# Effects of intravenous phosphodiesterase inhibitors and corticosteroids on severe meconium aspiration syndrome

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## Abstract

**Background:** Meconium aspiration syndrome (MAS) is a major cause of severe respiratory failure in near- and full-term neonates. Alleviating inflammation is key to successfully treating severe MAS. Phosphodiesterase (PDE) inhibitors are known to play a role in airway smooth muscle relaxation and alveolar inflammation inhibition. This study aimed to investigate the effects of various intravenous (IV) PDE inhibitors and corticosteroids on MAS.

**Methods:** MAS was induced in newborn piglets by instilling human meconium in them. The piglets were randomly divided into five groups (n = 5 in each group): (1) control (sham treatment); (2) dexamethasone (Dex) (IV 0.6 mg/kg of dexamethasone); (3) aminophylline (Ami) (IV 6 mg/kg of aminophylline, followed by continuous infusion of 0.5 mg/kg/h of aminophylline; (4) milrinone (Mil) (IV 50 μg/kg of milrinone, followed by continuous infusion of 0.75 μg/kg/h of milrinone); and (5) rolipram (Rol) (IV 0.8 mg/kg of rolipram). The duration of the experimental period was 4 hours.

**Results:** Compared to the control group, all the four treatment groups revealed better oxygenation 3 hours and more after the start of treatment. The Rol group had a significantly elevated heart beat ($p < 0.05$) and relatively lower blood pressure compared to the other groups during the first 2 hours of the experiment. The Dex group had significantly lower interleukin (IL)-1β levels in the lung tissue compared to the other groups ($p < 0.05$) and significantly lower IL-6 levels compared to the Ami and Mil groups ($p < 0.05$). Lung histology showed slightly less inflammation and atelectasis in the Dex group compared to the other groups, but lung injury scores showed no significant between-group differences.

**Conclusion:** Using IV corticosteroids or any type of PDE inhibitors has some beneficial effects in improving oxygenation in MAS. PDE inhibitors are not superior to IV corticosteroids; in fact, adverse cardiovascular effects occur with the phosphodiesterase type 4 (PDE4) inhibitor. Further investigations are required before using IV corticosteroids and PDE inhibitors in future clinical application.

**Keywords:** Dexamethosone; Inflammation; Meconium aspiration syndrome; Neonate; Phosphodiesterase inhibitor

## 1. INTRODUCTION

Meconium aspiration syndrome (MAS) is one of the major causes of severe respiratory failure in near- and full-term neonates. Aspiration of the meconium might induce various complications in the respiratory system. The major known mechanisms of MAS include direct lung damage by aspirated human meconium;[1] obstruction of the airways, followed by gas diffusion impairment, patchy atelectasis, pulmonary vascular hypertension, and chemical pneumonitis;[2–4] persistent pulmonary hypertension of the newborn or secondary pneumonia;[5–9] and severe pulmonary inflammation and apoptosis of alveolar epithelial cells.[10–17]

To successfully treat MAS in critical neonates, it is crucial to alleviate pulmonary inflammation, in addition to providing the neonates with appropriate respiratory care. Therefore, it is important to look for an ideal therapeutic way to decrease pulmonary inflammation without increasing adverse effects and improve outcomes in neonates with severe MAS. Corticosteroids have well-known antiinflammatory effects, but their use in acute lung injury is controversial.[18,19] Studies have reported that prophylactic dexamethasone can attenuate structural damage in MAS,[10] and intravenous (IV) corticosteroids have also been reported to reduce inflammatory reactions in some meconium-instilled animals.[11,20–22] However, using corticosteroids in neonates can have potential acute adverse effects, such as hyperglycemia, sepsis, hypertension, hypertrophic obstructive cardiomyopathy, gastrointestinal hemorrhage or perforation, growth failure, and hypothalamic–pituitary–adrenal axis suppression.[21] These side effects make the use of corticosteroids in neonates controversial.

Various nonsteroidal antiinflammatory drugs (NSAIDs) can provide different degrees of antiinflammatory effects without the main side effects of corticosteroids. Among NSAIDs, phosphodiesterase (PDE) inhibitors have proved to be effective in decreasing inflammation in some diseases. PDE inhibitors are of

two types, selective and nonselective. Xanthine derivatives, such as theophylline and aminophylline, are nonselective PDE inhibitors that are used to treat asthma. Investigators have researched different NSAIDS for treating MAS and have also tried to use nonselective PDE inhibitors to treat lung injuries.[23–25] However, nonselective PDE inhibitors have a narrow therapeutic window and side effects, which are of serious concerns. Mokra et al.[24] reported increased blood pressure, increased heart rate (HR), and HR variability in meconium-instilled animals. These results showed that nonselective PDE inhibitors might not be a good choice for treating MAS.

Research on using PDE inhibitors for neonatal lung injuries is still ongoing.[2,8,26–28] Some studies used the phosphodiesterase type 3 (PDE3) inhibitor in a meconium-instilled neonatal animal model and found some merits.[23] Other studies demonstrated that the phosphodiesterase type 4 (PDE4) inhibitor can prevent mortality and lung inflammation in hyperoxia-induced lung injuries in rat pups.[27–29] Therefore, both selective PDE3 or PDE4 inhibitors might have the potential to treat acute neonatal pulmonary diseases. However, before future clinical application, further studies are required on the therapeutic efficacy or administration techniques of PDE3 or PDE4 inhibitors in neonatal lung diseases.

In this study, we hypothesized that IV selective or nonselective PDE inhibitors might diminish inflammatory reactions, like corticosteroids do, in meconium-injured neonatal lungs and improve pulmonary outcomes. Therefore, this study aimed to compare the short-term therapeutic and adverse effects of IV corticosteroids and various PDE inhibitors in a MAS animal model.

## 2. METHODS

### 2.1. Meconium preparation
We obtained human meconium from <24-hours-old healthy neonates. The meconium was pooled, homogenized, diluted with 0.9% saline to a 20% (by weight) slurry, and frozen at –20ºC in a refrigerator until use.[30,31]

### 2.2. Animal preparation
Neonatal piglets (n = 25; <2-weeks old ) were prepared according to our laboratory model procedures used with meconium-injured lungs.[30] In brief, before the experiment, the piglets were anesthetized with isofluorane via inhalation and, then, injected intramuscularly with a 0.1 mg/dose of atropine and a 10 mg/kg/dose of ketamine. Lidocaine hydrochloride (2%) was used as local anesthesia. The piglets were sedated and paralyzed by continuous infusion of 5 mg/kg/h of ketamine, 0.5 mg/kg/h of midazolam, and 0.2 mg/kg/h of cisatracurium until the end of the experiment.

We placed a 3.5-mm-diameter Portex uncuffed tracheal tube (SIMS Portex Limited, Hythe, Kent, UK) via tracheotomy and established controlled mechanical ventilation using a volume-controlled ventilator Model 683 (Harvard, MA, USA). The ventilator settings were as follows: tidal volume = 9 mL/kg; initial respiratory rate = 40 breaths/min; inspiratory:expiratory (I:E) ratio = 1:1; positive end-expiratory pressure (PEEP) = 5 cm $H_2O$; and fractional concentration of inspired oxygen ($FiO_2$) = 1.0. These settings were kept constant throughout the experiment, except the ventilator rate, which was increased by 2 to 5 breaths/min when arterial blood gas analysis showed a partial pressure of carbon dioxide ($pCO_2$) > 50 mmHg and pH < 7.25. One 3.5 Fr Argyle umbilical vessel catheter (Sherwood Medical Company, St. Louis, MO, USA) was placed in the right femoral vein for medication and anesthesia[30,31] and another in the right femoral artery for continuous recording of arterial blood pressure and blood sampling. A solution of 0.33% saline in 5% dextrose

was infused at a 5 mL/kg/h rate throughout the experiment. Body temperature was constantly maintained at 38ºC to 39ºC using a servo-controlled heating blanket.[30]

### 2.3. Physiological monitoring
Throughout the experiment, the piglets' mean arterial blood pressure (MBP), oxygen saturation ($SpO_2$; detected with pulse oximetry), and anal temperature were continuously monitored using HP M1205A OmniCare Model 24/24C (Hewlett-Packard Company, Palo Alto, CA, USA). An electrocardiograph continuously monitored the piglets' hearts. Respiratory flow was measured using a Fleisch No. 3 heated capillary tube pneumotachograph (Phipps & Bird, Richmond, VA, USA) coupled with an MP-45-16 differential pressure transducer (Validyne, Northridge, CA, USA). All pressure transducers were calibrated using a water or mercury manometer. All signals for respiratory flow and airway pressure were recorded using a PowerLab 16/30 data acquisition system (ADInstruments Pty Ltd, Bella Vista, NSW, Australia). The flow signals were integrated to obtain tidal volume readings, and compliance of the respiratory system (Crs) was measured on a breath-by-breath basis with an online computer system equipped with a DA100C analog–digital converter (BIOPAC System, Inc., Goleta, CA, USA) and Chart & Scope software (ADInstruments). Arterial blood samples were taken for blood gas analysis (STAT3, Nova Biomedical Corporation, Waltham, MA).[30]

### 2.4. Induction of meconium-injured lungs
Following the baseline measurements, all piglets were given 6 to 8 aliquots of 1 mL/kg of 20% meconium slurry via the tracheal tube using an 8 Fr feeding tube as an introducing catheter. The target was to reduce the arterial partial pressure of oxygen ($pO_2$) to <150 mmHg at an $FiO_2$ of 1.0 ($PaO_2/FiO_2 < 150$) and the Crs to less than two-thirds of the baseline value, which ensured the severity of the injury was moderate to severe, as defined in acute respiratory distress syndrome.[32]

### 2.5. Experimental protocol
The 25 meconium-instilled piglets were randomly divided into five groups (n = 5 in each group):

- Control: The piglets received sham treatment with an IV injection of 5 mL of 0.9% saline.
- Dexamethasone (Dex): The piglets were administered an IV bolus injection of 0.6 mg/kg of dexamethasone (Methasone; Veterans Pharmaceutical Ltd., Taipei, Taiwan) in 5 mL of 0.9% saline. This dosage was based on the dosage for treating pediatric asthma.[33]
- Aminophylline (Ami): The piglets were administered an IV infusion of aminophylline (loading dose: 6 mg/kg), a nonselective PDE inhibitor, in 5 mL of 0.9% saline for 20 minutes, followed by continuous infusion with 0.5 mg/kg/h of aminophylline for 4 hours. This dosage was also based on the dosage for treating pediatric asthma.[34]
- Milrinone (Mil): The piglets were administered an IV injection of 50 μg/kg of milrinone, a selective PDE3 inhibitor, in 5 mL of 0.9% saline for 20 minutes, followed by continuous infusion with 0.5 μg/kg/h of milrinone for 4 hours. This dosage was based on the dosage for treating pediatric congestive heart failure.[35]
- Rolipram (Rol): The piglets were administered an IV injection of 3 mg/kg of rolipram, a selective PDE4 inhibitor. Rolipram was purchased from the R&D company. This dosage was referenced and selected from previous published reports.[36,37]

The total experimental observation period was 4 hours. During this period, piglets showing metabolic acidosis (pH < 7.20) with a base deficit >8 meq/L were administered 1 to 2 meq/kg/dose of sodium bicarbonate. Piglets with bradycardia (HR < 100 beats/min) were administered 0.01 mg/kg/dose of epinephrine. Piglets with significant hypotension (MBP < 40 mmHg) received 10 mL/kg of 0.9% saline. If any piglet's heart stopped beating before the end of the experiment, no cardiac massage was performed.

Arterial blood samples (0.2 mL) were obtained hourly for blood gas analysis (Chiron; Ciba Corning Diagnostics Corporation, E. Walpole, MA, USA). Simultaneously, cardiopulmonary profiles were continuously monitored and recorded. At the end of the observation period, an additional 1 mL of blood was drawn and centrifuged, and the serum was stored at −80°C in a refrigerator until analysis for cytokines.

Finally, the piglets were euthanized under deep anesthesia with 5 to 10 mL of 15% potassium chloride.

## 2.6. Histological preparations and examinations

Each piglet's chest was opened, and the lungs and thorax were inspected to assess their gross morphology. Within 5 minutes after death, the ventilator was stopped and a positive airway pressure, equivalent to 5 cm $H_2O$ PEEP, was applied. The trachea and right bronchus were clamped, and the airways and right lungs were incised and fixed in 4% formaldehyde solution for histological preparation and examination. The lung tissues were prepared for paraffin embedding. Thin sections (4 μm) were stained with hematoxylin and eosin and examined by using light microscopy. Lung injuries were scored from 0 to 4 as follows: <0 and >1/4, at least 1/4 and <1/2, at least 1/2 and <3/4, or at least 3/4 covering the microscopic field. Multiple fields (≥5) in sections from each lung tissue sample were checked to minimize variations.[30]

To collect bronchoalveolar lavage (BAL) fluid, the left lungs were lavaged using 4 mL/kg of 0.9% saline at 37°C. The BAL fluid was collected and stored at −80°C in a refrigerator until analysis.[30] Next, the left lungs were perfused by phosphate buffer solution by inserting a catheter into the pulmonary artery and cutting a hole in the left atrium until the outflow fluid was clear. Two pieces of lung tissue, ~1 cm in diameter, including the middle and lower lobes, were cut out. The pieces were weighed and homogenized at 4°C (MagNA Lyser Green Beads; Roche Diagnostics, Indianapolis, IN, USA) at a ratio of 0.1 g of tissue to 1 mL of cell lysis buffer containing 100 mM phenylmethysulfonyl fluoride. Then, the pieces were centrifuged twice at 6500 rpm for 20 seconds and spun down at 3000 rpm for 30 minutes. The supernatant was further centrifuged at 14 000 rpm for 30 minutes at 4°C. The final supernatant was stored at −80°C in a refrigerator until analysis.

## 2.7. Cytokine analysis

We measured proinflammatory cytokines, including interleukin (IL)-1β, IL-6, and IL-8, in duplicates using the commercially available porcine-specific Quantikine P enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems, Minneapolis, MN, USA). A Sunrise Absorbance Reader microplate spectrophotometer (Tecan Austria GmbH, Salzburg, Austria) was used to measure absorbance at a wavelength of 450 nm. Background absorbance of the blank wells was subtracted from the standards and unknowns before we determined sample concentrations.[30]

## 2.8. Phospholipase A2 activity detection

Phospholipase A2 (PLA2) activity might increase in inflammatory tissues;[10,12] therefore, we measured enzyme activity in lung tissue homogenates using the porcine-specific PLA2G1B ELISA Kit (ARP American Research Products, Inc., Waltham, MA, USA) according to the manufacturer's instructions. The Sunrise Absorbance Reader microplate spectrophotometer was used to measure absorbance at a wavelength of 450 nm. Background absorbance of the blank wells was subtracted from the standards and unknowns before we determined sample concentrations.

## 2.8. C-Reactive protein detection

C-reactive protein (CRP) levels in the piglets' serum, lung tissue, and BAL fluid were detected using the STA3932 CRP ELISA Kit (Cell Biolabs, Inc., San Diego, CA, USA) according to the manufacturer's instructions. The Sunrise Absorbance Reader microplate spectrophotometer was used to measure absorbance at a wavelength of 450 nm.

## 2.9. Data analysis

Values were presented as the mean ± SEM or the median (interquartile range), as appropriate. Data were analyzed, and graphs were plotted using SigmaPlot 12.0 (Systat Software, Inc., San Jose, CA, USA). We used the paired *t* test to determine statistical differences within groups for pre and postinjury cardiopulmonary data. One-way analysis of variance or the Kruskal–Wallis test was used to compare data among the five groups, when appropriate, and post hoc analysis was performed using the Student–Newman–Keuls test for pairwise comparisons, if necessary. $p < 0.05$ was considered statistically significant.

# 3. RESULTS

In this study, we found no significant between-group differences in the ages and weights of the piglets and the volume of instilled meconium among the five groups (Table 1). We also found no significant between-group differences in the arterial blood gas and Crs, MBP, and HR before and after lung injury (Table 2). However, there were significant changes before and after lung injury in the same group, such as a lower $pO_2$, higher $pCO_2$, and lower Crs (Table 2).

Gas exchange changes showed that $pO_2$ was significantly lower (Fig. 1A) and Alveolar-arterial oxygen gradient (A-a$DO_2$) was significantly higher in the control group compared to treatment groups (Fig. 1B) at hours 3 and 4 of the experiment. There was no significant difference in $pCO_2$ and pH during the 4 hours of the experiment (Figure 1C, D). We also found no significant differences in lung compliance and peak inspiratory pressure during the 4 hours of the experiment (Fig. 2).

Regarding cardiovascular conditions, compared with other groups, the HR of the Rol group was significantly higher, starting at 30 minutes and lasting up to hour 2 of the experiment (Fig. 3A). The blood pressure of the Rol group was relatively lower compared to other groups; however, we found no significant difference ($p > 0.05$; Fig. 3B).

**Table 1**

**Experimental piglets' age, weight, and meconium amount for inducing lung injuries**

| Groups | Age, d | Weight, kg | Meconium, mL/kg |
|---|---|---|---|
| Control (n = 5) | 6 (5-6) | 1.4 (1.4-1.6) | 6 (6-6) |
| Dex (n = 5) | 5 (4-6) | 1.4 (1.4-1.5) | 6 (6-7) |
| Ami (n = 5) | 4 (4-5) | 1.4 (1.4-1.6) | 6 (6-7) |
| Mil (n = 5) | 6 (5-6) | 1.4 (1.4-1.5) | 6 (6-7) |
| Rol (n = 5) | 9 (7-7) | 1.4 (1.5-1.6) | 6 (6-6) |

Data are presented as the median (interquartile range). There was no significant between-group difference.

Ami = aminophylline; Dex = dexamethasone; Mil = milrinone; Rol = rolipram.

## Table 2

**Pre and postinjury cardiopulmonary parameters of experimental piglets**

| Group | pH | $p\mathrm{CO_2}$, mmHg | $p\mathrm{O_2}$, mmHg | PEEP, cm $H_2O$ | Crs, mL/cm $H_2O$/kg | MBP, mmHg | HR, beats/min |
|---|---|---|---|---|---|---|---|
| Preinjury | | | | | | | |
| Control | 7.43 ± 0.03 | 43 ± 3 | 482 ± 38 | 15 ± 2 | 1.07 ± 0.07 | 81 ± 10 | 182 ± 13 |
| Dex | 7.44 ± 0.03 | 44 ± 3 | 517 ± 45 | 14 ± 1 | 1.09 ± 0.11 | 74 ± 7 | 181 ± 30 |
| Ami | 7.50 ± 0.02 | 40 ± 2 | 527 ± 25 | 15 ± 1 | 0.98 ± 0.06 | 75 ± 10 | 170 ± 6 |
| Mil | 7.47 ± 0.02 | 41 ± 2 | 548 ± 9 | 14 ± 1 | 0.93 ± 0.06 | 73 ± 4 | 171 ± 17 |
| Rol | 7.51 ± 0.02 | 37 ± 2 | 523 ± 12 | 14 ± 1 | 1.03 ± 0.09 | 75 ± 6 | 175 ± 18 |
| Postinjury | | | | | | | |
| Control | 7.40 ± 0.04 | 46 ± 2[a] | 107 ± 10[a] | 22 ± 2[a] | 0.61 ± 0.07[a] | 85± 4 | 194 ± 22 |
| Dex | 7.38 ± 0.03 | 49 ± 3[a] | 92 ± 15[a] | 20 ± 0[a] | 0.59 ± 0.04[a] | 75 ± 8 | 181 ± 35 |
| Ami | 7.46 ± 0.02 | 44 ± 2[a] | 86 ± 4[a] | 25 ± 5[a] | 0.53 ± 0.05[a] | 84 ± 13 | 198 ± 12 |
| Mil | 7.41 ± 0.02 | 46 ± 2[a] | 86 ± 6[a] | 21 ± 2[a] | 0.53 ± 0.03[a] | 74 ± 5 | 174 ± 23 |
| Rol | 7.43 ± 0.02 | 45 ± 2[a] | 81 ± 5[a] | 21 ± 1[a] | 0.54 ± 0.03[a] | 75 ± 4 | 171 ± 15 |

Data are presented as mean ± SEM.

Ami = aminophylline; Crs = compliance of the respiratory system; Dex = dexamethasone; HR, heart rate; MBP, mean arterial blood pressure; Mil, milrinone; $p\mathrm{CO_2}$, partial pressure of carbon dioxide; PEEP = positive end-expiratory pressure; $p\mathrm{O_2}$ = partial pressure of oxygen; Rol = rolipram.

[a]$p < 0.05$ vs the preinjury data of the same group.
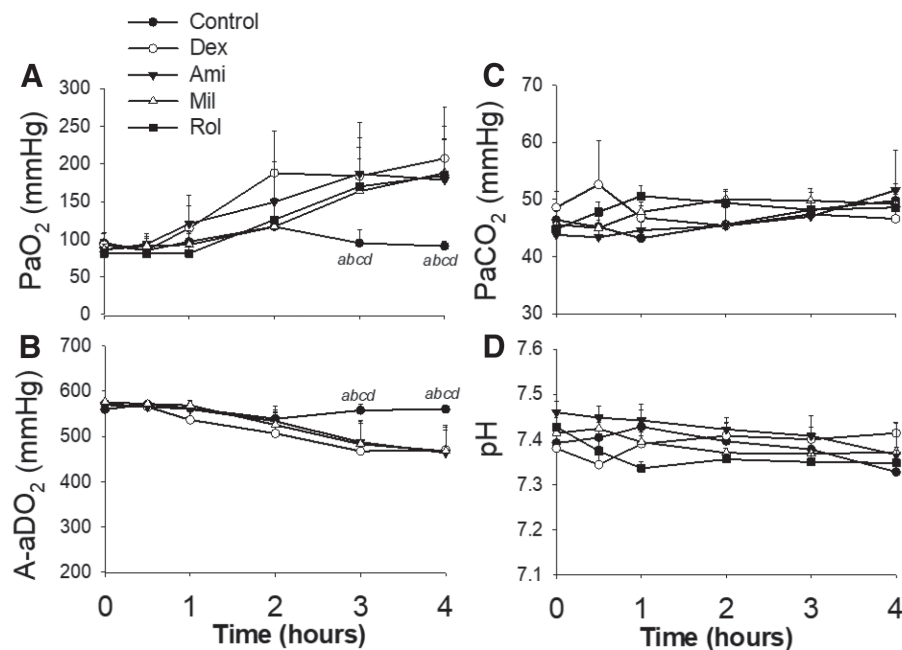


**Fig. 1** Gas exchange changes in the five groups during the 4-h experimental period. Data are presented as mean ± SEM. [a]$p < 0.05$ vs the Dex group; [b]$p < 0.05$ vs the Ami group; [c]$p < 0.05$ vs the Mil group; [d]$p < 0.05$ vs the Rol group. Ami = aminophylline; Dex = dexamethasone; Mil = milrinone; Rol = rolipram.

Analysis of proinflammatory cytokines and CRP showed between-group differences in serum IL-1β and IL-6 and CRP levels at the end of the experiment ($p > 0.05$; Fig. 4A). BAL fluid analysis showed a trend of lower IL-1β, IL-6, IL-8, and CRP levels in the Dex group compared to other groups; however, the difference between the five groups was not significant ($p > 0.05$; Fig. 4B). In lung tissues, the Dex group had significantly lower IL-1β levels compared to other groups ($p < 0.05$; Fig. 4C, top left). In addition, the Dex group had significantly lower IL-6 levels compared to the Ami and Mil groups ($p < 0.05$; Fig. 4C, top right). The lung tissue IL-8, CRP, or PLA2 levels ($p > 0.05$) were not significant (Fig. 4C, middle and bottom).

Lung histology revealed marked inflammation, hemorrhage, and atelectasis in the control group, with the dependent site being more severe than the nondependent site. In the four treatment groups, too, lung damage was more severe in the dependent

site, while inflammation and atelectasis were less severe in the Dex group compared to the control group (Fig. 5). However, we found no significant between-group differences in lung injury scores ($p > 0.05$; Fig. 5, bottom).

## 4. DISCUSSION

This study showed that IV injection of a PDE inhibitor or corticosteroid improves oxygenation when compared with untreated animals; however, our pathophysiological findings did not show significant evidence of a decrease in pulmonary inflammation. Transient tachycardia and relatively hypotension were also noted in piglets administered with PDE4 inhibitor (the Rol group).

PDEs are a family of 11 enzyme subtypes that catalyze the metabolism of intracellular cyclic nucleotides (cyclic adenosine
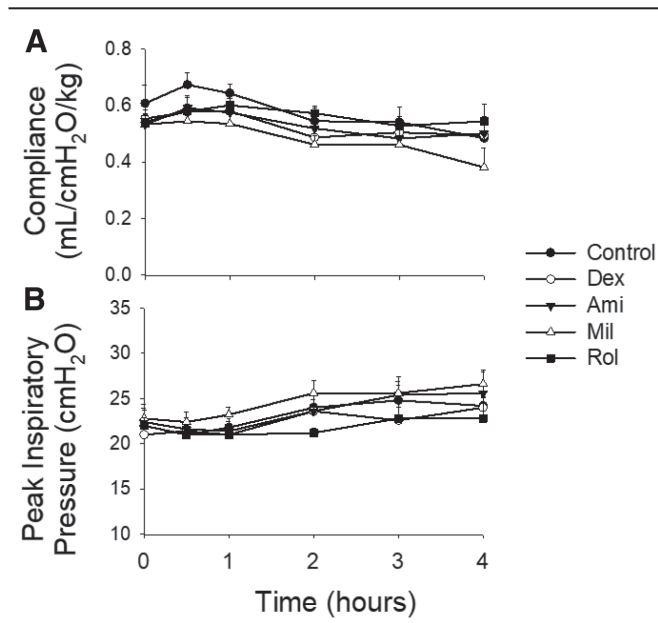
**Fig. 2** Changes in lung compliance and peak inspiratory pressure in the five groups during the 4-h experimental period. There was no significant difference at each time point. Data are presented as mean ± SEM. Ami = aminophylline; Dex = dexamethasone; Mil = milrinone; Rol = rolipram.

monophosphate [AMP] and cyclic guanosine monophosphate) that are expressed in a variety of cell types and respiratory diseases.[38,39] An increase in cyclic AMP is known to be associated with airway smooth muscle relaxation and antiinflammatory action. Therefore, PDE inhibitors are considered to play a role in airway smooth muscle relaxation and inhibition of cellular inflammation or other immune responses[40] and may also
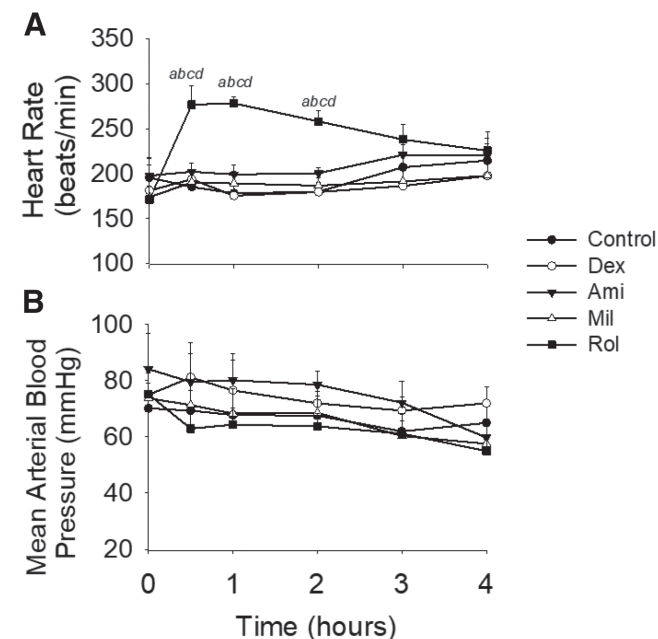


**Fig. 3** Changes in HR and MBP in the five groups during the 4-h experimental period. Data are presented as mean ± SEM. [a]$p < 0.05$ vs the control group; [b]$p < 0.05$ vs the Dex group; [c]$p < 0.05$ vs the Ami group; [d]$p < 0.05$ vs the Mil group. Ami = aminophylline; Dex = dexamethasone; Mil = milrinone; Rol = rolipram.

be helpful in treating severe respiratory diseases. Selective PDE inhibitors have more specific effects and less severe side effects compared with nonselective PDE inhibitors.

Among selective PDE inhibitors, PDE3 and PDE4 inhibitors are considered effective in treating airway diseases.[39,40] PDE3 inhibitors selectively inhibit the action of the PDE3 enzyme and, because of their positive inotropic effect, are commonly used in acute heart failure and cardiogenic shock therapy. PDE3 is also the predominant enzyme regulating the cyclic AMP concentration in airway smooth muscles. PDE4 is the most important PDE isoenzyme within inflammatory cells (eg, macrophages and monocytes, lymphocytes, dendritic cells, and neutrophils) because of its ability to inhibit inflammatory cell activation.[38,41,42] Currie et al. summarized the immunomodulatory and anti-inflammatory effects of PDE4.[38] PDE4 inhibitors have the high potential to enhance smooth muscle relaxation, attenuate fibroblast activity, reduce proinflammatory cytokine production, and decrease mucous secretion in airway cells.[38] Patel et al.[43] demonstrated that PDE4, but not PDE3, inhibitors can increase β-agonist-induced expression of antiinflammatory mitogen-activated protein kinase phosphatase 1 in airway smooth muscle. Therefore, PDE4 inhibitors may be more effective than PDE3 inhibitors in treating pulmonary inflammation and merit further investigation for their efficacy in eliminating inflammation in MAS. Studies have also reported beneficial results of using either PDE3 or PDE4 inhibitors to treat asthma or chronic obstructive pulmonary disease patients.[44–46]

Some studies have investigated combination therapy using different PDE inhibitors or PDE inhibitors plus other medications in pulmonary diseases. For example, the combination of PDE3 and PDE4 inhibitors had a synergistic effect in treating acute pulmonary inflammatory diseases.[47,48] The combination of a PDE4 inhibitor and a β2 agonist was effective in inhibiting proinflammatory and profibrotic mediator release from human lung fibroblasts.[49] However, no published study has focused on such combination use in treating MAS, which can be a topic of future studies.

In this study, the Rol group presented with significant tachycardia compared to other groups during the first 2 hours of the experiment. The Rol group also showed a relatively lower blood pressure, although there was no significant difference compared to other groups. Since the administration route was IV infusion, we could undoubtedly observe systemic side effects. The dosage and administration route should influence pulmonary outcomes and cardiovascular responses. Since the major inflammation site in MAS animal models is the lungs, intrapulmonary antiinflammatory administration is theoretically effective as the drugs directly go into the lungs and pulmonary inflammation decreases without an increase in side effects. Future studies can further adjust the PDE4 inhibitor dosage to ensure beneficial pulmonary effects and reduce adverse cardiovascular effects.

In addition to IV antiinflammatory drugs, such as PDE inhibitors and corticosteroids, the use of other therapeutic methods may also help improve pulmonary outcomes in MAS. Surfactant lavage has been used to decrease pulmonary inflammation and improve pulmonary outcomes.[31,50] Future studies can consider applying surfactant lavage with a diluted surfactant, in addition to PDE inhibitors, to treat moderate to severe MAS.

IV or intratracheal administration of antiinflammatory drugs is another issue to be investigated. As shown here, systemic adverse effects of rolipram, a selective PDE4 inhibitor, were obvious, although it improved oxygenation. However, its side effects do not make it a good choice for clinical use. In addition, there are many other issues to be investigated, such as IV dosage, direct intratracheal administration, or combination with other medications, before making a final decision with regard to rolipram's role in MAS.
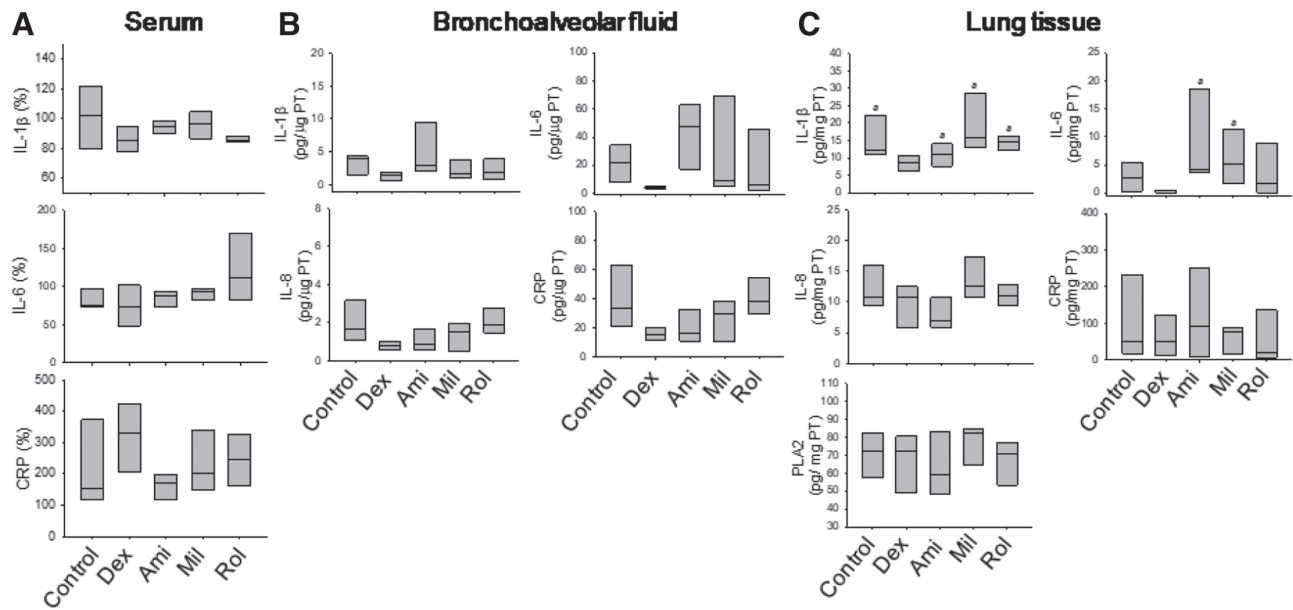
**Fig. 4** Comparisons of proinflammatory ILs, CRP, and PLA2 levels in (A) serum (percentage of postinjured values; *p* > 0.05 in each graph); (B) bronchoalveolar fluids (*p* > 0.05 in each graph); and (C) lung tissues (*p* > 0.05 in IL-8, CRP, and PLA2; *p* < 0.05 in IL-1β and IL-6) at the end of the 4-h experimental period in the five groups. Data are presented as a median (interquartile range). [a]*p* < 0.05 vs the Dex group by post hoc pairwise Stuent–Newman–Keuls test. Ami = aminophylline; CRP = C-reactive protein; Dex = dexamethasone; IL = Interleukin; Mil = milrinone; PT = protein; PLA2 = phospholipase A2; Rol = rolipram.
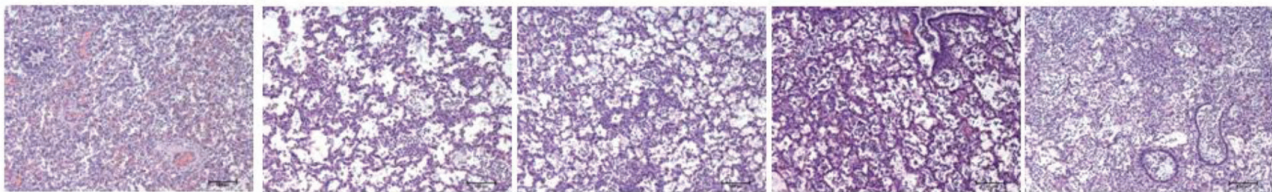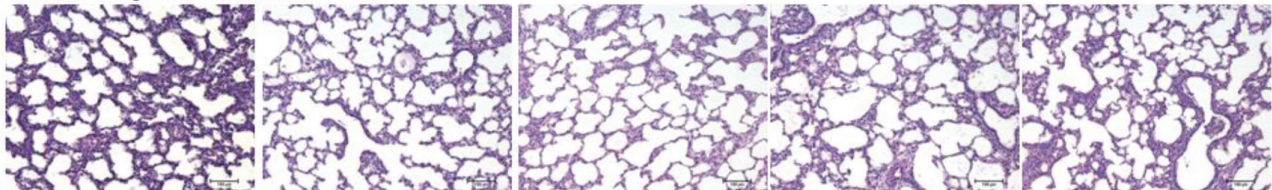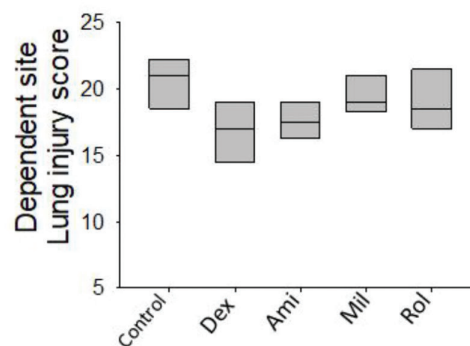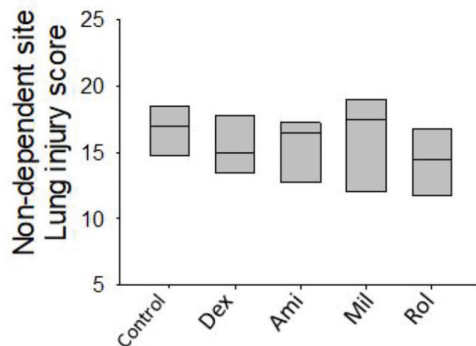


**Fig. 5** Representative pulmonary histology (H&E stain) of the five groups. Top: Representative histological graphs of dependent and nondependent sites of lung tissues. Bottom: Graphic lung injury scores of dependent and nondependent sites. Data are presented as the median (interquartile range; *p* > 0.05 in each graph). Ami = aminophylline; Dex = dexamethasone; H&E = hematoxylin and eosin; Mil = milrinone; PT = protein; Rol = rolipram.

Comparing corticosteroids and nonselective or selective PDE inhibitors, the Dex group had a general tendency of less pulmonary inflammation and proinflammatory cytokines compared to other groups. The Ami group had lower serum CRP levels but had no other benefits compared to the control group. Therefore, further investigation on aminophylline might not be necessary in the future. We also did not find any significant reduction of inflammatory profiles or histological inflammation in the Mil and Rol groups, and there was a potential of adverse cardiovascular effects. The Dex group had the lowest lung tissue IL-6 levels among all groups, but it was significantly lower than only the Ami and Mil groups. Further studies to elucidate the potential benefits and deficits of PDE inhibitors in severe MAS are still required.

This study had a few limitations. First, the small number of subjects (n = 5 in each group) and wide variations of data might have contributed to our statistical findings and not present the true significance. Second, we observed hyperacute outcomes only within 4 hours of lung injury. We noted that all four treatment groups showed improved oxygenation starting at hour 3 of the experiment and lasted until the end of 4 hours. Whether improved oxygenation could be maintained still requires further investigation. Third, the dosages of the drugs investigated might not be the best in treating severe pulmonary dysfunction. The dosage for the IV route might be different from that of intratracheal administration. Therefore, further investigation is necessary to answer these questions.

In conclusion, IV corticosteroids or selective and nonselective PDE inhibitors might improve oxygenation in severe MAS. However, this study failed to demonstrate conclusive evidence of any benefits of using IV PDE3 or PDE4 inhibitors in severe MAS. The dosage and administration route might influence the outcomes. Further investigations using different dosages, administration routes, or combination therapeutic methods are required before reaching a conclusion about the role of selective PDE inhibitors in treating MAS.

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