RADIOPROTECTION OF MICE BY SUPEROXIDE DISMTUASE

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SUMMARY The distribution in sensitivity to X-rays of female Swiss mice was log-normal following a single intravenous injection of superoxide dismutase at 35 μg/g body weight. The X-ray dose required to kill 50% of the enzyme-treated mice within 30 days ($LD_{50(30)}$) was 700 rads as compared to 627 rads for the saline-injected control group of mice which exhibited a normal distribution in sensitivity to radiation. Inactivated superoxide dismutase, when given intravenously in the amount of 35 μg/g, did not produce a significant radioprotective effect on mouse survival.

INTRODUCTION Superoxide dismutase reacts specifically with superoxide anions (1), generated in biological systems by a host of oxidoreductases (2) and in water by radiolysis (3). In aerobic cells this enzyme is present in amounts that vary from one type of tissue to another (4). At elevated oxygen tension under otherwise physiological conditions the tissue concentration of this enzyme is increased and the toxicity of oxygen in animals reduced (5, 6).

In radiobiology, explanations of the oxygen effect have previously emphasized the mechanistic involvement of superoxide or its complimentary acid, the hydroperoxyl radical (7). Contrary opinion (8), however, discounted these explanations and alternative accounts of the oxygen effect(s) have since been advanced. Nevertheless, the specificity with which superoxide dismutase catalyses the destruction of superoxide anions, together with the recent demonstration of the radioprotective effect of the enzyme on membranes (9), enzymes, bacteriophage,

bacteria (10), mycoplasma (11), and mammalian cells (12, 13), suggest that a superoxide-mediated component of the oxygen effect exists which is discernible and to an extent inhibitable. These observations prompted the present experiments in which the beneficial effect of a prophylactic dose of superoxide dismutase on survival of irradiated mice was examined.

MATERIALS AND METHODS Six weeks old, white, female, Swiss mice, obtained from Bio Breeding Laboratories of Canada Ltd., Ottawa, were used. The animals were housed in groups of 6 in plastic cages and received food and water ad libitum.

The superoxide dismutase was obtained as a lyophilized powder from Truett Laboratories, Dallas, Texas. Enzyme administration was done by intravenous infusion (tail vein) 1 h before the radiation exposure since preliminary tests suggested a time delay of this length between the prophylactic dose of enzyme and the irradiation. To test the effectiveness of varying amounts of enzyme at a constant radiation dose, fresh solutions were prepared by dissolving the enzyme in sterile saline (0.1 N) at concentrations varying between 0.6 - 4 mg/ml, so that for a 20 g mouse, receiving either 15, 35, 70, or 100 µg/g body weight, the volume of solution administered was 0.5 ml, while heavier and lighter mice received proportionately greater and lesser volumes, respectively. Control mice of comparable weight received equivalent volumes of saline (0.1 N). The results of this test (Table 1) suggested that 35 µg/g body weight be used in the $LD_{50(30)}$ experiments. The solutions for this series were freshly prepared as required by dissolving the enzyme in saline at a concentration of 1.4 mg/ml.

Tests were also made to determine whether the therapeutic effect of the enzyme was attributable to its inactivated form. For this purpose the enzyme solution was divided into two parts, one of which was then inactivated in an autoclave for 10 min. The active

Table 1. Thirty-day lethality of Swiss white mice exposed to X-rays (650 rad) 1 h after receiving an intravenous dose of saline (0.1 N) or varying amounts of superoxide dismtuase (15 - 100 µg/g body weight).

Treatment	No. of mice exposed	Dead at 30 days (%)
0.1 N Saline	48	85
Superoxide dismutase 15 µg	48	26
" " 35 µg	48	21
" " 70 µg	48	45
" " 100 µg	48	32

and inactive enzyme solutions, as well as the saline, were injected as before into three treatment groups, each consisting of 20 replicates of 6 mice each, for a total of 120 animals per treatment. The latter animals were exposed to 650 rads at a dose rate of 100 rad/min with 250 KV, 15 mA X-rays passing through a 0.5 mm Cu + 1 mm Al filter. The mice from one replicate of each treatment group were transferred in lots of three to pint-sized paper cartons which were then placed in fixed positions on a turntable centrally located in the beam and rotating at 0.5 rpm.

In the $LD_{50(30)}$ studies, the animals were similarly exposed to graded doses at the same dose rate. After the exposure, the mice were returned to their cages and observed on a regular basis. The dead mice were removed promptly and their numbers recorded.

RESULTS The data for the $LD_{50(30)}$ experiments are tabulated in Table 2 and presented in graphical form in Fig. 1 on linear-probability paper. It is observed that a normal distribution in sensitivity to radiation was exhibited by the population treated with saline but not by the group of mice given superoxide dismutase. The data for the latter

Table 2. Lethality data for Swiss white mice exposed to graded doses of X-rays 1 h after an intravenous dose of 0.1 N saline (Control group) or superoxide dismutase.

Dose (rad)	Treatment			
	Saline (0.1 N)		Superoxide dismutase (35 μg/g)	
	No. exposed	Dead at 30 days(%)	No. exposed	Dead at 30 days(%)
800			72	87.5
760			72	73.6
720	60	98.3	72	65.3
680	96	86.5	72	31.9
650	312	69.6	294	27.2
625	69	42.0	135	14.9
600	69	24.6	135	8.8
575	69	15.9	69	2.9
550	120	5.8	72	0.8

group are best fitted by a log-normal distribution in radiation sensitivity (14), thus indicating that the radioprotective effect of the exogenous superoxide dismutase was due to a multiplicative process involving a combination of random terms. As well, the line through the data (as fitted by eye in Fig. 1) for this group does not parallel that for the control (saline) group. This implies that the enzyme-mediated effect is not simply dose modifying.

In Table 3 the degree of protection afforded by superoxide dismutase against an X-ray dose of 650 rads is compared with the action of the inactivated enzyme. The effect of the latter on lethality was not significantly different from that of saline whereas the active enzyme reduced lethality to a significant extent when its effect is compared with those of the other two treatments. In this instance, therefore, radioprotection by superoxide dismutase is specifically derived from its enzyme function.

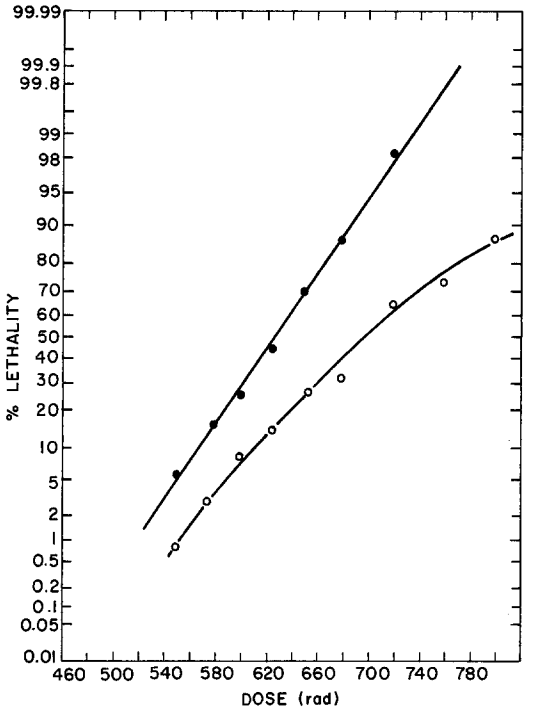

Figure 1. % Lethality vs X-ray dose (rad) for Swiss mice given an intravenous injection of 0.1 N saline (●——●) or 35 µg/g body weight of superoxide dismutase (o——o) 1 h before irradiation. The former data were best fitted by a normal distribution in radiation sensitivity (chi^2 = 2.29, degrees of freedom = 5) with an $LD_{50(30)}$ = 627 (626-628) rads (95% confidence limits). The latter data were best fitted by a log-normal distribution in radiation sensitivity (chi^2 = 4.38, degrees of freedom = 7) with an $LD_{50(30)}$ = 700 (688-713) rads (95% confidence limits).

DISCUSSION The in vivo protection of mice against radiation damage by superoxide dismutase is unlikely to be due, to a major extent, to its ability to quench singlet oxygen (15). Although a mechanism for the conversion of superoxide anions to singlet oxygen has been proposed (16), the yield in biological systems is at present uncertain. In any case, the quenching of singlet oxygen by superoxide dismutase is a function of its amino acid composition and not a specific characteristic of its enzymic activity (15). Accordingly, if singlet oxygen were involved, the inactivated enzyme should have shown a radioprotective

Table 3. Radioprotection of mice given superoxide dismutase intravenously at 35 μg/g body weight before irradiation with 250 KV X-rays to 650 rads at 100 rads/min.

Treatment Group	No. in Group	% Lethality* after 30 days
Saline (0.1 N)	120	72.5
Superoxide dismutase	120	20.8
Inactivated superoxide dismutase	120	65.0

* Mean of two randomized block experiments each demonstrating differences between the superoxide dismutase group and the two control groups that were significant at the 1% level.

effect comparable to that of the active form and this was not observed (Table 3).

In an alternative account of superoxide anion toxicity, the generation of hydroxyl (OH) radicals from hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-) is invoked (17). It has been previously pointed out, however (18), that the intracellular concentrations of superoxide dismutase and catalase are too high to allow the existence of H_2O_2 and O_2^- in sufficient concentrations to generate OH in appreciable quantity by reaction 1 (19):

$$O_2^- + H_2O_2 + H^+ \longrightarrow O_2 + H_2O + OH \qquad (1)$$

Furthermore, recent studies of the mechanism of damage of intracellular membranes by O_2^- have confirmed that the OH radical is not an important initiating species (20, 21). Therefore, alternative mechanisms appear to be required. Some of the reactions by which superoxide anions may inflict cellular damage directly include lipid peroxidation by iron catalysis (20), sulfhydryl oxidation (11), and chain oxidation of nicotinamide-adenine nucleotide (NADH) (22). The latter, together

with the essential function of superoxide anions in the electron transfer chain of respiration (2) suggests that the administration of a prophylactic dose of enzyme must be adjusted within a range that is adequate to remove some or all of the superoxide anions, generated radiolytically, but low enough not to seriously interfere with biochemical functions dependent on the superoxide anion. As the data in Table 1 suggest, this range may be quite narrow, a feature not uncommon in therapeutics.

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