THE PRODUCTION OF SUPEROXIDE RADICALS IN REACTIONS OF THE HERBICIDE DIQUAT

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1. Introduction

The herbicide diquat (fig. 1) causes lens opacities when it is fed to rats as 0.05% of their diet [1], but the mechanism of its toxicity is unknown. The rats appear normal in other respects.

Diquat can be reduced to an autoxidisable free radical, solutions of which are green in colour [2], by NADPH and glutathione reductase [EC 1.6.4.2] and photochemically in the presence of EDTA or other electron donors [3, 4].

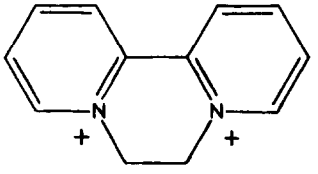

Fig. 1. Diquat, (1,1'-ethylene-2,2'-dipyridylium ion).

After intraperitoneal injection, diquat reaches the rat lens, which contains systems capable of reducing it [3, 4].

Hydrogen peroxide is formed in the aerobic autoxidation of diquat free radical [5]. The formation and oxidation of diquat free radical may be a factor in the toxicity of diquat to the lens, and a closer study has been made of the mechanism of this reaction. Evidence is given that superoxide radicals ($O_2^{\cdot-}$) are produced by the air oxidation of diquat free radicals.

2. Materials and methods

Diquat dichloride was the gift of Dr. K. Fletcher.

NADPH and glutathione reductase (90 U/mg) were obtained from Boehringer Corporation.

Catalase (20,000 U/mg) was obtained from the Sigma Chemical Co.

Superoxide dismutase was prepared by the method of McCord and Fridovich [6], and a sample of authentic material was the gift of Mr. E. Wood.

(a) Light-dependent reactions were carried out in 10 mm quartz spectrophotometer cells immersed in a glass water-bath at 20° illuminated by a Philips MLU 300 W lamp at a distance of 30 cm. The light passed through the glass sides of the water-bath and through 1 cm of water before entering the reaction mixture.

Adrenalin oxidation was measured by change in absorbance at 480 nm. The reaction mixture contained 0.13 mM diquat, 1 mM adrenalin, 10 μg catalase, with additions and omissions described in fig. 2, in 3.0 ml of 0.1 M potassium phosphate pH 8.5.

Cytochrome *c* reduction was measured by change in absorbance at 550 nm. The reaction mixture con-

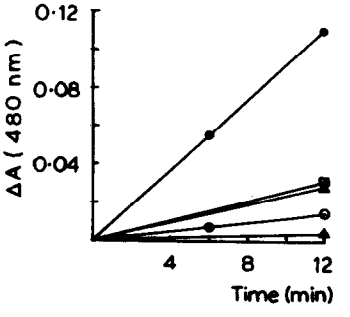

Fig. 2. Effect of superoxide dismutase on light-dependent adrenalin oxidation. Alterations to the reaction mixture described in Methods section: non •; 50 μg superoxide dismutase added, ○; diquat omitted, □; incubation in the dark △; incubation in vacuo, ▲.

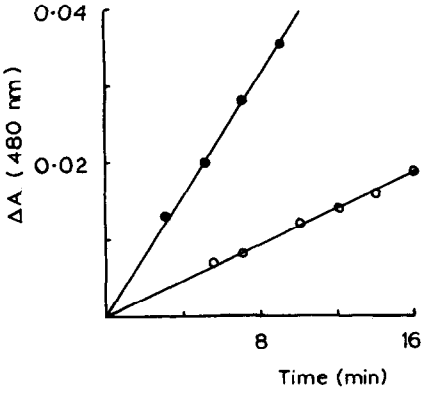

Fig. 3. Effect of superoxide dismutase on NADPH-linked adrenalin oxidation. Additions to the reaction mixture described in Methods section: non, •; 6 μg superoxide dismutase ○.

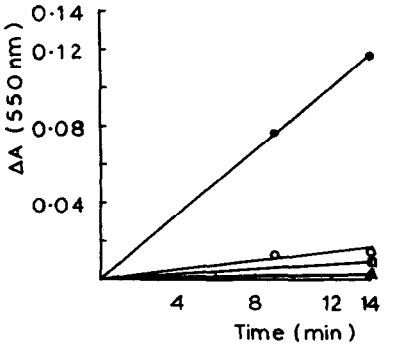

Fig. 4. Effect of superoxide dismutase on light-dependent cytochrome *c* reduction. Alterations to the reaction mixture described in Methods section: none, •; 100 μg superoxide dismutase added, ○; diquat omitted, □; dark incubation, △.

tained 0.13 mM diquat, 0.72 mg ferricytochrome *c*, 10 μg catalase, 10 mM EDTA with additions and omissions described in fig. 4 in 3.0 ml of 0.1 M potassium phosphate pH 7.5.

(b) NADPH-linked reactions were carried out at 20°–22° in a Unicam SP500 spectrophotometer. The reaction mixture for adrenalin oxidation (fig. 3) contained 0.53 mM diquat, 1 mM adrenalin, 0.08 mg/ml NADPH, 10 μg yeast glutathione reductase, with or without 6 μg superoxide dismutase, in 3.0 ml of 0.1 M triethanolamine-HCl, pH 8.0.

The reaction mixture for cytochrome reduction contained 0.53 mM diquat, 0.72 mg cytochrome *c*, 0.08 mg/ml NADPH, 10 μg catalase, 10 μg glutathione reductase and superoxide dismutase as indicated in fig. 5, in 3.0 ml of 0.1 M potassium phosphate pH 7.5. No cytochrome reduction was observed in the absence of diquat.

3. Results and discussion

Diquat free radical is formed either by NADHP and glutathione reductase or photochemically in the presence of EDTA or amino acids [4, 7]. Aerobically the free radical autoxidises and does not accumulate, and under these conditions both systems catalyse the oxidation of adrenalin to adrenochrome (figs. 2 and 3) or the reduction of ferricytochrome *c* (figs. 4 and 5).

Both reactions are inhibited by superoxide dismutase (figs. 2–5). Superoxide radicals are known to reduce ferricytochrome *c* and oxidise adrenalin [6, 8, 9] and the inhibition of these reactions by superoxide dismutase [6] has been used to detect the presence of superoxide. The inhibitory effect of superoxide dismutase in these experiments indicates that superoxide radicals are formed by the air oxidation of diquat free radicals.

The reduction of diquat to give diquat free radical is brought about by flavoproteins from liver [7], by glutathione reductase from lens [4], or photochemically, and thus the present results indicate that superoxide radicals can be formed in these tissues in diquat-fed animals.

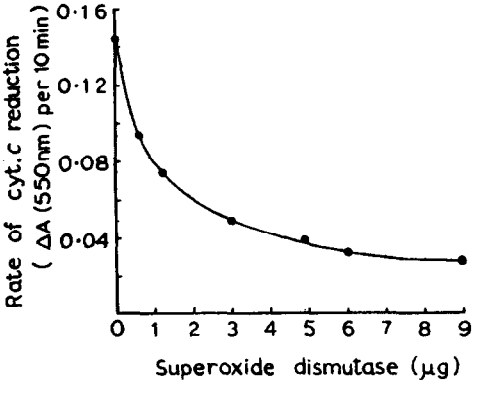

Fig. 5. Effect of superoxide dismutase on the rate of NADPH-linked cytochrome *c* reduction, under conditions described in Methods section.

Harman [10] and Dormandy [11] have suggested that peroxy and other free radicals may play a part in degenerative disease. It remains to be investigated whether the reactions of superoxide radicals with lens constituents play a necessary part in the development of experimental diquat cataracts.

Davenport [12] has described the formation of diquat free radical from diquat by extracts of chloroplasts, and thus the production of superoxide radicals may also be involved in the action of diquat as a herbicide.

Acknowledgements

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References

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