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# Pharmacokinetics and tolerability of ventricularly administered superoxide dismutase in monkeys and preliminary clinical observations in familial ALS

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**Abstract**

The discovery of mutations in the gene for Cu/Zn superoxide dismutase (SOD) in some cases of familial amyotrophic lateral sclerosis (FALS) provides a rationale for enzyme replacement therapy. The inability of SOD to cross the blood–brain barrier motivated this study of the safety, tolerability and pharmacokinetics of bovine SOD (bSOD) administered into the CSF of rhesus monkeys and one late-stage, SOD-deficient FALS patient. Kinetic analyses in the patient indicated that intracerebroventricular (i.c.v.) administration, but not lumbar administration, delivered bSOD to the entire CSF pathway. Daily bolus i.c.v. injections (32 mg/day) and continuous i.c.v. infusion (30 mg/day) were well tolerated by the patient. During the period of daily bolus injections, the patient's performance on manual muscle tests was nearly stable, in contrast with the rapid decline before and after that period. These results justify further investigation of bSOD therapy in SOD-deficient FALS patients.

*Keywords:* Amyotrophic lateral sclerosis; Cerebrospinal fluid; Intracerebroventricular infusion; Muscle; Neurodegeneration; Orgotein; Superoxide dismutase

## 1. Introduction

Amyotrophic lateral sclerosis (ALS) results from the progressive degeneration of motor neurons in the cortex, brain stem and spinal cord and occurs in both sporadic and familial forms. Estimates of the percentage of ALS cases that are familial (FALS) range from 3% (calculated from Mulder et al., 1986) to 10% (Deng et al., 1993). Most FALS cases show an autosomal dominant inheritance pattern (Kurland and Mulder, 1955a,b; Horton et al., 1976; Emery and Holloway, 1982) and some have mutations in the gene for Cu/Zn superoxide dismutase (SOD, EC 1.15.1.1) (Rosen et al., 1993; Deng et al., 1993; Rosen et al., 1994). The discovery of these mutations provides a rationale for attempting enzyme replacement therapy in such patients. Moreover, the similar clinical course and histopathology of sporadic ALS and FALS, whether or not there is an SOD gene mutation, suggest that common molecular mechanisms, and hence a common approach to treatment, may apply to all forms of this devastating disease (Bowling et al., 1993).

Damage due to free radicals has been invoked as an etiologic factor in a number of neurodegenerative diseases including ALS, Parkinson's disease, and Alzheimer's disease (Halliwell, 1992; Olanow, 1992; Jenner, 1994; Figlewicz et al., 1994; Hensley et al., 1994). Free radicals are chemical species which contain unpaired electrons and are generally highly reactive. Non-enzymatic molecules involved in protection against free radical and oxidative damage, e.g. $\alpha$-tocopherol (vitamin E), have not been shown to modify the course of ALS (Doyle and Merritt, 1941; Dorman et al., 1969).

Cu/Zn superoxide dismutase plays a central role as a scavenger of free radicals (McCord and Fridovich,

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1969). This cytoplasmic enzyme, like the mitochondrial and extracellular forms of SOD, catalyzes the conversion of the superoxide radical ($O_2^{-\cdot}$) to hydrogen peroxide ($H_2O_2$), which can be converted to water by glutathione peroxidase or catalase (Warner, 1994). Superoxide is formed both as a normal product of oxidative metabolism and by the reaction of oxygen with a variety of organic radicals (Winterbourn, 1993). Superoxide is involved in the generation of hydroxyl radicals ($\cdot OH$) and reacts with nitric oxide ($NO\cdot$) to form the powerful oxidant, peroxynitrite ($ONOO^-$) (Beckman, 1991; Beckman et al., 1993). Hydroxyl radicals and peroxynitrite are more reactive with proteins, nucleic acids and unsaturated fatty acids than superoxide itself (Warner, 1994). Thus, by scavenging superoxide radicals, SOD can decrease the concentrations of both the organic radical precursors of superoxide and certain reactive oxygen species derived from it. The selective vulnerability of neurons to deficits in SOD may be due, in part, to the destructive effects of peroxynitrite and the relatively high concentrations of its precursor, nitric oxide, in neurons (Warner, 1994).

The use of SOD and other proteins to treat CNS disorders such as ALS is complicated by the presence of the blood–brain barrier (Smith et al., 1985), but it is possible to achieve high concentrations of bovine SOD (bSOD) in the CNS by direct administration into the spinal fluid (Huber and Saifer, 1977). Therefore, this study was undertaken to assess the safety, tolerability and pharmacokinetics of bSOD administered into the cerebrospinal fluid (CSF) of rhesus monkeys and a FALS patient with an SOD gene mutation. Dose levels for this preliminary study were chosen to provide CSF concentrations corresponding to normal SOD concentrations in brain tissue, i.e. 60 $\mu$g/g (calculated from McCord and Fridovich, 1969), which are two to three orders of magnitude higher than the SOD concentrations reported for spinal fluids (Marklund, 1980; Lund-Olesen, 1985; Bracco et al., 1991; Laboyrie et al., 1992; Iwasaki et al., 1993). Both intracerebroventricular (i.c.v.) and lumbar routes of administration were investigated in an effort to provide pharmacologic levels of bSOD throughout the CSF pathway.

Clinical experience with bSOD includes more than 12 million injections of 4 or 8 mg, most of which were intra-articular or intramuscular (Oxis International, 1994). In addition, 82 patients in a Danish study received up to six intrathecal injections of 2–8 mg (Lund-Olesen, 1985) and 15 patients in Spanish studies received continuous intrathecal infusions of up to 66 mg/day for up to 5 years (Richard Penn, personal communication). Other than rare hypersensitivity reactions, independent of route, and transient discomfort following intrathecal bolus administration, the bSOD treatments were well tolerated. This report describes the first experience with i.c.v. administration of bSOD.

## 2. Methods

### 2.1. SOD preparation and assay

The formulation of bovine Cu/Zn SOD used in this study was Orgotein for Injection, supplied by Oxis International, Inc., Mountain View, CA. The US Food and Drug Administration (FDA) has designated orgotein an Orphan Drug for the treatment of FALS associated with an SOD mutation. Total SOD activity was measured by a colorimetric nitroblue tetrazolium reduction assay (Sun et al., 1989). Enzyme units were converted to SOD concentrations ($\mu$g/ml) based on a reference standard (McCord and Fridovich, 1969). The presence of significant endogenous human or monkey SOD was excluded by gel electrophoresis with zymogram staining (Beauchamp and Fridovich, 1971).

### 2.2. Monkeys

Rhesus monkeys (*Macacca mulatta*) weighing 7–12 kg had two intraventricular catheter systems implanted, as described by McCully et al. (1990). A Pudenz catheter, implanted in the fourth ventricle and connected to a subcutaneous Ommaya reservoir, was used for bolus administration of bSOD and repetitive CSF sampling in unanesthetized animals. A lateral ventricular catheter, connected to a Model 600 Infusaid pump (Infusaid, Inc., Norwood, MA), was used for continuous infusion of bSOD solutions. Lumbar CSF was sampled through a temporary catheter. The monkeys were prepared and maintained at the Pediatric Branch of the National Cancer Institute, Bethesda, MD, in accordance with the Department of Health Education and Welfare Guide for the Use and Care of Laboratory Animals.

### 2.3. Clinical studies

The 46-year-old patient, who had a family history of ALS, first became symptomatic in early 1993 and was found to have a mutation in exon 1 of the SOD gene (Deng et al., 1993). Rather rapidly, the patient began to lose function, first in the upper extremities and then in the lower limbs. By the middle of 1993, the more affected arm was completely paralyzed and bulbar symptoms were evident. A variety of treatments, including antioxidants, dextromethorphan and ciliary neurotrophic factor (Brooks et al., 1994) were of no apparent benefit. By the end of 1993, the patient's condition had deteriorated to the point that the full-time use of a respirator and nutrition via gastrostomy were required. After receiving the informed consent of the patient, an Investigational New Drug exemption from the FDA, and approval of the Institutional Review Board of Scripps Memorial Hospital, La Jolla,

CA, a surgical team placed a catheter in the right lateral ventricle which was connected to an Ommaya reservoir. A lumbar catheter was used to administer the drug intrathecally and to obtain spinal fluid samples.

Prior to the start of bSOD treatment, a skin test for hypersensitivity was performed. On Study Day 1, a 1-mg i.c.v. bolus test dose of bSOD was administered. Thereafter, the daily bolus dose was increased, generally in 2-fold steps, to 16 mg and, after 20 days at that dose, to 32 mg. On Study Day 51, a Medtronic Model 8611H SynchroMed infusion pump (Medtronic Inc., Minneapolis, MN) was implanted subcutaneously in the abdomen. After removal of the Ommaya reservoir, a catheter was tunneled subcutaneously to connect the pump to the remaining intraventricular catheter. Instructions were communicated to the pump via radiotelemetry. The pump was filled with 18 ml of bSOD solution (32 mg/ml), usually at 2-week intervals, and was adjusted to deliver between 25 and 50 mg/day.

Due to the degree of functional impairment at the start of bSOD therapy, it was not possible to monitor the patient's response to treatment using a quantitative neuromuscular examination, e.g. the Tufts Quantitative Neuromuscular Exam (Andres et al., 1986). Accordingly, the principal method of quantification was a physical therapist's assessment of the patient's strength using a modification of the Medical Research Council (1942) scoring system, in which 0 = no muscle strength, 1 = no movement against gravity, 2 = mild resistance, 3 = firm resistance and 4 = normal muscle strength. These measurements were performed approximately once each month during the 5-month study on muscles of the right and left shoulder, elbow, wrist hip, knee and ankle.

## 3. Results

### 3.1. Safety and tolerability studies

It was first established that single bolus i.c.v. doses of 0.5 or 2.5 mg bSOD were well tolerated in monkeys. A subchronic i.c.v. bolus study was then undertaken, in which 0.5-mg doses of bSOD (equivalent to about 5 mg in an adult human) were administered twice weekly for four weeks. During six weeks of monitoring cage activity, blood chemistry and hematology, and ventricular CSF chemistry and hematology, there were no clinical signs of toxicity or significant changes in body weight ($7.3 \pm 0.1$ kg). Clinical chemistry, including CSF protein concentration (6–16 mg/dl), and hematology, including leukocyte counts in CSF (1–7 cells/$mm^3$), were normal for monkeys with indwelling ventricular catheters (McCully et al., 1990). In a separate study, continuous infusion of bSOD (2.5 mg/day) into a lateral ventricle for 28 days was well tolerated by the monkey. There were no significant abnormalities in blood chemistry or hematology or in the tested properties of ventricular CSF samples.

Administration of bSOD was also well tolerated by the patient and there was no apparent systemic reaction. At no time was the patient febrile, and at no time did (s)he complain of headache, nausea, or malaise. No signs of hypersensitivity to bSOD were observed. Interpretation of the analyses of the patient's CSF samples was compromised by the occurrence of microscopic bleeding associated with placement of the intraventricular catheter. The time course of this bleeding indicated that it was not causally linked to the administration of bSOD. An increased leukocyte count (up to 10 cells/$mm^3$) was occasionally seen in the lumbar or ventricular CSF. While the protein concentration in ventricular CSF remained normal, the protein concentration in lumbar CSF increased gradually, reaching a maximum of 165 mg/dl on Study Day 139.

### 3.2. Pharmacokinetics in monkeys

In two rhesus monkeys given bolus i.c.v. doses of 0.5 or 2.5 mg bSOD, the initial ventricular half-life was about 1.3 h, and the second kinetic component had a half-life of 7–8 h. Within two hours of ventricular administration, the bSOD concentration in lumbar CSF exceeded the contemporaneous ventricular concentration. Thereafter, the bSOD half-life in lumbar CSF (7 h) was similar to the second clearance half-life in ventricular CSF. In another study, one monkey received a continuous i.c.v. infusion of bSOD (2.5 mg/day, equivalent to 25 mg/day in an adult human) for 28 days into a lateral ventricle. Steady-state levels were achieved within 10 days, after which the lumbar and ventricular CSF levels were similar, ranging from approximately 70 to 80 $\mu$g/ml. All measurements of bSOD concentration in lumbar CSF were within one standard deviation of the contemporaneous concentration in ventricular CSF.

### 3.3. Pharmacokinetic and functional studies in the patient

Clearance kinetics were evaluated following both lumbar and i.c.v. administration of bSOD to the FALS patient. Following a bolus injection of 16 mg of SOD through a lumbar catheter, the half-life of bSOD in the lumbar space was 7–8 h and very little bSOD was detected in the lateral ventricle. In contrast, within 2 h of the introduction of 16 mg of bSOD intraventricularly, a high concentration of bSOD was detected in the lumbar CSF (Fig. 1). Under these conditions, the

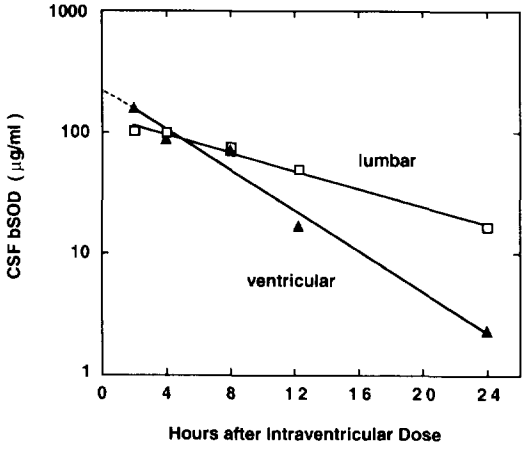


Fig. 1. Clearance of bSOD from CSF of a familial ALS patient after a bolus injection of 16 mg into the right lateral ventricle. ▲ = ventricular concentrations ($r^2 = 0.977$); □ = lumbar concentrations ($r^2 = 0.989$).

observed pseudo-first order half-lives were 3–4 h in ventricular CSF and 8 h in lumbar CSF.

Because the pump that was implanted in the patient had no sampling port, ventricular concentrations of bSOD could not be monitored during continuous infusion. Seven samples of lumbar CSF for bSOD assay were obtained during infusion of 25 or 50 mg/day, between Study Days 65 and 81 (see Fig. 2, top). Unexpectedly, analyses of these samples revealed bSOD concentrations of $< 1$ μg/ml. Subsequently, after surgical revision of the ventricular catheter on Study Day 102 and continuous infusion of bSOD (30 mg/day) starting on Study Day 109, nearly identical, high concentrations ($82 \pm 5$ and $84 \pm 5$ μg/ml) were found in lumbar CSF samples taken on Study Days 124 and 138, respectively.

During the period of daily bolus bSOD administration, muscle strength, tested on Study Days 12 and 42, was nearly stable, but it declined rapidly during the initial period of continuous infusion, before revision of the ventricular catheter (Fig. 2, bottom).

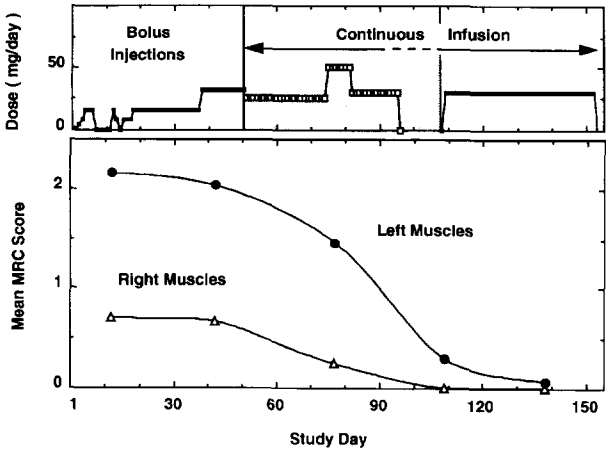


Fig. 2. Muscle testing during i.c.v. treatment of a familial ALS patient with bSOD. Top: daily doses of bSOD. From Day 1 to 50, bSOD was administered as bolus injections into the right lateral ventricle via an Ommaya reservoir. From Day 51 to 95, the indicated doses were infused continuously by a SynchroMed pump, but the catheter outlet was apparently not in the ventricle. After surgical revision of catheter placement on Day 102, the pump was refilled on Days 109, 124 and 138, and delivered 30 mg/day into the ventricle until the study was terminated on Day 153. Shading indicates the period when the bSOD concentration in lumbar CSF was $< 1$ μg/ml. Bottom: mean scores, based on a modification of the Medical Research Council (MRC) scale, of tests of the following right and left muscle groups: shoulder flexor, extensor and abductors; elbow flexor and extensor; wrist flexor and extensor; hip flexor and extensor; knee flexor and extensor, and ankle plantarflexor and dorsiflexor.

## 4. Discussion

This preliminary study of SOD replacement therapy in ALS was motivated by reports of decreased SOD activity in the CSF of some patients with sporadic ALS as well as familial ALS (Bracco et al., 1991; Iwasaki et al., 1993) and the discovery of defects in the gene encoding this enzyme in some of the familial cases (Rosen et al., 1993; Deng et al., 1993; Rosen et al., 1994). However, therapy of CNS disorders using a locally-acting enzyme like SOD requires the use of intrathecal or intraventricular delivery to by-pass the blood–brain barrier. The present results indicate that it is possible to maintain stable, high concentrations of bSOD in the CSF by continuous i.c.v. infusion. This administered enzyme might protect the neurons from free radical damage by serving as an extracellular free radical "sink" (Winterbourn, 1993), even if it is not internalized by the neurons.

Injection of bSOD intrathecally and intraventricularly was well tolerated by both the monkeys and the patient, whether the drug was administered as a bolus or by continuous infusion. The finding of elevated protein concentrations in the patient's lumbar CSF samples after several weeks of continuous i.c.v. infusion may not be related to the bSOD treatment, since high protein concentrations in the CSF of nine of 26 untreated FALS patients have been reported previously (Mulder et al., 1986). Indeed, the drug was so well tolerated that the patient described here has requested and received i.c.v. infusions of bSOD during more than seven months following the completion of the present study.

Clearance of bSOD administered intraventricularly to monkeys and the patient was slower than that observed previously in rats (Huber and Saifer, 1977). Clearance of bSOD from monkey ventricular CSF showed complex kinetics, with an initial phase having a half-life of 75–80 min (as in rats) and a second phase

(not observed in rats) with a half-life of 7–8 h. The available data from the patient, obtained starting 2 h after a bolus i.c.v. dose (Fig. 1) or 4 h after a bolus lumbar dose (not shown), provided no evidence for an initial rapid clearance phase from either compartment, and indicated a half-life of 7–8 h for bSOD in lumbar CSF, regardless of the route of administration.

Following lumbar administration to the patient, very little bSOD was detected in the ventricular CSF ( < 8% of lumbar concentrations). On the other hand, high lumbar CSF concentrations were achieved after i.c.v. administration (Fig. 1). These results imply that i.c.v. administration is the preferred route for delivering bSOD to the entire CSF pathway. This conclusion is consistent with the downward flow of CSF from ventricular to lumbar spaces (di Chiro et al., 1976). Continuous i.c.v. infusion made it possible to maintain steady, high bSOD concentrations (70–80 $\mu$g/ml) in both the lumbar and ventricular CSF of a monkey that was receiving 2.5 mg/day and approximately 83 $\mu$g/ml in the lumbar CSF of the FALS patient who was receiving 30 mg/day.

While ALS treatment programs have been universally disappointing, there is reason to hope that a new therapy, based on a firm scientific rationale, may prove effective. However, the functional status of the patient in this study deteriorated rapidly, with the exception of a 1-month period while (s)he was receiving daily bolus administrations of bSOD (Fig. 2). Clearly, it is impossible to assess the efficacy of bSOD treatment for FALS based on results obtained with a single, late-stage patient, particularly since intraventricular treatment was interrupted for nearly two months due to displacement of the catheter. It is also obvious that the administered doses of bSOD could have been either insufficient or excessive at times during this study (McCord, 1994). Future dose-ranging studies should address this issue.

Gurney et al. (1994) have shown that transgenic mice which over-express FALS mutant SOD develop motoneuron degeneration although they produce normal quantities of murine SOD. Unfortunately, these mice may not serve as a suitable model for testing SOD therapy of FALS, since their total SOD activity is extremely high, unlike that of the relevant subgroup of FALS patients.

Although many issues remain unresolved, the results of this preliminary investigation appear to justify further clinical studies of bSOD in FALS. Some aspects of the proposed treatment that cannot readily be studied in patients, e.g. evaluation of CNS tissue penetration by bSOD, can be pursued in laboratory animals. Finally, future clinical studies must enroll patients whose disease is less advanced than that of the patient described here, so that, if their functional status could be maintained, they could enjoy a higher quality of life.

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