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AGE-DEPENDENT RESISTANCE TO THE TOXIC EFFECTS OF PARAQUAT IN RELATION TO SUPEROXIDE DISMUTASE ACTIVITY IN RAT LUNG

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Young rats are much more resistant to the toxic effects of orally-administered paraquat than are older rats. The possible relation of this phenomenon to the activity of superoxide dismutase in lung is discussed.

Key words: paraquat; superoxide dismutase; lung; fibrosis; age-dependent toxicity

INTRODUCTION

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) is the active ingredient of many commercially-available herbicides. It has been used in agriculture for more than 20 years and continues to be the cause of fatal poisonings. It is reported[13] that paraquat accumulates in the lung much more than in other tissues. The most characteristic effect of paraquat toxicity in humans and animals is cyanosis caused by inadequate oxygenation of blood.[3] Histologically, paraquat-intoxicated lungs show regions of alveolar collapse and areas of advanced pulmonary oedema with alveolar flooding.[5] Other toxic effects noted are ulceration of the mouth and oesophagus, necrosis of the kidneys and liver, and haemorrhage and oedema in the brain. The lethal dose of paraquat for an adult man has been estimated to be approximately 10–15 ml of the commercially (29% paraquat) available material.[6] In rodents given a lethal parenteral dose, most animals die between 48 and 120 hr with a few deaths occurring as late as 240 hrs.[12,4] With an oral lethal dose the mortality time course is delayed by an additional 24 to 48 hr.[3,14]

The biochemical mechanism by which paraquat causes cellular damage in the lungs has not been completely established. However, it has been shown that paraquat sometimes accelerates lipid peroxidation in lung microsomes[2] and, *in vivo*, causes depletion of NADPH via its redox cycling activity (Smith *et al.* 1979). Recent studies of the effect of paraquat on *Escherichia coli*[8] and on *Salmonella typhimurium*[9] have shown that, in these organisms, paraquat acts by increasing the production of superoxide anion. That this mechanism is relevant to animal toxicity is supported by the observation that oxygen potentiates paraquat toxicity[5], whereas hypoxia affords some protection.[12]

In the present paper we have examined the role of lung superoxide dismutase in the survival of paraquat treated animals of different ages.

MATERIALS AND METHODS

Mill-Hill hybrid hooded rats were housed in individual plastic cages in a room with a constant temperature of 20–23°C and a regime of 12 h darkness/12 h light. They were given pelleted rat diet ad. libitum. Paraquat (from Imperial Chemical Industries, Bracknell, England) was given dissolved in the drinking water (tap water) at a concentration of 170 mg/litre. At the beginning of the experiment rats were 1, 2 or 3 months old and they all received paraquat until they were 120 days old. Hence the youngest animals received paraquat for 90 days and the oldest for 30 days. Control animals received tap water to drink.

Animals were killed at 120 days. The liver was perfused to remove blood and the lung and liver washed, minced in buffer (composition as below) and then homogenised at 4°C in a buffer containing 50 mM potassium phosphate and 10^{-4}M EDTA, pH 7.8. The homogenate was centrifuged for 15 min at 12000 g and the supernatant further centrifuged for 90 min at 85000 g. SOD activity was determined in the final supernatant by inhibition of adrenalin oxidation.[11] The protein content of fractions was measured by the method of Lowry *et al.* (1951).[10]

RESULTS

Three groups of rats were used, each containing 9 animals and each accompanied by a control group of the same age. Paraquat was administered to the experimental groups in the drinking water until the rats were 120 days old. The paraquat in 90-day-old rats had produced a 78% mortality by 30 days of treatment. In rats 60 days old at the start

TABLE I

The body mass (in grams) and the mortality rate of paraquat-treated experimental animals. Masses are expressed as mean ± one standard deviation.

Age of animals at start of treatment (days)	30	60	90
Duration of paraquat treatment (days)	90	60	30
No. of survivors at 120 days	7	6	2
% mortality rate	22	33	78
Mass of animals surviving at 120 days [no. of survivors]	261 ± 35 [7]	300 ± 57 [6]	240 ± 28 [2]
Mass of control animals (no paraquat given) [no. of animals]	281 ± 42 [9]	327 ± 35 [9]	373 ± 33 [9]

TABLE II
Superoxide dismutase activity in the lung of 90-day-old rats treated with paraquat for 30 days. The SOD activity of lung extract (prepared as described in the materials and methods section) was measured. Lungs from the two surviving animals were homogenised all together. One unit of SOD activity inhibits adrenalin oxidation by 50% under the assay conditions of Misra and Fridovich (1972).

Animals used	SOD activity	
	units/gm fresh weight of lung tissue	units/mg protein
Controls [n = 9]	521.3 ± 37.6	12.84 ± 1.29
Two survivors after 30 day treatment	297.6	9.83

of treatment the mortality at 120 days was only 33%, even though paraquat treatment was for 60 days (Table I). The youngest rats (30 days old at the start of treatment) showed a mortality of only 22% at 120 days, even though they had been treated with paraquat for 90 days. Hence the younger the animal, the lower the mortality produced by paraquat. This difference was not due to a difference in the amount of paraquat consumed by rats of different ages; the daily intake of drinking water was measured and it was found that each rat in the experimental groups consumed between 3.89 and 4.16 mg of paraquat daily, irrespective of age. The paraquat had only a slight, if any, effect on the body mass of the surviving animals at 120 days (Table I), although the animals who died, especially those 90 days old at the start of treatment, lost considerable weight before death.

The superoxide dismutase activity and protein content of the lungs of the rats were measured after sacrifice at 120 days. The lung tissue was also examined by light microscopy. The two surviving animals 90 days old at the start of treatment showed no visible lung abnormalities, although the lungs of those 7 animals who died during treatment showed gross histological abnormalities. However, the specific activities of superoxide dismutase in the lung of these two surviving animals was less than that of the controls (Table II). The six animals surviving after 60 days of paraquat treatment (60 days old at the start of treatment) showed microscopically-visible lung abnormalities in two cases, but no observable changes in the other four. The activity of SOD was

TABLE III
Superoxide dismutase activity in the lung and liver of 60-day-old rats treated with paraquat for 60 days. The SOD activity of lung and liver extracts was measured by the adrenalin method.

Animals used	SOD activity			
	in lung		in liver	
	units/g fresh weight of tissue	units/mg protein	units/g fresh weight of tissue	units/mg protein
Controls [n = 9]	402.9 ± 11.8	10.78 ± 1.42	5345.8 ± 220.0	129.0 ± 10.0
Survivors without pathological lung changes [n = 4]	438.4 ± 13.4	13.19 ± 2.75	7530 ± 405.8	159.0 ± 17.0
Survivors with pathological lung changes [n = 2]	137.9 ± 70.9	5.85 ± 2.76	5049.9 ± 302.9	130.0 ± 10.0

TABLE IV
Superoxide dismutase activity in the lung of 30-day-old rats treated with paraquat for 90 days. The SOD activity of lung extract was measured by the adrenalin method.

Animals used	SOD activity	
	units/gm fresh weight of tissue	units/mg protein
Controls [n = 9]	400.6 ± 49.4	8.60 ± 2.38
Survivors after 90 day paraquat treatment [n = 7]	325.9 ± 110.3	8.42 ± 0.16

strikingly reduced in the lungs of the first two animals, but seemed slightly increased in the lungs of the four animals showing no abnormalities (Table III) although tests of significance are not valid on such a small sample. For comparison, the specific activity of SOD in the liver was not much different in the controls and survivors with pathological lung changes (Table III).

For the seven animals surviving after 90 days of paraquat treatment (30 days old at the start of treatment), none showed microscopically-visible lung abnormalities. There was no obvious difference in the lung SOD activity as compared with controls (Table IV).

DISCUSSION

It seems clear from studies upon bacteria (Hassan and Moody, 1982) that paraquat acts by increasing intracellular O_2^- generation and that a raised level of SOD in the cells is protective.

The results in the present paper have shown that young rats are far less susceptible to the lethal effects of paraquat administered in the drinking water than are older animals. Of 30-day-old rats treated with paraquat for 90 days, most survived and none of the survivors showed pathological lung changes by light microscopy. Autor's group (for a review see Autor, 1982)[1] has shown that newborn rats survive exposure to increased oxygen concentrations much better than adult rats because the younger rats can raise the SOD activity in their lungs much more quickly in response to elevated O_2. However, the results in Table IV show that induction of SOD is not an explanation of the increased resistance of young rats to paraquat. Perhaps instead there are increases in lung glutathione peroxidase activity, an enzyme of importance in protection against paraquat toxicity in the lung (for a review see Halliwell and Gutteridge, 1985).[7] Similarly, the two 90-day-old animals surviving after treatment with paraquat for 30 days clearly showed depressed lung SOD activity, even though they were still alive and showed no lung abnormalities under the light microscope (Table II).

The results of paraquat treatment of the 60-day-old animals were particularly interesting. Those survivors that showed no lung abnormalities had increased lung SOD activity, whereas those with lung damage showed decreased SOD activity. This might be taken to mean that increase in SOD activity is an important protective mechanism against paraquat toxicity in animals, but the decrease is SOD seen in two animals could merely be a consequence of the lung damage rather than a cause of it.

Our results do not disprove the view that SOD is an important protective enzyme against paraquat-induced death in rats, but they do not support this view. Perhaps other antioxidant enzymes are of greater importance in these animals. However, it should be noted that our assay for SOD was performed at a pH of 10.1, and thus measures largely the copper-zinc enzyme (CuZn SOD). Further, assays of crude homogenates of lung measure average activities in the large number of different cell types present, and may conceal substantial changes in enzyme activity in only one cell type.

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