Reduced Fertility in Female Mice Lacking Copper-Zinc Superoxide Dismutase*

(Received for publication, September 4, 1997, and in revised form, December 10, 1997)

Ye-Shih Ho‡§, Mary Gargano‡, Jin Cao‡, Roderick T. Bronson‡, Ira Heimler‡, and Reinhold J. Hutz‡

From the ‡Institute of Chemical Toxicology and Department of Biochemistry and Molecular Biology, Wayne State University, Detroit, Michigan 48201, the §Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111, and the ¶Department of Biological Sciences, University of Wisconsin, Milwaukee, Wisconsin 53211.

Copper-zinc superoxide dismutase (CuZn-SOD) is believed to play a major role in the first line of antioxidant defense by catalyzing the dismutation of superoxide anion radicals to form hydrogen peroxide and molecular oxygen. Recent studies have shown that mutations in this gene contribute, evidently through a gain-of-function mechanism, to about 20% of familial amyotrophic lateral sclerosis. To define further the physiologic role of this enzyme, a model of mice deficient in this enzyme was generated using gene targeting technology. Mice lacking this enzyme were apparently healthy and displayed no increased sensitivity to hyperoxia. However, they exhibited a pronounced susceptibility to paraquat toxicity. Most surprisingly, female homozygous knock-out mice showed a markedly reduced fertility compared with that of wild-type and heterozygous knock-out mice. Further studies revealed that although these mice ovulated and conceived normally, they exhibited a marked increase in embryonic lethality. These data, for the first time, suggest a role of oxygen free radicals in causing abnormality of female reproduction in mammals.

Reactive oxygen species (ROS),† which are produced as by-products of normal metabolism, are capable of causing cellular damage, leading to cell death and tissue injury (for review, see Ref. 1). Mammalian cells are equipped with both enzymatic and nonenzymatic antioxidant defense mechanisms to minimize the cellular damage resulting from interaction between cellular constituents and ROS (for review, see Ref. 2). Despite the presence of these delicate cellular antioxidant systems, an overproduction of ROS in both intracellular and extracellular spaces often occurs upon exposure of cells or individuals to radiation, hyperoxia, and certain chemicals. An unbalanced production of ROS has been postulated to play a role in the pathogenesis of a number of clinical disorders such as acute respiratory distress syndrome, ischemia/reperfusion injury, atherosclerosis, neurodegenerative diseases, and cancer (for review, see Ref. 3). This understanding illustrates the importance of the antioxidant defense system in maintaining normal cellular physiology. However, due to the overlapping activity among some of the antioxidant enzymes, it is generally difficult to define the role of each individual antioxidant enzyme. We are interested in understanding the physiologic relevance of copper-zinc superoxide dismutase (CuZn-SOD) under normal physiologic conditions and in defending cells and animals against the pathogenesis of ROS-mediated diseases. The results from previous studies for defining the protective function of this enzyme using cells and animals with augmented enzyme expression have been controversial (4–9), since some of them develop an increased susceptibility to certain oxidants relative to that of parental cells and control animals. It is not clear whether the detrimental effect of CuZn-SOD overexpression is a result of the associated free radical generating activity of this enzyme or of its capability in enhancing nitration of tyrosine by peroxynitrite (10–15). Therefore, overexpression of this enzyme may not provide a suitable model to address the nature of this enzyme in cellular antioxidant mechanisms. To define further the role of CuZn-SOD in cellular antioxidant defense mechanisms, we generated, by gene targeting technology, mice lacking this enzyme.

MATERIALS AND METHODS

Targeted Disruption of the Mouse Sod1 Gene—Eleven mouse Sod1 genomic clones were isolated from a 129/SvJ genomic library purchased from Stratagene (La Jolla, CA) by screening with a rat Sod1 cDNA probe (16). An approximately 7.2-kb SauI genomic fragment from clone 30 was found to contain the entire mouse Sod1 gene with a sequence very similar to that published by Benedetto et al. (17). To inactivate the mouse Sod1 gene, the Smal and HindIII restriction sites flanking the Smal-HindIII fragment, which contains sequences from intron 1 to intron 4, were converted into XhoI sites by linker ligation and then inserted into the XhoI site in plasmid vector pPNT (see Ref. 18, Fig. 1a). Similarly, linker ligation was also used to clone the EcoRI-Sod1 fragment containing the 3′-flanking sequence of the gene into the BamHI site in the pPNT vector.

The Sod1 targeting vector, in which exon 5 was deleted, was linearized by HindIII digestion and transfected into R1 embryonic stem cells (19). Clones resistant to G418 and ganciclovir were screened by Southern blot analysis using a probe 5′ external to the genomic sequence present in the targeting vector. Fifty-two clones were identified from 666 clones screened to contain the expected targeted Sod1 allele. Targeted clones were microinjected into C57BL/6 blastocysts following the standard procedure (20). Thirty chimeric mice with near 100% chimerism were generated using Sod1 knock-out clones 5 and 8. Chimeric mice derived from either clone showed 100% transmission of the 129SvJ chromosomes.

Breeding of Sod1 Knock-out Mice—The heterozygous CuZn-SOD knock-out (Sod1−/−) mice were initially derived from breeding between the chimeric mice and C57BL/6 mice. They, therefore, are F1 hybrid between the 129SvJ and C57BL/6 inbred genetic backgrounds. These Sod1−/− mice were interbred to generate mice with three Sod1 geno-
types (Sod1<sup>+/+</sup>, Sod1<sup>+/−</sup>, and Sod1<sup>−/−</sup>). These F2 littersmates were used in expression studies including RNA and protein analysis. Since the female Sod1<sup>−/−</sup> mice are not very fertile, breeding was performed between F2 male Sod1<sup>−/−</sup> and female Sod1<sup>+/−</sup> littersmates as well as between F2 Sod1<sup>+/−</sup> male and female littersmates to generate a large number of wild-type and knock-out mice for various pathologic and physiologic studies described in this report.

**RNA Blot Analysis**—Total RNA was isolated from tissues by the

---

**Fig. 1. Generation and characterization of CuZn-SOD-deficient mice.**

- **a.** Schematic diagram showing the genomic and partial restriction map of the mouse Sod1 locus (top), the targeting vector (middle), and the predicted structure of the targeted locus (bottom). Numbered black boxes represent exons. Striped box represents the 5′ external sequence used as a hybridization probe. neo, neomycin resistance gene cassette; TK, herpes thymidine kinase gene cassette. B, BamHI; E, EcoRI; H, HindIII; P, PstI; S, SacI; Sa, SalI; Sm, SmaI. The approximate sizes of hybridizing PstI genomic fragments of the wild-type allele and the targeted allele are indicated at the top and bottom of the figure, respectively. kb, kilobase pairs.
- **b.** DNA blot analysis of mouse offspring. Mouse tail DNA was digested with PstI and probed with the 5′ external probe shown in a. +/+ , +/− , and −/− represent wild-type, heterozygous, and homozygous knock-out mice, respectively.
- **c.** RNA blot analysis of total cellular RNA isolated from tissues of Sod1<sup>+/+</sup>, Sod1<sup>+/−</sup>, and Sod1<sup>−/−</sup> mice. Twenty-five micrograms of total RNA from each tissue were separated on agarose gel for blot analysis. The RNA blot was initially hybridized with a rat CuZn-SOD cDNA probe (top panel), and then re-hybridized with a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH, bottom panel) cDNA to check the potential variation in sample loading.
- **d.** A native polyacrylamide gel showing activity staining for SOD in tissues of Sod1<sup>+/+</sup>, Sod1<sup>+/−</sup>, and Sod1<sup>−/−</sup> mice (24). The positions of CuZn-SOD and Mn-SOD migration are indicated to the left.
CuZn-SOD activities were detected. Sod1 with plugs were then housed individually and the birth date and nontoxic plugs were checked at around 9 a.m. each morning. Female mice 12-h off light cycle at all times. The numbers of surviving animals were initially soaking the gel in 2.43 mM nitro blue tetrazolium for 20 min, hundred micrograms of tissue protein were separated on a non-denaturing polyacrylamide gel. The SOD activity was then visualized by initially soaking the gel in 2.43 mM nitro blue tetrazolium for 20 min, followed by incubating in a solution of 50 mM potassium phosphate buffer, pH 7.8, containing 0.028 mM riboflavin, and 280 mM TEMED.

Assay for Superoxide Dismutase—Activities of CuZn-SOD and Mn-SOD were determined using a xanthine oxidase/cytochrome c assay (25). However, acetylated cytochrome c instead of cytochrome c was used in the reaction mixture to minimize interference from cytochrome c reductases and oxidases present in the tissue homogenates (26).

Detection of SOD Activity in a Native Polyacrylamide Gel (24)—One hundred micrograms of tissue protein were separated on a non-denaturing polyacrylamide gel. The SOD activity was then visualized by initially soaking the gel in 2.43 mM nitro blue tetrazolium for 20 min, followed by incubating in a solution of 50 mM potassium phosphate buffer, pH 7.8, containing 0.028 mM riboflavin, and 280 mM TEMED.

Histological Analysis—Wild-type and age-matched homozygous knock-out mice were fixed by systemic perfusion with Bouin’s fixative through the left ventricle. The tissues were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissue sections were examined under a light microscope.

Measurement of Estrous Cyclicity (27)—Vaginal smears were taken daily using a moisturized cotton tip and examined under a light microscope. Entry into the estrous cycle is indicated by an increase in the number of nucleated epithelial cells in vaginal samples.

Fecundity Index Determination—Female mice of three Sod1 genotypes were housed with either male Sod1+/+ or Sod1−/− mice. Copulatory plugs were checked at around 9 a.m. each morning. Female mice with plugs were then housed individually and the birth date and number of pups recorded.

Hyperoxic Exposure of Mice—Ten-week-old Sod1+/+ and Sod1−/− mice were used for exposure to >99% oxygen in several Plexiglas chambers. The oxygen concentration varied less than 2%, and CO2 concentration was maintained at less than 0.5% by providing approximately 12 complete gas changes per h. During the exposure, food and water were provided ad libitum.

Inactivation of the functional mouse Sod1 gene by gene targeting was then demonstrated by expression study. RNA blot analysis revealed an approximate 40–60% reduction of Sod1 mRNA in tissues of Sod1−/− mice compared with that of wild-type (Sod1+/+) mice (Fig. 1c). Furthermore, no CuZn-SOD mRNA could be found in the tissues from Sod1−/− mice, indicating that the truncated CuZn-SOD or CuZn-SOD-neo fusion mRNA was degraded rapidly in these tissues. Reduction of CuZn-SOD activity in tissues of Sod1−/− and Sod1+/− mice was also confirmed by activity staining on a native polyacrylamide gel (Fig. 1d) and the enzyme assay (Table I). It should be noted that whereas no CuZn-SOD activities were found in brain and liver of Sod1−/− mice, a very low level of CuZn-SOD activity was present in the lung samples. This activity presumably represents the activity of extracellular superoxide dismutase, as expression of this SOD isozyme is relatively high in the lungs compared with other tissues (30). A decrease in CuZn-SOD activity apparently had no effect on the activity of other cellular antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD) (Fig. 1d and Table I), catalase, and glutathione peroxidase and the enzymes that participate in the recycling of oxidized glutathione including glutathione reductase and glucose-6-phosphate dehydrogenase in these tissues

Table 1

| Enzyme | Sod1 genotype | Brain | Liver | Lung |
|--------|---------------|-------|-------|------|
| CuZn-SOD | +/+ | 23.0 ± 5.4 | 61.2 ± 15.9 | 21.9 ± 1.7 |
|         | +/- | 10.7 ± 1.2 | 28.5 ± 4.5 | 11.4 ± 1.3 |
|         | −/− | NAa | NAa | 0.6 ± 0.4 |
| Mn-SOD  | +/- | 11.3 ± 3.1 | 13.0 ± 2.7 | 9.4 ± 0.9 |
|         | −/− | 11.1 ± 2.2 | 12.9 ± 3.1 | 9.5 ± 1.3 |
|         | −/+ | 11.4 ± 1.4 | 115.1 ± 3.3 | 8.3 ± 1.1 |

p < 0.01 when comparing +/+ and −/− mice.

p < 0.001 when comparing +/+ and −/− mice.

p < 0.0001 when comparing +/+ and −/− mice.

p < 0.0001 when comparing +/+ and −/− mice, or +/−, and −/− mice.

RESULTS AND DISCUSSION

Generation and Characterization of Sod1 Knock-out Mice—As shown in Fig. 1a, exon 5 of the mouse Sod1 gene (which encodes the C-terminal of the protein from amino acid residues 120–145 that constitute both the structure and function of the active site channel (28)) and some of the flanking intron sequences were replaced by a neomycin resistance cassette (neo). Insertion of the neo in the mouse Sod1 gene creates a new PstI restriction site, resulting in a shorter PstI genomic fragment from the targeted allele (∼12.5 kb) than that from the wild-type allele (∼16.5 kb). Mice heterozygous (Sod1+/−) for the targeted allele were interbred to generate homozygous knock-out (Sod1−/−) mice. An example of DNA blot analysis of mouse DNA is shown in Fig. 1b. In addition to the ∼16.5-kb wild-type and the ∼12.5-kb targeted genomic fragments, the 5′ external probe containing exon 1 sequence also hybridized with a PstI fragment of 6.6 kb. This is believed to result from cross-hybridization between the probe and the mouse Sod1 pseudogene(s) (29).

Inactivation of the functional mouse Sod1 gene by gene targeting was then demonstrated by expression study. RNA blot analysis revealed an approximate 40–60% reduction of Sod1 mRNA in tissues of Sod1−/− mice compared with that of wild-type (Sod1+/+) mice (Fig. 1c). Furthermore, no CuZn-SOD mRNA could be found in the tissues from Sod1−/− mice, indicating that the truncated CuZn-SOD or CuZn-SOD-neo fusion mRNA was degraded rapidly in these tissues. Reduction of CuZn-SOD activity in tissues of Sod1−/− and Sod1+/− mice was also confirmed by activity staining on a native polyacrylamide gel (Fig. 1d) and the enzyme assay (Table I). It should be noted that whereas no CuZn-SOD activities were found in brain and liver of Sod1−/− mice, a very low level of CuZn-SOD activity was present in the lung samples. This activity presumably represents the activity of extracellular superoxide dismutase, as expression of this SOD isozyme is relatively high in the lungs compared with other tissues (30). A decrease in CuZn-SOD activity apparently had no effect on the activity of other cellular antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD) (Fig. 1d and Table I), catalase, and glutathione peroxidase and the enzymes that participate in the recycling of oxidized glutathione including glutathione reductase and glucose-6-phosphate dehydrogenase in these tissues.

Statistical Analysis—One-way analysis of variance was used to examine differences in each measurement performed on wild-type, heterozygous, and homozygous knock-out mice. If a significant difference was observed (p < 0.05), then pairwise comparisons among mice were made using Duncan’s test. Fecundity indices of mice were analyzed by one-sided Fisher’s exact test. Survival of wild-type and knock-out mice exposed to >99% oxygen or following intraperitoneal administration of paraquat at 10 mg/kg body weight was analyzed using the Kaplan-Meier method.

Fig. 2. Increased susceptibility in Sod1−/− mice to paraquat but not hyperoxia. a, survival analysis of Sod1+/+ and Sod1−/− mice under hyperoxia. The survival times of age-matched Sod1+/+ and Sod1−/− mice of both genders under >99% oxygen were measured. b, survival curves of age-matched male Sod1+/+, Sod1−/−, and Sod1−/− mice following intraperitoneal administration of paraquat at a dose of 10 mg/kg body weight.

Table 1

| Enzyme | Sod1 genotype | Brain | Liver | Lung |
|--------|---------------|-------|-------|------|
| CuZn-SOD | +/+ | 23.0 ± 5.4 | 61.2 ± 15.9 | 21.9 ± 1.7 |
|         | +/- | 10.7 ± 1.2 | 28.5 ± 4.5 | 11.4 ± 1.3 |
|         | −/− | NAa | NAa | 0.6 ± 0.4 |
| Mn-SOD  | +/- | 11.3 ± 3.1 | 13.0 ± 2.7 | 9.4 ± 0.9 |
|         | −/− | 11.1 ± 2.2 | 12.9 ± 3.1 | 9.5 ± 1.3 |
|         | −/+ | 11.4 ± 1.4 | 115.1 ± 3.3 | 8.3 ± 1.1 |
CuZn-SOD-deficient Mice

Mice Lacking CuZn-SOD Exhibit a Marked Increase in Post-implantation Embryonic Death—During the study, we intended to generate a large number of Sod1−/− mice for various pathologic and physiologic studies by interbreeding between Sod1−/− mice. To our surprise, the reproductive performance of female Sod1−/− mice was inferior to that of female Sod1+/− mice. As shown in Table II, while 10 female Sod1−/− mice gave birth to 26 litters (mean litter size 7.5 ± 2.5) in a period of 3 months, only 16 litters (mean litter size 1.6 ± 1.0) were yielded from an equal number of female Sod1+/− mice. Of these 16 litters, 6 litters contained only 1 pup, 3 litters contained 2 pups, and one litter contained 4 pups. It should be noted that the drastic reduction in reproduction of Sod1−/− females is not a result of a defect in the development of Sod1−/− fetuses, since of the 194 pups derived from the breeding between Sod1−/− female and Sod1+/− male mice, 52% were heterozygous and 48% homozygous for the targeted Sod1 allele.

To understand further this unexpected observation, the reproductive performance of the female mice with three Sod1 genotypes was closely followed. As shown in Table III, male Sod1−/− mice were as fertile as Sod1+/− males, and female Sod1−/− and Sod1+/− mice were similarly fertile when bred with either Sod1−/− or Sod1+/− males. However, the fecundity index (number of litters/number of copulations) and size of the litters of Sod1−/− females were much less than those of Sod1+/− females.

The mechanism underlying the poor reproductive performance of Sod1−/− females was further investigated. Examination of vaginal smears indicated that all types of mice had similar estrous cycles, with an average length of 4 to 5 days. The frequency of female mice that became receptive to males was also measured. For 6 weeks, eight Sod1+/−, Sod1−/−, and Sod1−/− female mice mated 17, 20, and 18 times with vasectomized males, respectively. Apparently, the reduced fertility in Sod1−/− mice was not a result of altered estrous cycles. The numbers of ova ovulated by these three types of females at each estrous cycle were also found to be equivalent (Table IV). In addition, female Sod1+/− mice exhibited a normal ovarian histology including the number, size, or morphology of antral follicles and corpora lutea compared with that of Sod1+/− and Sod1−/− mice (data not shown). These results suggested that the reduced fertility in Sod1−/− female mice might result from

### Table II

| Sod1 genotype | Number of progeny produced from breeding of female Sod1 knock-out mice |
|---------------|---------------------------------------------------------------------|
| Male          | Female                                                              |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
| +/-           | +/-                                                                |
CuZn-SOD-deficient Mice

Sod1 embryonic death that occurred in Sod1–/– female mice and unrelated to the paraquat. Drosophila with an earlier study reported by Phillips and colleagues (40) be catalyzed by the enzyme NADPH-dependent cytochrome b6f, a bipyridyl herbicide capable of generating oxygen radicals and our results suggest that Mn-SOD may play a more critical role in normal physiologic conditions (for review, see Ref. 1), and the rate of radical production is further enhanced in radicals under normal physiologic conditions (for review, see Ref. 1). Our data reveal that although the role of CuZn-SOD in defending against oxygen radicals in normal subcellular sites producing oxygen radicals under normal physiologic conditions (for review, see Ref. 1), and the rate of radical production is further enhanced in mitochondria of hyperoxic lungs (35–37). This understanding and our results suggest that Mn-SOD may play a more critical role than does CuZn-SOD in antioxidant defense mechanisms (26) under normal physiologic conditions, and in defending against lung injury resulting from hyperoxic insults. This notion is supported by the recent findings that mice lacking Mn-SOD die at very young ages (38–39). However, CuZn-SOD is apparently critical for animals to survive under a lethal exposure to paraquat, a bipyridyl herbicide capable of generating oxygen radicals through the redox cycling mechanism. This reaction is believed to be catalyzed by the enzyme NADPH-dependent cytochrome P-450 reductase, primarily located in the endoplasmic reticulum. Our data suggest that both the cytosolic and microsomal enzymes may be the primary targets of superoxide radicals generated during paraquat toxicity. This conclusion is in agreement with an earlier study reported by Phillips and colleagues (40) that Drosophila deficient in CuZn-SOD is hypersensitive to paraquat.

The most intriguing observation made in this study is the reduced fertility of female mice lacking this enzyme. Apparently, this defect is associated with CuZn-SOD deficiency in the female mice and unrelated to the Sod1 genotypes of the fetuses. Since the female Sod1–/– mice exhibited a normal estrous cycle and generated comparable numbers of ova compared with those of Sod1+/– and Sod1+/– females, the reduced fertility might not have been a result of a gross defect in the hypoxia-pamititary axis in these mice. However, post-implantation embryonic loss did occur, and this could certainly be endocrine-related. Interestingly, male CuZn-SOD-deficient fruit flies are sterile, and females show a markedly reduced fertility (40). These and our results suggest that CuZn-SOD plays a critical role in female reproduction. The exact mechanism(s) underlying the observed reduced fertility in female CuZn-SOD-deficient mice as well as its implication in human reproductive dysfunction remain to be defined.

Acknowledgments—We thank Dr. Richard Mulligan of Massachusetts Institute of Technology for the gift of plasmid pNT, Dr. Andras Nagy of Mount Sinai Hospital at Toronto for the gift of R1 embryonic stem cells, and Syntex Inc. (Palo Alto, CA) for supplying ganciclovir.

REFERENCES

1. Freeman, B. A., and Crapo, J. D. (1982) Lab. Invest. 47, 412–426
2. Forman, H. J., and Fisher, A. B. (1982) in Oxygen and Living Processes: An Interdisciplinary Approach (Gibert, D. L., ed) pp. 235–249, Springer-Verlag Inc., New York
3. Cross, C. E., Halliwell, B., Borish, E. T., Pryor, W. A., Ames, B. N., Saul, R. L., and McCord, J. M. (1987) Ann. Intern. Med. 107, 526–545
4. Elroy-Stein, O., and Groner, Y. (1988) Cell 52, 259–267
5. Elroy-Stein, O., Bernstein, Y., and Groner, Y. (1986) EMBO J. 5, 615–622
6. Krall, J., Bagley, A. C., Mullenbach, G. T., Halliwell, R. A., and Lynch, R. E. (1988) J. Biol. Chem. 263, 1914–1914
7. Scott, M. D., Meshnick, S. R., and Eaton, J. W. (1989) J. Biol. Chem. 264, 2498–2501
8. Keiner, M. J., and Bagnell, R. (1990) J. Biol. Chem. 265, 10872–10875
9. Bar-Peled, O., Korkotian, E., Segal, M., and Groner, Y. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8530–8535
10. Hodgson, E. K., and Fridovich, I. (1975) Biochemistry 14, 5924–5928
11. Hodgson, E. K., and Fridovich, I. (1975) Biochemistry 14, 5929–5933
12. Yim, M. B., Chock, P. B., and Stadtman, E. R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5006–5010
13. Yim, M. B., Chock, P. B., and Stadtman, E. R. (1991) J. Biol. Chem. 266, 4099–4105
14. Ischiropoulos, H., Zhu, L., Chen, L., Tsai, M., Martin, J. C. Smith, C. D., and Beckman, J. S. (1992) Arch. Biochem. Biophys. 298, 431–437
15. Ischiropoulos, H., Zhu, L., and Beckman, J. S. (1992) Arch. Biochem. Biophys. 298, 446–451
16. Ho, Y.-S., and Crapo, J. D. (1987) Nucleic Acids Res. 15, 6746
17. Benedetto, M., Ansai, Y., and Gordon, J. W. (1991) Gene (Amst.) 99, 191–195
18. Tybulewicz, V. L. J., Crawford, C. E., Jackson, P. K., Bronson, R. T., and Mulligan, R. (1991) Cell 65, 1153–1163
19. Nagy, A., Rassart, J., Nagy, R., Abersam-Newly, W., and Roder, J. C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8424–8428
20. Bradley, A. (1987) in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach (Robertson, E. J., ed) pp. 113–151, IRL Press at Oxford University Press, Oxford
21. Chirgwin, J. M., Przybyla, A. E., Macdonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
22. McMaster, G. R., and Carmichael, G. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4835–4838
23. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5201–5205
24. Beauchamp, C., and Fridovich, I. (1971) Anal. Biochem. 44, 276–287
25. Crapo, J. D., McCord, J. M., and Fridovich, I. (1978) Methods Enzymol. 53, 382–393
26. Azzi, A., Montecucco, C., and Richter, C. (1975) Biochem. Biophys. Res. Commun. 65, 597–603
27. Rugh, R. (1990) The Mouse: Its Reproduction and Development, pp. 24–43, Oxford University Press, Oxford
28. Fisher, C. L., Halliwell, R. A., Roberts, V. A., Tainer, J. A., and Getzoff, E. D. (1991) Free Radical Res. Commun. 12–13, 287–296
29. Danicger, E., Dafni, N., Bernstein, Y., Laver-Rudich, Z., Neer, A., and Groner, Y. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5619–5623
30. Matsumoto, S. L. (1984) Biochem. Biophys. Res. Commun. 120, 468–473
31. McCord, J. M., and Fridovich, I. (1969) J. Biol. Chem. 244, 6049–6055
32. Crapo, J. D., Barry, B. E., Foscue, H. A., and Shelburne, J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4501–4505
33. White, C. W., Avraham, K. B., Shanley, P. F., and Groner, Y. (1991) Cell 80, 1910–1914
34. Lebovitz, R. M., Zhang, H., Vagel, H., Cartwright, J., Dione, L., Yu, N., Huang, S., and Matzuk, M. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9782–9787
35. Phillips, J. P., Campbell, S. D., Michaud, D., Charbonneau, M., and Hilliker, A. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2761–2765
Reduced Fertility in Female Mice Lacking Copper-Zinc Superoxide Dismutase
Ye-Shih Ho, Mary Gargano, Jin Cao, Roderick T. Bronson, Ira Heimler and Reinhold J. Hutz

J. Biol. Chem. 1998, 273:7765-7769.
doi: 10.1074/jbc.273.13.7765

Access the most updated version of this article at http://www.jbc.org/content/273/13/7765

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 37 references, 16 of which can be accessed free at http://www.jbc.org/content/273/13/7765.full.html#ref-list-1