

Long-Term Follow-up of Premature Infants Treated With Prophylactic, Intratracheal Recombinant Human CuZn Superoxide Dismutase

Jonathan M. Davis, MD
Susan E. Richter, MPA
Saumitra Biswas, MD
Warren N. Rosenfeld, MD
Lance Parton, MD
Ira H. Gewolb, MD
Richard Parad, MD
Waldemar Carlo, MD
Robert J. Couser, MD
Stephen Baumgart, MD
Vijaya Atluru, MD
Lisa Salerno, BS
Nadim Kassem, MD

OBJECTIVE:

To examine the long-term effects of treatment with recombinant human CuZn superoxide dismutase (rhSOD) in infants enrolled previously in two placebo-controlled trials.

STUDY DESIGN:

Records for 46 (88%) infants were examined, with 19 infants having received either single or multiple intratracheal (i.t.) doses of placebo, 12 having received a single i.t. dose of rhSOD, and 15 having received multiple i.t. doses of rhSOD. Mean age at follow-up was 28 months corrected age. Records were examined for neurologic dysfunction, developmental delay, and any significant medical disorders.

RESULTS:

Four placebo infants (21%) had evidence of neurodevelopmental abnormalities and four infants developed asthma. Four single-dose rhSOD infants (33%) had neurodevelopmental abnormalities and two infants developed asthma. One multiple-dose rhSOD infant had evidence of

neurodevelopmental abnormalities and one developed asthma. No other differences were found between the placebo and rhSOD groups.

CONCLUSION:

Preliminary data suggest that rhSOD is safe and not associated with any long-term adverse effects. Further results will depend on the results of multicenter trials of rhSOD in preterm infants.

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Oxygen free radical injury has been implicated in the pathogenesis of a wide variety of conditions that may affect newborns. This is not surprising, because newborns (especially premature infants) are uniquely susceptible to free radical injury. Premature infants are exposed to supraphysiological oxygen concentrations compared with the intrauterine environment and may experience reperfusion injury after periods of hypoxia or ischemia. In addition, newborns may have inadequate defenses against free radical injury. Free radicals can cause damage to both the pulmonary and central nervous systems, resulting in significant long-term sequelae. Bronchopulmonary dysplasia (BPD) can develop in up to 40% of premature neonates with birth weights of <1500 gm who are treated with oxygen and mechanical ventilation.¹ The development of BPD is associated with increased mortality and morbidity, such as repeated hospitalizations and neurodevelopmental handicaps.² Intraventricular hemorrhage (IVH) and/or periventricular leukomalacia can also develop in 20% to 40% of very low birth weight infants, resulting in long-term neurodevelopmental deficits such as cerebral palsy.³ Treatment directed at preventing free radical injury may decrease the incidence and severity of these conditions and improve long-term outcome.

Superoxide dismutases (SODs) are ubiquitous antioxidant enzymes that have been shown to prevent free radical injury due to hyperoxia and reperfusion. Several animal studies have demonstrated that SOD treatment mitigated lung injury from prolonged hyperoxia and mechanical ventilation.^{4–6} SOD administration has also been found to decrease the incidence of IVH and brain injury in animal studies.⁷ rhSOD is a human recombinant CuZn SOD that has been given to premature infants with respiratory distress syndrome (RDS) in an attempt to prevent oxidant-induced lung injury and the development of BPD.

Department of Pediatrics (J. M. D., S. E. R., S. B., W. N. R., V. A.), Winthrop University Hospital, Mineola, NY; University Hospital (L. P.), Stony Brook, NY; University of Maryland Medical Center (I. H. G.), Baltimore, MD; Brigham and Women's Hospital (R. P.), Boston, MA; University of Alabama Medical Center (W. C.), Birmingham, AL; Children's Healthcare (R. J. C.), Minneapolis, MN; Thomas Jefferson University (S. B.), Philadelphia, PA; and Bio-Technology General Corporation (L. S., N. K.), Iselin, NJ.

Address correspondence and reprint requests to Jonathan M. Davis, MD, Department of Pediatrics, Winthrop University Hospital, 259 First Street, Mineola, NY 11501.

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Table 1 Patient Population

	Single-dose rhSOD (750–1250 gm)			Multiple-dose rhSOD (700–1300 gm)		
	Infants enrolled (<i>n</i>)	Survivors (<i>n</i>)	Records available (<i>n</i>)	Infants enrolled (<i>n</i>)	Survivors (<i>n</i>)	Records available (<i>n</i>)
Placebo	11	10	10	11	10	9
Low-dose rhSOD	8	6	5	11	9	7
High-dose rhSOD	7	7	7	11	10	8
Total	26	23	22 (96%)	33	29	24 (83%)

Two previous placebo-controlled pilot studies have been conducted in premature infants (700 to 1300 gm) with RDS using either single or multiple intratracheal (i.t.) doses of rhSOD.^{8,9} These studies have established the short-term safety of rhSOD and have suggested that early inflammatory changes in the lung, which appear to be associated with the development of BPD, can be significantly reduced. The current study prospectively examines the longer-term neurodevelopmental and pulmonary outcomes of infants who received i.t. rhSOD.

METHODS

Subjects

Premature infants who were enrolled previously in two placebo controlled rhSOD trials were studied. The first study was conducted at two participating hospitals from April to December of 1993. A total of 26 infants with RDS received a single i.t. dose of placebo ($n = 11$), a very low (test) dose of rhSOD (0.5 mg/kg per dose; $n = 8$), or a therapeutic dose of rhSOD (5.0 mg/kg per dose; $n = 7$) within 2 hours of surfactant administration. The 5.0-mg/kg dose had been established as an effective therapeutic dose in animal studies.⁴ The second study was conducted at six participating hospitals from November of 1994 to June of 1995. A total of 33 infants received multiple i.t. doses of placebo or rhSOD (2.5 or 5.0 mg/kg per dose) within 2 hours of surfactant therapy and every 48 hours thereafter (while requiring intubation and mechanical ventilation) for up to seven doses.

Survivors were initially evaluated prospectively in each center's neonatal follow-up program. Examinations included comprehensive neurodevelopmental and medical assessments. Only one of the infants in the study had no formal follow-up data available. Neurodevelopmental assessments were conducted by pediatric neurologists, pediatricians, or developmental psychologists who were not aware of treatment assignment. Neurologic examinations included evaluation of muscle tone and of the power and persistence of newborn reflexes and were considered abnormal if hypertonia or hypotonia, spastic diplegia, quadriplegia, increased reflexes, and/or delays in speech or language were detected. Formal developmental assessments varied depending on the center and included Bayley Scales of Infant Development or Denver Developmental Assessments. Both the neuro-

logic examination and developmental testing were included in the grading of neurodevelopmental abnormalities. Neurodevelopmental problems were graded as none, mild, moderate, or severe. Mild abnormalities were defined as mild motor abnormalities or mild speech/language delay without any significant functional impairment. Moderate abnormalities were defined as more significant motor abnormalities with evidence of developmental delay (<6 months). Severe abnormalities were defined as significant motor abnormalities (spastic diplegia or quadriplegia) with global developmental delay (>6 months). All infants with suspected neurodevelopmental abnormalities were classified in the mild category, because there are no accepted minimal diagnostic criteria for mild cerebral palsy.

To improve accuracy, we continued to obtain neurodevelopmental data as the infants got older. Frequently, formal follow-up data were no longer available (family had moved, infant had been discharged from the follow-up program or enrolled in an early intervention program, etc.), so detailed information was obtained from parents and confirmed by their pediatricians. This was critical, because a few infants who had significant neurodevelopmental delays at 1 year of age had only mild neurodevelopmental abnormalities by 3 to 5 years of age.

The development of any significant medical disorders requiring therapeutic intervention or rehospitalization was also recorded. Diagnoses of asthma or other chronic pulmonary disorders were made using criteria described previously by Palta et al.¹⁰ and included the use of bronchodilators and/or steroids for treatment of wheezing and respiratory distress.

Statistical Analyses

Differences in the incidence of neurodevelopmental abnormalities and medical disorders between the three experimental groups were analyzed with Fisher's exact test, with 3×2 tables being constructed. Analyses were also performed to confirm that the experimental groups were comparable for birth weight, gestational age, race, gender, use of antenatal and postnatal steroids, incidence of IVH, and other complications in the perinatal period (analysis of variance with multiple comparison testing, correlation analyses).

Table 2 Classification of Neurodevelopmental Abnormalities

	<i>n</i>	Neurodevelopmental problems			
		None	Mild	Moderate	Severe
Placebo	19	15	3	0	1
rhSOD (single dose)	12	8	2	0	2*
rhSOD (multiple dose)	15	14	1	0	0

* All in the single, low-dose (0.5 mg/kg) group.

RESULTS

In the two studies, 52 of 59 infants (88%) survived to hospital discharge. Follow-up data for 46 (88%) of the 52 surviving infants were available (Table 1). The mean birth weight and gestational age for infants in the placebo group was 931 ± 263 gm and 27.5 ± 1.8 weeks, respectively, and this was not significantly different between the other groups. In addition, there were no differences between groups with respect to gender and race. Sixty-one percent of placebo babies were exposed to antenatal steroids compared with 18% of single-dose infants and 79% of multiple-dose infants ($p < 0.01$, Fisher's exact test, 3×2 tables, with group 2 different from groups 1 or 3). Fifty-five percent of placebo infants received postnatal dexamethasone for an average of 10.1 ± 2.8 days, 100% of single-dose infants were treated for 24.4 ± 1.3 days, and 36% of multiple-dose infants were treated for 1.9 ± 1.1 days ($p < 0.001$ analysis of variance, with group 2 different from groups 1 or 3). The incidence of IVH and other complications (i.e., pneumothorax, etc.) was not different between the groups.

Mean age at follow-up was 28.1 months corrected age (range 6 to 60 months). Four infants (21%) from the placebo group had demonstrable neurodevelopmental abnormalities (Table 2). Three infants had mild abnormalities, which included one patient with upper extremity hypotonia, one with lower extremity hypertonia, and one with speech/language delay. The patient with severe involvement had spastic quadriplegia with global developmental delay. Four infants (33%) from the single-dose rhSOD group had neurodevelopmental abnormalities, with two infants having mild disabilities and two infants having severe disabilities. One infant had mild hypertonia, one infant had mild speech/language delay, and two infants had spastic diplegia. It is important to note that the two infants in this group with the most severe functional abnormalities all received a single i.t. test dose of 0.5 mg/kg of rhSOD at birth (with 5.0 mg/kg being the target dose). One of the 15 infants who had received multiple i.t. doses of rhSOD had neurodevelopmental abnormalities. This infant had evidence of mild speech/language delay requiring weekly therapy. There was no correlation between the presence of an IVH and the development of any neurodevelopmental disorder.

Pulmonary problems that developed along with the need for repeated hospitalizations are shown in Table 3. Four infants (21%) in the placebo group, two infants (17%) in the single-dose rhSOD treat-

Table 3 Total Neurodevelopmental and Medical Problems

	Total neurodevelopmental problems <i>n</i>	Medical problems		
		Asthma	Hypothyroid	Hospital admissions*
Placebo	4	4	1	2
rhSOD (single dose)	4	2	0	4
rhSOD (multiple dose)	1	1	1	2

* Bronchiolitis, presumed sepsis, Kawasaki's disease.

ment group, and one infant (7%) in the multiple-dose rhSOD group were treated for chronic respiratory disorders (asthma). Two infants were rehospitalized in the placebo group, four in the single-dose rhSOD group, and two in the multiple-dose rhSOD group. Infants were re-admitted for conditions such as bronchiolitis, presumed sepsis, and Kawasaki's disease. One infant in the placebo group and one in the single-dose rhSOD group had both mild neurodevelopmental and chronic pulmonary abnormalities.

When the presence of neurodevelopmental and/or chronic pulmonary abnormalities were combined (Table 3), differences approached significance. In the placebo group, 7 of 19 (37%) infants had either neurodevelopmental or chronic respiratory problems, whereas 5 of 12 (42%) infants in the single-dose group and 2 of 15 (13%) infants in the multiple-dose group had similar difficulties (Fisher's 3×2 contingency table: $p = 0.06$; one-tailed Student's *t*-test).

DISCUSSION

Oxygen free radical injury has been implicated in the pathogenesis of a wide variety of medical conditions that may affect newborn infants. This includes chronic lung disease and central nervous system injury.¹¹ At birth, premature infants are exposed to significantly higher oxygen concentrations compared with the intrauterine environment and may develop medical conditions (i.e., RDS) that require treatment with even higher concentrations of inspired oxygen. In addition, premature infants may experience reperfusion injury after periods of hypoxia and/or ischemia and may have inadequate antioxidant defenses to handle these oxidant challenges.

A rational approach for the prevention of such injury is the augmentation of antioxidant enzyme activity. The use of rhSOD in particular is based on extensive cell culture, animal experiments, and limited experience in premature infants.¹² Two pilot studies have shown that single and multiple i.t. doses of rhSOD given to premature infants (700 to 1300 gm) with RDS resulted in significant increases in SOD concentration and activity in serum, tracheal aspirates, and urine.^{8,9} A variety of cell and biochemical markers of inflammation in tracheal aspirates that appear to be associated with the development of BPD

were significantly reduced in rhSOD-treated infants compared with placebo controls. The drug appeared to be well tolerated in the dosage ranges used (0.5 to 5.0 mg/kg) and not associated with any demonstrable short-term abnormalities. However, the long-term effects of the administration of pharmacological doses of rhSOD have never been evaluated.

A variety of other therapeutic strategies have been used in an attempt to prevent or ameliorate the development of BPD. Vitamin E therapy was not found to be efficacious and significantly increased the incidence of sepsis and necrotizing enterocolitis in pharmacological doses.¹² The early use of high-frequency jet ventilation has been shown to reduce the incidence of BPD and neurologic injury in premature infants, but may actually worsen neurodevelopmental outcome if not used properly.¹³ Dexamethasone is a therapeutic agent that has been extensively studied in premature infants at risk for developing BPD.¹ Dexamethasone has been shown to improve short-term pulmonary status but appears to increase the risk of death and/or neurodevelopmental abnormalities at long-term follow-up.¹⁴

In the present study, neither single nor multiple doses of rhSOD appeared to increase the risk for adverse pulmonary or neurodevelopmental outcome. Although the present studies represent the first time that premature infants have received recombinant human antioxidants, definitive results are limited by the relatively small number of patients enrolled in these pilot studies. However, the results are encouraging and warrant continued evaluation of rhSOD to ameliorate short- and long-term morbidity in this vulnerable group of infants.

Pulmonary injury caused by oxygen radical injury has been shown to be ameliorated by pretreatment with SOD,^{4–6} which may be important in the development of chronic respiratory disorders such as asthma in infants. SOD has also been shown to prevent ischemia/reperfusion injury and neurologic damage in animal models of IVH, suggesting that oxygen radical injury is important in the pathogenesis of neurologic injury in premature infants.^{7,15,16} Preliminary reports from a recently completed multicenter, placebo-controlled trial of multiple doses of rhSOD in 301 very low birth weight infants indicate that rhSOD treatment is associated with approximately a 60% decrease in the incidence of severe (grades III and IV) IVH and periventricular leukomalacia, which is known to affect long-term neurodevelopmental outcome.^{3,11,12,17} Further demonstrable evidence of the role of rhSOD in preventing severe neonatal pulmonary and neurologic injury will need to await both the short-term and long-term results of multicenter controlled trials with larger number of enrolled infants.

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