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## EFFECTS OF VITAMINS AND MINERALS ON CHRONIC PARAQUAT TOXICITY IN RATS

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### ラットパラコート中毒に及ぼすビタミンとミネラルの効果

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### Summary

The effects of nutrients on chronic paraquat toxicity seem worthwhile studying to find out ingredients effective for protection against environmental pollution of the herbicide. Effects of restriction of vitamins and/or minerals on chronic paraquat toxicity were studied using Wistar and ODS rats. Both strains of rats were fed with diets deficient in vitamins and/or minerals, and containing 125 mg paraquat/kg for 6 to 14 days. The following measurements were made; body weight changes, levels of two acute phase reactant proteins, such as cysteine proteinase inhibitor and  $\alpha$ 1-proteinase inhibitor, and levels of plasma ascorbyl radical. Appreciable changes were observed in the onset day of toxicosis and cysteine proteinase inhibitor levels for animals treated with the mineral-deficient diet. It was concluded that mineral deficiency enhanced the paraquat toxicity, but vitamin deficiency did not in both normal and ODS rats.

*Key words:* Paraquat; Mineral deficiency; Vitamin C; Cysteine proteinase inhibitor;  $\alpha$ 1-Proteinase inhibitor; ODS rats

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## **Introduction**

The toxicity of the herbicide paraquat (PQ) is known to be attributable to generation of reactive oxygen species [1]. Oxidative stress results in diseases when antioxidant defenses are defeated, and reactive oxygen species are not sufficiently removed. Many studies were performed to find out effects of antioxidants such as vitamin C, vitamin E and selenium (Se) on PQ toxicity. For example, deficiency of either vitamin E or Se significantly enhanced lethality due to PQ in mice [2]. In another work the acute PQ toxicity was highly influenced by nutritional Se, but not by vitamin E in chicks [3]. Vitamin C increased survival rate of mice [4], but it potentiated acute pathological and toxicological effects of PQ in rats [5]. These experiments all dealt with the effects of severe deficiency of nutrients on acute PQ toxicity.

Our studies have been being designed to examine the effects of moderate deficiency of various nutrients on toxicity induced by small amounts of PQ, because these mild conditions are more realistic in human life. Our previous results, however, were not satisfactory; vitamin E deficiency had no influence on chronic PQ toxicity in rats [6]. The effect of vitamin C was not clear; ODS rats, which cannot synthesize vitamin C, exhibited PQ toxicosis much earlier than normal rats as expected [7], but neither restriction nor oversupply of vitamin C changed the onset of toxicosis. In the present work, therefore, the effects of restriction of mixture of vitamins A, D, K, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, biotin, folic acid, pantothenic acid, nicotinic acid and choline on PQ toxicity have been studied; the effects of restriction of mineral mixture of Ca, K, Na, Mg, Mn, Fe, Zn, Cu and Cr have been also tested. Our study may give a clue to find out a dietary component, which protects humans from chronic PQ intoxication or which is useful for treatment of the intoxication.

## **Materials and method**

Male Wistar rats denoted by NOR in Table 1 were purchased from Nihon SLC, Hamamatsu; male ODS rats from Seiken Shizai K.K., Shizuoka. The concentration of vitamin E was 50 mg/kg diet in all groups and that of vitamin C supplemented to only ODS rats was 300 mg/kg diet. The concentrations of other vitamins or minerals were either those recommended by American Institute of Nutrition (AIN)-76 or half of them, expressed as 1 or 0.5, respectively in Table 1. The amounts of sucrose and starch were increased in accordance with the decreased amounts of vitamins and minerals. The maximum non-toxic dose of PQ administered to normal rats for two years was reported to be 250 mg/kg diet in 1970 [9], but the dose was greatly reduced to 35 mg/kg diet in 1986 [10]. In our previous work, we observed that 125 mg PQ/kg diet did not cause PQ poisoning for up to 14 days in both ODS and normal rats [8];

**Table 1.** Experimental design with various combinations of strain, animal number, doses of vitamin and mineral mixtures in the diet, onset day of toxicosis, and killing day

Exp	Group	Strain	Rat number	PQ mg/kg	Vitamin	Mineral	Onset day	Killing day
1	1	NOR	6	125	0.5	0.5	6	14
"	2	"	6	125	1	0.5	6	14
"	3	"	7	125	0.5	1	-*	14
"	4	"	6	125	1	1	-	14
"	5	"	6	0	1	1	-	14
2	6	ODS	6	125	0.5	0.5	3	6
"	7	"	6	125	1	0.5	3	6
"	8	"	6	0	1	0.5	-	7
"	9	"	6	125	1	1	-	14
"	10	"	6	0	1	1	-	14

\* No symptoms appeared

**Table 2.** Results of various parameters during chronic intoxication by paraquat under deficiencies of vitamins and/or minerals in rats

Exp	Group	Initial body weight g	Final body weight g	CPI unit/ml	$\alpha$ 1-P1 unit/ml	Ascorbyl radical unit/ml
1	1	134 $\pm$ 3	162 $\pm$ 12*	1.54 $\pm$ 0.21*	2.32 $\pm$ 0.23	1.34 $\pm$ 0.21
"	2	134 $\pm$ 3	164 $\pm$ 17*	1.54 $\pm$ 0.22*	2.21 $\pm$ 0.16	1.42 $\pm$ 0.18
"	3	133 $\pm$ 4	189 $\pm$ 13	0.92 $\pm$ 0.10	2.12 $\pm$ 0.16	1.14 $\pm$ 0.12
"	4	133 $\pm$ 4	193 $\pm$ 18	0.90 $\pm$ 0.12	1.85 $\pm$ 0.19	1.24 $\pm$ 0.29
"	5	134 $\pm$ 4	197 $\pm$ 9	0.80 $\pm$ 0.20	1.95 $\pm$ 0.20	1.00 $\pm$ 0.10
2	6	166 $\pm$ 12	138 $\pm$ 9**	4.35 $\pm$ 0.21**	2.84 $\pm$ 0.10**	1.74 $\pm$ 0.38**
"	7	171 $\pm$ 8	141 $\pm$ 9**	4.28 $\pm$ 0.40**	3.16 $\pm$ 0.23**	1.94 $\pm$ 0.16**
"	8	170 $\pm$ 6	201 $\pm$ 16	0.80 $\pm$ 0.08	1.95 $\pm$ 0.18	0.71 $\pm$ 0.10
"	9	168 $\pm$ 14	237 $\pm$ 14	0.83 $\pm$ 0.18	2.07 $\pm$ 0.34	0.76 $\pm$ 0.10
"	10	167 $\pm$ 12	221 $\pm$ 16	0.80 $\pm$ 0.18	2.00 $\pm$ 0.29	0.80 $\pm$ 0.16

\*: significantly (P<0.05) different from either group 3, 4 or 5. \*\*: significantly (P<0.05) different from either group 8, 9 or 10.

thus 125 mg PQ /kg diet was chosen in the present experiments.

The experimental design with various combinations of strains and doses of vitamin and mineral mixtures are shown in Table 1. Groups 5, 8 and 10 served as controls.

Animals were housed in individual cages at a controlled temperature (22°C) under a 12 h light-dark cycle. The feed and distilled water were given freely and the consumption of feed was measured everyday to assess anorexia. All intoxicated and healthy rats were killed on the day listed in Table 1 by cardiac puncture under light nembutal anesthesia. The initial and final body weights were recorded as shown in Table 2.

PQ, ficin and trypsin were purchased from Sigma, St. Louis, MO, USA, and other reagents used were of analytical grade. The levels of cysteine proteinase inhibitor (CPI),  $\alpha$ 1-proteinase inhibitor ( $\alpha$ 1-PI) and ascorbyl radical in plasma were measured by the methods described in our previous report [8]. The significance of the difference was evaluated using Student's *t*-test,  $P < 0.05$ .

## Results

PQ toxicosis in rats includes anorexia, diarrhea, hypokinesia and epistaxis. The first day, when these symptoms were noted, was recorded as onset day of toxicosis as shown in Table 1. The results obtained for each group are summarized in Table 2. In Exp. 1, mineral-restricted rats in groups 1 and 2 exhibited mild diarrhea and their feed intake was about 80% of that of the control from day 6, irrespective of vitamin restriction. Because their toxicosis progressed slowly, they were killed on day 14 together with mineral-adequate rats in groups 3 to 5, which appeared healthy. In accordance with the appearance of the symptoms, mineral-restricted groups 1 and 2 showed statistically higher CPI than those of mineral-adequate groups 3 to 5 (Table 2).  $\alpha$ 1-PI and ascorbyl radical levels tended to elevate, but did not reach statistical significance. The body weights of mineral-restricted rats (groups 1 and 2) increased by only about 30 g and those of mineral-adequate rats (groups 3 to 5) increased by about 60 g during two-week feeding. These results in Exp. 1 suggest that mineral-restriction enhances PQ toxicity, but vitamin-restriction does not, in this experimental model.

In Exp. 2 with ODS rats incapable of vitamin C biosynthesis, mineral-restricted rats in groups 6 and 7 exhibited diarrhea on day 3. Since their feed intake decreased day by day to zero on day 5, they were sacrificed on day 6 (Table 1). To assess the degree of mineral depletion, group 8 (mineral-deficient diet, no PQ) were sacrificed on day 7. Despite the treatment with PQ, mineral-adequate rats in group 9 did not show any symptom, and were sacrificed on day 14 together with control rats in group 10. CPI levels of groups 6 and 7 (mineral deficient, PQ) were about 5 times the control value, whereas those of group 8 (mineral-deficient, no PQ) and group 9 (mineral-adequate, PQ) were not different from control value (group 10) (Table 2). Significant difference was also true for both  $\alpha$ 1-PI and ascorbyl radical levels. The body weights of mineral-restricted rats with PQ (groups 6 and 7) decreased by about 30 g in one week of treatment, whereas that of mineral-restricted rats without PQ (group 8) increased by 30 g after the same time (Table 2). The body weights of

mineral-adequate rats (groups 9 and 10) increased by about 60 g during two-week feeding, irrespective of PQ treatment. These results in Exp. 2 are essentially similar to those in above Exp. 1 except that the mineral-restriction enhanced PQ toxicity more markedly in ODS rats than in normal Wistar rats.

### Discussion

If we ascribe the toxicity of PQ to peroxidation, antioxidants such as vitamins E and C are expected to act as antidote for PQ poisoning. However, conflicting results, such as protection, ineffectiveness and even worsening for PQ toxicity have been reported on these vitamins [2–8]. The effects of other vitamins have not been studied extensively. Niacin did not protect rats from hyperoxia induced by PQ [11]. Administration of either riboflavin or vitamin C alone had no effects, but the combined administration of both caused a significantly higher survival of rats [12]. Our present data suggest that restriction of vitamins other than E and C has no effects on chronic PQ toxicity (Table 2).

In both normal and ODS rats given adequate minerals, we were unable to find any behavioral or biochemical evidence of PQ toxicity at 125 mg/kg diet. However, severe toxicity was observed in mineral-restricted ODS rats at this dose of PQ (Table 2). We have already observed that Se-restriction to half the standard amount has no effects on chronic PQ toxicity in rats (unpublished data). Combs and Peterson [3] reported that severe Se deficiency markedly enhanced PQ toxicity in chicks. Administration of an element at half the standard amount like in the present experiments does not usually induce severe deficiency of the element, because the dietary standard amount is much higher than that of physiological requirement. In Exp. 2 mineral-restricted ODS rats given PQ displayed symptoms beginning on day 3, while mineral-adequate rats given PQ were healthy up to day 14. This difference suggests that some important *in vivo* reactions might be affected by a small change of certain mineral(s). There are a large number of minerals necessary in body; minerals needed in minute amounts may not be responsible for the present PQ toxicity. The relationship between mineral deficiency and PQ toxicity in whole animals have not been reported yet, although overdoses of Fe and Cu were reported to potentiate PQ toxicity in rats [13]. We are now in progress for specifying mineral(s) responsible for affecting PQ toxicosis.

Since humans resemble ODS rats in their inability to synthesize vitamin C, the present results suggest that humans are more sensitive to PQ intoxication under conditions of mild depletion of minerals.

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