used low volumes of gastric fluid instilled into small areas of the lung with the aim of answering questions mainly related to gastric aspiration and lung transplant rejection.[14–16]

Although valuable to our understanding of the pathophysiology of aspiration-induced lung injury, existing

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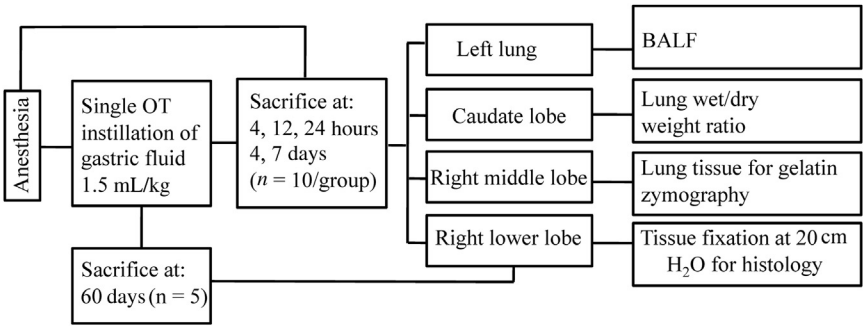

Figure 1 Diagram showing animal groups, tissue sampling, and analysis. BALF, bronchoalveolar lavage; OT, orotracheal.

models have not addressed the study of the continuum of changes after a single event of bilateral aspiration of whole gastric contents. This approach could help to understand what determines the histological pattern in human aspiration-induced acute lung injury (ALI), because either diffuse alveolar damage or organizing pneumonia (OP), the two main patterns described under the term ALI,[17] can be found in lung biopsy specimens from patients who have aspirated.[18–20] Whether the OP pattern in this context represents a *de novo* process or, instead, originates from an earlier inflammatory reaction is unknown.

Moreover, it is still unknown how and to what extent the lung repairs in individuals who survive the early vascular and mechanical derangements induced by a single event of aspiration. In mice models of hydrochloric acid–induced ALI in which resolution of injury markers allowed for evaluation of subacute changes, fibrosis was found.[4,7] However, it has not yet been answered whether this fibrosis is persistent (ie, pathological fibrosis) or, instead, is reversible as part of a normal healing process that ends with restoration of normal lung architecture.

We postulate that an animal model of bilateral instillation of a single dose of whole gastric fluid to study the time course of changes during ALI, its resolution and repair, could contribute to a better understanding of the full range of lung responses after aspiration in humans.

The purposes of this study, therefore, were as follows: First, to develop a rat model of single orotracheal instillation of whole gastric contents. Second, to characterize ALI, using a method recently published by the American Thoracic Society.[21] Third, to evaluate key elements known to participate in lung injury resolution and repair but not included in American Thoracic Society guidelines, such as organization of the exudate, presence of myofibroblasts, collagen deposition, and activity of matrix metalloproteinases (MMPs). Finally, we studied histological changes at a later time to evaluate long-term consequences of a single instillation of gastric fluid.

Because lung biopsy specimens from patients represent a single snap-shot of the events that occur after aspiration, a study of early and late changes induced in this model will lead to a better understanding of the full range of lung responses, allow for the identification of markers of injury and repair likely to be evaluated in humans who aspirate, and improve treatment strategies.

Materials and Methods

Model of Orotracheal Instillation of Rat Gastric Contents

The study was performed according to a protocol submitted to and approved by the Animal Research Ethics Committee of the Pontificia Universidad Católica de Chile (Santiago, Chile).

Gastric Contents Pool

Adult male Sprague-Dawley rats (weight, 300 ± 10 g) fasted overnight were anesthetized i.p. with xylazine-ketamine (5.1 and 55.1 mg/kg, respectively) to obtain gastric fluid through a gastrotomy. Gastric fluid samples were pooled, filtered through a 100-μm mesh, and kept at −80°C. Animals were euthanized thereafter by exsanguination under anesthesia.

Orotracheal Instillation

Under the same anesthetic protocol, another set of animals was orotracheally intubated with a 22 gauge wire-fed catheter. Visualization of the glottis was achieved using a modified human otoscope (Welch Allyn, Skaneateles Falls, NY). A volume of gastric fluid previously determined by the authors (data not shown) to distribute evenly (1.5 mL/kg, pH = 1.69) was instilled, and animals were allowed to recover spontaneously from anesthesia.

Study Groups

Histological and biochemical studies were performed at 4, 12, and 24 hours, and 4 and 7 days after instillation ($n = 10$ per group). Physiological measurements were performed in a small group of animals at 12 to 24 hours ($n = 6$) and at day 7 ($n = 6$).

Table 1 Acute Lung Injury Scoring System

	Score per field		
Parameter of acute lung injury	0	1	2
Inflammatory cells in the alveolar space	0	1–5	>5
Inflammatory cells in the interstitial space	0	1–5	>5
Hyaline membranes	0	1–10	>10
Proteinaceous debris filling the airspaces	0	1–10	>10
Alveolar septal thickening	<2×	2× to 4×	>4×

Adapted and modified from Matute-Bello et al,[21] with permission from the American Thoracic Society (copyright 2015).