

Perfluorochemical Liquids Enhance Delivery of Superoxide Dismutase to the Lungs of Juvenile Rabbits

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ABSTRACT: Previous studies suggest acute lung injury (ALI) in premature newborns is associated with relative deficiency of antioxidant enzymes that may be ameliorated by recombinant human superoxide dismutase (rhSOD). Perfluorochemicals (PFCs) are distributed homogeneously and support gas exchange in diseased lungs. We investigated whether PFCs could provide an effective delivery system for rhSOD. Juvenile rabbits were lung-lavaged, treated with surfactant, and randomized: group I: fluorescently labeled rhSOD (5 mg/kg in 2 mL/kg saline); group II: fluorescently labeled rhSOD (5 mg/kg in 18 mL/kg PFC). Animals were ventilated with oxygen for 4 h; the lungs were harvested for analysis of SOD distribution and oxidative injury. Cardiopulmonary indices remained stable and similar between groups. Qualitative assessment (QA) showed a more homogeneous lung SOD distribution in group II and a better histologic profile. QA of lung SOD distribution showed significant increase in SOD concentrations in group II ($7.37 \pm 1.54 \mu\text{g}/\text{mg}$ protein) compared with group I ($1.65 \pm 0.23 \mu\text{g}/\text{mg}$ protein). Oxidative injury as assessed by normalized protein carbonyl was $149.1 \pm 26.8\%$ SEM in group II compared with $200.5 \pm 7.3\%$ SEM in group I. Plasma SOD was significantly higher in group II. Administration of rhSOD with or without PFCs does not compromise cardiovascular function or impede lung recovery after ALI. PFCs enhance rhSOD delivery to the lungs by 400% while decreasing lung oxidative damage by 25% compared with rhSOD alone. These data suggest that PFCs optimize lung rhSOD delivery and might enhance the beneficial effects of rhSOD in preventing acute and chronic lung injury. (*Pediatr Res* 60: 65–70, 2006)

Preterm infants have incomplete structural lung development, rendering the lung prone to oxygen-mediated toxicity. Furthermore, the lung is uniquely exposed to higher oxygen tensions compared with most organs. Room air oxygen tension in the alveolus is approximately 100 mm Hg compared with 45 mm Hg in venous blood and as low as 1 mm Hg in some sites within other organs. The molecular mechanisms responsible for oxygen toxicity include formation

of free radical intermediates and the subsequent damage of lung proteins, lipids, and DNA (1).

To counteract the detrimental effects of oxygen, a number of antioxidant systems have evolved. The antioxidant enzymes catalase, glutathione peroxidase, and SOD are strategic components of such complex defense mechanisms. SOD is capable of converting the toxic superoxide anion radical to potentially less toxic hydrogen peroxide and water. Three SOD isoenzymes have been identified in mammals. The major intracellular SOD is a low molecular weight copper/zinc-containing protein (Cu/Zn SOD) present in the cytoplasm of all mammalian cells (2). Mitochondrial SOD (MnSOD) is a manganese-containing protein that is synthesized in the cytoplasm and translocated to the mitochondria (3). The last mammalian SOD discovered is a high molecular weight Cu/Zn-containing protein primarily located in extracellular spaces (EC-SOD) (4).

The premature lung lacks not only the late gestational maturation of the surfactant system, but also the antioxidant enzyme systems. The lungs of different animal species show a dramatic rise in activity of the major antioxidant enzymes around the time of birth (5,6). Human studies show a similar developmental trend (7,8). Furthermore, at least in the rabbit, only term animals are capable of up-regulating enzymatic antioxidant expression when faced with oxygen stress (9). Therefore, it appears attractive to supplement the lung with SOD in an attempt to modulate oxidant stress and injury around the time of birth.

rhSOD is a human recombinant Cu/Zn SOD that has been shown to ameliorate ALI and chronic lung injury in animal studies (10,11). Two placebo-controlled pilot studies have been conducted in premature infants with respiratory distress syndrome using single or multiple intratracheal doses of rhSOD (12,13). These studies have established pharmacoki-

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Abbreviations: ALI, acute lung injury; C_R , respiratory compliance; OI, oxygenation index; PEEP, positive end-expiratory pressure; PFC, perfluorochemical; PIP, peak inspiratory pressure; rhSOD, recombinant human CuZn superoxide dismutase; VEI, ventilation efficient index

netic profiles and short-term safety for rhSOD. They have also suggested that rhSOD might reduce the early lung inflammatory changes associated with the subsequent development of bronchopulmonary dysplasia (BPD). Subsequent studies have suggested that rhSOD may improve long-term pulmonary and neurodevelopmental outcome in critically ill preterm neonates (14,15). One central factor during the rhSOD clinical trials was that intratracheal rhSOD administration was not always well tolerated by extremely preterm infants. Furthermore, because rhSOD was administered in a saline suspension using small volumes, it was unlikely that the rhSOD was distributed homogeneously to the entire lung.

PFC liquids offer potential for biomedical use and ventilatory support. PFC fluids act as a vehicle for delivering oxygen and removing carbon dioxide (16,17). Air-liquid interfaces are eliminated, thereby increasing lung compliance and recruiting alveoli. PFC liquids can be distributed homogeneously throughout the lungs at low pressures because of low surface tension properties. Furthermore, PFC liquids are biologically inert and appear to have no deleterious histologic, cellular, or biochemical effects (18). There are several commercially available PFC fluids with physicochemical properties and purity specifications appropriate for respiratory applications (19,20). Our laboratory has shown that PFC fluids are attractive respiratory media for pulmonary administration of various drugs (19,20). Therefore, PFC liquids may provide an excellent vehicle for the distribution of rhSOD while simultaneously supporting gas exchange in an already diseased lung.

In the current study, we hypothesized that rhSOD could be safely administered either with or without PFCs in our juvenile rabbit model of ALI. Also, we examined whether PFC-mediated administration of rhSOD would enhance lung distribution of the enzyme and, in the short term, decrease markers of oxidative damage. Finally, physiologic and histologic comparisons of SOD treatment groups are discussed within the context of published data from untreated animals and those treated with PFC alone (21–27).

METHODS

Animals were managed according to the National Institutes of Health regulations "Guide for the Care and Use of Laboratory Animals". In addition, all procedures were approved by the Institutional Animal Care Use of Temple University.

Animal preparation. Twelve New Zealand White juvenile rabbits (8–10 wk old; weight, 1.3–2.1 kg) with normal lungs were anesthetized with an intramuscular injection of a mixture of ketamine (23 mg/kg), acepromazine (0.58 mg/kg), and xylazine (0.8 mg/kg) and an intramuscular injection of atropine (0.02 mg/kg) was given. This animal model was chosen based on our previous experience with the model, the suitability of the model as it applies to neonatal and pediatric medicine, and the history of this preparation for liquid ventilation (21–25). The skin and soft tissues were anesthetized with 0.5% lidocaine HCl (4 mg/kg), and the animals were instrumented with tracheostomy placement of a 3-mm-internal diameter endotracheal tube (Hi-Lo Jet Tube; Mallinkrodt, St. Louis, MO). Catheters were placed in the jugular vein and carotid artery. Anesthesia was maintained with an i.v. injection of sodium pentobarbital 5 mg/kg as needed or hourly. Muscle relaxation was induced by i.v. administration of pancuronium bromide (0.1 mg/kg) and maintained with a continuous infusion of 0.15 mg/kg/h throughout the experiments. Maintenance fluids were provided by a continuous infusion of 5% dextrose at a rate of 5 mL/kg/h. Arterial blood pressure was monitored by attaching the arterial catheter to a standard pressure transducer connected to a neonatal monitor (Athena/Neonatal 9040, S & W Medico Teknik, Albertslund, Denmark). ECG electrodes and a rectal temperature

probe were inserted for monitoring. The animal's rectal temperature was maintained at 37–38°C.

Tidal volume and respiratory compliance (C_R) were measured (Peds MAS, Hatfield, PA) with pneumotachography and airway manometry (21,26). Arterial blood gases (Stat profile, Nova Biomedical, Waltham, MA) were also measured in all groups.

Mechanical ventilation was provided with a time-cycled, pressure-limited ventilator (V.I.P. BIRD Gold, Bird Products Corp.) and adjusted to maintain a tidal volume of 6 mL/kg and to keep the arterial pCO_2 below 60 mm Hg (7.9 kPa). The inspired oxygen fraction was maintained at 1.0 throughout the experiment. Initial and maximal values for the remaining settings were peak inspiratory pressure (PIP) of 1.1–3 kPa (11–30 cm H_2O); frequency of 30–60 breaths/min; inspiratory time of 0.3–0.5 s; and positive end-expiratory pressure (PEEP) of 0.4–0.8 kPa (4–8 cm H_2O). The PFC liquid was a mixture of 25% perfluoromethylcyclohexane (PP2) and 75% perfluoromethyldecalin (PP9) (F2 Chemicals Corp., Preston, U.K.). This mixture provides better gas exchange, compliance, and histologic findings after ALI as described in our laboratory (27).

Protocol. After obtaining baseline data for arterial blood gases, ventilatory parameters, pulmonary function tests, and physiologic parameters in all animals, lung injury was induced by repeated lung lavage with 10 mL/kg of warm normal saline until injury criteria were achieved: arterial $PO_2 < 100$ mm Hg (13.3 kPa) with a C_R decreased by more than 50% and less than 0.5 mL/cm H_2O /kg maintained for more than 30 min (21). All animals were treated with 4 mL/kg of surfactant (Survanta[®], Ross Laboratories, Columbus, OH) and then randomly assigned to one of two groups: group I, treated with rhSOD (5 mg/kg) suspended in saline (2 mL/kg), and group II, treated with rhSOD (5 mg/kg) suspended in PFC (18 mL/kg). rhSOD was given in all animals within 10 min after surfactant administration. Half the animals in each group received rhodamine-conjugated rhSOD. We have previously reported physiologic and histologic data from this lung injury model over 4 h in an untreated mechanically ventilated group, as well as a PFC (25% PP2/75% PP9) alone group (21,27).

The rhSOD/PFC suspension was instilled into the lungs via the side port of the endotracheal tube over 5–10 min. During instillation, the animals were repositioned to optimize PFC distribution using four equal increments of the total dose with Trendelenburg, reverse Trendelenburg, and left and right lateral decubitus positions. After instillation, the animal position (supine), airway temperature (35°C), and humidity (100%) were kept constant. The arterial blood gases, ventilatory parameters, pulmonary function tests, and physiologic data were measured and/or recorded again after lung injury and then every 30 min for 4 h or until death.

The oxygenation index (OI) and the ventilation efficient index (VEI) were computed as described (21,28,29): $OI = [(\text{mean airway pressure} \times \text{inspired oxygen fraction}) / Po_2 (\text{mm Hg})] \times 100$; $VEI = 3800 / [\text{respiratory rate} (PIP - PEEP) \times Pco_2 (\text{mm Hg})]$, where VEI relates alveolar ventilation to respiratory input, and 3800 is a constant for estimating alveolar ventilation (3800/ Pco_2) with the assumption of CO_2 production near normal minimal volumes of 5 mL/kg/min in resting animals.

At the end of the experiments, the animals were euthanized with sodium pentobarbital. The thorax was opened, and the lungs were inspected to assess gross morphology. Within 5 min of death, the ventilator was stopped and continuous positive airway pressure equivalent to the final PEEP was applied. Lungs were immediately perfused with Millinogs buffer, and samples within the dependent and nondependent regions of the lungs were obtained (0.5- to 1.0-cm³ blocks) and snap frozen for qualitative and quantitative analysis of rhSOD distribution and lung oxidative injury as assessed by protein carbonyls.

rhSOD/PFC suspension. The creation of nanocrystal suspensions of rhSOD with PFC fluids was carried out as described (30). Briefly, the suspensions were made by combining 5 mg/kg rhSOD with 18 mL/kg of 25% PP2/75% PP9 in an ultrasonic unit (Bronson B-220H) for 5–10 min.

SOD analyses. Lung homogenates or plasma samples were incubated with ~20,000 cpm of ¹²⁵I-rhSOD and a mouse anti-rhSOD1 monoclonal antibody at room temperature followed by an overnight incubation at 4°C. Rabbit anti-mouse serum was added and incubated for at room temperature. Antibody-SOD complexes were precipitated by the addition of 20% PEG, incubation at room temperature, and centrifugation. Counts were determined using a gamma counter.

Protein carbonyl analyses. Proteins were extracted from lung homogenates and yield determined using the Bio-Rad assay. An aliquot of each sample was derivatized with 2,4-dinitrophenylhydrazine (DNP). Serial dilutions of DNP-derived and nonderived were dot blotted on to nylon membranes. Dot blots were incubated with an anti-DNP polyclonal antibody followed by a HRP-conjugated secondary antibody per the manufacturer's instructions (Intergen Co). Antibody complexes were detected by chemiluminescence and visualized on x-ray film. Films were scanned, and densitometry

etry was determined using the Sigmagel program. Values were normalized to nonderived protein.

Histomorphometric analysis. Tissues were formalin fixed and embedded in paraffin. Thin sections (5 μ m) were cut and stained with hematoxylin and eosin. The specimens were analyzed qualitatively with gross light microscopy at $\times 100$ magnification as described (31).

Statistics. One-way analysis of variance (ANOVA) was used to compare basic physiologic data among groups. A paired *t* test was used to compare pre- and postinjury data within groups. Two-way repeated measures ANOVA was used to compare data of gas exchange, OI, and respiratory compliance as a function of time and group. *Post hoc* testing with the Student-Newman-Keuls test was used to determine significance. Significance was accepted at the $p < 0.05$ level. Values are presented as mean \pm SEM.

RESULTS

As shown in Table 1, body weights and the number of saline lavages did not differ significantly between groups. Saline lavage resulted in a similar pattern of lung injury in both studied groups. The values of postinjury OI, VEI, and C_R were significantly ($p < 0.05$) different from their corresponding preinjury values for each group (Table 1). The heart rate showed significant increase at the time of injury and/or 1 h after injury (Table 2). However, there was no significant difference between groups (Table 2). Mean blood pressure remained stable with no significant difference between groups (Table 3). C_R , OI, P_{aO_2} , and P_{aCO_2} were not significantly different at the various times between the two experimental groups (Fig. 1). C_R , OI, and P_{aO_2} postinjury were significantly different compared with preinjury values in both groups ($p < 0.01$). OI and P_{aO_2} then improved significantly by 4 h in both groups ($p < 0.01$).

Representative regional histomicrographs of the lung are presented in Figure 2. As shown, both groups (Fig. 2A and B) demonstrated a significant degree of injury associated with saline lavage; however, the rhSOD/saline delivery group (Fig. 2A) demonstrated more marked atelectasis and inflammatory cell infiltration. In contrast, there appeared to be less atelectasis and inflammation in the rhSOD/PFC group (Fig. 2B). Grossly, there was a greater number of open exchange units in sections from the rhSOD/PFC animals compared with the animals breathing rhSOD/saline.

Qualitative assessment of lung SOD by fluorescent microscopy of the rhodamine-conjugated rhSOD lung sections showed a more homogeneous and abundant distribution in the rhSOD/PFC group both within and between lung regions

Table 1. Characteristics of animals pre- and post-injury of lungs (mean \pm SE)

	Group I (n = 6)	Group II (n = 6)
Body weight (kg)	1.85 \pm 0.10	1.79 \pm 0.15
No. of lavages	5 \pm 1	5 \pm 1
Pre-injury		
OI	1.1 \pm 0.2	1.1 \pm 0.2
VEI	0.38 \pm 0.15	0.33 \pm 0.13
C_R	0.99 \pm 0.14	1.05 \pm 0.23
Post-injury		
OI	15.7 \pm 6.1*	17.8 \pm 8.0*
VEI	0.11 \pm 0.03*	0.08 \pm 0.03*
C_R	0.35 \pm 0.05*	0.34 \pm 0.10*

Values are mean \pm SEM. Group I: + SOD; group II: + SOD/PFC.

* $p < 0.05$ vs corresponding data of pre-injury. No significant difference between groups.

Table 2. Heart rate (beats/min) in the two treatment groups (mean \pm SE)

	Group I (n = 6)	Group II (n = 6)
Baseline	199 \pm 13	190 \pm 23
Injury	190 \pm 30	233 \pm 16*
1 h	218 \pm 21*	230 \pm 17*
2 h	207 \pm 36	209 \pm 14
3 h	197 \pm 30	202 \pm 17
4 h	192 \pm 49	200 \pm 39

Values are mean \pm SEM. Group I: + SOD; group II: + SOD/PFC. No significant difference between groups.

* $p < 0.01$ vs corresponding baseline data.

Table 3. Mean blood pressure (mm Hg) in the two treatment groups (mean \pm SE)

	Group I (n = 6)	Group II (n = 6)
Baseline	55 \pm 8	57 \pm 10
Injury	76 \pm 4*	76 \pm 5*
1 h	75 \pm 8	65 \pm 9
2 h	72 \pm 7	68 \pm 3
3 h	70 \pm 8	68 \pm 10
4 h	74 \pm 18	62 \pm 16

Values are mean \pm SEM. Group I: + SOD; group II: + SOD/PFC. No significant difference between groups.

* $p < 0.01$ vs corresponding baseline data.

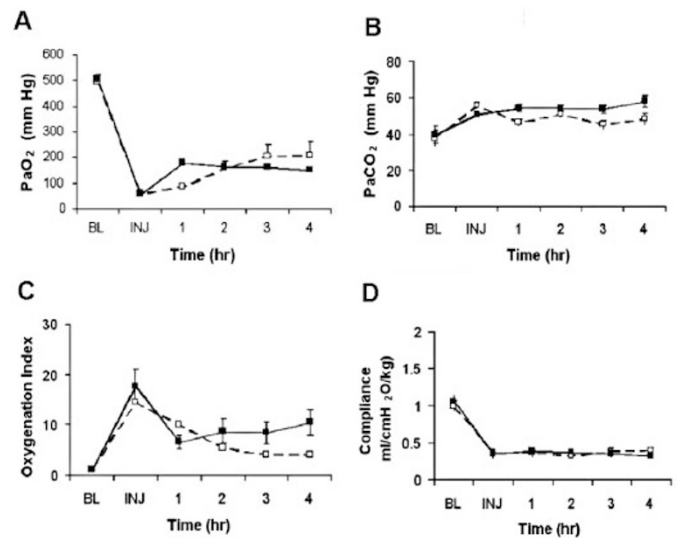


Figure 1. P_{aO_2} (A) and P_{aCO_2} (B) Arterial blood gas measurements pulmonary, OI (C), and pulmonary compliance (D) in animals administered rhSOD (\square) in saline vs SOD/PFC (\blacksquare) suspension. Values are expressed as mean \pm SEM. To convert mm Hg to kPa, multiply the value by 0.1333. SOD group, $n = 6$; SOD/PFC group, $n = 6$. No significant differences were noted between groups.

compared with the rhSOD/saline group. Figure 2C and D are representative illustrations of such findings. Quantitative analysis of overall lung SOD distribution showed a significant increase in SOD concentrations in the rhSOD/PFC group ($7.37 \pm 1.54 \mu\text{g}/\text{mg}$ protein) compared with the rhSOD group ($1.65 \pm 0.23 \mu\text{g}/\text{mg}$ protein) ($p < 0.01$) (Fig. 3).

Lung oxidative damage, as expressed by the normalized overall protein carbonyl, was $149.1 \pm 26.8\%$ SEM in the rhSOD/PFC group compared with $200.5 \pm 7.3\%$ SEM in the rhSOD group ($p < 0.03$) (Fig. 4).

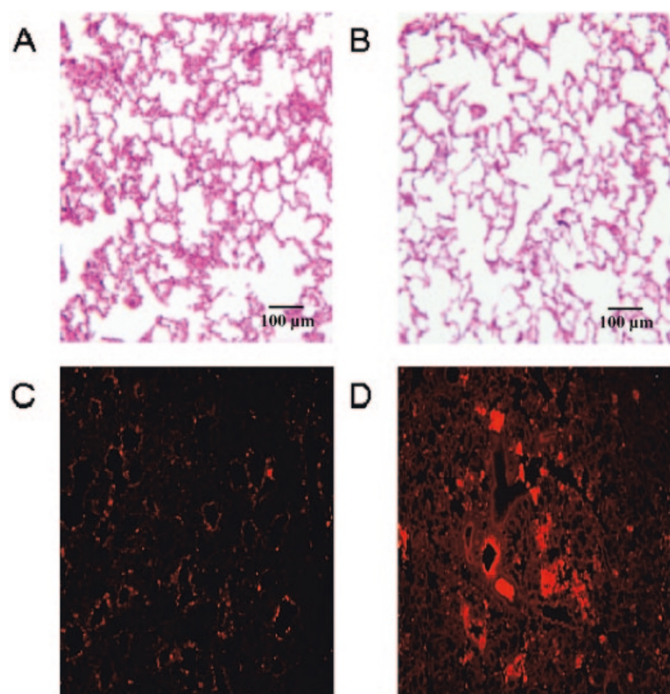


Figure 2. Histomicrographs illustrating qualitative assessment of lung expansion by light microscopy of hematoxylin/eosin-stained lung sections (A,B) and rhSOD distribution by fluorescent microscopy of rhodamine-conjugated rhSOD lung sections (C,D). Lung sections are representative of typical patterns observed over many different lung sections. A and C: Saline-delivered SOD. B and D: PFC suspension of SOD ($\times 100$ magnification).

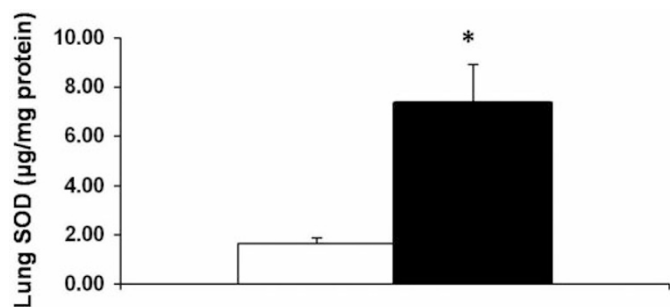


Figure 3. Quantitative lung rhSOD ($\mu\text{g}/\text{mg}$ protein) distribution [+ SOD (open column); + SOD/PFC (solid column)]. Values are expressed as mean \pm SEM. $*p < 0.01$ vs SOD group.

Plasma SOD at 4 h was $3.30 \pm 0.55 \mu\text{g}/\text{mL}$ in the rhSOD/PFC group compared with $1.45 \pm 0.64 \mu\text{g}/\text{mL}$ in the rhSOD group ($p < 0.05$) (Fig. 5).

When evaluating oxidative injury and tissue SOD over a matched SOD range between 0 and $5 \mu\text{g}/\text{mg}$ protein, only 8% of lung sections in the rhSOD group had a protein carbonyl below the normalized 100% level compared with 22% in the rhSOD/PFC group (Fig. 6). This indicates a three times likelihood that a lung section in the rhSOD/PFC group with an SOD level below $5 \mu\text{g}/\text{mg}$ protein would have a normalized protein carbonyl level below 100% compared with the rhSOD group.

DISCUSSION

Initial clinical attempts at augmenting the antioxidant defenses of the premature lung have been encouraging (12–15).

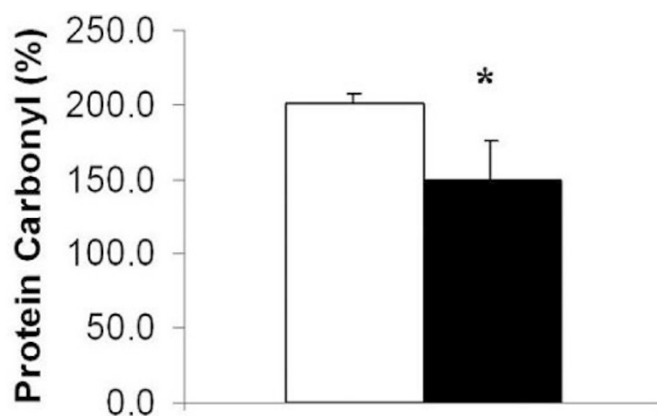


Figure 4. Lung oxidative damage expressed as normalized lung protein carbonyl (%) level [+ SOD (open column); + SOD/PFC (solid column)]. Values are expressed as mean \pm SEM. $*p < 0.01$ vs SOD group.

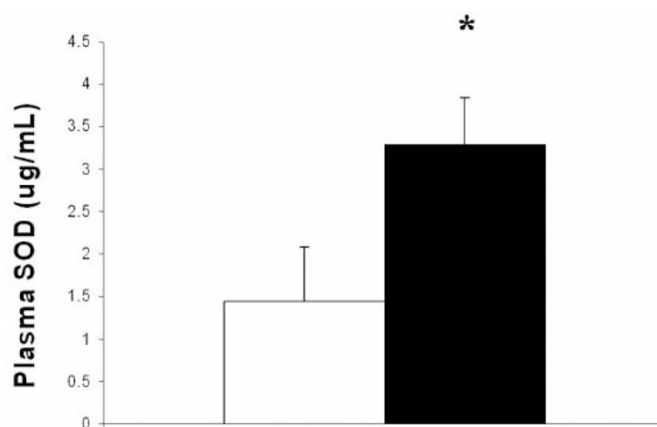


Figure 5. Quantitative rhSOD levels ($\mu\text{g}/\text{mL}$) in plasma at 4 h post-delivery [+ SOD (open column); + SOD/PFC (solid column)]. Values are expressed as mean \pm SEM. $*p < 0.01$ vs SOD group.

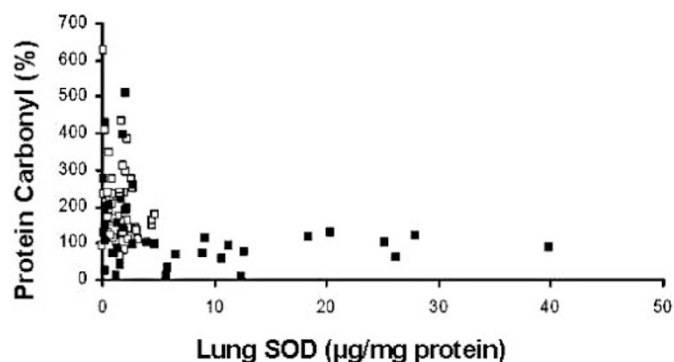


Figure 6. Relationship between oxidative injury protein carbonyls (PC) and tissue rhSOD over a matched SOD range between 0 and $5 \mu\text{g}/\text{mg}$ protein. + SOD (\square); + SOD/PFC (\blacksquare). The curve represents the best fit regression analysis.

One central factor evident during these clinical trials was that some extremely premature infants did not tolerate intratracheal rhSOD administration well. Furthermore, it was unlikely that rhSOD in a small-volume saline suspension would distribute homogeneously to the entire lung, which may have had a negative impact on pulmonary outcome. In the current study, we demonstrated that rhSOD can be safely administered either with or without PFC in a juvenile rabbit model of ALI treated

with surfactant to simulate clinical management. Importantly, we showed that the PFC-mediated administration of rhSOD allows a more homogeneous distribution of the enzyme accompanied by a synergistic decrease in lung oxidative damage. Taken together, these findings suggest that PFC might optimize rhSOD delivery, better expand and recruit the lung, and enhance the beneficial effects of rhSOD after ALI.

The lung is exposed to high oxygen tensions compared with other organs and is therefore particularly prone to oxidant stress. Oxidative stress triggers inflammatory mediators thus activating neutrophils and alveolar macrophages (32). Such inflammatory changes may be the link between ALI and the development of chronic lung disease. When such events occur, improvements in lung function will require significant amounts of time to occur. Our study was carried out over a short time period, and it should be noted that we did not observe any improvement in lung physiology in the rhSOD/PFC group compared with the rhSOD alone group despite less oxidative damage and an improved histologic profile. We can speculate that less oxidative injury and better lung expansion in the rhSOD/PFC group may translate over time to improved physiologic lung parameters. However, to confirm this issue, studies of longer duration are warranted to determine whether rhSOD could protect surfactant from oxidative injury and result in physiologic improvements in lung function.

More recently, the combination of two PFC fluids has been shown to be particularly effective for treatment of ALI (27). As shown in this study, the rhSOD/PFC group had similar physiologic and histologic profiles as in previously treated PFC (25% PP2/75% PP9) alone groups (27). Compared with untreated lung-injured rabbits (21), groups treated with PFC alone, rhSOD/PFC, or rhSOD/saline demonstrated better gas exchange and lung function over the 4-h study period. In addition to the favorable physiologic and histologic outcomes, combinations of PFC liquids and drugs have proven very effective in promoting the nanocrystal suspension of biologic agents in the lungs (30). Here we show that such PFC combination promotes more uniform distribution of rhSOD and actually improved lung rhSOD delivery by 400%. Less rhSOD delivered to the lungs in the rhSOD group probably implies more rhSOD remaining in the larger airways. The determination of a 25% decrease in oxidative injury in the rhSOD/PFC group suggests that the bulk of the oxidative damage in our model of ALI occurs in the peripheral regions of the lung.

Numerous studies have investigated whether SOD enzymes given either by injection or inhalation can protect lung tissue against oxidant injury. Most models have used Cu/Zn SOD, and intratracheal instillation has generally been shown to provide better protection than i.v. injection, although some conflicting results have been obtained (33–35). Recently recombinant human Cu/Zn SOD has become available and intratracheal rhSOD has been shown to protect neonatal piglet lungs from damage from hyperoxia and mechanical ventilation (36). Furthermore, rhSOD was shown to rapidly incorporate into cells with a subsequent increase in lung SOD activity (37). In these studies, rhSOD was administered by instillation with saline or by aerosolization and did not distribute homo-

geneously to the lungs (38). Here we show that PFC enhances rhSOD administration by facilitating higher plasma SOD concentrations, more optimal rhSOD lung distribution, and a better histologic profile. This might have additional pulmonary and systemic protective effects because lung inflammation has the potential to lead to systemic inflammation and possibly multiple system organ failure (39).

Most cells generate free radicals under normal conditions and increased amounts under conditions of stress. The first reactive oxygen species produced is the superoxide anion, and SOD is the only enzymatic system capable of degrading superoxide to hydrogen peroxide and water. Here we show that lung oxidative damage is decreased when rhSOD is delivered by PFC and that this finding could be due to a synergistic effect between rhSOD and PFC. Although, rhSOD-mediated decreases in oxidative damage seems a desirable target, we currently do not know whether this correlates necessarily with improved lung function. There could be many reasons for this incongruity. Although SOD has a central function in lung protection, many other antioxidant systems are active in the lung. For example, mitochondrial MnSOD has also been shown to be a key element in protection against injury (40). Furthermore, reactive oxygen species have recently been recognized to play a role in normal cell growth and signaling (41,42). These complex interactions explain why it is always important to assess the relationship between oxidative stress and lung physiologic parameters. Despite these uncertainties, our data show that PFC optimizes the delivery of rhSOD after lung injury and that improved PFC-mediated SOD distribution is associated with decreased lung oxidative injury.

In summary, the current study demonstrates an effective way to deliver SOD to the lungs of juvenile rabbits after ALI. The PFC-augmented SOD delivery enhances the antioxidant effects of the enzyme on the lungs. We speculate that this rhSOD delivery strategy might be critical in improving the antioxidant defenses of premature babies with acute lung disease and further improve long-term clinical pulmonary status.

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