

Short communication

## CuZn-superoxide dismutase (CuZnSOD) transgenic mice show resistance to the lethal effects of methylenedioxyamphetamine (MDA) and of methylenedioxymethamphetamine (MDMA)

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### Abstract

We have used female and male transgenic (Tg) mice that carry the complete sequence of the human copper-zinc (CuZn) superoxide dismutase (SOD) gene in order to assess the lethal effects of methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA). In contrast to non-Tg mice, both heterozygous and homozygous SOD-Tg mice showed resistance to the lethal effects of both drugs. Females of both SOD-Tg and non-Tg strains were somewhat more resistant to the effects of these drugs in comparison to males. In general, homozygous animals show greater resistance to the effects of the two drugs. These results suggest that the acute lethal effects of amphetamine-substituted analogs might involve the intracellular overproduction of the superoxide radicals secondary to hypoxic injury. The gender differences suggest that there might be hormonal-free radical scavenger interactions that offer better protection to female mice. This might be related both to the lifespan of and to the lower prevalence of Parkinson's disease in women. Future studies will need to address these issues further.

**Key words:** Transgenic mouse; Superoxide dismutase; Methylenedioxyamphetamine; Methylenedioxymethamphetamine; Lethality; Free radical

Oxidative stress and oxygen free radicals are thought to play an important role in both the acute and chronic effects of a number of neurotoxic processes. These include administration of some drugs, radiation-induced injury, as well as oxygen-induced injury to the central nervous system [1,3] (see refs. for a comprehensive review). Despite the belief that oxygen free radicals are of importance in a variety of pathological processes affecting the nervous system, it has not always been possible to examine their roles directly. However, transgenic animal technology has made it possible to constitutively increase the level of cytosolic CuZnSOD [9], an enzyme which is in the first line of defense against oxygen-based radicals such as the superoxide anion ( $O_2^-$ ) [10]. We have used these mice in order to evaluate the role of oxygen-based radicals in

the toxic manifestations of methamphetamine [3] and MPTP [20] and have shown that the toxic effects of these drugs are attenuated in these animals.

Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) are drugs of abuse that affect the monoaminergic systems of rodents [8,15,22] and of non-human primates [21]. In addition to their neurotoxic effects in the brain, these drugs cause acute lethality at high doses [12]. The mechanisms for this acute toxicity are not understood but might involve deleterious effects on the cardiovascular or central nervous system [17]. In the central nervous system, these could include seizures with secondary anoxic events. These occurrences might be accompanied by the intracellular generation of high levels of superoxide radicals, hydrogen peroxide, or hydroxyl radicals [7]. As a first step towards assessing the role of free radicals in the neurotoxic events associated with the use of MDA and MDMA, we have used CuZnSOD-Tg mice in order to investigate acute reactions to various doses of MDA and MDMA. We also

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sought to determine if there were any gender differences in these responses.

Male and female transgenic mice of strain 218/3 carrying the complete human CuZnSOD gene were used in these experiments. These animals were produced as previously described [9]. Heterozygous and homozygous SOD-Tg mice and non-transgenic (non-Tg) mice were used in these experiments. The heterozygotes had a mean increase of 2.6 fold whereas the homozygotes had a mean increase of 5.7 fold in comparison to non-Tg mice. All animal use procedures were according to the NIH guide for the Care and Use of Laboratory Animals and were approved by the local NIDA Animal Care Committee.

On the day of experiments, the animals were given saline or various doses of either MDA or MDMA via the intraperitoneal route. The total number of animals that survived during the interceding 24 h was enumerated. The lethality data were analyzed by a two-way analysis of variance (ANOVA), with strains of mice and doses of drugs as factors. Post-hoc analysis was done using the Duncan multiple range test after a one-way ANOVA in order to compare the survival rate of the three strains at each dose of the drug. Criteria for significance were set at the 0.05 level.

Fig. 1 shows the results of the acute administration of MDA to male and female mice. For the males, there were significant main effects of dose of MDA ( $F = 22.24$ ,  $P < 0.0001$ ) and of strains of mice ( $F = 12.50$ ,  $P < 0.0001$ ). The interactions were also significant ( $F = 3.04$ ,  $P < 0.01$ ). Post-hoc analyses showed that the homozygous mice showed more resistance to the lethal effects of the drugs whereas the non-Tg and the heterozygous animals showed a lack of survival at the higher doses of MDA (Fig. 1A). At lower doses, the heterozygous SOD-Tg mice were less resistant than the non-Tg mice. Fig. 1B shows the results of the acute administration of MDA to female mice. There were significant main effects of doses of MDA ( $F = 16.8$ ,  $P < 0.001$ ) and of strains of mice ( $F = 12$ ,  $P < 0.0001$ ). The interactions were also significant ( $F = 2.1$ ,  $P < 0.05$ ). Interestingly, in contrast to the males, the female mice of all strains were more resistant to the lethal effects of MDA. For example, at 80 mg/kg of MDA, 50% of the non-Tg female mice survived whereas only 10% of the male non-Tg survived. Similar patterns were observed with the other strains of mice, with the homozygous animals showing 80% survival even at the highest dose (120 mg/kg) of MDA used in the females (Fig. 1B).

Fig. 2 shows the results of the acute administration of MDMA to male and female mice. There were significant main effects of strains of mice ( $F = 28.12$ ,  $P < 0.0001$ ) but not of doses ( $F = 1.91$ ,  $P = 0.154$ ). The interactions were not significant ( $F = 0.460$ ,  $P < 0.765$ ). Post-hoc analyses showed that both the homozygous

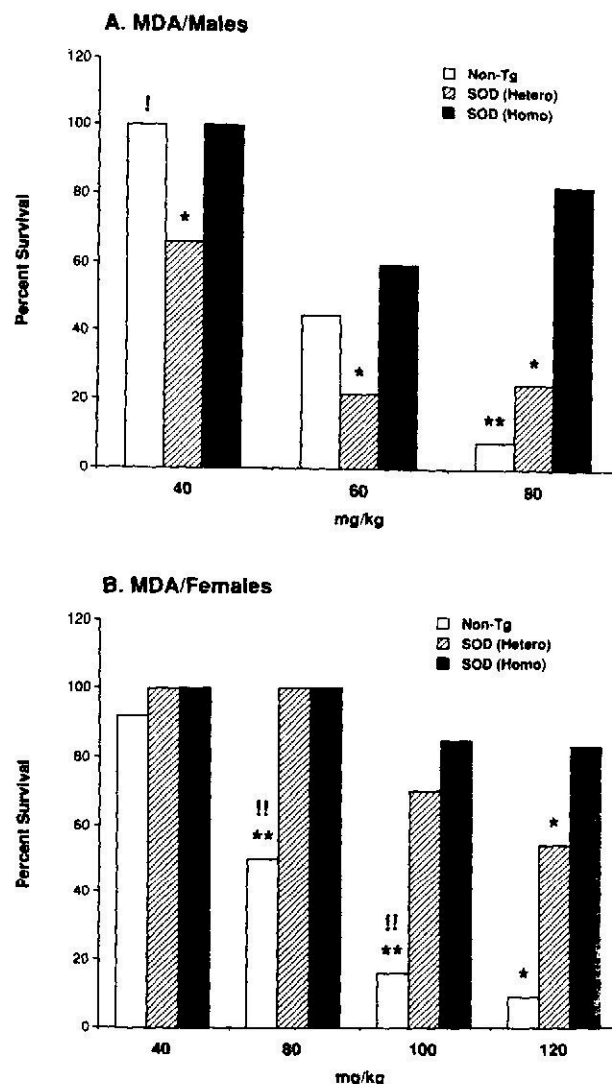


Fig. 1. Acute lethal effects of MDA in male (A) and female (B) mice. The mice were administered the different doses of MDA as described in the text. The values represent percent survival of 6–25 animals per group. \*  $P < 0.05$ , \*\*  $P < 0.01$  in comparison to the homozygous SOD-Tg mice; †  $P < 0.05$ , ‡  $P < 0.01$  in comparison to the heterozygous SOD-Tg mice (by Duncan).

(90%) and the heterozygous (70%) SOD-Tg mice showed resistance to the lethal effects of the drugs unlike the non-Tg animals which showed only 10% survival at the highest dose (80 mg/kg) used for the males (Fig. 2A). Fig. 2B shows the results of the acute administration of the two drugs to female mice. There were significant main effects of doses of MDMA ( $F = 42.26$ ,  $P < 0.0001$ ) and of strain of mice ( $F = 4.76$ ,  $P < 0.01$ ). The interactions did not reach significance ( $F = 0.135$ ,  $P = 0.33$ ). Post-hoc analyses revealed that the homozygous animals were somewhat more resistant than the other two strains (Fig. 2B). At very toxic doses of MDMA (120 mg/kg), the strain differences were no longer obvious in the female mice (Fig. 1B). As in the case for MDA, the female mice of all strains showed

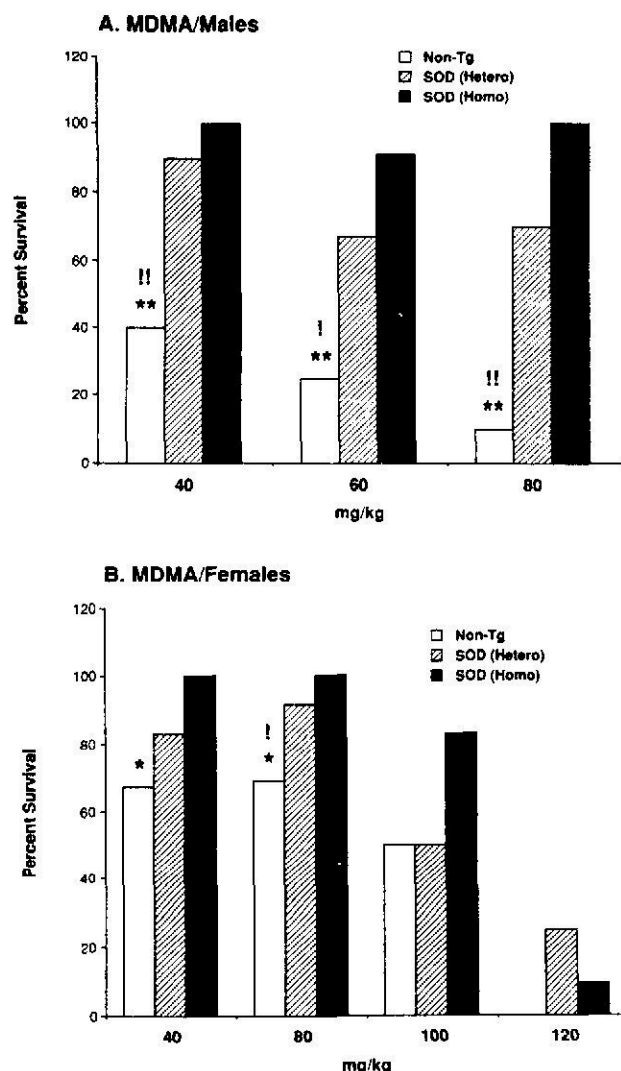


Fig. 2. Acute lethal effects of MDMA in male (A) and female (B) mice. The mice were injected with different doses of MDMA as described above. The values represent percent survival of 6–18 animals per group. \*  $P < 0.05$ ; \*\*  $P < 0.01$  in comparison to the homozygous SOD-Tg mice; †  $P < 0.05$ , ‡  $P < 0.01$  in comparison to the heterozygous SOD-Tg mice (by Duncan).

more resistance to the lethal effects of MDMA. Specifically, only 10% of the male non-Tg survived whereas 70% of the female non-Tg mice survived after being given 80 mg/kg of MDMA. In contrast to MDA, almost no animals survived when the highest dose of MDMA (120 mg/kg) was used in the female mice of any strain (Fig. 2B).

This is the first demonstration that SOD-Tg mice which have high levels of CuZnSOD in the brain are protected against the lethal effects of substituted amphetamines. These results suggest that the acute effects of MDA and MDMA may be mediated partially by the overproduction of the superoxide anion, possibly secondary to anoxic damage to various brain regions. Together with our recent demonstration that metham-

phetamine-induced neurotoxicity is attenuated in SOD-Tg mice [3], the present data provide further support for a role of oxidative stress in the pathological changes that are observed after the administration of amphetamine analogs. Nevertheless, because there are some differences in the response profile to the two drugs, the present data suggest that the lethal mechanisms are not identical for MDA and MDMA. In vitro studies will be necessary in order to dissect the molecular basis of these differences.

It is, also, of interest that transgenic mice that express an extracellular form of the CuZnSOD enzyme are more susceptible to the effects of increased oxygen tension [19] whereas the SOD-Tg mice, used in the present study, express the intracellular form of the enzyme and are more resistant to the effects of the MDA and MDMA (present study) and to the effects of glutamate-induced toxicity [4] and oxygen-based injuries [5,6,14]. When taken together, these studies suggest that increasing CuZnSOD activity and secondary decrease in the intracellular  $O_2$  levels may be beneficial while increasing the extracellular CuZnSOD activity with associated decrease in the extracellular  $O_2$  levels may be detrimental to host survival.

In contrast to males, female mice of all groups showed more resistance to the lethal effects of both MDA and MDMA. These data suggest possible hormonal effects of the metabolism of these drugs or a sexual dimorphism in the handling of oxidative stress. Epidemiological reports have documented a lower prevalence of Parkinson's disease in women [16], a disease which is thought to be secondary to oxygen-based radicals [2,18]. These results might also be related to the fact that, as a group, women tend to show less cortical atrophy with increasing age [11] since the aging process has also been attributed to oxidative mechanisms [1]. Further studies are needed to clarify these points. In any case, the present results suggest that oxidative stress might play an important role in the toxic effects of amphetamine and its derivatives [3].

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