Journal of Gerontology: BIOLOGICAL SCIENCES
2000, Vol. 55A, No. 1, B5–B9

Ubiquitous Overexpression of CuZn Superoxide Dismutase Does Not Extend Life Span in Mice

Ting-Ting Huang, Elaine J. Carlson, Anne Marie Gillespie, Yuping Shi, and Charles J. Epstein

Division of Medical Genetics, Department of Pediatrics, University of California, San Francisco.

Oxidative damage has been implicated in the aging process and in a number of degenerative diseases. To investigate the role of oxygen radicals in the aging process in mammals, the life spans of transgenic mice on a CD-1 background expressing increased levels of CuZn superoxide dismutase (CuZnSOD), the enzyme that metabolizes superoxide radicals, were determined. Homozygous transgenic mice with a two- to five-fold elevation of CuZnSOD in various tissues showed a slight reduction of life span, whereas hemizygous mice with a 1.5- to 3-fold increase of CuZnSOD showed no difference in life span from that of the nontransgenic littermate controls. The results suggest that constitutive and ubiquitous overexpression of CuZnSOD alone is not sufficient to extend the life spans of transgenic mice.

BECAUSE of the increasing evidence for the role of oxidative damage in the aging process and in a number of age-related degenerative diseases (see references 1–5 for review), enzymes that metabolize reactive oxygen species have received special attention for their potential to reduce age-related oxidative damage and consequently to extend life span. Recently, extension of life span up to 48% was achieved in transgenic *Drosophila* with elevated levels of CuZn superoxide dismutase (SOD) alone (6), or of CuZnSOD and catalase (7), and in *Drosophila* with motorneuron-specific overexpression of CuZnSOD (8). Similarly, *Drosophila* selected for longevity and long-lived *Caenorhabditis elegans* with the *age-1* mutation were found to have increased activities of CuZnSOD and catalase (9–11). By contrast, several earlier transgenic *Drosophila* studies with generalized increases in CuZnSOD activity showed only a small increase in life span (12–15).

Given these divergent results in invertebrates, it could not be predicted how alterations in CuZnSOD activity would affect life span in mammals. Therefore, we have used human CuZnSOD transgenic mice, TgN<*SOD1*>Cje3 [previously designated TgHS-218(3)], to assess the effect of increased CuZnSOD activity on longevity (16). We now report that life span was not increased in the transgenic mice and, if anything, was decreased in the animals with the highest levels of CuZnSOD activity.

Methods

Generation of Mice

Hemizygous *SOD1* transgenic mice on the CD1 background were intercrossed to generate a cohort of female homozygous (homo-tg), hemizygous (hemi-tg), and nontransgenic (non-tg) mice for the life span studies. To identify transgenic mice, red cell lysates were separated by nondenaturing PAGE, and the gels stained for SOD activity (17). The presence of the human CuZnSOD band in the gel indicated the transgenic status of the mice. SOD activity assays and fluorescence in situ hybridization (FISH) of metaphase spreads using genomic *SOD1* as the probe were then used to distinguish between hemi-tg and homo-tg mice (18).

Housing Conditions

Animals were usually housed with their own littermates at five to six mice per cage in a nonbarrier facility for the pilot study and in a pathogen-free facility for the full-scale aging study. We periodically consolidated cages with less than three mice remaining to prevent mice from being caged alone. The mice in both studies were maintained on a 12-hour light-dark cycle at a constant temperature of 20 to 22°C. The mice in the pilot study were fed nonsterilized Formulab chow (6.5% fat, PMI Nutrition Intl., Brentwood, MO) from weaning. The mice in the full scale study were fed sterilized PicoLab Mouse Diet 20 5058 (9% fat, PMI Nutrition Intl.) from weaning to 9 months of age. Because the animals were gaining too much weight, they were then switched to sterilized PicoLab Mouse Diet 20 5053 (4.5% fat). The mice in both studies were given food and water ad libitum. Mice were checked daily by animal care personnel and weekly by veterinary nurses. The study was carried out under the approval of Committee on Animal Research of the University of California, San Francisco.

Statistical Analyses

The statistical analysis program JMP, version 3 (SAS Institute Inc., Cary, NC), was used to carry out the analysis of variance (ANOVA) test on the body weights, and the Kaplan-Meier estimates (univariate survival analysis) and the proportional hazards analysis (Cox model) on life spans.

Results

Ubiquitous Transgene Expression

Human CuZnSOD transgenic mice, CD1-TgN<*SOD1*>Cje3, with ubiquitous and constitutive expression of the human transgene (Figure 1 and Table 1) were used in this study. Published and unpublished CuZnSOD activities in different tissues are summarized in Table 1. The activity of CuZnSOD ranged from 1.5- to 3- and 2- to 5-fold, respectively, in various tissues of hemizygous (hemi-tg) and homozygous (homo-tg) transgenic mice, with the higher activities being found in most organs other than kidney, liver, and lung.

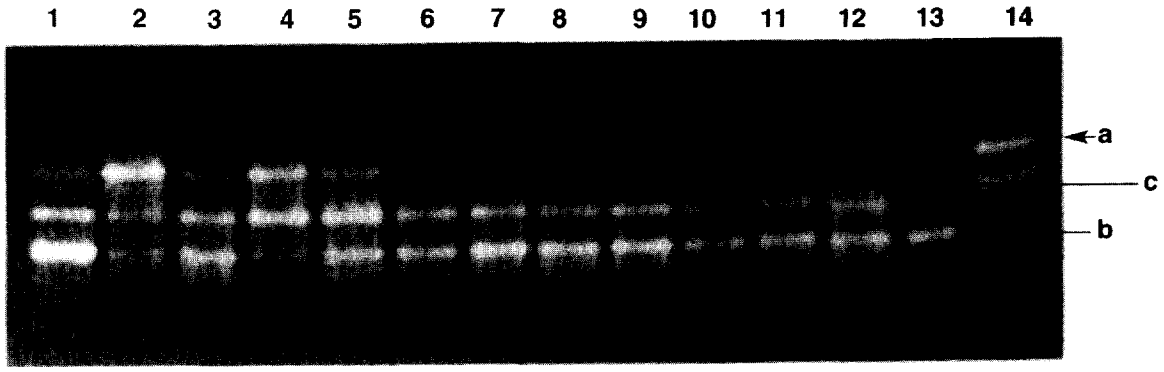

Figure 1. Ubiquitous expression of human CuZnSOD gene. Tissues were homogenized (*w:v* = 1:5) in a buffer containing 1 mM EDTA, 0.5% NP40, and Complete protease inhibitor cocktail (1 tablet per 50 mL) from Boehringer Mannheim. The tissue lysates were centrifuged at 10,000*g* for 5 minutes and the resulting supernatants were used to load the acrylamide gel. Electrophoresis and gel staining were carried out as described (17). Lane 1, skeletal muscle; 2, kidney; 3, spleen; 4, liver; 5, lung; 6, heart; 7, caudet-putamen; 8, hippocampus; 9, brain stem; 10, cerebellum; 11, cortex; 12, blood; 13, human control (blood); 14, nontransgenic mouse control (blood). The enzyme bands in the gel are a, mouse homodimer; b, human homodimer; c, human/mouse heterodimer.

Table 1. CuZnSOD Activities of Different Tissues of CD1-TgN<*SOD1*>Cje3

Tissues	Nontransgenic*	*N*	Transgenic* (Hemizygous)	*N*	Ratio to Control	Reference
Heart	40.3 ± 5.1	3	91.8 ± 5.0	3	2.3	†
Lung	52.0 ± 3.0	3	84.0 ± 8.6	3	1.6	†
Liver	90.0 ± 6.0	3	165.0 ± 13.0	3	1.8	†
Spleen	35.8 ± 2.7	3	91.0 ± 3.6	3	2.5	†
Kidney	76.0 ± 5.7	3	118.0 ± 7.9	3	1.6	†
Blood	10.5 ± 1.9	3	35.0 ± 2.5	3	3.3	†
Skeletal muscle	4.8 ± 0.9	5	23.1 ± 0.8	5	4.8‡	27
Fibroblasts	11.0 ± 0.8	23	38.1 ± 2.4	5	3.5	19
Frontal cortex	3.4 ± 0.6	12	9.6 ± 0.4	12	2.8	38
Cerebral cortex	7.9 ± 0.5	3	22.7 ± 1.4	3	2.9	39
Caudate-putamen	5.8 ± 0.4	12	13.4 ± 0.5	12	2.3	38
Hippocampus	4.2 ± 0.6	12	11.2 ± 0.9	12	2.7	38
Cerebellum	2.1 ± 0.2	12	7.2 ± 0.8	12	3.4	38
	9.0 ± 2.0	3	25.8 ± 1.3	3	2.9	39
Brainstem	4.8 ± 0.2	12	9.6 ± 0.4	12	2.0	38
Spinal cord	15.4 ± 2.0	3	34.9 ± 1.5	3	2.3	39

*CuZnSOD activities are expressed as U/mg protein; mean ± *SEM*.

†E. Carlson, *unpublished results*.

‡Homozygous transgenic mice were used for this study.

Shorter Survival Time for homo-tg Mice

The pilot study was initially set up with 15 nontransgenic (non-tg), 13 hemi-tg, and 17 homo-tg mice. Subsequently, the full-scale aging study was set up with 119 non-tg, 200 hemi-tg, and 98 homo-tg mice. The animals were weighed once a month, and there were no differences in body weight among the three groups of mice (Figure 2). All of the animals in the pilot study were allowed to die without intervention. The mean life spans in this study were 23.5 ± 2.3 months (mean ± *SD*) for non-tg, 22.5 ± 2.1 months for hemi-tg, and 17.8 ± 1.8 months for homo-tg mice (Figure 3*a*). The mean life span of non-tg mice is similar to that of CD1 mice (706 days) published by Liddle and colleagues (19). Life span comparisons of the entire population indicated that survival was not the same across the three groups of mice (Wilcoxon's chi-square analysis; $DF = 2$, $p = .0462$). Subsequent pair-wise comparisons indicated a statistical difference in survival between non-tg and homo-tg mice (Wilcoxon's chi-square analysis; $DF = 1$, $p = .0297$) and a marginal difference between hemi-tg and homo-tg mice ($p = .0773$). There was no difference in survival between non-tg and hemi-tg mice ($p = .595$). Proportional hazards analysis (Cox model) indicated a risk ratio of 1.78 between homo-tg and non-tg, implying a shorter survival time for the homo-tg mice.

In the full-scale aging study, 69.7% (83/119) non-tg, 70.5% (141/200) hemi-tg, and 69.4% (68/98) homo-tg mice died without intervention; and 30.3% (36/119) non-tg, 29.5% (59/200) hemi-tg, and 30.6% (30/98) homo-tg mice were euthanized when the animals were considered to be suffering from terminal illness. The animals were considered to be terminally ill when they had lost more than 10% body weight within 2 weeks, had difficulty breathing or moving around, had obvious tumors larger than 1 cm in diameter, or had skin lesions (scratch wounds) greater than 5% of total body surface. The euthanized animals were treated as censored subjects in the statistical analysis. The differences in life span between the censored and noncensored calculations are 0.6, 0.3, and 0.6 month, respectively, for non-tg, hemi-tg, and homo-tg mice. The mean, median, and maximum (when 95% of the population had died) life spans were 20.1 ± 0.6, 19, and 31 months, respectively, for the non-tg mice; 19.8 ± 0.5, 19, and 31 months for hemi-tg mice; and 18.5 ± 0.7, 18, and 30 months for homo-tg mice (Figure 3*b*). There was no statistical difference in survival across the three groups of mice (Wilcoxon's chi-square test; $DF = 2$, $p = .145$). Pair-wise comparisons indicated a marginal difference in survival between non-tg and homo-tg ($p = .0863$) and between hemi-tg and homo-tg ($p = .0823$), but there was no difference between non-tg and hemi-tg mice ($p = .771$). The proportional hazards test showed no difference in the risk ratio among the three groups of mice.

Discussion

Extensive in vivo and in vitro studies have been carried out with the strain of transgenic mice used for the present studies.

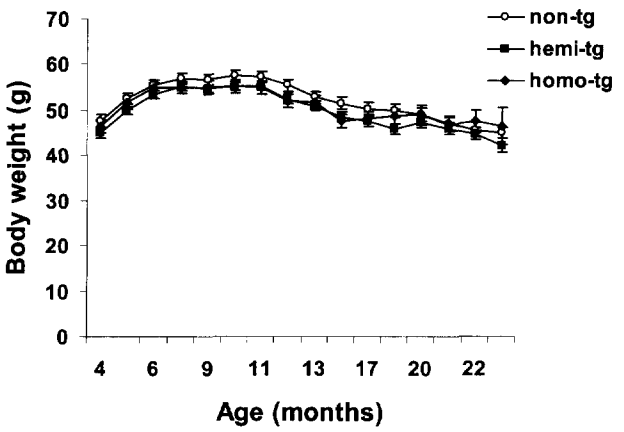

Figure 2. Animals were weighed once a month from 3 months of age. We only present weight data from 3 to 23 months as the animal numbers were too low after 23 months for statistical analysis. No significant differences in body weight were observed at each time point.

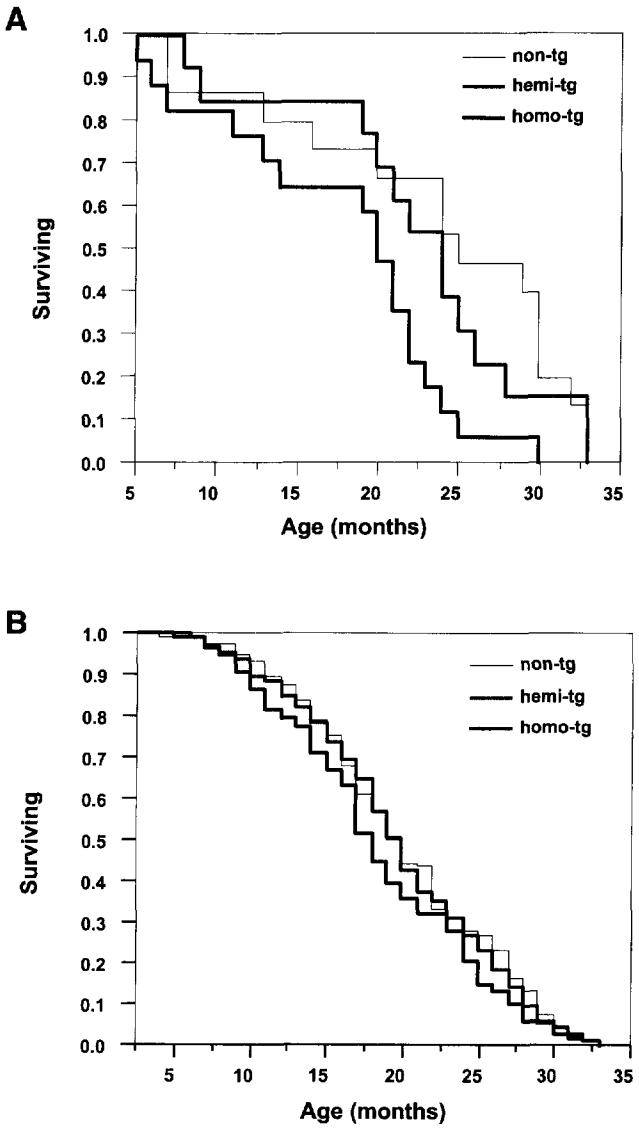

Figure 3. Kaplan-Meier survival analyses. *A*, Pilot study; *B*, Full-scale study. The life spans of the mice were rounded up to the nearest months.

In most cases, increased levels of CuZnSOD activity have been shown to be protective against various forms of acute oxidative stress produced by insults as diverse as brain trauma, ischemia and reperfusion, chemicals and drugs, and, in dopaminergic cells, the toxic effects of 3,4-dihydroxyphenylalanine (DOPA) autooxidation (20–25). The only negative effect in acute experiments was observed when neonatal transgenic animals were exposed to ischemia and hypoxia; in this instance, increased H_2O_2 production could be demonstrated (26). However, long-term studies with the same strain of transgenic mice showed a higher level of age-related muscle atrophy (27), implying an adverse effect of having increased CuZnSOD activity. Similar results were obtained from studies with a separate set of *SOD1* transgenic mice (28). Other negative effects of elevated levels of CuZnSOD activity in other transgenic strains of mice include premature aging changes in neuromuscular junctions (29,30), thymic abnormalities, and enhanced apoptosis of thymocytes and bone marrow cells (31), and increased basal levels of lipid peroxidation in the brain (32). Taken in the aggregate, these findings indicate that the effects of increased CuZnSOD activity can be beneficial or deleterious, depending on the particular circumstances.

It is not yet clear how the disparate results of the many investigations with CuZnSOD transgenic mice, including the present longevity studies, can be reconciled. It is commonly believed that increasing CuZnSOD activity without a simultaneous increase in either catalase or glutathione peroxidase activity may result in increased levels of H_2O_2 and consequently, result in an increased level of •OH. By inference, it is assumed that such an increase will be deleterious. However, this is not the only pathway for O_2^- metabolism. Superoxide radicals can also interact with NO and form the highly toxic peroxynitrite (33,34). To complicate the issue further, CuZnSOD actually has the capacity to catalyze this process (35,36). Therefore, the consequences of having more CuZnSOD may be difficult to predict, depending on the type of oxidative insult and the different components involved in the process.

In addition, genetic background may be a contributing factor to the lack of positive effect of CuZnSOD on the life spans of transgenic mice on the CD1 background. In *Drosophila* studies, genetic background affects the degree of life span extension that could be achieved. Thus, no life span extension was observed in some strains of transgenic *Drosophila* (12–15), whereas up to a 48% life span extension was observed in other strains (6). In our hands, different genetic backgrounds have a profound effect on the life span of MnSOD deficient mice (*Sod2–/–*) (37). Therefore, increased CuZnSOD might affect the life span of transgenic mice on genetic backgrounds other than that used in the current study.

Over the life of an organism, it is likely that increased levels of CuZnSOD activity may be beneficial for certain tissues or cell types and deleterious for others, depending on the rate of O_2^- generation, the presence of other oxygen radicals, and the capacity for H_2O_2 metabolism. Because life span is the outcome of the way in which all of the tissues work together, the effect of the transgene in some tissues may mask the opposite effect

in others so that no positive (or negative) change in life span is observed. The findings of increased longevity in *Drosophila* with the motorneuron-specific CuZnSOD transgene versus the lack of life span extension in *SOD1* transgenic *Drosophila* with generalized increase of CuZnSOD would be compatible with this notion. However, it would be expected that this balance in effects will be influenced by the degree of CuZnSOD overexpression. This may be the situation in the homo-tg animals in which there may be a reduction in life span, even though no change in life span was noted in the hemi-tg animals.

In summary, the current study suggests that constitutively and ubiquitously elevated levels of CuZnSOD alone are not sufficient to extend the life span of mice. Transgenic mice with balanced increases of CuZnSOD and catalase (or glutathione peroxidase) or transgenic mice with tissue-specific and/or temporal-specific expression of CuZnSOD on different genetic backgrounds will need to be tested to determine whether the manipulation of oxygen free radical metabolism can positively affect the aging process.

ACKNOWLEDGMENTS

We thank Dr. Peter Bacchetti for helpful consultation on statistical analyses and Rhoda Gacayan for weighing the mice and animal care. This work is supported by NIH grants AG08938 and AG16998.

Address correspondence to Ting-Ting Huang, PhD, Division of Medical Genetics, Department of Pediatrics, Box 0546, HSW 1429, University of California, San Francisco, San Francisco, CA 94143-0546. E-mail tthuang@itsa.ucsf.edu

REFERENCES

1. Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Exp Gerontol.* 1998;33: 95–112.
2. Harman D. Extending functional life span. *Biochem Pharmacol.* 1997; 1:533–539.
3. Multhaup G, Ruppert T, Schlicksupp A, et al. Reactive oxygen species and Alzheimer's disease. *Physiol Rev.* 1998;78:547–581.
4. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998;78:547–581.
5. Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. *Biochim Biophys Acta.* 1998;1366:211–223.
6. Sun J, Tower J. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol Cell Biol.* 1999;9:216–228.
7. Orr WC, Sohal RS. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster. Science.* 1994; 236:1128–1130.
8. Parkers TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL. Extension of *Drosophila* life span by overexpression of human SOD1 in motorneurons. *Nat Genet.* 1998;19:171–174.
9. Dudas SP, Arking R. A coordinate upregulation of antioxidant gene activities is associated with the delayed onset of senescence in a long-lived strain of *Drosophila. J Gerontol Biol Sci.* 1995;50A:B117–B127.
10. Hari R, Burde V, Arking R. Immunological confirmation of elevated levels of CuZn superoxide dismutase protein in an artificially selected long-lived strain of *Drosophila melanogaster. Exp Gerontol.* 1998;33:227–237.
11. Larsen P. Aging and resistance to oxidative damage in *Caenorhabditis elegans. Proc Natl Acad Sci USA.* 1993;90:8905–8909.
12. Seto NO, Hayashi S, Tener GM. Overexpression of Cu-Zn superoxide dismutase in Drosophila does not affect life-span. *Proc Natl Acad Sci USA.* 1990;87:4270–4274.
13. Staveley BE, Phillips JP, Hilliker AJ. Phenotypic consequences of copper-zinc superoxide dismutase overexpression in *Drosophila melanogaster. Genome.* 1990;33:867–872.
14. Reveillaud I, Niedzwiecki A, Bensch KG, Fleming JE. Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance of oxidative stress. *Mol Cell Biol.* 1991;11:632–640.
15. Orr WC, Sohal RS. Effects of Cu-Zn superoxide dismutase overexpression on life span and resistance to oxidative stress in transgenic *Drosophila melanogaster. Arch Biochem Biophys.* 1993;301:34–40.
16. Epstein CJ, Avraham KB, Lovett M, et al. Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome. *Proc Natl Acad Sci USA.* 1987;84:8044–8048.
17. White CW, Nguyen DH, Suzuki K, et al. Expression of manganese superoxide dismutase is not altered in transgenic mice with elevated level of copper-zinc superoxide dismutase. *Free Radic Biol Med.* 1993;15:629–636.
18. Shi YP, Huang TT, Carlson EJ, Epstein CJ. The mapping of transgenes by fluorescence in situ hybridization on G-banded mouse chromosomes. *Mamm Genome.* 1994;5:337–341.
19. Liddle CG, Putnam JP, Huey OP. Alteration of life span of mice chronically exposed to 2.45 GHz CW microwaves. *Bioelectromagnetics.* 1994; 15:177–181.
20. Huang TT, Carlson EJ, Leadon SA, Epstein CJ. Relationship of resistance to oxygen free radicals to CuZn-superoxide dismutase activity in transgenic, transfected, and trisomic cells. *FASEB J.* 1992;6:903–910.
21. Nakao N, Frodl EM, Widner H, et al. Overexpressing Cu/Zn superoxide dismutase enhances survival of transplanted neurons in a rat model of Parkinson's disease. *Nat Med.* 1995;1:226–231.
22. Beal MF, Ferrante RJ, Henshaw R, et al. 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgenic mice. *J Neurochem.* 1995;65:919–922.
23. Mikawa S, Kinouchi H, Kamii H, et al. Attenuation of acute and chronic damage following traumatic brain injury in copper, zinc-superoxide dismutase transgenic mice. *J Neurosurg.* 1996;85:885–891.
24. Andrews AM, Ladenheim B, Epstein CJ, Cadet JL, Murphy DL. Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2'-NH2-MPTP on serotonergic and noradrenergic nerve terminals. *Mol Pharmacol.* 1996;50:1511–1519.
25. Murakami K, Kondo T, Epstein CJ, Chan PH. Overexpression of CuZn-superoxide dismutase reduces hippocampal injury after global ischemia in transgenic mice. *Stroke.* 1997;28:1797–1804.
26. Fullerton HJ, Ditelberg JS, Chen SF, et al. Copper/zinc superoxide dismutase transgenic brain accumulates hydrogen peroxide after perinatal hypoxia ischemia. *Ann Neurol.* 1998;44:357–364.
27. Rando TA, Crowley RS, Carlson EJ, Epstein CJ, Mohapatra PK. Overexpression of copper/zinc superoxide dismutase: a novel cause of murine muscular dystrophy. *Ann Neurol.* 1998;44:381–386.
28. Peled-Kamar M, Lotem J, Wirguin I, Weiner L, Hermalin A, Groner Y. Oxidative stress mediates impairment of muscle function in transgenic mice with elevated level of wild-type Cu/Zn superoxide dismutase. *Proc Natl Acad Sci USA.* 1997;94:3883–3887.
29. Yarom R, Sapoznikov D, Havivi Y, Avraham KB, Schickler M, Groner Y. Premature aging changes in neuromuscular junctions of transgenic mice with an extra human CuZnSOD gene: a model for tongue pathology in Down's syndrome. *J Neurol Sci.* 1988;88:41–53.
30. Avraham KB, Sugarman H, Rotshenker S, Groner Y. Down's syndrome: morphological remodelling and increased complexity in the neuromuscular junction of transgenic CuZn-superoxide dismutase mice. *J Neurocytol.* 1991;20:208–215.
31. Peled-Kamar M, Lotem J, Okon E, Sachs L, Groner Y. Thymic abnormalities and enhanced apoptosis of thymocytes and bone marrow cells in transgenic mice overexpressing Cu/Zn-superoxide dismutase: implications for Down syndrome. *EMBO J.* 1995;14:4985–4993.
32. Ceballos-Picot I, Nicole A, Clement M, Bourre JM, Sinet PM. Age-related changes in antioxidant enzymes and lipid peroxidation in brains of control and transgenic mice overexpressing copper-zinc superoxide dismutase. *Mutat Res.* 1992;275:281–293.
33. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol.* 1996;271:C1424–C1437.
34. Crow JP, Beckman JS. The importance of superoxide in nitric oxide-dependent toxicity: evidence for peroxynitrite-mediated injury. *Adv Exp Med Biol.* 1996;387:147–161.
35. Crow JP, Ye YZ, Strong M, Kirk M, Barnes S, Beckman JS. Superoxide dismutase catalyzes nitration of tyrosines by peroxynitrite in the rod and head domains of neurofilament-L. *J Neurochem.* 1997;69:1945–1953.
36. Crow JP, Sampson JB, Zhuang Y, Thompson JA, Beckman JS. Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite. *J Neurochem.* 1997;69:1936–1944.

37. Huang TT Carlson EJ, Gillespie AM, Epstein CJ. Genetic modification of the dilated cardiomyopathy and neonatal lethality phenotype of mice lacking manganese superoxide dismutase. *Age.* 1998;21:83–84.
38. Przedborski S, Jackson-Lewis V, Kostic V, Carlson E, Epstein CJ, Cadet JL. Superoxide dismutase, catalase, and glutathione peroxidase activities in Copper/Zinc-superoxide dismutase transgenic mice. *J Neurochem.* 1992;58:1760–1767.
39. Chan PH, Yang GY, Chen SF, Carlson E, Epstein CJ. Cold-induced brain edema and infarction are reduced in transgenic mice overexpressing CuZn-superoxide dismutase. *Ann Neurol.* 1991;29:482–486.

Received December 23, 1998
Accepted May 26, 1999
Decision Editor: Jay Roberts, PhD