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Paraquat toxicity is enhanced by iron and reduced by desferrioxamine in laboratory mice

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Desferrioxamine, a specific iron chelator [1, 2] used clinically to treat cases of iron overload [3, 4], successfully increased the survival of laboratory mice poisoned by paraquat. On the other hand, loading the mice with iron prior to the poisoning led to a severe reduction in their life span. We therefore conclude that iron plays a major role in paraquat toxicity.

Paraquat (methyl viologen; 1,1′-dimethyl-4,4′-bipyridinium (cation), di-chloride; PQ) is a widely used herbicide [5, 6]. Since the early sixties, when its use spread all over the world, hundreds of cases of death from paraquat poisoning have been reported [7–9]. This has prompted extensive research using animal studies and other models, both *in vivo* and *in vitro*. Using bacterial systems, it has been shown that the manifestation of paraquat toxicity requires molecular oxygen and a readily metabolizable electron source [10, 11]. However, despite voluminous research, the exact mechanisms of paraquat toxicity in mammals is still not fully understood [12]. It is known that paraquat causes its toxicity through a pulmonary injury [13], regardless of the method of contact [14]. Clinical manifestations of paraquat toxicity include edema (higher water content) of the lungs [15], fibrosis of the lungs, and change in activity of lung enzymes [16], all leading to mortality.

Metal chelators such as desferrioxamine have been shown to reduce and even prevent paraquat toxicity in *E. coli* (R. Kohan and M. Chevion, submitted), where a catalytic amount of copper ion (1 μM) dramatically enhanced the rate of inactivation of the cells by paraquat. These findings are similar to results in other systems, indicating the protective effect of chelators against biological damage induced by redox cycling compounds such as streptonigrin [17, 18, 10], bleomycin [20] and ascorbate [21]. Consequently, we studied the effects of desferrioxamine on paraquat poisoned mice.

Three parameters were studied: survival; activity of an enzyme in the lungs; water content of the lungs in paraquat intoxicated mice.

Male mice (Balb/C; 30 g in weight) were used. All the injections were given intraperitoneally (i.p.). Each experiment was repeated three times. The reported results are an average of the replications.

In the survival experiment, four groups of mice were tested (10 mice in each group). Mice that received a single dose of paraquat (17 mg/kg) (Sigma) began to die 24 hr after the injection, 50% of the mice were dead by 63 hr after the injection (Fig. 1), and no survivors were left by the fourth day (Fig. 1). In contrast, mice that received the repeated injections of desferrioxamine (5 mg/inj) (Desferal, Ciba–Geigy) once 24 hr prior to, and twice daily, for two days following the same dose of paraquat, began to die 40 hr after injection, 50% of these mice were dead only 90 hr after injection, and 20–30% of the mice survived for at least three more months (Fig. 1). Thus, desferrioxamine therapy significantly increased the life span of poisoned mice. Two other groups were used as controls; one group was given only desferrioxamine twice daily for five days, and the second group received injections of water only; both control groups survived.

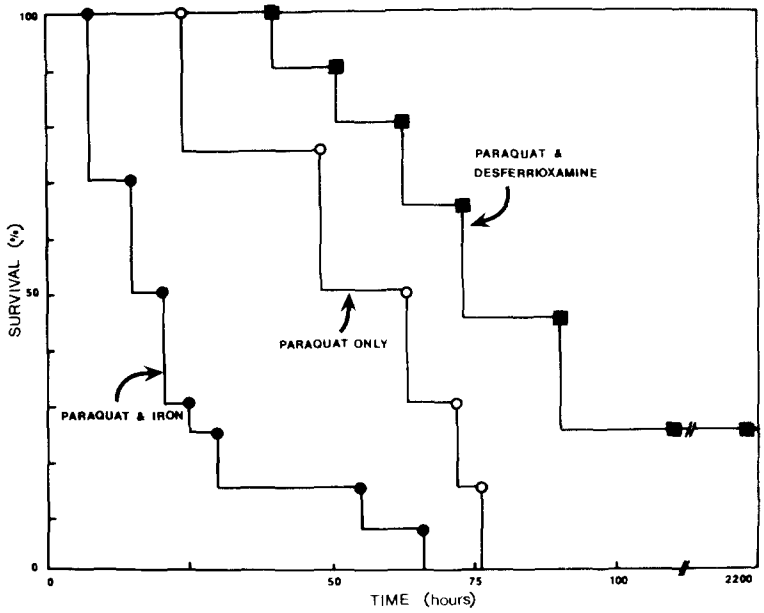

Fig. 1. Effects of treatment with desferrioxamine or iron on the survival of paraquat-poisoned mice. Male Balb/c mice, approximatetly 30 g each, were given unlimited amounts of food and water. Six groups of mice were tested (10 mice in each group in each experiment). Each experiment was repeated three times, and the results are the average of the replications. All the injections were given intraperitoneally, using solutions of paraquat (17 mg/kg), desferrioxamine (5 mg/inj.) and ferrous sulfate (75 mg/kg) that had been prepared in autoclave-sterile distilled water and were subsequently filtered through 0.45 μm pore membranes (Schleicher and Schull Corporation). Three groups that were used as controls received injections of desferrioxamine alone, of ferrous sulfate alone, or water and showed no mortality during the experiments and 3 months afterwards (not shown).

Treatment of paraquat poisoned mice with desferrioxamine also resulted in a smaller weight loss. Animals given only paraquat lost 19% (±3%) by day 2 and 25% (±4%) by day 3, whereas those treated with desferrioxamine showed a loss of 4% (±0.8%) by day 2 and 10% (±0.8%) by day 3.

To support our results with direct evidence that iron is involved in paraquat toxicity, a series of experiments on another four groups of mice (10 mice in each group) was carried out. The survival time of these mice was measured. In one group, ferrous sulphate (75 mg/kg) (Sigma) was given i.p. once daily for five days prior to the paraquat (17 mg/kg) injection. A significant shortening of the life span of the animals was recorded (Fig. 1). Thus, 21 and 30 hr after paraquat was injected into the iron loaded animals, 50 and 75% respectively of the mice had died, whereas the unloaded mice receiving paraquat showed no mortality within this time period.

A second series of experiments dealt with the deleterious effects to the lungs consequent to paraquat intoxication. As a model we chose to follow the activity of acetylcholine esterase in the lungs. It has been reported that the activity of acetylcholine esterase in the lung is reduced after exposure to paraquat [22]. Supposedly, paraquat acts as a competitive inhibitor of this enzyme [22]. Our results (not shown) suggest that the reduction in the activity of the enzyme is not due to simple competitive inhibition, because *in vitro* paraquat failed to block the enzyme. We rather suggest that paraquat decreased enzyme activity by destroying it in a mechanism involving oxygen-derived free radicals [23, 24] analogous to that occurring in the ascorbate–copper system [21]. Alternatively, the reduction of acetylcholine esterase activity may be secondary to the direct damage inflicted by free radicals to other components of the lungs. In mice poisoned with paraquat (20 mg/kg) and treated with desferrioxamine (5 mg), the reduction in the enzyme activity of the whole lung was much smaller than in the group that was given paraquat alone (Fig. 2). When the enzymatic activities were calculated per mg lung protein, the result obtained remained the same (not shown).

In a third set of experiments we studied the water content of the lungs after exposure to high dose of paraquat (25 mg/kg) [25]. The mice were killed at intervals, the lungs weighed, then freeze-dried for 72 hr, and weighed again. The water content was calculated as the difference between the two weights. Four groups (5 mice in each group) were used. The groups received injections of water (group I), desferrioxamine (group II), PQ (group III), and a combination of PQ + desferrioxamine (group IV). The water content of the lungs of groups I, II and IV did not change 2 and 4 hr following injections (75.0 ± 0.2%), ($P < 0.05$) while the water content in the lungs of group III increased by 1.0% (±0.2%) and 2.0% (±0.2%) after 2 and 4 hr, respectively. Six hours after the injection, the difference between group III and the other groups had reached its maximum (2.5%).

In summary, chelation therapy with desferrioxamine increased the life span of paraquat intoxicated mice, increased the rate of survival, totally prevented the loss in activity of lung acetylcholine esterase, and fully maintained the water content of the lungs. Furthermore, iron preloading led to a severe shortening of the life span of intoxicated mice by 68% (20 and 63 hr), and to a more pronounced elevation (+2.5%) in the water content of the lungs six hours after paraquat injection. All these effects may be due to the reaction of free radicals directly with lung acetylcholine esterase, or with other lung components, or may result from an enhanced inflammatory process. We expect that experiments with larger animals permitting continuous chelation therapy whether by infusion or inhalation together with additional supportive treatment in an intensive care unit will prove even more efficient.

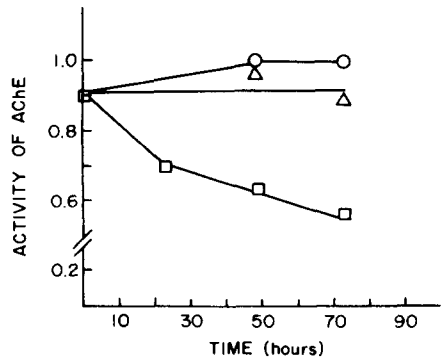

Fig. 2. Activity of lung acetylcholine esterase from paraquat-poisoned mice. Four groups of mice, five animals in each group, were used in each experiment. Experimental conditions were similar to those described in Fig. 1. Injections were given intraperitoneally with 1 hr intervals. The first group (control) was injected five times with sterile water (not shown). The second group (○) was treated with desferrioxamine (three injections of 5 mg followed by two injections of 2 mg of Desferal). The third group (□) was treated with a single dose of paraquat (20 mg/kg), and the fourth group (△) was treated with a combination of paraquat and desferrioxamine (a single dose of paraquat (20 mg/kg) followed by multiple treatment of desferrioxamine, 3 injections of Desferal 5 mg each, two injections of Desferal 2 mg each). Lungs from each group were excised daily and individually analysed for the enzymatic activity of acetylcholine esterase, determined according to Brown and Mailing [22] and Ellman *et al.* [26].

It has been suggested that the toxicity of paraquat is due to oxygen-derived free radicals such as the superoxide anion [10]. We have shown in a bacterial model that transition metal ions are an essential requirement for paraquat toxicity, in a mechanism analogous to that of superoxide or ascorbate [23, 21]. In this mechanism we hypothesize that hydroxyl radicals, produced via the "site-specific metal-mediated Haber-Weiss" reaction, are highly efficient in destroying biological macromolecules. The results in this communication are in full accord with the mechanism suggested for the bacterial system. Thus, it is anticipated that chelation therapy should have a marked beneficial effect on paraquat intoxicated human subjects.

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Enzymatic synthesis of sarin and soman

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There are two groups of organophosphate inhibitors that inactivate cholinesterases, namely G-group inhibitors $CH_3(RO)P(O)F$ and V-group inhibitors $CH_3(RO)P(O)$-$SCH_2CH_2NR_2'$. Hydrolysis of G-group organophosphate inhibitors is specifically catalyzed by G-group organophosphate hydrolase (phosphorylphosphatase) [1–4]. According to the theory of catalysis, enzymatic reactions might proceed in both directions. In this paper, enzymatic syn-