Exogenous surfactant instillation attenuates inflammatory response to acid-induced lung injury in rat

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ABSTRACT

The present study was performed to investigate the role of exogenous surfactant on hydrochloric acid (HCL) - induced lung injury in rats. Six-week-old male Sprague-Dawley rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg) and HCL (0.1 N, 2 mL/kg) or normal saline (NS, 2 mL/kg) was instilled into the trachea. Thirty minutes after HCL instillation, surfactant at a dose of 60 mg (=2 mL)/body or NS (2 mL) was instilled into the rat lungs. Animals in another experimental group were also treated with the same dose of surfactant supplement 2 hours after the first administration. Bronchoalveolar lavage fluid (BALF) was obtained 5 hours after HCL instillation. In BALF, increases in total nuclear cell counts, neutrophil counts, optical density at 412 nm as an indicator of pulmonary hemorrhage, neutrophil elastase activity, concentrations of albumin and cytokine-induced neutrophil chemoattractant (CINC) induced by HCL instillation were significantly attenuated by surfactant treatment. The wet-to-dry weight (W/D) ratio in the lung and partial oxygen tension (P_{O2}) were also estimated; surfactant treatment significantly attenuated the W/D ratio and improved deteriorated P_{O2} induced by HCL. Additional surfactant supplementation did not show further beneficial effects on HCL-induced lung injury compared with a single treatment. These results suggest that surfactant shows an anti-inflammatory effect on acid lung injury in rats but the beneficial effects may be dose limited.

INTRODUCTION

Pulmonary surfactant is composed of 90% phospholipid and 10% specific surfactant proteins (SP)-A, B, C, and D. Surfactant reduces the surface tension and prevents collapse of the alveoli, and it is therefore important to maintain normal lung function [1]. Administration of exogenous surfactant has markedly improved survival rate and is now routine therapy for surfactant-deficient infants [2]. It is also well known that synthesis and/or secretion of surfactant are impaired in acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS) [3 – 6]. Thus, surfactant administration has been applied in patients with ALI/ARDS and shows improvement of oxygenation [7 – 11].

Previous experimental and clinical studies suggested that surfactant administration were associated with anti-inflammatory effects in lungs. Surfactant blocks lipopolysaccharide (LPS) signaling and inhibits proinflammatory cytokine secretion in human alveolar macrophages [12,13], and deficiency of surfactant protein increases the inflammatory response to LPS *in vitro* [14,15]. Several experimental and clinical studies indicated that surfactant proteins attenuate acute lung injury and/or production of inflammatory cytokines in the lungs [16-20].

Acid aspiration directly damages the alveolar-capillary membrane, resulting in the influx of protein-rich edema fluid into the alveolar space [21], which causes surfactant dysfunction and degradation [5,6]. Lung inflammation by acid aspiration is also characterized of activation and recruitment of inflammatory cells and elaboration of inflammatory cytokines [21, 22]. However, the precise therapeutic role of pulmonary surfactant in the development of acid aspiration-induced lung injury is not yet clear. In particular, the optimal dose and effect of surfactant on the anti-inflammatory response in acid-induced lung injury remain unclear.

Our hypothesis is that exogenous surfactant can attenuate acid-aspiration lung injury by preventing inflammatory responses in rats. We focused on examining the effects of a single exogenous surfactant supplement on inflammatory response to intratracheal instillation of HCL, including oxygenation and inflammatory mediator production. Furthermore, we evaluated the effects of serial and cumulative surfactant treatment on the inflammatory responses.

MATERIALS AND METHODS

This study was carried out in accordance with the Guidelines for Animal Experimentation of the Shinshu University School of Medicine, Matsumoto Japan.

Animal preparation and agents:

Six-week-old male Sprague-Dawley rats weighting 250 – 300 g were purchased from Japan SLC Inc. (Hamamatsu, Japan). Each rat was anesthetized by intraperitoneal pentobarbital sodium (40 mg/kg). The right jugular venous line was prepared for fluid maintenance (normal saline (NS) 1 mL/h). A tracheostomy was performed and animals were then mechanically ventilated (Shinano Respirator Model SN-480-7; Shinano Tokyo, Japan) at a tidal volume (Vt.) of 10 mL/kg, frequency of 50 breaths/min, and fraction of inspired O_2 (FiO_2) of 1.0. We used Surfacten (Mitsubishi Pharma Co., Osaka, Japan), a powder isolated from bovine lungs containing SP-B and C, which was suspended in NS just before use.

Experiments and protocols

After reaching a stable baseline, NS (2 mL/kg) or HCL (0.1 N 2 mL/kg) was instilled into the trachea *via* the tracheostomy. Thirty minutes after HCL or NS instillation, surfactant at a dose of 60 mg (=2 mL)/body or NS (2 mL) was instilled into the rat lungs. Observation was continued for 5 hours after HCL or NS administration. Animals were divided into four groups: 1) NS group, NS instilled into the trachea; 2) HCL group, HCL instilled into the trachea; 3) HCL plus single surfactant treatment group (HCL+S), HCL instillation and 60 mg of surfactant treatment administered intratracheally into the right and left lungs in a volume of 1 mL; 4) HCL plus double surfactant treatment group (HCL+SS), two hours after the first surfactant administration, the same dose was instilled again into the trachea. As a surfactant control, 2 mL of NS, the same as the volume of surfactant, was administered into the trachea in experiments 1) and 2). An air bolus of 10 mL/kg was injected immediately following surfactant or NS instillation to facilitate peripheral distribution of the agent. After that, no maneuver procedures were done during experiments in any groups.

The following two experiments were performed in the present study. Experiment 1: Bronchoalveolar lavage was performed to collect bronchoalveolar lavage fluid (BALF) 5 hours after HCL or NS instillation. The lungs of each animal were lavaged with 20 mL of NS (5 ml of NS four times, $n = 8$ in each group). Experiment 2: Lung tissue samples were taken to evaluate histopathological changes and to assess wet-to-dry weight (W/D) ratio ($n = 8$ in each group).

Measurements:

1) BALF

Total nuclear cell counts (NCC) in BALF were measured using a hemocytometer (Sysmex F-520; Sysmex, Kobe, Japan). Cell monolayers were prepared by cytocentrifugation to determine neutrophil counts. Differential counts were performed on 200 cells from smears stained with May-Giemsa. The absorbance of BALF was measured to determine pulmonary hemorrhage (UVIDEC-610A; Japan Spectroscopic Co., Ltd., Tokyo, Japan). Neutrophil elastase (NE) activity in BALF was determined using a specific substrate, MeO-Suc-Ala-Pro-Val-pNa [22,23]. Samples were incubated with Tris-HCL buffer (pH 8.0) containing 0.5 M NaCl and 1 mM substrate for 24 hours at 37°C, after which the absorbance at 405 nm was measured to determine NE activity. The albumin and cytokine-induced neutrophil chemoattractant (CINC) concentration in BALF was also determined by nephelometric immunoassay and enzyme immunoassay

(EIA) using a rat GRO/CINC-1 measurement kit IBL (Immuno-Biological Laboratories Co. Ltd., Gunma Japan), respectively.

2) Blood gas analysis

Blood samples for measurement of partial oxygen tension (P_{O2}) were taken from the left ventricle of the heart at the end of each experiment.

3) Assessment of pulmonary edema and histopathological findings

Eight rats in each group were used to evaluate the W/D ratio of the lung and histopathology. The animals were sacrificed, and the lungs were removed immediately. The lungs were weighed and then heated at 80°C to a constant weight in a convection oven (Programmable Incubator IC-300P; Iuchi, Osaka, Japan) for 72 hours and the residue was weighed.

Statistical analysis:

All data are expressed as means ± SD. Differences between the mean values of two groups were evaluated with analysis of variance (ANOVA) followed by Bonferroni's test. P values less than 0.05 were considered significant. STATVIEW 5.0 software (Abacus Concepts, Berkely, CA) was used for all statistical tests.

RESULTS

Recovery rates of BALF were greater than 90% in all groups. There were no significant differences in the recovery rates among the groups.

NCC, the percentage of neutrophil counts, and NE activity in BALF are shown in Figure 1. NCC in the HCL group was significantly higher than that in the NS group. The increased cells in the HCL group were mainly accounted for by neutrophils (85%). NCC and neutrophil counts in BALF were significantly lower in rats treated with surfactant compared with the HCL group. NE activity was markedly increased by HCL instillation and was significantly attenuated by surfactant treatment. There were no significant differences in these parameters between single and double surfactant treatment groups.

Absorbance, albumin, and CINC concentrations in BALF are shown in Figure 2. Absorbance in the HCL group was significantly higher than in the other groups, suggesting marked hemorrhage in lung tissue after exposure to HCL. Surfactant treatment significantly improved the lung tissue hemorrhage induced by HCL instillation. Albumin concentration in BALF was significantly higher in the HCL group than the NS group, suggesting increased pulmonary vascular permeability. Surfactant supplementation of only double treatment group significantly attenuated the permeability induced by HCL instillation. CINC concentration in BALF was extremely high in the HCL group. Both single and double surfactant treatments attenuated the increased CINC in BALF. Although the attenuation by double surfactant treatment was slightly better with regard to absorbance, albumin, and CINC compared with the single surfactant treatment group, the improvements were not statistically significant.

The W/D ratio in the lung and P_{O2} are shown in Figure 3. Increased lung edema and deteriorated oxygenation were observed in the HCL group. Both single and double surfactant treatment significantly attenuated the increased W/D ratio and impaired P_{O2} induced by HCL instillation. Double treatment with surfactant tended to show further improvement, but the differences did not reach statistical significance.

Microscopic examination showed that the alveolar wall was thickened and damaged in the HCL group (Fig. 4). Erythrocytes and infiltration of neutrophils were observed in the alveolar spaces, while hyaline membrane formation was also observed in the HCL group. These changes were diminished in both standard and double surfactant treatment groups (Fig. 4). No apparent differences were observed between single and double surfactant treatment groups, at least based on the histological findings.

DISCUSSION

The results of the present study indicated that exogenous surfactant administration improves the deteriorated oxygenation and inflammatory responses to acid aspiration in rats. However, subsequent additional surfactant treatment resulted in little further improvement in acid aspiration - induced ALI in rats.

Exogenous surfactant administration in patients with ALI/ARDS and experimental ALI has been tested about two decades ago. These trials indicated that surfactant improved oxygenation [8 – 10]. Similarly, rats treated with surfactant showed better oxygenation than the HCL group in the present study. It has been reported that acute lung injury induces endogenous surfactant alterations and functional inactivation, which cause progressive lung dysfunction and lower oxygenation [4 – 6]. Alveolar edema is further exacerbated by the quantitative reduction and qualitative abnormalities of surfactant synthesis due to direct damage of endogenous surfactant and secondary inactivation of surfactant activity [4,6]. Thus, the improved oxygenation may have been mainly due to the direct action of exogenous surfactant in the present study.

The protective effects of surfactant observed in the present study were associated with diminished inflammatory response to acid aspiration, such as cell infiltration and cytokine production in the lung. Surfactant proteins inhibit neutrophil recruitment and function in lung tissue during inflammation [24,25]. Surfactant blocks LPS signaling and inhibited proinflammatory cytokine secretion in human alveolar macrophages [12 – 15]. Epaud *et al.* [20] reported that endotoxin-increased total cell counts, neutrophil influx, protein leakage, and production of cytokines [interlukin-6 (IL-6), tumor necrosis factor-α (TNF-α), *etc.*] were significantly lower in mice overexpressing SP-B than in wild-type controls. Furthermore, surfactant treatment reduced NE activity in infants with ARDS [26] and IL-6 in BALF of patients with ARDS [9]. In the present study, surfactant treatment significantly attenuated neutrophil filtration, NE activity, protein leakage, and CINC in BALF compared with those in rats with acid aspiration. These findings suggested that surfactant decreases inflammation in lung injury, although the present study yielded no data indicating the direct effects of exogenous surfactant on inflammatory cells or cytokine production.

However, additional administration of surfactant failed to show further protective effects on inflammatory responses compared with single administration in the present study. Instillation of surfactant into sepsis-induced ARDS patients improved gas exchange and mortality rate only in the group receiving four doses of 100 mg/kg compared with other groups receiving eight doses of 50 mg/kg or 100 mg/kg, respectively [8], suggesting that the benefits of subsequent dosing regimens are random. Spragg *et al.* [9] evaluated the anti-inflammatory effects of SP-C-based surfactant in patients with ARDS. They reported that IL-6 levels in BALF were significantly decreased at 48 hours in patients treated with the high dose, but not the standard dose, of surfactant in comparison with the untreated control group. In addition, there were no differences in neutrophil counts, NE, or TNF-α in BALF between the SP-C-treated group and controls. Thus, more doses of exogenous surfactant administration were not always associated with further protective outcome in the inflammatory response in patients with ALI/ARDS. In general, the surfactant dose of 100 mg/kg is appropriate in infants with respiratory distress syndrome [2]. The dose of 60 mg/rat (about 200-240mg/kg) used in the present study was markedly higher than the standard dose [2,27]. Based on the results in the double surfactant administration group, the initial

dose of 60 mg/body may be sufficient and limited the anti-inflammatory effect in rats with acid lung injury during the 5 hours of the experiment. In addition, the present results suggested little therapeutic benefit of “rescue” treatment with additional surfactant in the development of acid-induced lung injury. However, the efficacy of exogenous surfactant administration is influenced by a number of factors, including the administration schedule, delivery method, type of agent, and the severity of targeted lung injury [17,27]. If additional treatment of surfactant is essential in this ALI model, the optimal timing of subsequent administration should be determined. Further studies are needed to determine endogenous surfactant clearance during the development of HCL-induced lung injury and to assess the necessary dose of exogenous surfactant.

In summary, we reported that exogenous supplementation with surfactant, which contained SP-B and C, could provide protection against lung damage induced by acid aspiration in rats. The present study indicated that protection for acid-induced lung injury could be mediated by an anti-inflammatory response of exogenous surfactant as well as the direct function of alveolar distension, but the effects may be dose limited.

Figure legends

Figure 1. Nuclear cell counts, neutrophil differential ratio, and neutrophil elastase activity in bronchoalveolar lavage fluid of all groups. NS group, rats were instilled with normal saline and treated with normal saline (n = 8); HCL group, rats were instilled with hydrochloric acid (HCL, 2 mL/kg) and treated with normal saline (n = 8); HCL+S group, rats were instilled with HCL and treated with standard dose (60 mg/body) of surfactant, (n = 8); HCL+SS group, rats were instilled with HCL and treated twice with standard dose (60 mg/body) of surfactant (n = 8). Values are expressed as means ± SD. * P < 0.05 vs. NS group; # P < 0.05 *vs.* HCl group.

Figure 2. Absorbance, albumin concentration, and CINC in bronchoalveolar lavage fluid of all groups. Groups were as described in Fig. 1. Values are expressed as means ± SD. * P < 0.05 vs. NS group; # P < 0.05 *vs.* HCL group.

Figure 3. Wet-to-dry weight ratio of lung and partial oxygen tension in all groups. Groups were as described in Fig. 1. Values are expressed as means ± SD. * P < 0.05 vs. NS group; # P < 0.05 *vs.* HCL group.

Figure 4. Light photomicrographs of lung tissue in the groups at 200× magnification. Sections were stained with hematoxylin and eosin. Groups were as described in Fig. 1. In the HCL group, congested alveolar walls, alveolar edema and hyaline membranes with alveolar wall disruption were observed. Numerous neutrophils and red blood cells were present in the alveolar spaces. In the HCL+S and SS groups, few neutrophils and red blood cells were seen in the airspaces. No alveolar edema or hyaline membranes were seen and the integrity of the alveolar walls was better preserved.

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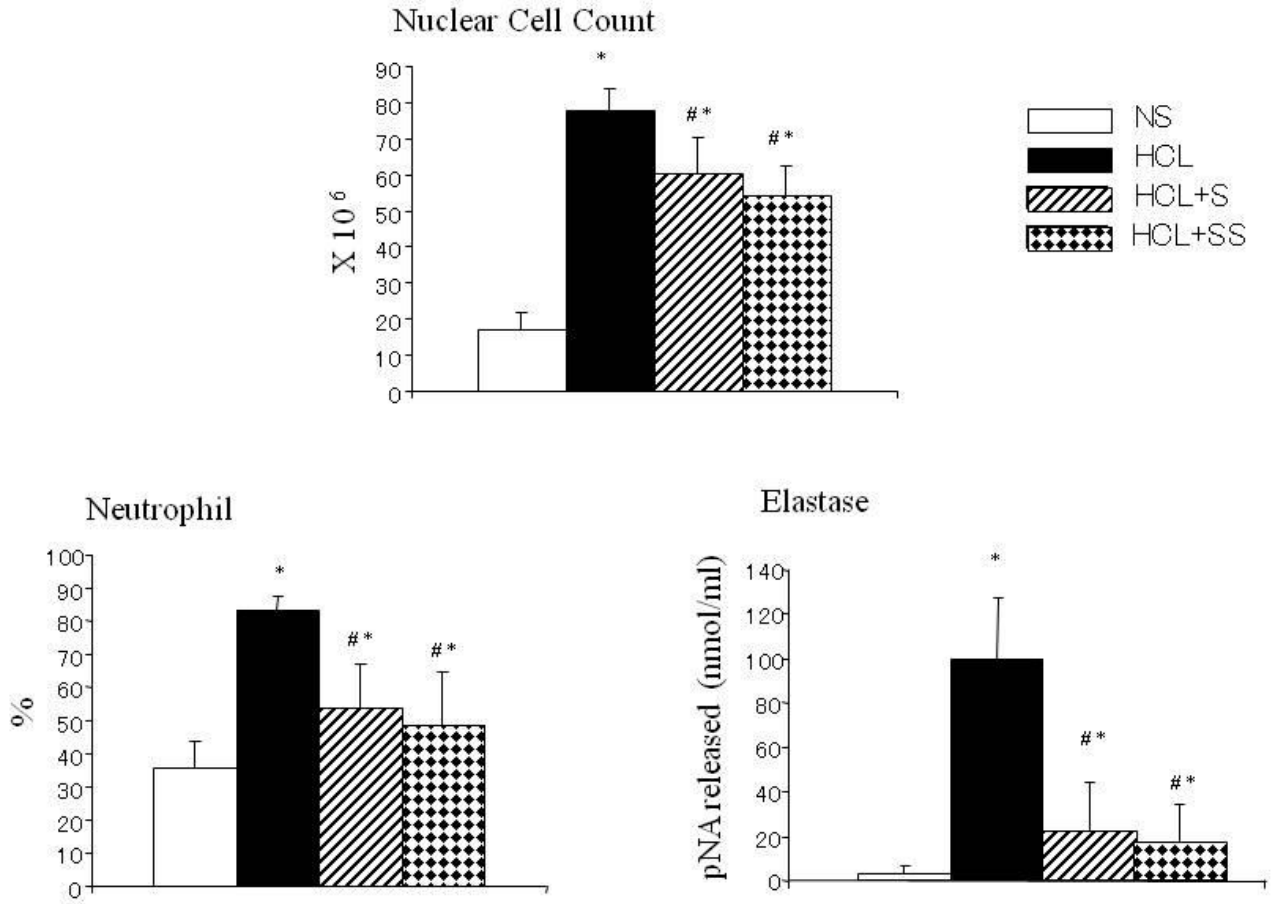
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Fig. 1

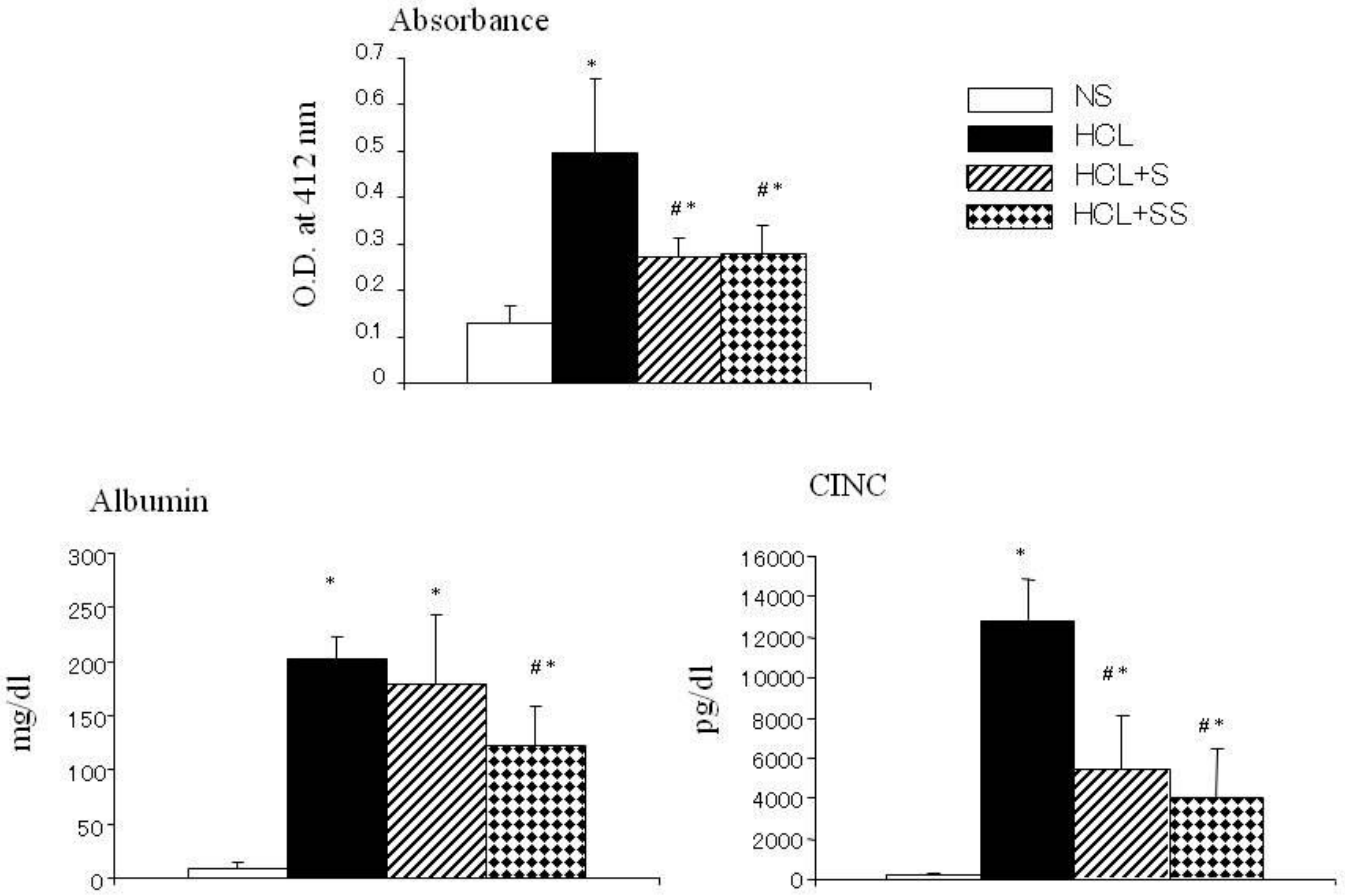
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Fig. 2

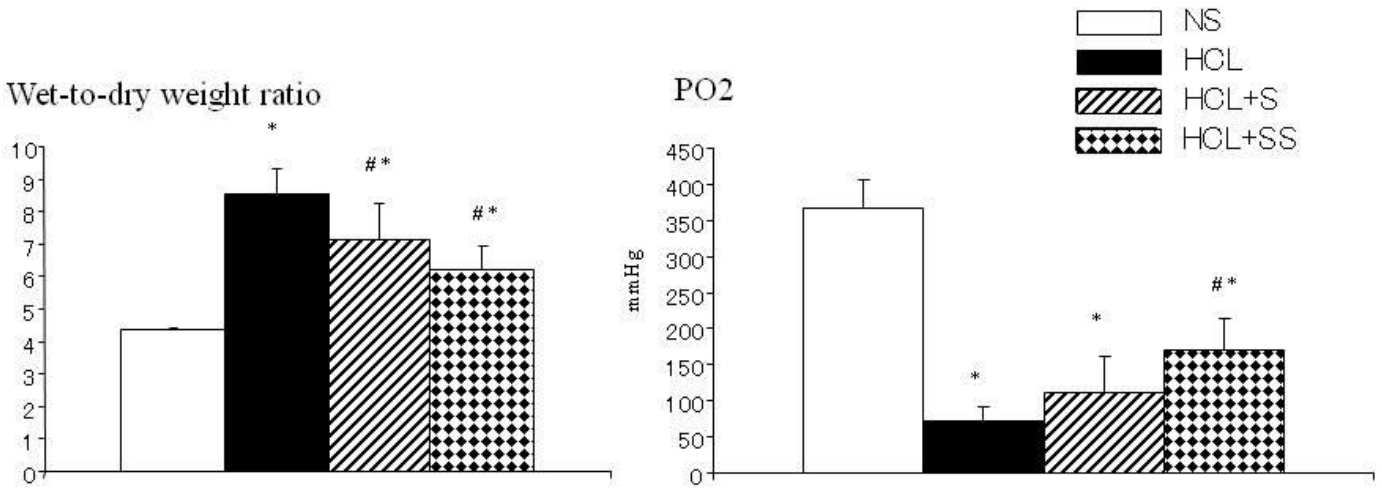
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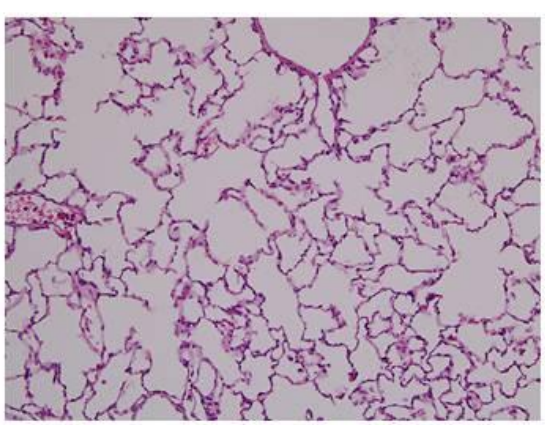
Fig. 3



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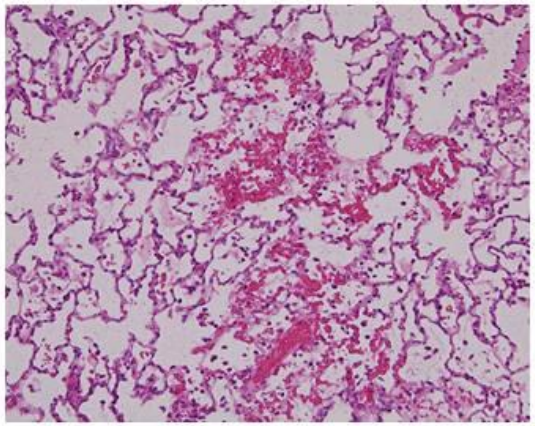

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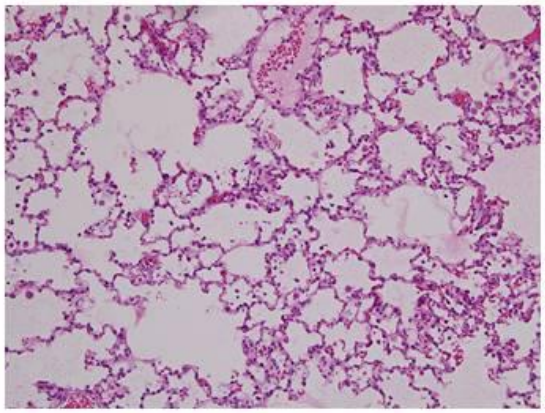
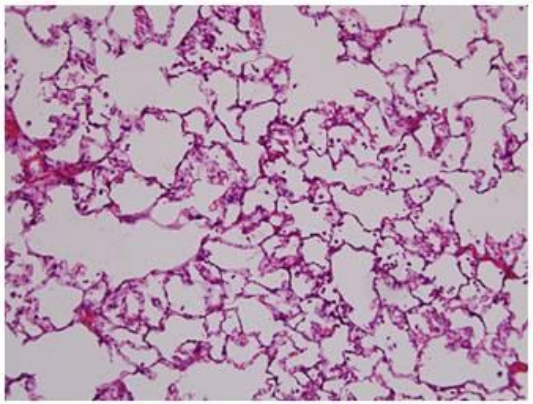


HCL+S



HCL+ SS

Fig. 4