

Pentoxifylline Reduces Regional Inflammatory and Ventilatory Disturbances in Meconium-Exposed Piglet Lungs

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ABSTRACT

Neonatal meconium aspiration frequently produces severe respiratory distress, which is associated with patchy pulmonary neutrophil influx and inflammatory injury. To examine the effects of pentoxifylline (PTX), a potent anti-inflammatory agent, on regional pulmonary inflammation and ventilation after meconium aspiration, we studied 17 anesthetized and ventilated neonatal piglets (age <2 d) for 12 h. After unilateral intrapulmonary instillation of meconium, PTX treatment was started in nine animals, and eight untreated animals served as controls. Bronchoalveolar lavage (BAL) fluid and lung tissue were studied for inflammatory variables at the end of the study, and changes in regional ventilation were serially analyzed with a dynamic pulmonary x-ray imaging method. Meconium insufflation increased BAL fluid total cell, neutrophil, and macrophage counts and tumor necrosis factor- α (TNF- α) and protein concentrations as well as lung tissue myeloperoxidase activity in the instilled lungs, compared with the noninstilled side. PTX treatment prevented the increase of BAL fluid alveolar macrophage count and TNF- α and protein concentrations in the meconium-instilled lungs but had no significant effect on the pulmonary neutrophil accumulation. Ventilation of the meconium-insulted lung was

initially disturbed similarly in both study groups, but PTX administration prevented the sustained local ventilatory perturbation at 4, 6, and 12 h after meconium instillation. The results thus indicate that PTX treatment may attenuate meconium-induced regional ventilation derangements, mainly through its effects on local alveolar macrophages and TNF- α production as well as alveolocapillary permeability rather than *via* significant prevention of accumulation of active neutrophils in the insulted lungs. (*Pediatr Res* 56: 901–906, 2004)

Abbreviations

AaDO₂, alveolo-arterial oxygen difference
AMP, range of variation of pulmonary transmittance
AVG, average of pulmonary transmittance
BAL, bronchoalveolar lavage
DPI, dynamic pulmonary imaging
MAS, meconium aspiration syndrome
PMN, polymorphonuclear neutrophil
PTX, pentoxifylline
R/L, right to left lung ratio
TNF- α , tumor necrosis factor- α

Perinatal aspiration of meconium frequently produces respiratory failure with hypoxemia, hypercapnia, and acidosis in the newborn infant and may be complicated by air leaks, pulmonary hypertension, hypoxic organ damage, and long-term pulmonary sequelae (1,2). The pathophysiology of the meconium aspiration syndrome (MAS) is complex and includes initial mechanical obstruction of the airways, ventilation/perfusion mismatch, surfactant inactivation, and progressively develop-

ing inflammatory lung injury (1–6). Aspirated meconium may directly damage the alveolar-capillary membrane and may also augment pulmonary injury through activation of alveolar macrophages, inflammatory cytokine production, and accumulation of polymorphonuclear neutrophils (PMNs) (1,2,6,7). Whereas the uneven distribution of inhaled meconium leads to localized areas of injury with variable aeration of the affected lungs, the contribution and mechanisms of the patchy inflammatory processes to the pulmonary dysfunction induced by meconium aspiration is still unclear. Consequently, the therapeutic value of anti-inflammatory agents in the management of MAS remains controversial (6,8–12).

Pentoxifylline (PTX), a methylxanthine derivative and phosphodiesterase inhibitor, has hemorheologic and immunomodulatory properties and is proposed to have a therapeutic role in

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the suppression of inflammatory reactions in different tissues, including the lungs (13,14). Increasing evidence suggests that PTX administration decreases the activation of inflammatory cells and inhibits production of proinflammatory cytokines within the insulted lungs (14–17). These anti-inflammatory effects, together with reduction of high-permeability pulmonary edema, may contribute to attenuation of several forms of acute lung injury after PTX treatment (16–18). Consequently, there are experimental data indicating that PTX is able to attenuate endotoxin, oxygen, tumor necrosis factor (TNF), saline lavage, and acid aspiration–induced acute lung injuries (19–23), but its effect on the inflammatory injury in the meconium-exposed lungs is still unknown. We hypothesized, that the regional inflammatory reactions and the resulting local ventilatory disturbances in the neonatal lungs after meconium aspiration could be diminished by the anti-inflammatory properties of PTX. Therefore, we induced a unilateral lung injury with local meconium instillation, as previously described (24), and examined the effects of PTX on pulmonary inflammation and ventilatory function in both lungs.

METHODS

Animal preparation. Seventeen newborn (age <2 d; weight ~2 kg) piglets were studied. Animals were sedated with diazepam (1.5 mg/kg intramuscularly) and anesthetized with sodium pentobarbital (30 mg/kg intravenously). Tracheotomy was performed, a 3.5-mm internal diameter endotracheal tube was inserted with the distal tip located at least 1 cm proximal to the carina, and animals were placed on a pressure-controlled neonatal mechanical ventilator (Baby Bird; Bird Productions Corp., Palm Springs, CA). The initial respiratory settings were as follows: fraction of inspired oxygen 0.21, rate 20 breaths/min, peak inspiratory pressure 20 cm H₂O, and positive end expiratory pressure 4 cm H₂O. Anesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (6 mg · kg⁻¹ · h⁻¹). Paralysis was induced with i.v. pancuronium bromide (0.3 mg/kg) and maintained with a continuous infusion (0.6 mg · kg⁻¹ · h⁻¹). A polyethylene catheter was inserted through the umbilical artery into the descending aorta to obtain blood gas samples and to continuously measure the blood pressure. Catheter insertion *via* the umbilical artery was not successful in four control piglets, and their arterial blood sampling thus was performed from the femoral artery. Alveolo-arterial oxygen difference (AaDO₂) was evaluated using the equation $AaDO_2 = 700 \times F_{iO_2} - PaCO_2/0.8 - PaO_2$ (mm Hg), where F_{iO_2} is fraction of inspired oxygen, PaO_2 is arterial oxygen tension (mm Hg), and $PaCO_2$ is arterial carbon dioxide tension (mm Hg).

Experiments were approved by the Committee of Animal Care in Research of the University of Turku. Animals were cared for in accordance with procedures outlined in the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publications No. 85-23).

Meconium preparation. Meconium was collected from the first stools of several healthy term human neonates. The samples were initially frozen and later pooled, lyophilized, and irradiated for sterility. Endotoxin was quantified using Limulus

Amebocyte Lysate assay (LAL Kinetic-QCL, 50–650 U; Bio-Whittaker/Camprex, Walkersville, MD). Before the experiment, meconium was diluted with sterile saline to a concentration of 65 mg/mL, clinically corresponding to thick meconium in amniotic fluid (25).

Study protocol. As described earlier (24), after 1 h of stabilization, a bolus (1.5 mL/kg) of meconium was instilled into the lower lobe of the right lung. In a supine position, an x-ray positive catheter was inserted through the intubation tube under fluoroscopic guidance into the right main bronchus, 2 cm distal from the tracheal bifurcation. The piglets then were turned to the right lateral position, and meconium was instilled slowly through the catheter. Remaining of meconium in the right lung was confirmed in preliminary roentgenographic studies by unilateral instillation of meconium mixed with barium, and in the present study macroscopically and histologically after each experiment. After 15 min of ventilation, the animals were turned back to the supine position for the rest of the study. An i.v. PTX bolus (20 mg/kg), followed by continuous infusion (20 mg · kg⁻¹ · h⁻¹), was started in nine piglets at 15 min after the pulmonary insult. Control piglets ($n = 8$) with unilateral intrapulmonary meconium instillation received the same amount of vehicle. Inspired oxygen was adjusted to keep P_{aO_2} between 8 and 12 kPa. After the stabilization period, ventilatory frequency, peak inspiratory pressure, and positive end expiratory pressure were kept constant throughout the study. Body temperature was maintained >38°C with a radiant warmer. Five percent glucose in saline was infused intravenously at 6 mL · kg⁻¹ · h⁻¹. Regional ventilation was assessed noninvasively with a dynamic pulmonary imaging (DPI) method before and 30 min and 1, 2, 4, 6, and 12 h after meconium instillation. At the end of the study, the animals were killed with an overdose of potassium chloride, and bronchoalveolar lavage (BAL) of both lungs was performed separately and samples of lung tissue were obtained.

Inflammatory variables. At the end of the study, the heart and lungs were removed *en bloc*. Both main bronchi were alternately tied, and lavage of both lungs was performed separately by instillation of 15 mL of normal saline three times to each lung followed by immediate aspiration. BAL fluid was centrifuged (350 G, 4°C for 10 min), and total alveolar cell counts from cellular debris were performed in Bürker chamber. Differential cell counts were performed after cytocentrifugation and May-Grünwald-Giemsa staining. Supernatant was collected, frozen at –70°C, and later used for measurements of TNF- α concentration with the ELISA system (BioSource International, Camarillo, CA) and total protein concentration with BCA protein assay (Pierce Biotechnology, Rockford, IL). Lung tissue samples (2 × 2 cm) were excised in a consistent manner from the lower lobes of both lungs, frozen at –70°C, and later used for the spectrophotometric assay of myeloperoxidase activity (26).

Analysis of regional ventilation. Regional ventilation was assessed with the DPI method, an image acquisition system based on digital capture and analysis of sequential fluoroscopic chest x-ray images (27). The imaging system consisted of a standard fluoroscopic unit connected to a computer with custom-programmed software.

During a ventilation study, animals were supine on the examination table in a custom-built plastic holder. Care was taken to maintain the position between studies. Neither contrast media nor radioactive isotopes were used. Fluoroscopic antero-posterior chest images of 384×288 pixels with 256 gray shades were collected at 25 frames/s for 4 s and digitally stored on the computer. High voltage (90–110 kVp) with a fixed mA value and a thick copper filtration (1–3 mm Cu) were used to get the best contrast between aerated lung and soft tissues and avoid attenuation of the moving ribs. The total radiation dose (~ 0.1 mGy) is approximately the same as that in a normal chest x-ray examination.

The image analysis of the fluoroscopic images was performed using a specially developed software (Venobs, version 4.0). One respiratory cycle was used for ventilation analysis to allow standardized procedure. Lungs were outlined from the end-expiratory image of the image series. Tracheal bifurcation was visualized, and lung regions cephalic to bifurcation were excluded from the analysis. An automatic lung detection mode then was used to roughly outline the lungs. After that, when necessary, the lungs were manually delineated to exclude areas outside the rib cage and diaphragm, and finally cardiac shadow was removed from the analysis. The outlined area was automatically divided into four regions of equal number of pixels, and the sum of the two most caudal regions was used in the analysis. From one ventilatory cycle of fluoroscopic anteroposterior chest images, the average (AVG) and the range of variation of pulmonary transmittance [amplitude (AMP)] in the aforementioned lower lung regions was measured. AVG is suggested to represent the average amount of alveolar gas and AMP the maximal change in pulmonary gas content during one respiratory cycle. Subsequently, the ratios in right to left lung (R/L) AVG and AMP ratios were calculated. Low ratio values indicate poor regional ventilation of the right lung.

Data analysis. Longitudinal data first were studied with ANOVA for repeated measures design with one between factor (group) and one within factor (time), and *post hoc* comparisons between groups were made with a nonpaired *t* test. Significance between means of other data first were tested by ANOVA for repeated measurements with one between factor (group) and one within factor (side). When significant differences were noted, a Bonferroni adjusted paired *t* test was used to compare right and left lungs, and a nonpaired *t* test was used to compare instilled and noninstilled lungs between the study groups. A $p < 0.05$ was considered statistically significant.

RESULTS

Mean arterial blood pressure was similar at baseline, decreased in both study groups ($p < 0.001$), and was lower in the PTX-treated than nontreated animals from 30 min onward (Fig. 1). In contrast, heart rate was higher in the PTX group than in the untreated group at 6 and 12 h (Fig. 1). The AaDO₂ was not different at baseline and was equally increased during the study period ($p < 0.001$) in both groups (Fig. 1). Arterial pH was lower in the nontreated than in the PTX-treated piglets at 30 min and 1 h, but thereafter no differences were found (data not

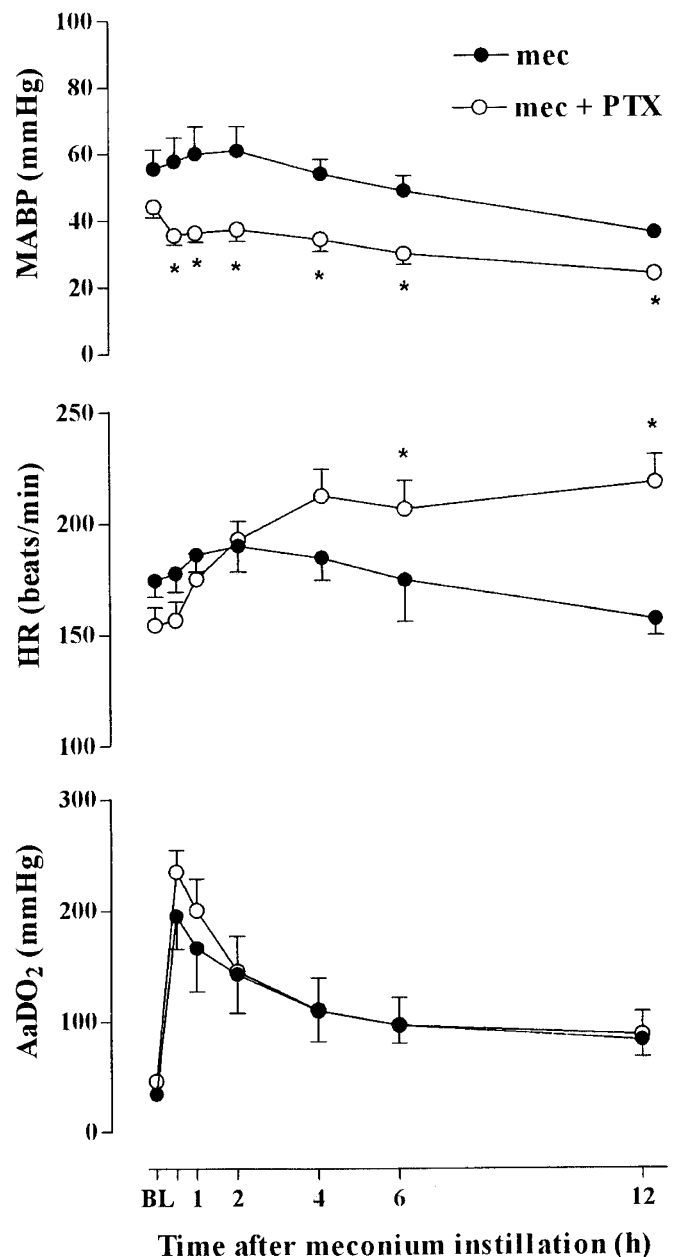


Figure 1. Mean arterial blood pressure, heart rate, and AaDO₂ in eight untreated (mec) and nine PTX-treated (mec + PTX) newborn piglets after unilateral intrapulmonary instillation of meconium. Mean \pm SEM; * $p < 0.05$ vs untreated group. BL, baseline.

shown). The meconium batch was sterile in culture, and its endotoxin content was 24 ng/mg of meconium.

Unilateral meconium instillation increased BAL fluid total cell and PMN counts locally in the instilled lungs, when compared with the contralateral lungs, but there were no differences in the counts between the PTX-treated and nontreated study groups (Table 1). Meconium also increased alveolar macrophage count in the contaminated lungs, compared with contralateral lungs, but this effect was not seen after PTX treatment (Table 1). Unilateral meconium contamination increased BAL fluid TNF- α and protein concentrations locally in the exposed lungs, when compared with contralateral lungs, but the values were significantly lower in the PTX-treated

Table 1. BAL fluid total cell, PMN, and macrophage counts ($\times 10^6/\text{mL}$); BAL fluid TNF- α (pg/mL) and total protein (mg/mL) concentrations; and lung tissue MPO activity (units/mg prot) in eight untreated (mec) and nine PTX-treated (mec + PTX) newborn piglets 12 hours after unilateral intrapulmonary instillation of meconium

	Total cell count	PMN count	Macrophage count	TNF- α concentration	Protein concentration	MPO activity
Mec						
Instilled lung	12.0 \pm 7.3* (3.6–24.2)	7.5 \pm 5.8* (3.0–19.9)	3.1 \pm 2.8* (0.4–7.7)	42.2 \pm 22.1* (21.1–86.8)	1.1 \pm 0.3* (0.7–1.7)	1.6 \pm 1.0* (0.4–3.5)
Noninstilled lung	2.1 \pm 2.2 (0.3–6.9)	1.0 \pm 1.2 (0.1–3.8)	0.6 \pm 0.5 (0.1–1.5)	21.6 \pm 4.9 (16.1–28.7)	0.5 \pm 0.3 (0.2–1.2)	0.4 \pm 0.3 (0.1–0.9)
mec + PTX						
Instilled lung	6.2 \pm 3.5* (2.3–11.7)	4.7 \pm 2.7* (1.9–10.3)	0.9 \pm 0.7 (0.1–2.3)	17.4 \pm 5.0*† (9.8–25.4)	0.6 \pm 0.2*† (0.4–0.9)	1.5 \pm 0.2* (1.1–1.7)
Noninstilled lung	1.4 \pm 1.1 (0.1–4.0)	1.0 \pm 0.9 (0.1–2.8)	0.3 \pm 0.3 (0.0–1.0)	8.2 \pm 1.9‡ (5.9–10.4)	0.2 \pm 0.1 (0.1–0.4)	0.4 \pm 0.1 (0.2–0.7)
	group, $p = 0.244$ side, $p < 0.001$ interaction, $p = 0.428$	group, $p = 0.679$ side, $p < 0.001$ interaction, $p = 0.339$	group, $p = 0.020$ side, $p < 0.001$ interaction, $p = 0.470$	group, $p = 0.002$ side, $p < 0.001$ interaction, $p = 0.361$	group, $p < 0.001$ side, $p < 0.001$ interaction, $p = 0.036$	group, $p = 0.446$ side, $p < 0.001$ interaction, $p = 0.609$

Data are mean \pm SD (range). MPO, myeloperoxidase.

* $p < 0.05$ vs contralateral lung.

† $p < 0.05$ vs nontreated meconium instilled lung.

‡ $p < 0.05$ vs nontreated noninstilled lung.

group (Table 1). In fact, BAL fluid TNF- α concentration was undetectable in one PTX-treated/meconium-instilled lung, three nontreated/noninstilled lungs, and five PTX-treated/noninstilled lungs, and the concentrations were significantly lower also in the noninstilled lungs of the PTX-treated compared with the nontreated study group (Table 1). Similar to BAL fluid total cell and PMN counts, lung tissue myeloperoxidase activity was higher in the meconium-instilled than noninstilled lungs, and PTX treatment did not affect these values (Table 1).

R/L AVG decreased ($p < 0.001$) similarly ($p = 0.220$) in both groups during the study period (Fig. 2, top). Similarly, R/L AMP decreased ($p < 0.001$) initially in both groups, but there was an interaction ($p = 0.023$) between the groups and the values were lower in the nontreated than in the PTX-treated piglets at 4, 6, and 12 h (Fig. 2, bottom).

DISCUSSION

Initial deterioration in neonatal lung mechanics after aspiration of meconium is supposed to be mainly obstructive, caused by thick or particulate meconium blocking the airways partially or completely (3,6). Experimental investigations have shown that dynamic lung compliance drops rapidly during the first hour after meconium insufflation and remains low for the first days after the insult (3,6). Whereas the initial perturbation in global pulmonary mechanics after meconium insult may be due to widespread obstruction of the airways and uneven lung aeration (3), the subsequent phase of sustained pulmonary dysfunction is characterized by progressively developing inflammatory reaction within the lung tissue (6). In line with these findings, the earliest disturbance of regional pulmonary ventilation in the meconium-contaminated lung area in our study was reflected in ipsilaterally registered decrease of ventilation amplitude. This variable of the DPI examination, indicating the variation in the transmitted radiation during the ventilatory expansion of the defined lung region, is in fact demonstrated to be low when the specific lung region is functionally or anatomically obstructed (28). Similarly, the sustained low DPI amplitude after the initial obstructive phase

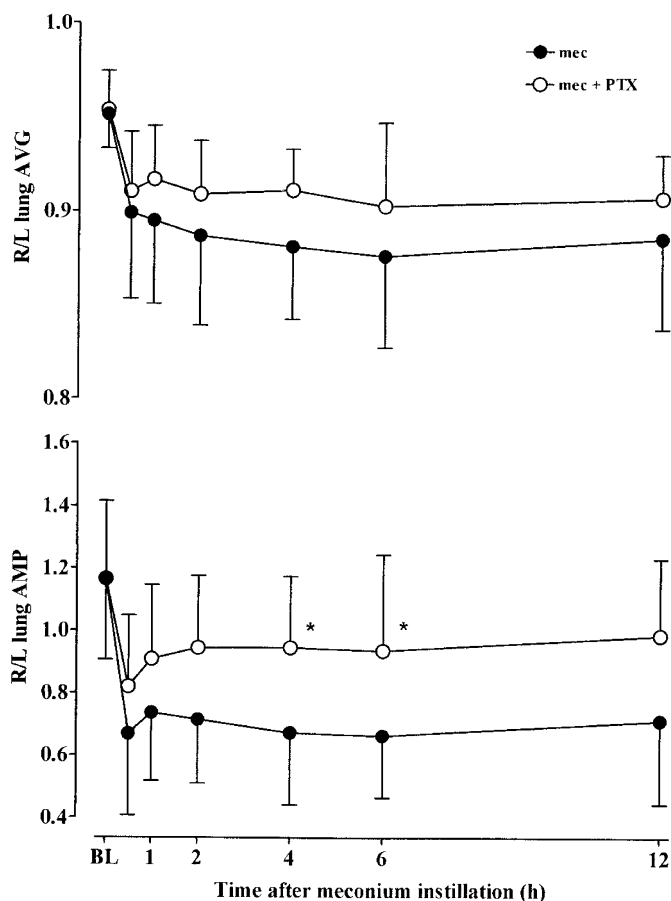


Figure 2. R/L ventilation AVG and AMP in eight untreated (mec) and nine PTX-treated (mec + PTX) newborn piglets after unilateral (right lung) meconium instillation. Mean \pm SD; * $p < 0.05$ vs untreated group. BL, baseline.

in meconium-exposed lungs may reflect the developing pulmonary inflammation with local neutrophil and fluid accumulation, reported to be present already at 4–6 h after meconium aspiration (6,29,30). The lowest regional aeration values, represented as reduced AVG in our study, are also compatible with this exudative phase of patchy consolidation or atelectasis in the meconium-contaminated pulmonary tissue. As evident

from our data, the inflammatory reaction is associated with migration of activated macrophages and neutrophils, production of inflammatory cytokines, and increased vascular permeability with potential inactivation of pulmonary surfactant contributing to significant impairment of lung function (6,31,32). Direct inhibition of lung surfactant by meconium supposedly also contributes to the ventilation insufficiency (4,5), but initially, during the first 2 h after aspiration, it is considered to be of minor importance (3). The endotoxin content of meconium was in the nanomolar range, which is well below the amount required for induction of significant pulmonary neutrophil influx.

The effects of anti-inflammatory agents, specifically glucocorticoids, in the treatment of MAS have been variable and often unsuccessful. Although steroids may improve oxygenation and lung function in clinical and experimental trials (10,33), the significance of their anti-inflammatory action for the improved ventilatory status in MAS is still unclear (11). In fact, there is evidence indicating that steroids may diminish vascular permeability in the meconium-exposed lungs, but no clear effect on the inflammatory cellular response in the pulmonary tissue has been shown (8,11,34). Similar to these findings, the present results demonstrate that PTX has no significant effect on the neutrophil number or activity in the meconium-exposed lung areas, despite being able to inhibit local activation and migration of inflammatory cells in various other lung injury models (19–23). However, PTX administration effectively suppressed the meconium-induced increase in alveolar macrophage number and local TNF- α production in the insulted lungs. The cause of this variable effect of PTX on the pulmonary inflammatory cells in our model remains uncertain, but it is probable that, unlike after acid aspiration (35), local TNF- α production by alveolar macrophages is not essential for the early neutrophil migration in meconium-exposed lungs. However, TNF- α is able to induce increased pulmonary permeability and reduced surfactant production and is thereby supposed to be involved in the pathogenesis of the various lung injuries, including meconium-induced pulmonary damage (36–40). As also evident from our data, these lung injury processes are effectively prevented by PTX (41,42) and thus may contribute to the improved ventilatory function in the meconium-contaminated areas of the treated neonatal lungs. Although PTX additionally may have significant hemodynamic properties, the reduction of permeability damage is unlikely attributed to alterations in pulmonary microvascular pressure (22). In fact, in line with our results, PTX has been shown to preserve lung compliance in an experimental injury model despite significant neutrophil sequestration (43). This mechanism of PTX on lung function is further underscored by the observation that PTX was unable to affect the initial meconium-induced obstructive phase but significantly prevented the impairment of local ventilation from 4 h onward after the meconium insult, a phase characterized by the progressive localized pulmonary inflammatory reaction (6).

Although our present data indicate that regional pulmonary inflammatory and ventilatory disturbances after meconium aspiration may be diminished by PTX treatment, prompt obstetric and pediatric suctioning of the upper and lower airways still

remains the critical procedure in reduction of the severity of MAS. This is especially important when amniotic fluid is stained with thick or particulate meconium (1). Once meconium has entered the airways, amelioration of surfactant dysfunction with surfactant replacement or lavage and augmentation of the developing lung inflammatory reaction with anti-inflammatory therapy, such as corticosteroid treatment, have been proposed to be beneficial (1). Our present results additionally indicate that early treatment of MAS with PTX could be a new, pathophysiologically plausible therapeutic alternative. However, as indicated by our data, systemic hemodynamics must be monitored closely in newborns with PTX treatment.

CONCLUSION

In conclusion, PTX treatment has no clear effect on neutrophil influx but decreases the number of intra-alveolar macrophages, TNF- α production, and alveolocapillary permeability in the meconium-contaminated areas of neonatal lungs. Because these local effects are associated with simultaneous improvement of the regional pulmonary ventilation, targeted anti-inflammatory approaches with PTX may be of benefit in the treatment of respiratory failure in severe MAS.

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