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ORIGINAL ARTICLE

# Efficacy of intratracheal budesonide-surfactant combined therapy in surfactant-insufficient rat lungs with lipopolysaccharide insult

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## Abstract

**Background:** Intratracheal steroid therapy for lipopolysaccharide (LPS)-induced acute lung injury (ALI) remains challenging particularly in surfactant-insufficient lungs, a common problem of neonatal or pediatric ALI. Surfactant has been used as a vehicle for intratracheal steroid in the treatment of other types of ALI. This study investigated the efficacy of intratracheal budesonide (BUD) delivered by two concentrations of surfactant in the treatment of LPS-induced ALI in surfactant-insufficient rat lungs.

**Methods:** Male adult rats were anesthetized and ventilated. Our ALI model was established by repeated saline lavage to produce surfactant insufficiency, followed by intratracheal LPS instillation. Five study groups (n = 5 for each) with different intratracheal treatments following ALI were used: control (no treatment), BUD (NS-BUD; BUD in saline), DS-BUD (BUD in diluted surfactant), FS-BUD (BUD in full-strength surfactant), FS (full-strength surfactant). Cardiopulmonary variables were monitored 4 hours post injury. Histological and immunohistochemical assessments of the lungs were performed.

**Results:** The FS-BUD and FS groups presented better gas exchange, less metabolic acidosis, less oxygen index, and more stable hemodynamic changes than the DS-BUD, NS-BUD, and control groups. The total lung injury scores assessed by histological examination were ordered as follows: FS-BUD < DS-BUD or FS < NS-BUD < control. The immunostaining intensities of lung myeloperoxidase showed the following order: NS-BUD, DS-BUD, or FS-BUD < control or FS. Only the FS-BUD group displayed a smaller immunostaining intensity of lung tumor necrosis factor (TNF)-α than the control group.

**Conclusion:** Among our therapeutic strategies, intratracheal BUD delivered by full-strength surfactant confers an optimal protection against LPS-induced ALI in surfactant-insufficient rat lungs.

**Keywords:** Acute lung injury; Budesonide; Intratracheal instillation; Lipopolysaccharide; Surfactant; Surfactant-insufficient

## 1. INTRODUCTION

Pediatric pneumonia is a major cause of mortality and morbidity in children under 5 years old, especially in developing countries.[1,2] Among the cases of pediatric pneumonia, 21.7% occur during the neonatal stage,[3] and some of them may develop acute lung injury (ALI) and its severe form—acute respiratory distress syndrome (ARDS).[4,5] One major presentation of this form of ALI is lung inflammation mainly induced by lipopolysaccharide (LPS), a major pathogenic component of Gram-negative bacilli.[6] In particular, the inflammatory effect of the LPS insult accompanies the injurious impact of surfactant insufficiency in the lungs, a situation that generally occurs in preterm and sometimes newborn infants.[7,8] Thus, surfactant insufficiency and the lack of ability to cope with inflammation are the major disadvantages of these infants against LPS-induced ALI.[4,5,7,8] The therapy options for ALI or neonatal ARDS (NARDS) in this vulnerable population are currently limited.[5,9] Thus, investigations of effective therapeutic strategies to alleviate LPS-induced ALI in surfactant-insufficient lungs are warranted.

Steroid has been long considered a classical therapy for NARDS due to its anti-inflammatory effect; however, the side effects of systemic steroids remain a major concern.[10–12] For the prevention of side effects, several animal or clinical studies used intratracheal instillation[13,14] or inhalation[15–18] of steroids for the treatment of meconium aspiration syndrome (MAS) or prevention of bronchopulmonary dysplasia (BPD) and other respiratory morbidities. However, the local treatment with steroids remains challenging particularly in surfactant-insufficient lungs with alveolar collapse and diffuse atelectasis, which is a common problem in preterm infants.[7,19] To this end, intratracheal

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administration of surfactant is an effective treatment for surfactant-deficient lung diseases or NARDS.[19,20] Additionally, growing evidence suggests that surfactants can be used as a vehicle for intratracheal steroid administration.[20] This delivery mode distributes the steroid well in the lungs without changing the biophysical and chemical properties of the drug.[21–24] In animal studies, this steroid delivery mode has been used to investigate its efficacy in reducing ALI in animal models of surfactant-depleted lung diseases,[23,25] MAS,[26] hyperoxia,[27] and injurious mechanical ventilation.[28,29] In clinical studies, this steroid delivery mode has been used to prevent BPD[30–32] and chronic diseases[33] or to treat NARDS[34,35] in premature infants. However, no study has been conducted to investigate the therapeutic effect of this steroid delivery approach on LPS-induced ALI in surfactant-insufficient lungs.

This study aimed to investigate the therapeutic effects of intratracheally instilled budesonide (BUD) delivered by two concentrations of exogenous surfactant on LPS-induced ALI in surfactant-insufficiency rat lungs. Our ALI model was established by repeated saline lavage to produce surfactant insufficiency, followed by an intratracheal LPS insult.

## 2. METHODS

### 2.1. Ethic statement

The experimental protocols described in this study were approved by the Institutional Animal Care and Use Committee of National Yang Ming Chiao Tung University, Taiwan (YMIACUC number: 981239) and were in accordance with the recommendation in the Guideline for the Care and Use of Laboratory Animals published by the Council of Agriculture Executive Yuan, Taiwan.

### 2.2. Animal preparation

Male rats weighing 400 to 500 g at the age of 12 weeks received general anesthesia with isoflurane 2% at 2 L/min $O_2$ plus intraperitoneal injection with 25% urethane (5 mL/kg) before the surgical procedures. Anesthetic efficacy was determined by the lack of withdrawal from painful stimulus on the tail. All animals were placed in a supine position with a subcutaneous injection of lidocaine hydrochloride (2%) for local anesthesia. A midcervical tracheostomy was performed using a 16-gauge cannula. A polyethylene tube was placed into the femoral artery for the continuous recording of arterial blood pressure and blood sampling. Another catheter was inserted into the femoral vein for intravenous infusion. After these procedures, all animals were paralyzed with an intravenous injection of cisatracurium besylate (0.2 mg/kg), followed by a continuous intravenous infusion of the same agent (0.05 mg/kg/min). The animals were then sedated with a continuous intravenous infusion of propofol (0.7 mg/kg/min). The animals were connected to a volume-controlled animal ventilator (Model 683, Harvard Apparatus, Holliston, MA, USA) to establish conventional ventilation. A tidal volume of 6 mL/kg, an inspiratory versus expiratory ratio of 1:1, a positive end-expiratory pressure (PEEP) of 3 cm $H_2O$, and a fractional fraction of inspiration $O_2$ ($FIO_2$) concentration of 1.0 were maintained throughout the experiments. The original ventilation rate was set at 60 breaths/min, followed by an increment or decrement of 2 to 3 breaths/min to maintain an arterial blood partial pressure of carbon dioxide within 40 and 50 torr (5.33-6.67 kPa) and pH > 7.25. The body temperature was maintained at 38°C to 39°C via a servo-controlled heating blanket with a probe monitoring the anal temperature throughout the experiments.

### 2.3. Monitoring of physiological parameters

During the experiment, electrocardiography, mean arterial blood pressure, and the percentage of arterial hemoglobin oxygen saturation ($SpO_2$) were continuously measured via a monitor (M1205A Omni-Care 24/24C, Hewlett Packard, Essex, MA, USA). Respiratory flow and airway pressure were measured with a heated pneumotachograph coupled to a differential pressure transducer (MP-45-16, Validyne, Kenilworth, NJ, USA) and a pressure transducer (MP45-28, Validyne), respectively. The tidal volume was integrated from the flow signal. These signals were recorded by a recorder (TA11, Gould, Eastlake, OH, USA) and a data acquisition system (PowerLab 16/30, ADInstruments Pty Ltd, Bella Vista, NSW, Australia). Arterial blood gas levels were analyzed using an autoanalyzer (OPTIMedical, Roswell, GA, USA). Alveolar-arterial oxygen gradient ($AaDO_2$) was calculated.

### 2.4. Surfactant depletion by lavage and LPS-induced lung injury

After the baseline cardiopulmonary data had been collected, all rats received lung lavage with 10 mL/kg/dose normal saline intratracheally via a tracheostomy tube for both sides of the lungs. An additional saline lavage was given 5 minutes later if $SpO_2$ remained above 90%. When $PaO_2$ dropped to less than 150 torr (20 kPa) (at $FIO_2$ = 1.0), the rat was considered to have reached surfactant depletion. Subsequentially, a dose of 50 μg/kg LPS (*E. Coli* O111:B4, Sigma, USA) diluted by saline to a total volume of 1 mL was intratracheally instilled into the lungs to induce ALI. An additional 15-minute period of postinjury stabilization was allowed before the subsequent procedures.

### 2.5. Animal groups

The surfactant-depleted and LPS-injured rats were assigned randomly to one of the following five study groups (n = 5 for each group) and received different intratracheal treatments: (1) control (no treatment); (2) intratracheal treatment with BUD in 4 mL/kg of normal saline (NS-BUD; 0.5 mg/kg BUD in saline) (Pulmicort, 1 mg/2 mL, AstraZeneca Taiwan, Taipei, Taiwan); (3) DS-BUD (0.5 mg/kg BUD in a solution of diluted surfactant [concentration, 10 mg/mL; dosage, 40 mg/kg]) (Survanta, 25 mg/mL, Abbott Laboratories, Abbott Park, IL, USA); (4) FS-BUD (0.5 mg/kg BUD in a solution of full-strength surfactant (100 mg/kg)); (5) intratracheal treatment with full-strength surfactant alone (FS; 100 mg/kg). This treatment option in this study based on a previous animal model of MAS which suggested better oxygenation of treatment with 0.5 mg/kg of instilled BUD than with 0.25 mg/kg.[14] Therefore, DS-BUD and FS-BUD groups received an equal volume (4 mL/kg) of surfactant (diluted and full-strength surfactant) mixed with of 0.5 mg/kg of liquid-form BUD. Each intratracheal treatment was divided into two aliquots; these were instilled separately with a position in either the right or the left decubitus via a feeding tube following by six breaths of ambu bagging. The interval between aliquots was 30 seconds to 1 minute to allow stabilization.

### 2.6. Experimental protocol

After starting the above-mentioned intratracheal treatments, the PEEP of the ventilator setting was turned up to 5 cm $H_2O$. A total of 4 hours was used for observation for each experiment. During the study period, sodium bicarbonate (1-2 mEq/kg/dose) was given to the animals displaying metabolic acidosis (pH < 7.20) with a base excess lower than −8 meq/L. Epinephrine (0.01 mg/kg, one bolus injection) was given to the animals displaying bradycardia (heart rate < 100 beats/min) every 3 to 5 minutes if necessary. The arterial blood samples (0.2 mL) were obtained hourly for blood gas analysis (Chiron, Ciba Corning Diagnostics Corporation, MA, USA) at 0 and 30 minutes and then hourly until the end of experiment.

### 2.7. Pathological examinations

At the end of the experiments, the animals were euthanized under deep anesthesia with a high dose of 15% potassium chloride (3 mL). Within 5 minutes after death, the ventilator was stopped, and a positive airway pressure equivalent to PEEP of 5 cm $H_2O$ was applied. The chest wall was then opened, and the trachea was clamped. The airway and lung were incised and fixed in 10% formaldehyde solution for histological preparations and examinations. Routine techniques were used to prepare the lung tissues for paraffin embedding. Thin sections were stained with hematoxylin and eosin stain. The sections were examined under light microscopy (Olympus AX-80, Yuanyu Industry CO., Ltd, Taipei, Taiwan), and histology was scored using a quantitative system by an investigator blinded to the identity of the specimens. Injury scores were based on the following pathohistological characteristics: alveolar inflammation, interstitial inflammation, alveolar hemorrhage, interstitial hemorrhage, atelectasis, necrosis, and overdistension. Injury score was graded 0, 1, 2, 3, and 4 for abnormalities noted in 0%, 25%, 50%, 75%, and diffusely covering the lung field, respectively. Multiple (>10) fields of the lung section for each pathohistological characteristic were examined, and the scores were averaged to obtain a mean value.

### 2.8. Immunohistochemical assessments

Lung sections (5 μm) from paraffin-embedded tissues were used for immunohistochemical studies. After deparaffinization and dehydration, endogenous peroxidases were blocked with 5% $H_2O_2$, nonspecific protein-binding sites were blocked with Thermo Scientific Ultra V block (TA-060-PBQ, ThermoFisher Scientific, Pittsburg, PA, USA), and the slices were permeabilized with 0.5% Triton X-100 (Merck, Kenilworth, NJ, USA) in Tris-buffered saline. The slices were incubated with rabbit antimyeloperoxidase or anti-tumor necrosis factor (TNF)-α primary antibody (CAT: Abcam, ab6671, 1:50; Abcam, ab9535, 1:100, Cambridge, MA, USA) overnight at 4°C. The subsequent steps of incubation included an enhancer reagent and horseradish-peroxidase-linked secondary antibody, both of which are components of the Polink-2 Plus HRP Detection Kit (D39, GBI Labs, Bothell, WA, USA). The staining was visualized with diaminobenzidine. The slices were mounted on microscopic slides with Eukit® (Merck, Kenilworth, NJ, USA) and digitally recorded with a microscope slide scanner (Zeiss Mirax Midi Slide Scanner, Carl Zeiss MicroImaging GmbH, Germany) operated by a CaseViewer software (3D Histech Ltd., Budapest, Hungary).

Myeloperoxidase is a biomarker of neutrophil activation, and TNF-α is an important cytokine in LPS-induced lung inflammation.[36,37] The levels of myeloperoxidase and TNF-α expression in lung sections were assessed with a semiquantitative approach. The percentage of positive staining cells per slide (0%-100%) was multiplied by the dominant intensity pattern of staining (0, negative or trace; 1, weak; 2, moderate; 3, intense). The maximal score was 300. All the histological sections were examined by Aperio Color Deconvolution v9 (Leica Microsystems, Germany).

### 2.9. Statistical analysis

Continuous data with a normal distribution are presented as mean ± SEM. Ranked data or data that failed to follow a normal distribution are presented as medians with interquartile range. For within-group comparison, a paired Student *t* test was used to compare cardiopulmonary data between preinjury and immediately after injury. For between-group comparison, one-way analysis of variance followed by post hoc Student–Newman–Keuls test was performed for the comparison continuous variables, and the Kruskal–Wallis test followed by pairwise comparison was conducted for the comparison of lung injury scores among the five study groups. Statistical significance was defined as $p$ value less than 0.05. Two-tailed $p$ values of less than 0.05 were considered statistically significant for all analyses. All data management and analyses were performed using Statistical Package for Social Science version 22 (SPSS Inc., Chicago, IL, USA).

## 3. RESULTS

Table 1 shows the physiological conditions at pre- and postinjury (before intratracheal treatment). Severe acidosis with a concomitant increase in $Paco_2$ and a notable drop in $Pao_2$ were found for all animals the after induction of ALI by lavage and LPS ($p < 0.05$). All animal groups also presented significant increases in peak inspiratory pressure, $AaDo_2$, and oxygen index after ALI. Except for the DS-BUD group, all other groups showed a significant drop in the mean arterial blood pressure after ALI. However, these cardiopulmonary parameters at

**Table 1**

**Physiological variables of the five study groups at preinjury and postinjury (before intratracheal treatment)**

| Group | Weight (g) | pH | $Paco_2$ (torr) | $Pao_2$ (torr) | $AaDo_2$ | Peak inspiratory pressure (cm $H_2O$) | Oxygen index | Heart rate (beats/min) | Mean arterial blood pressure (mm Hg) |
|---|---|---|---|---|---|---|---|---|---|
| Preinjury | | | | | | | | | |
| Control | 422.0 ± 13.6 | 7.39 ± 0.02 | 43.6 ± 3.4 | 486.4 ± 12.6 | 172.1 ± 14.5 | 13.7 ± 1.2 | 1.42 ± 0.12 | 290 ± 33.7 | 46.4 ± 7.2 |
| NS-BUD | 403.3 ± 13.1 | 7.37 ± 0.01 | 47.0 ± 0.9 | 515.2 ± 20.5[a] | 139.1 ± 20.4[a] | 13.4 ± 1.1 | 1.24 ± 0.18 | 320 ± 28.8 | 65.5 ± 11.8 |
| DS-BUD | 420.0 ± 5.0 | 7.39 ± 0.02 | 43.0 ± 2.0 | 490.8 ± 12.2 | 168.5 ± 10.4 | 15.4 ± 0.9 | 1.54 ± 0.10 | 306 ± 22.6 | 51.2 ± 5.0 |
| FS-BUD | 391.0 ± 14.2 | 7.38 ± 0.01 | 45.4 ± 2.3 | 475.4 ± 14.5 | 180.9 ± 16.5 | 13.8 ± 1.2 | 1.44 ± 0.09 | 363 ± 20.7 | 72.2 ± 4.6[a] |
| FS | 450.8 ± 8.8 | 7.39 ± 0.01 | 43.7 ± 1.9 | 515.7 ± 20.1 | 142.8 ± 20.7 | 14.8 ± 0.9 | 1.46 ± 0.10 | 310 ± 13.6 | 74.5 ± 4.5[a] |
| Postinjury (before intratracheal treatment) | | | | | | | | | |
| Control | | 7.15 ± 0.02[b] | 75.8 ± 1.9[b] | 48.0 ± 4.4[b] | 570.3 ± 5.5[b] | 28.9 ± 1.6[b] | 27.0 ± 2.8[b] | 360 ± 11.5 | 34.6 ± 4.4[b] |
| NS-BUD | | 7.17 ± 0.02[b] | 73.2 ± 2.7[b] | 47.8 ± 1.7[b] | 573.7 ± 2.7[b] | 25.8 ± 0.4[b] | 23.8 ± 1.0[b] | 329 ± 19.0 | 42.8 ± 5.2[b] |
| DS-BUD | | 7.17 ± 0.02[b] | 70.4 ± 2.8[b] | 44.0 ± 1.4[b] | 581.0 ± 3.7[b] | 30.7 ± 0.9[b] | 29.8 ± 0.5[b] | 385 ± 22.6[b] | 42.6 ± 3.8 |
| FS-BUD | | 7.16 ± 0.02[b] | 74.8 ± 4.2[b] | 47.4 ± 2.7[b] | 572.1 ± 6.9[b] | 28.1 ± 1.1[b] | 26.1 ± 2.1[b] | 379 ± 9.3 | 40.8 ± 6.3[b] |
| FS | | 7.20 ± 0.02[b] | 70.0 ± 3.7[b] | 54.7 ± 6.3[b] | 570.8 ± 7.9[b] | 25.2 ± 2.1[b] | 21.8 ± 3.2[b] | 357 ± 26.9[b] | 61.9 ± 7.8[b] |

The study groups subsequently received different intratracheal treatments: (1) control group, no treatment; (2) NS-BUD group, BUD (0.5 mg/kg) in saline; (3) DS-BUD group, BUD (0.5 mg/kg) in a solution of diluted Survanta (10 mg/mL); (4) FS-BUD group, BUD (0.5 mg/kg) in a solution of full-strength Survanta (25 mg/mL); (5) FS group, a solution of full-strength Survanta (25 mg/mL) alone.

$AaDo_2$ = alveolar-arterial oxygen gradient; DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline; $Pao_2$ = oxygen tension in arterial blood; $Paco_2$ = carbon dioxide tension in arterial blood.

[a]$p < 0.05$ vs control group at the same time point.

[b]$p < 0.05$ vs preinjury data of the same group. Data in each group (n = 5) are presented as mean ± SEM.

postinjury (before intratracheal treatment) showed no statistical difference among the five study groups.

After intratracheal treatments, the postinjury deteriorations of $Pao_2$ and $AaDo_2$ significantly improved over time in the FS-BUD and FS groups (Fig. 1A, C). By contrast, these improvements were not observed in the control, NS-BUD, and DS-BUD groups (Fig. 1A, C). The base excess remained in the normal range during the study period in the FS-BUD and FS groups but worsened in the control, NS-BUD, and DS-BUD groups (Fig. 1D). The postinjury deteriorations of $Paco_2$ significantly improved over time in all the study groups (Fig. 1B). The FS-BUD and FS groups also had a lower oxygen index (Fig. 2A), higher mean arterial pressure (Fig. 2B), and lower peak inspiratory pressure (Fig. 2C) compared with the other three groups throughout the whole study period. Heart rate changed variedly over time among the five study groups, and no significance can be detected between any two groups at almost all time points (Fig. 2D).

A histological evaluation of the lung sections from the control and NS-BUD groups revealed extensive inflammatory cell infiltration, hemorrhage, edema, and atelectasis, and all of these pathohistological changes were lessened in lung sections from the DS-BUD, FS-BUD, and FS groups (Fig. 3). These observations were confirmed by comparison of the group data in terms of lung injury scores for each pathohistological characteristic or total lung injury scores (Table 2). Numerically, the total injury scores in the five study groups followed the order FS-BUD < DS-BUD or FS < NS-BUD < control (Table 2). Further immunohistochemical staining of the lung sections from the control and FS groups revealed marked signals of myeloperoxidase and TNF-α in the alveoli space and interstitials of the alveoli (Fig. 4). These signals were reduced in the lung sections from the NS-BUD, DS-BUD, and FS-BUD groups (Fig. 4). Comparisons of group data revealed that the immunostaining intensity of myeloperoxidase in the NS-BUD, DS-BUD, and FS-BUD groups was significantly smaller than that in the control and FS groups (Fig. 5). Additionally, the immunostaining intensity of TNF-α in the FS-BUD group was significantly smaller than that in the other four groups (Fig. 5).

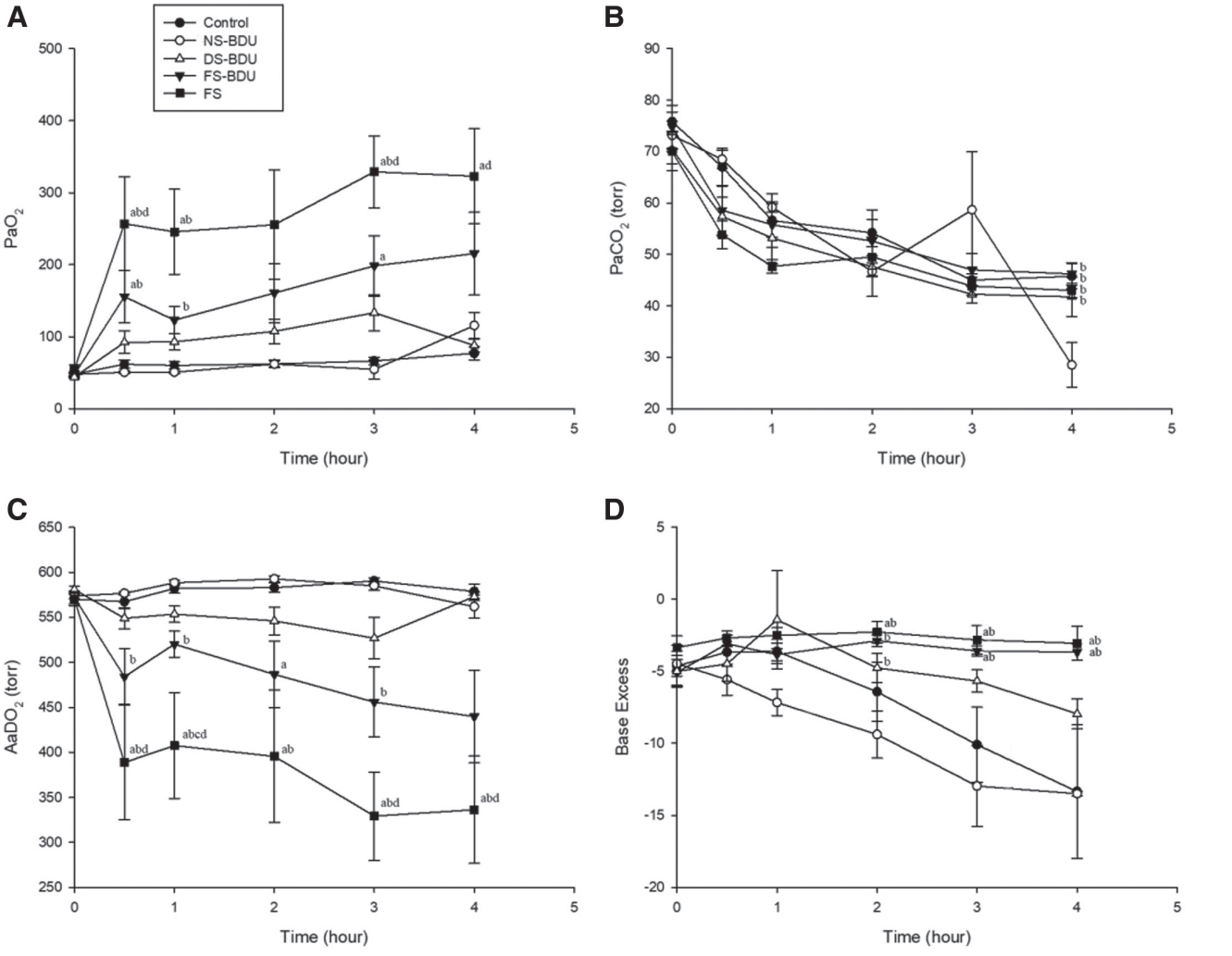


**Fig. 1** Changes in $Pao_2$ (A), $Paco_2$ (B), $AaDo_2$ (C), and base excess (D) over the 4 h postinjury period after different intratracheal treatments in the five study groups. Control group, no treatment; NS-BUD group, treatment with BUD (0.5 mg/kg) in saline; DS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of diluted Survanta (10 mg/mL); FS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of full-strength Survanta (25 mg/mL); FS group, treatment with a solution of full-strength Survanta (25 mg/mL) alone. $^{a}p < 0.05$ vs control group; $^{b}p < 0.05$ vs NS-BUD group; $^{c}p < 0.05$ vs FS-BUD group; $^{d}p < 0.05$ vs DS-BUD group. Data in each group (n = 5) are expressed as mean ± SEM. $AaDo_2$ = alveolar-arterial oxygen gradient; DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline; $Pao_2$ = oxygen tension in arterial blood; $Paco_2$ = carbon dioxide tension in arterial blood.

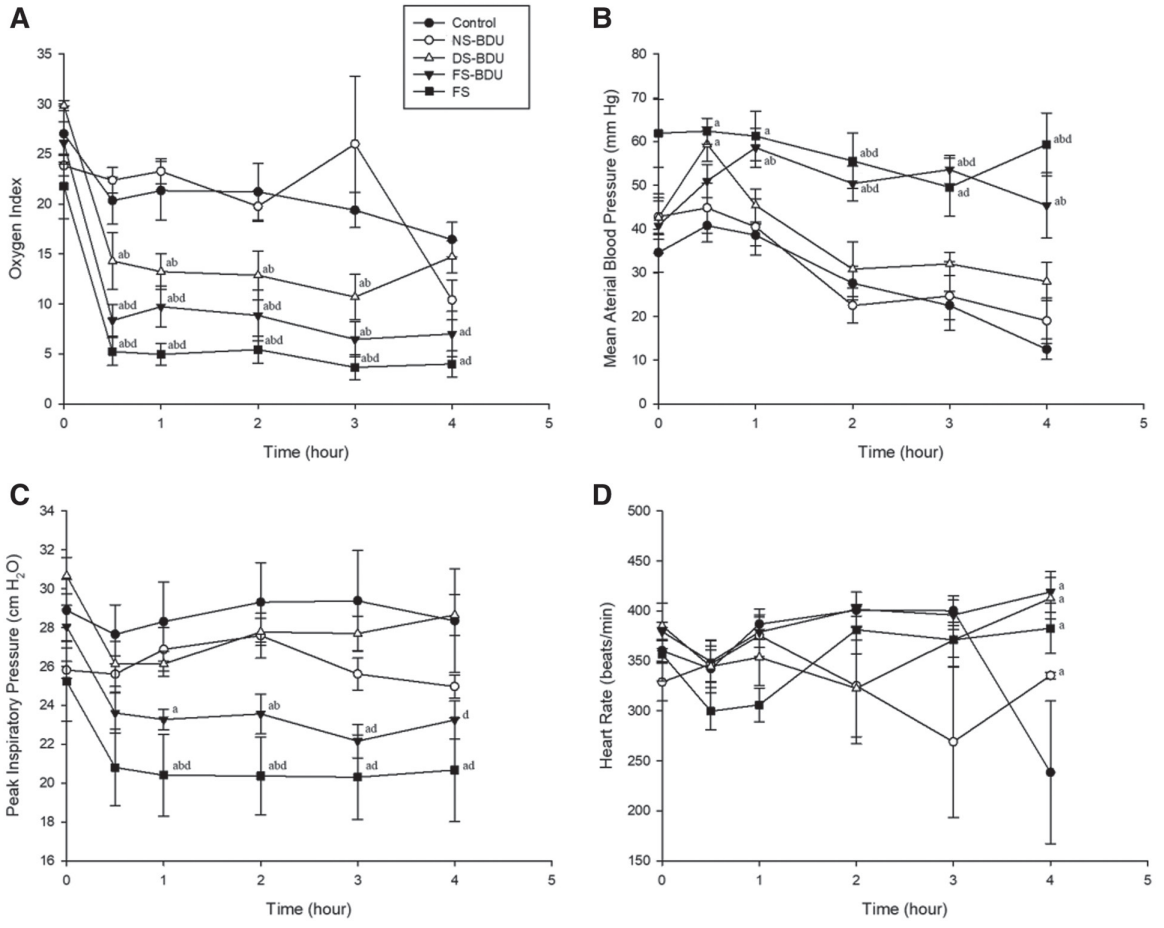


**Fig. 2** Changes in oxygen index (A), mean arterial pressure (B), peak inspiratory pressure (C), and heart rate (D) over the 4 h postinjury period after different intratracheal treatments in the five study groups. Control group, no treatment; NS-BUD group, treatment with BUD (0.5 mg/kg) in saline; DS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of diluted Survanta (10 mg/mL); FS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of full-strength Survanta (25 mg/mL); FS group, treatment with a solution of full-strength Survanta (25 mg/mL) alone. [a]$p < 0.05$ vs control group; [b]$p < 0.05$ vs NS-BUD group; [c]$p < 0.05$ vs FS-BUD group; [d]$p < 0.05$ vs DS-BUD group. Data in each group (n = 5) are expressed as mean ± SEM. DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline.

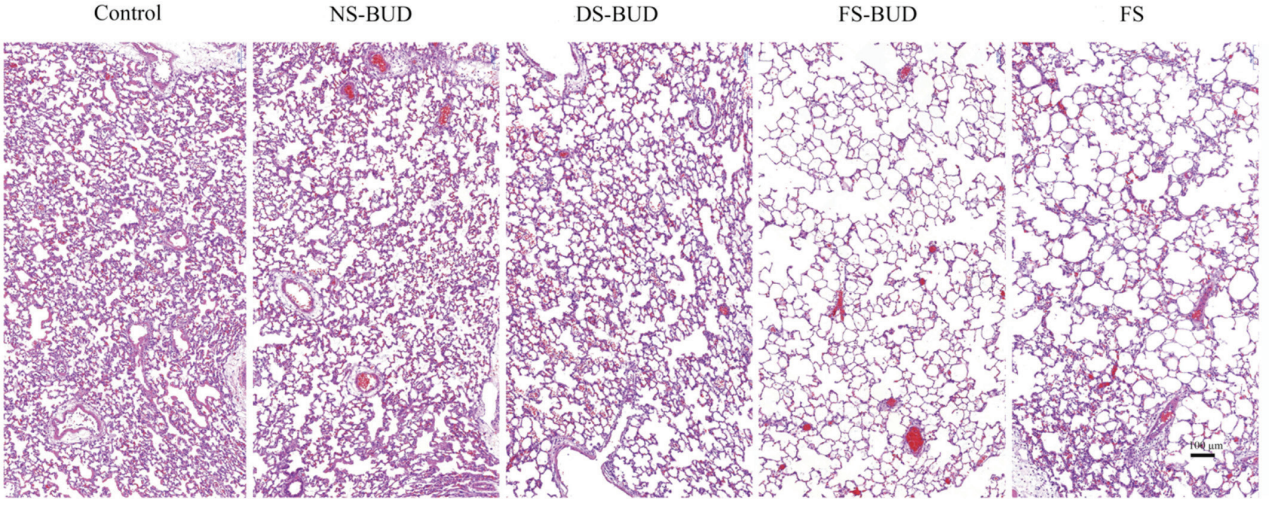


**Fig. 3** Representative pulmonary histological photomicrographs of the five study groups with different intratracheal treatments. Lung sections were stained with hematoxylin and eosin. Control group, no treatment; NS-BUD group, treatment with BUD (0.5 mg/kg) in saline; DS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of diluted Survanta (10 mg/mL); FS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of full-strength Survanta (25 mg/mL); FS group, treatment with a solution of full-strength Survanta (25 mg/mL) alone (×10 power, the total scale length = 1 cm). DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline.

**Table 2**

**Lung injury scores of different pathohistological characteristics in the five study groups**

| Group | Alveolar inflammation | Interstitial inflammation | Alveolar hemorrhage | Interstitial hemorrhage | Edema | Atelectasis | Necrosis | Total |
|---|---|---|---|---|---|---|---|---|
| Control | 1.2 (0.4-1.7) | 2.5 (2.0-2.8) | 0.7 (0.0-1.2) | 1.2 (0.9-2.2) | 1.1 (0.9-1.5) | 1.1 (0.6-1.7) | 0.7 (0.3-1.1) | 8.3 (6.8-10.6) |
| NS-BUD | 0.7 (0.4-1.1) | 1.8 (2.0-2.8) | 0.5 (0.0-0.8) | 0.9 (0.4-1.5) | 0.8 (0.6-1.0) | 0.7 (0.3-1.3) | 0.3 (0.1-1.0) | 5.8 (3.1-8.5) |
| DS-BUD | 0.5 (0.1-1.1)[a] | 1.3 (0.7-2.2)[a] | 0.4 (0.1-0.9) | 0.7 (0.2-1.4) | 0.6 (0.2-1.0)[a] | 0.5 (0.2-0.9)[a] | 0.3 (0.0-0.6) | 4.2 (2.3-7.4)[a] |
| FS-BUD | 0.3 (0.0-0.6)[a] | 0.9 (0.4-1.2)[a,b] | 0.3 (0.0–0.6) | 0.3 (0.0-0.7)[a,b,c] | 0.2 (0.0-0.7)[a,b] | 0.3 (0.1-1.1)[a] | 0.0 (0.0-0.1)[a,b] | 2.4 (1.2-3.7)[a,b] |
| FS | 0.4 (0.0-1.0)[a] | 1.3 (0.8-2.1)[a] | 0.3 (0.1-0.9) | 0.8 (0.3-1.4) | 0.5 (0.0-1.4)[a] | 0.7 (0.0-1.4) | 0.2 (0.0-0.4)[a] | 4.3 (2.4-7.2)[a] |

The study groups received different intratracheal treatments after induction of lung injury. Control group, no treatment; NS-BUD group, treatment with BUD (0.5 mg/kg) in saline; DS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of diluted Survanta (10 mg/mL); FS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of full-strength Survanta (25 mg/mL); FS group, treatment with a solution of full-strength Survanta (25 mg/mL) alone. Data in each group (n = 5) are presented as medium (interquartile range).

DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline.

[a] $p < 0.05$ vs control.

[b] $p < 0.05$ vs NS-BUD.

[c] $p < 0.05$ vs FS.

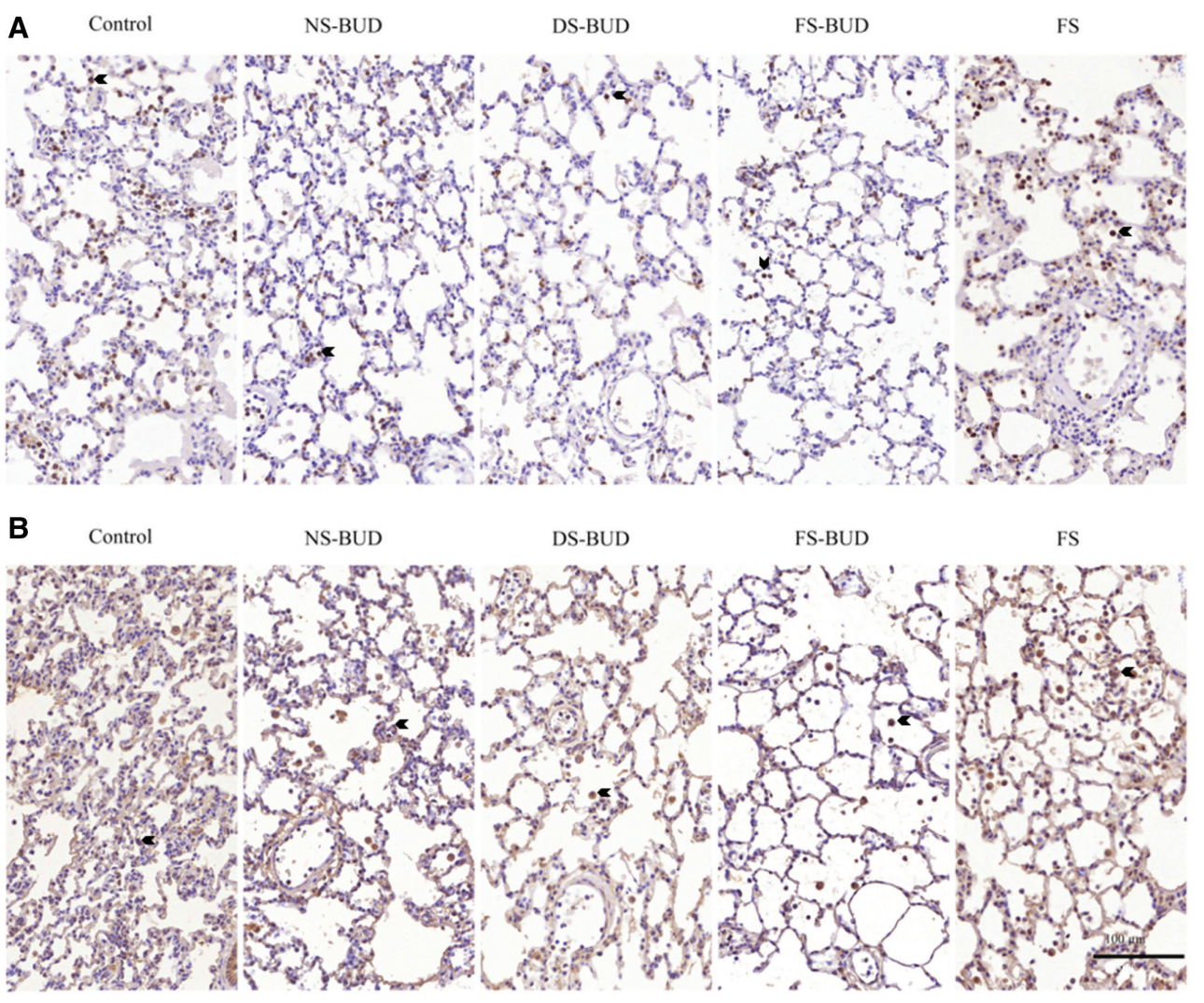


**Fig. 4** Representative pulmonary photomicrographs of immunohistochemical expression of myeloperoxidase (A) and TNF-α (B) in the five study groups with different intratracheal treatments. Myeloperoxidase is a marker of neutrophil activation, whereas TNF-α is an inflammatory cytokine. Control group, no treatment; NS-BUD group, treatment with BUD (0.5 mg/kg) in saline; DS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of diluted Survanta (10 mg/mL); FS-BUD group, treatment with BUD (0.5 mg/kg) in a solution of full-strength Survanta (25 mg/mL); FS group, treatment with a solution of full-strength Survanta (25 mg/mL) alone (×40 power, the total scale length = 4 cm). DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline; TNF = tumor necrosis factor.

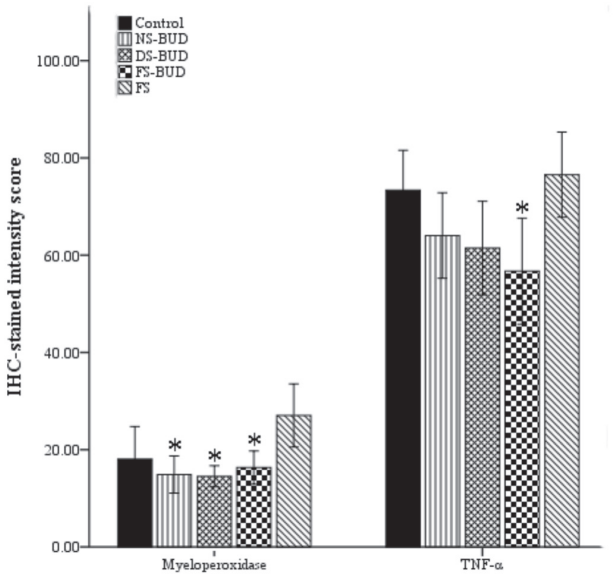


**Fig. 5** Semiquantitative analysis of immunohistochemical expression of myeloperoxidase and TNF-α in lung sections from the five study groups. The percentage of positive staining cells per slide (0%-100%) was multiplied by the dominant intensity pattern of staining (0, negative or trace; 1, weak; 2, moderate; 3, intense). The maximal score was 300. *$p < 0.05$ vs control group. DS-BUD = budesonide in diluted surfactant; FS-BUD = budesonide in full-strength surfactant; NS-BUD = budesonide in normal saline; TNF = tumor necrosis factor.

## 4. DISCUSSION

In this study, we investigated the therapeutic effect of BUD intratracheally delivered by two (diluted and full strength) concentrations of exogenous surfactant on the ALI in a rat model. The ALI in our model was established by repeated saline lavage, to produce surfactant insufficiency, followed by an intratracheal LPS insult. Our results demonstrated that intratracheal treatments with full-strength surfactant with or without BUD improved postinjury deteriorations of cardiopulmonary variables. Additionally, intratracheal treatments with surfactant (both concentrations) with or without BUD alleviated ALI, with FS-BUD being the most effective treatment, as evidenced by our data of lung injury scores. Furthermore, intratracheal treatments with BUD delivered by normal saline or surfactant (both concentrations) ameliorated lung inflammation, with FS-BUD being the most effective treatment, as evidenced by our data of immunohistochemical analysis. Collectively, among our therapeutic strategies, intratracheal BUD delivered by full-strength surfactant conferred the optimal protection against LPS-induced ALI in surfactant-insufficient rat lungs.

Preterm and newborn infants may concurrently have surfactant insufficiency and Gram-negative bacterial infections of the lungs, which are major causes of NARDS.[4,5,7] To avoid the side effects of systemic steroid,[10–12] several investigators advocated the use of intratracheal steroid for the treatment of ALI or NARDS.[13–18] However, surfactant insufficiency may lead to alveolar collapse and diffuse lung atelectasis,[7,19] which hinders the uniform distribution of drug within the lungs and reduces the efficacy of local steroid treatment. Surfactant is a surface tension-lowering agent that has been widely used to treat surfactant-insufficient lung diseases or NARDS.[8,19] When instilled into the trachea, the surfactant may spread throughout the lungs, driven by the surface tension gradient.[20] For this reason, surfactant has been proposed as a vehicle for intratracheal steroid administration.[20] This notion is supported by the stable biophysical and chemical properties of the surfactant/steroid mixtures under ex vivo condition[21–23] and enhanced pulmonary distribution of steroid in vivo when using a surfactant as the vehicle.[21–24] In this two-hit model of surfactant depletion following by LPS insult, FS provided certain benefits such as best oxygenation but was ineffective in reducing lung inflammation, which is well known as ani-inflammatory effect of steroid. On the other hand, NS-BUD was ineffective in improvement on gas exchange but had a slight effect on the reduction of lung inflammation. Our findings regarding the superiority of FS-BUD combined therapy in alleviating ALI and lung inflammation in our model provided a strong evidence for using full-strength surfactant as a vehicle for intratracheal steroid therapy.

The use of surfactant as a vehicle to deliver steroids to the lungs of premature infants has been the subject of increasing clinical interest.[20] Several clinical studies have investigated the efficacy of this steroid delivery mode in preventing BPD[32] and chronic respiratory diseases[33] or in treating NARDS[34,35] in premature infants. Two recent meta-analysis studies reported that intratracheal instillation of steroid-surfactant combination was an effective therapy for preventing BPD in preterm infants, but its benefit in reducing mortality was inconsistent.[30,31] Given the diverse etiology of NARDS,[5,7] several animal studies have investigated the efficacy of using a surfactant as a vehicle for intratracheal steroids in reducing ALI in animal models of surfactant-depleted lung diseases,[23,25] MAS,[26] hyperoxia,[27] and injurious mechanical ventilation.[28,29] Regardless of the type of insult to induce ALI, these studies reported that intratracheal instillation of steroid-surfactant combination can alleviate ALI[23,25–29] and reduce lung inflammation.[23,26–28] Our model is a type of ALI in surfactant-insufficient lungs, particularly with lung inflammation induced by LPS. Our results demonstrated for the first time that intratracheal steroid-surfactant therapy is promising in this particular setting.

This study exhibited several limitations. First, we did not use preterm rats as our animal model for lung surfactant deficiency because preterm rats have a high mortality rate in response to the insult of LPS when they are mechanically ventilated. Second, our observation period was 4 hours, and thus, we cannot be certain about the changes that might have occurred after that time. Third, the concentration of BUD in the systemic circulation was not measured, and thus, further studies targeting this topic are required. Fourth, FS-BUD and FS groups took advantages on less lung tissue inflammation and better oxygenation in this study, respectively. More instilled volume due to BUD administration might cause more fluid retention in lung shortly after treatment. Therefore, the improvement of oxygenation in FS-BUD group was not as good as FS group during the ultrashort period (4 hours) in this study. Further studies to investigate the long-term benefits deriving from anti-inflammatory effect of FS-BUD would be necessary. Fifth, we chosen immunohistochemical stain to express the localization of inflammatory protein in lung tissue, both normal and diseased tissues, in this study. However, the accuracy of quantification is not as good as Western blot or enzyme-linked immunosorbent assay.

In conclusion, intratracheal BUD delivered by full-strength surfactant effectively alleviates ALI and lung inflammation in surfactant-insufficiency rat lungs with LPS insult. This intratracheal steroid-surfactant treatment may be considered a potential therapy for preterm infants with Gram-negative bacteria lung infections.

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