An Evaluation Of Alveolar Cytokine Response To Aspiration Of Gastric Contents

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Citation

Abstract

Background: Aspiration injury is a difficult clinical diagnosis. Excessive cytokine is associated with adult respiratory distress syndrome (ARDS).

Design: We evaluated the cytokine response in bronchoalveolar lavage fluid associated with pulmonary aspiration of gastric juice as compared to saline.

Materials and Methods: Pulmonary aspiration injury was induced in 10 adult swine using 1 cc/kg of gastric juice (n=5) and saline (n=5). Bronchoalveolar lavage (BAL) was performed immediately before and one hour after aspiration injury in all animals.)?The BAL fluid was analyzed for tumor necrosis factor alpha (TNF- and interkeukin 6 (IL-6) levels using commercially available ELISA kits.

Results: and IL-6?The levels of TNF- in BAL fluid with gastric juice aspiration showed a significant increase over baseline levels as compared to saline aspiration.

Conclusions: Pulmonary aspiration with gastric juice is associated a strong local inflammatory response as compared to saline.

INTRODUCTION

Aspiration is a serious complication in intensive care units. The incidence of pulmonary aspiration of gastric contents in ICU populations receiving enteral feedings varies widely, ranging from 0.8 % to 77 % [1,2,3,4]. Aspiration is associated with two main detrimental sequelae, pneumonitis and pneumonia. The incidence of nosocomial pneumonia in mechanically ventilated intensive care unit patients has been found to range between 21-38 % [5]. The high morbidity and mortality rates (30 % to 60 %) associated with aspiration pneumonia and pneumonitis are a result of the combined effects of a predisposing illness, a degree of acute airway obstruction and a direct chemical pulmonary injury [6]. Aspiration may not be clinically recognized unless it is accompanied by respiratory distress. Symptoms associated with aspiration may occur hours after the episode [7] making correlation between the inciting event and the symptoms even more difficult. Aspiration has also been identified as a strong general risk factor in development of the adult respiratory distress syndrome (ARDS) [8]. Huxley et al have shown that patients with depressed levels of consciousness are at increased risk of aspiration as compared to normal patients (70 % vs. 45 %, respectively) [9]. Trauma and critical illness may further increase the risk of aspiration injury by increasing the gastric secretion rate and acidity level of gastric secretions. As treatment of pulmonary aspiration is largely supportive, early delineation of alveolar injury may allow more aggressive treatment with possible avoidance of detrimental sequelae.

Acid aspiration has been shown to increase alveolar protein content and pulmonary macrophage accumulation with subsequent activation in various models [10]-12. Tissue cytokine levels may be directly associated with further accumulation and activation of pulmonary macrophages, thus leading to further lung injury. Cytokines are low molecular weight proteins produced by activated immune cells (e.g. tumor necrosis factor, interleukins). These cytokines have been shown to mediate the induction and amplification of the inflammatory response to various types of injury including hemorrhagic and endotoxic shock [13,14]. However alveolar (tissue level) cytokines have not been studied as markers for detection of aspiration injury.

Levels of alveolar as well as systemic cytokines (TNF-a, IL-6) have been shown to change acutely (within 1-2 hours) in response to alveolar injury [11,12]. Preliminary data from cytokine levels in bronchoalveolar lavage (BAL) fluid have been shown to correlate with subsequent development and

severity of ARDS in high risk medical patients 16,17.

We hypothesized that cytokine levels in BAL fluid become acutely elevated in aspiration injury with gastric juice. To study this hypothesis we evaluated levels of TNF-? and IL-6 in BAL fluid as markers for detection of gastric juice induced lung injury in a porcine model.

MATERIAL AND METHODS

This protocol was approved by the University's laboratory animal utilization committee. Animals were cared for in accordance with the current guidelines of the National Institutes of Health. Ten mixed breed adult swine, weighing 75 to 85 kg, were fasted overnight with free access to water. On the day of the experiment, the animals were initially anesthetized with intramuscular telazol (4 mg/kg). Intravenous access was obtained by cannulation of an ear vein and anesthesia was continued with intravenous sodium pentobarbital (3-5 mg/kg/hour). The animals were intubated endotracheally and mechanically ventilated. The ventilator was adjusted to maintain eucarbia and partial pressure of oxygen of at least 100 mmHg.

Gastric juice (pH = 4.57) from one of the fasted pigs was withdrawn using an orogastric tube and this served as the study aspiration material (1 ml/kg) for pigs in the study group (n=5). Normal saline (1 ml/kg) was instilled in to the endotracheal tube of the control animals (n=5).

Bronchoalveolar lavage was performed with 50 ml normal saline using a non bronchoscopic lavage catheter (BALCATH, Ballard Medical Products, Draper, Utah) placed through the endotracheal tube. The bronchoalveolar lavage was performed immediately prior to and 1 hour after instillation of the saline or gastric juice. The aspirated fluid was assayed for TNF and IL-6 using commercially available ELISA kits (Genzyme, Cambridge, Mass). The cytokine levels (in pg/ml) are based on a standardization curve using human cytokines. All assays were performed in duplicate. Statistical analysis was performed using the averaged values for baseline and post aspiration values. Post aspiration values were compared with baseline values using one way analysis of variance with repeated measures. Statistical significance threshold was $p < 0.05$.

RESULTS

Two consecutive bronchoalveolar lavages before and after aspiration were successfully carried out in all animals. The average recovery volume from the bronchoalveolar lavage was 32 ml with recovery volumes ranging from 25 to 40 ml.

No significant change in oxygenation or ventilation was noted in the animals during the course of the study.

The levels of TNF were statistically unchanged before and after aspiration of saline (93.0 ± 15.0 pg/ml vs. 94.0 ± 15.0 pg/ml, p= 0.81). Gastric juice aspiration however resulted in a significant change with the TNF level increasing from 136.0 ± 15.0 pg/ml to 915.0 ± 15.0 pg/ml (p=0.003) (Table 1).

Figure 1

Table 1: BAL cytokine levels with acid aspiration injury

	TNF a (pg/ml)	Interleukin-6 (pg/ml)
Saline Pre-Aspiration BAL (n=5)	93 ± 15	148 ± 20
Saline Post-Aspiration BAL (n=5)	94 ± 15*	193 ± 20**
Gastric Juice Pre-Aspiration BAL (n=5)	136 ± 15	115 ± 20
Gastric Juice Post-Aspiration BAL (n=5)	915 ± 15+	1217 ± 20+

*p= n.s., **p=0.003, +p<0.0001, Mean ± SEM

Levels of IL-6 were elevated with both saline and gastric juice aspiration. With saline the rise in IL-6, from 148.0 ± 20.0 pg/ml to 193.0 ± 20.0 pg/ml, was small but statistically significant (p=0.003). In contrast gastric juice aspiration produced a large rise in the level of IL-6 from 115.0 ± 20.0 pg/ml to 1217.0 ± 20.0 pg/ml (p < 0.0001) (Table 1).

The gastric juice used for the pulmonary aspiration was checked for the presence of TNF and IL-6 and was found to have no detectable levels.

DISCUSSION

Aspiration is a serious complication in intensive care units. The incidence of pulmonary aspiration of gastric contents in ICU populations receiving enteral feedings varies widely, ranging from 0.8 % to 77 % 1-4. The high morbidity and mortality rates (30 % to 60 %) associated with aspiration are the result of the combined effects of a predisposing illness, a degree of acute airway obstruction and a direct chemical pulmonary injury 6. Symptoms associated with aspiration may occur hours after the event 8 making the correlation between the inciting event and the symptoms even more difficult. Trauma and critical illness may further increase the risk of aspiration injury by increasing the gastric secretion rate and acidity level of gastric secretions 1,3. As aspiration treatment is largely supportive, early delineation of alveolar injury may allow more aggressive treatment with possible

avoidance of detrimental sequelae.

The lack of change in oxygenation and ventilation seen in our animals with gastric aspiration was not surprising considering the insidious nature of aspiration injury. The elevation of alveolar cytokines may lead to delayed symptoms as described in the clinical literature 3,4.

Cytokine analysis has been vastly simplified by the recent introduction of commercially available enzyme linked immunosorbent assay (ELISA) kits. These ELISA kits are currently available for mouse and human cytokines. Porcine cytokines have been shown to be cross reactive with human cytokines. Verification of the utility of human cytokine ELISA with porcine models has been done with tumor necrosis factor-alpha (TNF-a) using a bioassay [15]. Porcine interleukin-6 (IL-6) shares a significant homology with human IL-6 and does cross react using the human ELISA kits, however bioassay verification has not been performed because of technical problems 15.

Levels of alveolar cytokines change acutely (within 1 hour) in response to high pressure and high volume ventilation 11. Systemic cytokines (TNF-a, IL-6) have been shown to change in response to acid aspiration related alveolar injury 12. Cytokines have been found to be useful markers of severity of injury in various clinical settings. In contradistinction to alveolar protein levels which have been shown in the past to be elevated in acid aspiration injury but not have any predictive value 10, cytokine levels may provide an effective window to look at subsequent increases in pulmonary macrophage accumulation and activation. If the direct correlation of aspiration with cytokine levels seen in our study is reproduced in patients, then this association of BAL cytokine levels with aspiration injury would allow for a more aggressive directed therapeutic approach. Stated differently, if alveolar cytokine levels are indeed elevated with significant aspiration injury and not elevated with insignificant injury an appropriate treatment algorithm can be instituted. This might include more aggressive pulmonary care and antibiotics in patients with significant injury. Conversely, in a majority of patients with less injury, our cytokine analysis approach would allow for a more cost effective approach with resource conservation.

Our preliminary data looking at forced aspiration of gastric juice has shown significant elevations in alveolar TNF-a and IL-6 levels as compared to baseline values. Acid aspiration injury has been shown to result in a rise in systemic TNF levels, 12 however our study is the first to look at alveolar

cytokine levels in this type of injury. The baseline levels of TNF-a and IL-6 were slightly different between the study group and control group. This may be related to subtle differences in anesthesia levels. These differences are insignificant when compared to the changes noted with gastric juice aspiration. The rise in IL-6 with saline aspiration in the control group may indicate IL-6 is a more sensitive marker. Once again the rise in BAL IL-6 levels was significantly greater with gastric juice aspiration as compared to saline aspiration. The association between BAL cytokine levels and gastric juice aspiration is based upon a relative change in levels rather than exact numbers as our standardization curves were based upon human cytokines provided with the ELISA kits. We would have to assume 100 % cross reactivity to rely on exact numbers. Stated differently the ELISA kits rely on light absorption at a particular wavelength. The exact levels of specific cytokines are based upon extrapolation from a standardized curve plotted using known levels of human cytokines. If we were checking for human cytokine levels the extrapolation along this standard curve would be exact, however we are sampling porcine cytokines using the human cytokine derived standard curve. We assume a large degree of cross reactivity although some degree of error may be present. This error should be inherently negated as we used each animal as its own control at baseline. Further the change in relative values as compared to baseline and saline aspiration is clear.

Another shortcoming of this data is that relative changes are clinically difficult to use as most injury does not announce itself before arrival. This is a common problem with using animal models to extrapolate to the patient's condition. However this problem may be overcome in certain high risk patients by obtaining baseline levels at initiation of mechanical ventilation. Then if a patient is subsequently suspected of having aspirated, a repeat BAL can be performed and subsequent cytokine levels can be checked for elevation. There may be other problems associated with using such sensitive markers as cytokine levels. Alveolar cytokine levels have been shown to be elevated early (at 60 minutes) in relation to injury by high volume high pressure ventilation 11. Since alveolar cytokine changes may be associated with ventilator changes this may pose a problem in the mechanically ventilated patient in whom we are trying to detect significant aspiration. Early elevations in alveolar cytokine levels were seen in preliminary data studying various ventilator modalities by our group as well [18] although the persistence of these changes over time is unclear.

Such an early rise in BAL cytokine levels has been seen in high risk human populations and this has been shown by several groups to be a predictive of subsequent severe lung injury [16,17]. The potential benefit of early detection of significant aspiration injury is that intervention (resuscitation and supportive therapy) can be more aggressive and focused. Further histologic studies of injury classification and delineation studies of the relationship between cytokine elevation and lung injury are needed.

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