LIPID PEROXIDATION IN HUMAN PARAQUAT POISONING

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Abstract.....The concentration of malondialdehyde (MDA) in tissues in paraquat poisoning was compared between humans and rats. Lung MDA increased in humans, but not in rats despite of development of histological change of the lung. On the other hand, liver MDA increased both in humans and in rats.

These findings suggest that lipid peroxidation may not be primary mechanism of paraquat toxicity.

Key words : Paraquat poisoning, lipid peroxidation, malondialdehyde.

INTRODUCTION

Paraquat (1, 1'-dimethyl-4, 4'-bipyridylium dichloride) has been widely used as a herbicide. Poisoning by this compound in humans and experimental animals results in damage to several organs, among which the lung is most severely affected (Thurlbeck & Thurlbeck, 1976; Wasan & McElligott, 1972; Smith et al., 1974). The mechanism of paraquat toxicity remains hitherto unsolved despite of extensive researches. Bus et al. (1975) proposed the hypothesis that paraquat causes the lung damage by inducing the peroxidation of microsomal lipids which was mediated by superoxide anions generated with paraquat radicals and by singlet oxygen derived from the anions. Thereafter, the effect of paraquat on peroxidation has been studied by other investigators. Their results, however, were not always coincident with the above hypothesis (Steffen et al., 1980; Kornbrust & Mavis, 1980; Shu et al., 1979; Ilett et al., 1974; Steffen & Metter, 1979).

In this paper, tissue malondialdehyde levels in fatal human paraquat poisoning and in experimental poisoning of rats were compared in attempt to elucidate the effect of paraquat on lipid peroxidation.

MATERIALS AND METHODS

1. Human paraquat poisoning

Lung, liver and kidney in 7 cases of paraquat intoxication were taken at autopsy and stored in a freezer. Tissues in 16 cases with other causes of death were also taken as

the control; 6 cases of bleeding and each one case of lobar pneumonia, cerebral laceration, poisoning with hydrogen sulfide, ventricular hemorrhage, electrothanasia, uterine cancer, gastric cancer, uremia, systemic lupus erythematosus and myocardial infarction.

Tissues were homogenized with cold 1.15% KCl solution to make a 10% homogenate, and centrifuged at 3000 rpm for 5 min. The supernatants were used for determination of malondialdehyde (MDA). MDA was determined according to the thiobarbituric acid test by Uchiyama and Mihara (1978). Tissue paraquat (PQ) levels were determined colorimetrically (Kurisaki & Sato, 1979). Concentration of protein was determined with Biuret reagent.

2. Experimental poisoning

Male rats (150–200 g) were used; they were allowed free access to food and water. Gramoxone (containing 24% paraquat, Nippon Noyaku Co. Ltd., Japan) was appropriately diluted with distilled water and given to rats orally at dosage of 75 mg cation/kg. After dosing, five animals were sacrificed by decapitation at 6 and 24 hours, and four at 72 hours. Lung, liver and kidney were taken and used for assays. Five non-treated rats were used as the control.

RESULTS

1. Human poisoning (Fig. 1)

Case histories and paraquat levels in tissues were summarized in Table 1. The doses

Fig. 1. Concentration of tissue MDA in human paraquat poisoning. ● : paraquat poisoning, ○ : control group.

*1: lung edema and hemorrhage by electrothanasia, *2: lung damage by traffic accident, *3: lobar pneumonia and cirrhosis, *4: liver cancer by metastasis of uterine cancer, *5: liver cancer by metastasis of gastric cancer.

Table 1. Cases of paraquat intoxication.

Case	Age	Sex	Dose*	Survival Time	Lung	Paraquat ($\mu\text{g/g}$) Liver	Kidney
1	60	F	100ml	19h	12	19	34
2	52	M	50	21 h	15.7	24.7	45.2
3	38	M	?	3 d ?	3.3	3.5	11.7
4	51	M	50	5 d	0.3	0.6	4.8
5	25	M	15	12 d	0.2	0.2	0.7
6	38	F	5	20 d	N.D.	N.D.	N.D.
7	51	M	50	39 d	N.D.	N.D.	N.D.

* presumed volumes

of herbicide were represented as the volume of Gramoxone.

As seen in Fig. 1, MDA level in the lung was significantly higher in PQ poisoning group than in the control group ($p < 0.05$). In the control group, three cases with higher MDA level had lung lesions as pneumonia and edema. In PQ poisoning group, higher MDA levels were found in cases of earlier death, that is, higher MDA levels were seen in cases of development of inflammatory changes such as congestion, edema and appearance of macrophage and neutrophil. On the other hand, lower MDA levels were seen in cases of development of fibrotic changes. In the liver, MDA level in PQ poisoning group was also higher than in the control group. In the control group, 5 cases with metastasis of cancer, liver cirrhosis and so on showed higher MDA level. No difference of MDA level in the kidney was found between the two groups, because MDA level varied widely in the control group. The reason why kidney MDA varied widely in the control group was not elucidated in this study.

2. Experimental poisoning (Fig. 2)

Fig. 2. Concentration of tissue MDA in paraquat-poisoned rats. The value indicates mean of 5 animals at 0, 6 and 24 hrs, and 4 animals at 3 days. ● : lung, ○ : liver, □ : kidney.

The histological changes were slight at 6 and 24 hours after dosing. At 72 hours, however, the so-called paraquat lung showed alveolar atelectasis, compensatory dilation, hemorrhage, exudate and macrophages in the alveoli, and edema and fibroblast in the alveolar walls in some of poisoned rats. Tissues other than the lung showed no significant changes in all groups.

The concentration of MDA in the lung and kidney showed no significant difference between the PQ poisoning and control groups, whereas that in the liver was enhanced from 6 to 72 hours in PQ poisoned rats.

DISCUSSION

Lipid peroxidation hypothesis for paraquat toxicity was proposed by Bus et al. (1975). Yasaka et al. (1981) reported that serum malondialdehyde level in paraquat poisoning was elevated in man and poisoned guinea pig, and others also observed experimentally the stimulation of lipid peroxidation by paraquat (Sata et al., 1983; Brigelius et al., 1980), while Steffen et al. (1980) and others (Kornbrust & Mavis, 1980; Shu et al., 1979; Ilett et al., 1974) observed the lack of lipid peroxidation using experimental animals.

It is very difficult to establish the normal composition of tissue components in man, because normal human tissue is not available. In spite of this difficulty, present study revealed that MDA level in human paraquat poisoning significantly increases in the lung and liver. On the other hand, MDA level in the poisoned rats did not significantly increase in the lung, in spite of the development of histological changes. The dose given to rats might be possibly too small to affect the lipid peroxidation, because the dose was fatal in human poisoning while the dose was not fatal in rats. But, MDA in the liver increased both in humans and in rats. MDA level is indicative of lipid peroxidation. Therefore, it is suggested that the stimulation of lipid peroxidation by paraquat may not be primary mechanism of paraquat toxicity.

Murry and Gibson (1972) compared the toxicity of paraquat in rats, guinea pigs and monkeys, and concluded that paraquat-induced pulmonary fibrosis in humans could be duplicated in monkeys and rats but not in guinea pigs. Accordingly, the difference of tissue MDA levels between humans and rats may be attributed to the clinical treatment after paraquat ingestion.

REFERENCES

- Brigelius R., Haslem A. and Lengfelder E. (1980): Paraquat-induced alteration of phospholipids and GSSG-release in the isolated perfused rat liver, and the effect of SOD-active copper complexes. *Biochem. Pharmacol.*, **30**, 349-354.
- Bus J. S., Aust S. D. and Gibson J. E. (1975): Lipid peroxidation: A possible mechanism for paraquat toxicity. *Res. Commun. Chem. Pathol. Pharmacol.*, **11**, 31-38.
- Ilett K. F., Stripp B., Menard R. H., Reid W. D. and Gillette J. R. (1974): Studies on the mechanism of the lung toxicity of paraquat: Comparison of tissue distribution and some biochemical parameters in rats and rabbits. *Toxicol. Appl. Pharmacol.*, **28**, 216-226.

- Kornbrust D. J. and Mavis R. D. (1980) : The effects of paraquat on microsomal lipid peroxidation in vitro and in vivo. *Toxicol. Appl. Pharmacol.*, **53**, 323-333.
- Kurisaki E. and Sato H. (1979) : Tissue distribution of paraquat and diquat after oral administration in rats. *Forensic Sci. Int.*, **14**, 165-170.
- Murry R. E. and Gibson J. E. (1972) : A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. *Exp. Med. Pathol.*, **17**, 317-325.
- Sata T., Takeshige K., Takayanagi R. and Minakami S. (1983) : Lipid peroxidation by bovine heart submitochondrial particles stimulated by 1, 1'-dimethyl-4, 4'-bipyridylium dichloride (paraquat). *Biochem. Pharmacol.*, **32**, 13-19.
- Shu H., Tallcott R. E., Rice S. A. and Wei E. E. (1979) : Lipid peroxidation and paraquat toxicity. *Biochem. Pharmacol.*, **28**, 327-331.
- Smith D., Heath D. and Key J. M. (1974) : The pathogenesis and structure of paraquat induced pulmonary fibrosis in rats. *J. Pathol.*, **114**, 57-67.
- Steffen C. and Metter K. J. (1979) : On the mechanism of paraquat action on microsomal oxygen reduction and its relation to lipid peroxidation. *Toxicol. Appl. Pharmacol.*, **47**, 593-602.
- Steffen C., Muliawan H. and Kappus H. (1980) : Lack of in vivo lipid peroxidation in experimental paraquat poisoning. *Arch. Pharmacol.*, **310**, 241-243.
- Thurlbeck W. M. and Thurlbeck S. M. (1976) : Pulmonary effects of paraquat poisoning. *Chest*, **69**, 276-280.
- Uchiyama M. and Mihara M. (1978) : Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.*, **86**, 271-278.
- Wasan S. M. and McElligott T. F. (1972) : An electron microscopic study of experimentally induced interstitial pulmonary fibrosis. *Amer. Rev. Resp. Dis.*, **105**, 276-282.
- Yasaka T., Ohya I., Matsumoto J., Shiramizu T. and Sasaguri Y. (1981) : Acceleration of lipid peroxidation in human paraquat poisoning. *Arch. Intern. Med.*, **141**, 1169-1171.