Pharmacologic Interactions of Exogenous Lung Surfactant and Recombinant Human Cu/Zn Superoxide Dismutase

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ABSTRACT. The effect of exogenous surfactant on the pharmacokinetics of intratracheally administered recombinant human superoxide dismutase (rhSOD) was studied. Five groups of rats received the following intratracheally: 1 mL/kg of saline; 5 or 25 mg/kg of rhSOD; or 4 mL/kg of exogenous surfactant followed in 30 min by 5 or 25 mg/kg of rhSOD. Animals were killed at 24, 48, and 72 h, and serum, bronchoalveolar lavage, and lung tissue were analyzed for rhSOD. rhSOD was not detected in the lungs of saline-treated animals or in serum from any animal. At 24 h, lung-tissue rhSOD was higher in rats treated with surfactant and rhSOD versus rhSOD alone (5 mg/kg: 6.8 ± 2.5 versus $0 \mu\text{g/whole lung}$, $p < 0.05$; 25 mg/kg: 29.9 ± 9.6 versus $0.1 \pm 0.1 \mu\text{g/whole lung}$, $p < 0.05$). Bronchoalveolar lavage fluid levels correlated well with lung tissue concentrations. By 48 h, lung tissue rhSOD concentrations were insignificant in all groups. rhSOD was still present in lavage fluid from rats treated with surfactant and rhSOD. No rhSOD was detected at 72 h. In separate *in vitro* experiments, physical and biological drug-drug interaction studies were performed. When radiolabeled rhSOD was combined with exogenous surfactant and centrifuged at $10\,000 \times g$ for 30 min, $81.3 \pm 2.5\%$ of rhSOD was found in the supernatant versus $18.7 \pm 2.5\%$ in the surfactant pellet. Serial washing of the surfactant pellet removed virtually all remaining rhSOD. This finding suggests that the rhSOD and surfactant were only weakly associated. Combining rhSOD and exogenous surfactant did not alter the activity of either agent. Data suggest that exogenous surfactant prolongs the $t_{1/2}$ of rhSOD in the lung. This finding may be important in determining future rhSOD administration strategies in preterm infants with respiratory distress syndrome who receive exogenous surfactant replacement therapy. (*Pediatr Res* 35: 37–40, 1994)

Abbreviations

rhSOD, recombinant human Cu/Zn superoxide dismutase
SOD, superoxide dismutase
RDS, respiratory distress syndrome
BPD, bronchopulmonary dysplasia
BAL, bronchoalveolar lavage

in controlled clinical trials (1–6). The beneficial effects occur despite variations in surfactant composition, dose, or administration strategies. However, the significant reductions in oxygen and ventilator requirements after surfactant therapy do not appear to have influenced the overall incidence of BPD.

A promising method for preventing BPD may be prophylactic supplementation of antioxidant enzymes. This strategy seems logical because oxygen radicals appear to play a major role in the pathogenesis of acute and chronic lung injury, and premature infants are known to be relatively deficient in antioxidants at birth (7–9). Exogenous antioxidant supplementation in addition to surfactant therapy may be especially relevant in premature infants because significant SOD activity has been shown to be present in natural lung surfactant and absent in exogenous surfactant preparations (10). Cu/Zn SOD is an antioxidant enzyme whose only known function is to catalyze the dismutation of the highly toxic superoxide radical to molecular oxygen and potentially less toxic hydrogen peroxide. Several animal studies have reported that intratracheal, intravenous, or intraperitoneal administration of SOD (with or without liposome encapsulation or conjugation with polyethylene glycol) can reduce lung injury caused by exposure to prolonged hyperoxia and mechanical ventilation (11–15). Davis *et al.* (11) demonstrated that intratracheal administration of rhSOD prevented the inflammatory changes and acute lung injury caused by exposure to 100% inspired oxygen and mechanical ventilation for 48 h in neonatal piglets. However, the effects of exogenous surfactant therapy on rhSOD activity and pharmacokinetics in the lung have not been well studied. Studying the effects is important because premature infants who are at risk for having BPD develop and who might benefit from rhSOD supplementation often receive surfactant replacement therapy. In this study we examined some of the functional and pharmacologic interactions of exogenous surfactant with rhSOD to determine whether the presence of surfactant alters the function or clearance of rhSOD from the lung.

MATERIALS AND METHODS

Seventy-eight weanling rats (39 males weighing 132.5–156.8 g; 39 females weighing 117.2–137.8 g) were studied as follows: 1) 18 rats received 5 mg/kg of rhSOD (in 1 mL/kg of normal saline) intratracheally, and 6 of them (3 male, 3 female) were killed 24, 48, and 72 h after rhSOD administration; 2) 18 rats received 25 mg/kg of rhSOD (in 1 mL/kg of normal saline) intratracheally, and 6 of them were killed at similar time points; 3) 18 rats received 4 mL/kg of exogenous surfactant intratracheally (Survanta, 25 mg/mL; Ross Laboratories, Columbus, OH) followed 30 min later by the administration of 5 mg/kg of rhSOD intratracheally, and 6 of them were killed 24, 48, and 72 h after rhSOD administration; 4) 18 rats received 4 mL/kg of surfactant followed 30 min later by the administration of 25 mg/kg of

The benefits of surfactant replacement therapy for the treatment of premature infants with RDS have been well documented

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rhSOD, and 6 of them were killed at similar time points; and 5) 6 animals received 1 mL/kg of normal saline and were killed immediately.

Surfactant and rhSOD administration. For exogenous surfactant and rhSOD dosing, animals were lightly anesthetized with methoxyflurane and vertically suspended in an acrylic plastic restrainer. A 6-cm-long speculum (which could fit into the trachea but was too large to enter the mainstem bronchi easily) containing a drop of sterile water was gently inserted into the trachea. Correct placement was confirmed by movement of the fluid in concert with the animal's breathing. A 7.5-cm catheter attached to a syringe containing the dosing material was inserted (no farther than the tip of the speculum) through the speculum, and the dose was administered. The catheter was then flushed with 0.5 mL of normal saline. The catheter and speculum were removed, and oxygen was administered until each animal had sufficiently recovered from the anesthesia.

Animals were killed with pentobarbital, and the chest cavity was opened. Blood samples (for serum rhSOD analyses) were obtained by cardiac puncture. A catheter was then placed into the main pulmonary artery, the left atrium opened, and saline infused into the lungs until the perfusate was clear (to remove rhSOD present in blood). The entire trachea and lungs were removed and then lavaged twice (7 mL/lavage for males and 6 mL/lavage for females). Sixty to 70% of the total lavage volume was always recovered. The lungs were then flash frozen in liquid nitrogen and stored at -20°C until analyzed.

rhSOD concentrations. Serum samples, lavage samples, and lung tissue (homogenized) were analyzed for rhSOD concentrations by RIA using MAb specific for rhSOD. The RIA was performed by first labeling 100 μg rhSOD with ^{125}I using 1 mCi Bolton Hunter Reagent (Amersham, Arlington Heights, IL). The solution was then diluted to 15 mL with PBS with 0.1% BSA. A total of 100 μL of sample or standard (10–2500 μg rhSOD/L) was added to 100 μL of the ^{125}I -labeled rhSOD solution and 100 μL of mouse anti-rhSOD MAb (Bio-Technology General Corp., New York, NY). After the solution was incubated at 4°C for 12 h, 50 μL of rabbit antimouse whole serum (Sigma Chemical Co., St. Louis, MO; 1:40 dilution) was added. This solution was incubated at room temperature for 30 min, and then 300 μL of 20% polyethylene glycol 8000 (Sigma Chemical Co.) was added. After the solution was incubated at room temperature for 20 min, centrifugation was performed at $1500 \times g$ for 20 min. The supernatant was counted in an LKB 1282 Compugamma RIA program.

In vitro studies of surfactant and rhSOD. *In vitro* experiments examining the interaction between surfactant and rhSOD were also performed. The first experiment was designed to test the degree of binding between these two agents when combined. Two mg (in 0.4 mL) of ^{125}I -labeled rhSOD was added to 1 mL (25 mg/mL) of Survanta. After gentle mixing, the suspension was centrifuged at $10\,000 \times g$ at 4°C for 30 min. Concentrations of rhSOD were determined in both the surfactant pellet and the supernatant as previously described. The second series of experiments examined the activity of these two agents after they had been combined. rhSOD (3600 U activity/mg protein) was added to Survanta to achieve final concentrations of 0.5, 5.0, and 50 g/L. rhSOD activity was then analyzed using the reduction of cytochrome C as previously described by McCord and Fridovich (16). Surfactant function was measured using a pulsating bubble surfactometer (17).

Data were analyzed using unpaired *t* tests comparing values from animals treated with rhSOD alone versus those treated with exogenous surfactant followed by rhSOD.

RESULTS

rhSOD was not detected in serum from any of the animals (at any time point) or from lung tissue or BAL in the saline-treated group. This finding suggests that rhSOD was rapidly cleared from

the serum. It also confirmed that the MAb used to detect rhSOD was specific and did not cross-react with rat SOD. Figure 1 shows lung tissue concentrations from the remaining groups of animals. rhSOD was initially measured separately in both the right and left lungs for all animals killed at 24 h to confirm that the drug was delivered to both lungs. Concentrations of drug were similar in both lungs, so the 48 and 72 h groups had measurements made on the entire lung. Data are presented as micrograms of rhSOD detected in the entire lung. At 24 h, lung tissue concentrations were significantly higher (for both dosage groups) in rats treated with surfactant followed by rhSOD compared with rhSOD alone ($p < 0.05$). BAL concentrations of rhSOD correlated well with lung tissue concentrations and were significantly higher ($p < 0.05$) in the surfactant plus rhSOD groups compared with rhSOD alone (Fig. 2).

At 48 h, lung tissue concentrations were negligible in all animals tested. However, rhSOD concentrations were higher only in BAL from animals treated with surfactant plus rhSOD compared with rhSOD alone, but the differences were not statistically different because of wide variability ($p < 0.09$). rhSOD was not detected at 72 h. When rhSOD concentrations were normalized for protein concentration, results were similar for all groups studied.

Because surfactant appeared to delay rhSOD clearance from the lung, *in vitro* experiments examining the drug-drug interaction were performed. When labeled rhSOD was combined with surfactant and sedimented at $10\,000 \times g$ for 30 min, $81.3 \pm 2.5\%$ of counts were found in the supernatant compared with

Fig. 1. The presence of exogenous surfactant significantly increases lung tissue rhSOD concentration 24 h after administration for both the 5 mg/kg and 25 mg/kg groups (* $p < 0.05$). rhSOD was not detected in lung tissue at 48 and 72 h.

Fig. 2. The presence of exogenous surfactant significantly increases BAL rhSOD concentration 24 h after administration (* $p < 0.05$). BAL rhSOD concentration appears to correlate well with lung-tissue rhSOD concentration.

18.7 $\pm$ 2.5% in the surfactant pellet ($p < 0.001$). Resuspending the surfactant pellet in 1 mL of buffered saline and repeating the sedimentation a second time removed virtually all remaining rhSOD. Combining rhSOD and surfactant did not alter the activity of either agent. rhSOD activity remained at 100%, and the rhSOD-surfactant suspension was still able to reduce surface tension to <1 dyne/cm on the oscillating bubble apparatus.

DISCUSSION

The present study demonstrates that rhSOD treatment resulted in definite (equivalent) distribution to both lungs whether or not exogenous surfactant was present. The administration of surfactant 30 min before rhSOD resulted in higher concentrations of rhSOD in both BAL and lung tissue than when rhSOD was given without surfactant. We have previously demonstrated in newborn piglets that exogenous surfactant appears to distribute completely and relatively homogeneously within 30 min of administration (18). In the present study, rhSOD was administered 30 min after exogenous surfactant to minimize the impact of the surfactant administration process on rhSOD pharmacokinetics. Surfactant appears to delay the clearance or metabolism of rhSOD from the lung for at least 24–48 h. It is possible that rhSOD becomes entrapped in the lipid bilayer of surfactant. This association appears to be weak and reversible because virtually all the rhSOD can be easily separated from the surfactant by simple sedimentation. Notably, although surfactant administration was able to increase pulmonary concentrations of rhSOD significantly at 24 h, only 4–5% of the total administered dose could be recovered (in lung tissue and BAL) from the surfactant and rhSOD group *versus* only 1% in rats treated with rhSOD alone. This result is most likely related to the accelerated clearance or metabolism of rhSOD in more mature animal species compared with neonatal animals.

The success of pulmonary surfactant preparations in decreasing mortality and morbidity in premature neonates with RDS has resulted in their widespread use. However, surfactant therapy has not eliminated associated complications from oxygen therapy and mechanical ventilation. Among these complications is BPD, the incidence of which has remained unchanged in controlled studies of surfactant efficacy (1–4, 6). To prevent complications such as BPD, further refinements in the treatment of neonates receiving surfactant therapy will be necessary. The initial development of surfactant centered on its surface-reducing properties necessary for proper ventilation. However, it has become increasingly apparent that natural surfactant's structure and function is complex and that further modifications of exogenous surfactant could improve efficacy. For example, several different proteins found associated with surfactant (*i.e.* surfactant-associated proteins) have been shown to influence function (19). Other protein constituents associated with natural surfactants such as SOD and catalase may be important in protection from the toxic effects of oxygen by scavenging free radicals and enhancing intracellular antioxidant enzyme activity (10). Because natural surfactant appears to have significant antioxidant properties (which are absent in commercial surfactant preparations), it seems logical that the next generation of exogenous surfactants should possess rhSOD activity (10).

Oxygen derived free radicals probably have an important role in the pathogenesis of acute and chronic lung injury (7, 9, 20). The premature neonate may be at increased risk because hyperoxia, mechanical ventilation, and inflammation provide increased exposure of the lung to free radicals. In addition, the premature lung may not have developed sufficient antioxidant defenses required for the transition to the relative hyperoxia of extrauterine life (7). The use of rhSOD in premature infants with RDS represents a rational approach to the prevention of further lung damage in neonates who also receive exogenous surfactant. The interactions between surfactants and rhSOD must be better

understood to design the most efficacious regimen of administration.

Some investigators have suggested that antioxidants, including SOD and catalase, should be delivered to the lung entrapped in liposomes (21, 22). This delivery is thought to be necessary to delay the rapid clearance of SOD and to enhance cellular uptake. The clearance of SOD from blood is through the kidneys and is rapid in most species ($t_{1/2}$ 3–8 min) (23). In the present study, rhSOD was not detected in serum even when it was present in lung tissue and BAL fluid. This finding suggests that the rhSOD is cleared rapidly from serum because renal excretion is the only known pathway for SOD elimination. However, clearance or metabolism of rhSOD in the lung appeared to be far slower. When given with surfactant, rhSOD was detected in lung tissue and BAL fluid at 24 h with both the 5 and 25 mg/kg doses and at 48 h in BAL fluid only with the 25 mg/kg dose. In previous studies, neonatal piglets given a single 5 mg/kg intratracheal dose of rhSOD (without surfactant) and mechanical ventilation with 100% oxygen had 25.0 $\pm$ 6.7 mg/L of rhSOD in BAL and 0.5 $\pm$ 0.2 μ g rhSOD/mg protein in lung tissue 48 h after administration (11). rhSOD was detected in serum (peaking 12 h after administration) and urine, which suggests that significant amounts of rhSOD move from the lung to the blood and are eventually cleared via the kidney.

Variations in pulmonary rhSOD levels may be the result of differences in clearance and metabolism of the species tested. As previously mentioned, mature animals (*i.e.* rats) may clear or metabolize rhSOD more rapidly than neonatal animals such as piglets. In newborn animals (*i.e.* neonatal piglets), where clearance or metabolism is slower, lung tissue, BAL fluid, and serum levels are maintained for longer periods of time. Previous studies examining the pharmacokinetics of *s.c.* administered bovine SOD in human premature neonates showed a much longer $t_{1/2}$ of SOD than expected, ranging from 3–6 h (24, 25). Bovine SOD is cleared more slowly in premature neonates through the blood and kidney, so the antioxidant may be present both in blood and lung for even longer periods compared with full-term animal models. On the basis of data in the present study, extensive pharmacokinetic studies examining the interaction of surfactant and rhSOD are currently being performed in premature neonates to determine whether doses of rhSOD will need to be adjusted in neonates who received surfactant replacement therapy for treatment of RDS.

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