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Jpn. J. Forensic Toxicol. Vol.19, No.3, 2001

原　　報

MAGNESIUM RESTRICTION AFFECTS TOXICITY OF PARAQUAT AND TISSUE COPPER AND IRON IN PARAQUAT-DOSED RATS

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Received May 24, 2001
Accepted Jyly 18, 2001

パラコート摂取ラットにおける組織中CuとFeの濃度に及ぼすMg減食の効果

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Summary

It seems very useful to find out nutrients including metals effective for chronic paraquat (PQ) toxicity, because pollution of the herbicide is one of the worldwide problems. The present work deals with the effects of Mg restriction on tissue Cu and Fe levels and on PQ toxicity in PQ-dosed rats. Wistar-ODS rats were fed with either Mg restricted diet containing PQ or mineral adequate diet containing PQ. The concentration of PQ in the diet was 125 ppm not to cause PQ toxicosis at least for 14 days in the rats with the adequate diet. The mineral adequate diet was based on the American Institute of Nutrition-76, and in the Mg restricted diet, the concentration of Mg was a half of that in the adequate diet. The rats fed with the Mg restricted diet began to display the symptoms of PQ toxicosis from day 4; the Mg-restricted and control rats were sacrificed on day 8. In the restricted rats, Cu concentrations increased about 2-fold in the liver and 1.5-fold in the lung and plasma, but decreased 0.6-fold in the kidney; Fe concentrations increased 1.8-fold in the liver, but decreased 0.7-fold in plasma. The restriction of Mg in the diet did not cause decrease in tissue Mg levels, but caused 1.3-fold increase in the Mg levels in the kidney and 1.2-fold increase in the spleen and plasma.

Key words: Paraquat; Magnesium; Copper; Iron; Mineral restriction

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Introduction

Paraquat (PQ) forms a monocation radical by enzymatic reduction in tissues and the radical reacts with oxygen to produce superoxide radical. The superoxide radical serves as a source of hydrogen peroxide and can also reduce Cu(II) and Fe(III) to Cu(I) and Fe(II), respectively. These reduced transition metals further react with hydrogen peroxide to yield hydroxyl radical. The superoxide radical and hydroxyl radical can be repeatedly generated by redox cycling of PQ, resulting in severe oxidative injuries in tissues by PQ [1]. There are several works dealing with the effect of metals on paraquat toxicity; Cu and Fe enhanced paraquat toxicity in E. coli [2] and in mice [3], whereas Zn protected E.coli from injury by PQ [4]. We found that Mg and/or K restricted diet enhanced PQ toxicity [5]. Although these works suggest strong correlation between PQ toxicity and metal concentrations in organs, only Cu concentrations in the lung and plasma were examined [6]. In the present work, we have measured concentrations of Cu, Fe and Mg in the lung, kidney, spleen, liver, heart and plasma in PQ-dosed rats, and tested the effects of Mg restriction on PQ toxicity and on Cu and Fe levels in the organs. We have used Wistar-ODS rats which cannot synthesize vitamin C like humans.

Materials and methods

Twelve male Wistar-ODS rats weighing 182 ± 10 g were purchased from Seiken Shizai K. K., Shizuoka. The animals were divided into two groups, fed with Mg restricted diet containing 125 ppm PQ (restricted group) and fed with mineral adequate diet containing 125 ppm PQ (control group). The mineral adequate diet was based on the American Institute of Nutrition-76 and the restricted diet contained a half amount of Mg. The equal weight of sucrose was added to the Mg-restricted diet according to the decrease of Mg. Supplemented amount of vitamin C was 300 ppm for both diets, which was reported to be sufficient to maintain normal growth of the Wistar-ODS rats. The ingredients of the diets were obtained from Oriental Yeast, Tokyo. The rats were housed in individual cages in a temperature controlled room (22 ℃) under 12 h light-dark cycle. The feed and distilled water were given freely and the feed consumption of each rat was measured every day to assess the degree of anorexia. The rats fed with the Mg restricted diet displayed symptoms of PQ intoxication, such as anorexia, hypokinesia, diarrhea and epistaxis from day 4. The restricted and control rats were sacrificed on day 8 by cardiac puncture under light nembutal anesthesia. Blood was collected with heparinized syringes and plasma was separated by centrifugation. Then the liver, kidney, lung, heart and spleen were excised, weighed, and stored frozen until analysis. A 0.2-g aliquot of each frozen tissue was mixed with 0.2 ml of conc. nitric acid, and 1 ml of plasma with 0.1 ml of conc. nitric acid in long quartz test tubes having loose quartz caps. They were kept at room temperature overnight. By this pretreatment, tissues except the heart were digested to small-particle suspension. Acid digestion was further carried out in a deep heating block for 8 h at 100℃ to give clear digested solution, which required little attention and permitted handling of a large number of samples. A Shimadzu flame atomic absorption spectrometer (type AA6200, Kyoto) was used for quantitation of Fe and Mg. To measure Cu concentrations, we used a sensitive ESR method [7]. Nitric acid and standard metal solutions used were of atomic absorption grade, and other reagents were of analytical grade. The ultra-pure water having resistance of 18 MΩ cm was used. All glassware or plastics were soaked in conc. HNO_3 or 0.3 N HNO_3, respectively, overnight, and rinsed more than ten times with the ultra-pure water.

The significance of the difference between the values obtained from the restricted and control rats was determined by the Student's *t*-test [8], and *P*-values less than 0.05 were regarded as being significant.

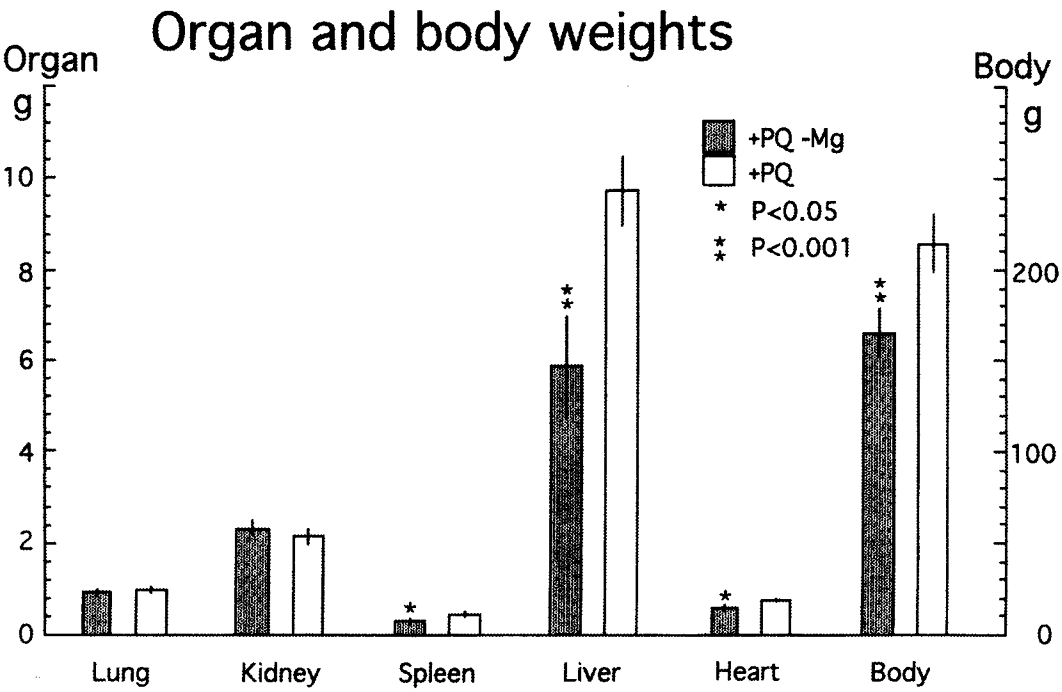

Fig. 1 . Organ and body weights of the Mg-restricted and control rats (n=6 for each group). Histogram of mean values with S.D. is shown. *P*-values less than 0.05 and 0.001 are indicated by * and * *, respectively.

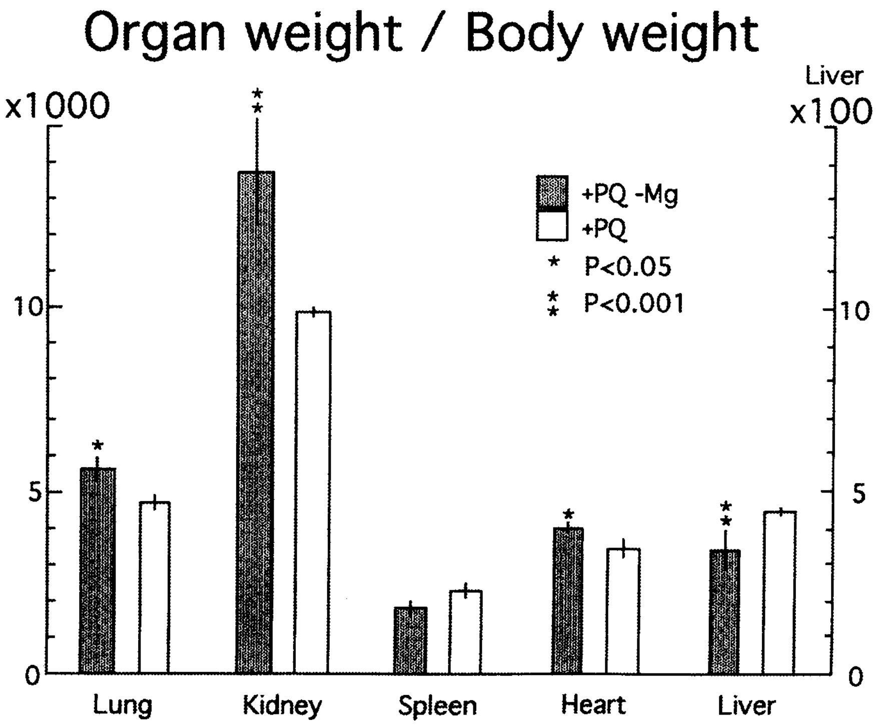

Fig. 2 . Weight ratios of organ to body for the 5 organs of the Mg-restricted and control rats. The explanations are the same as in Fig. 1.

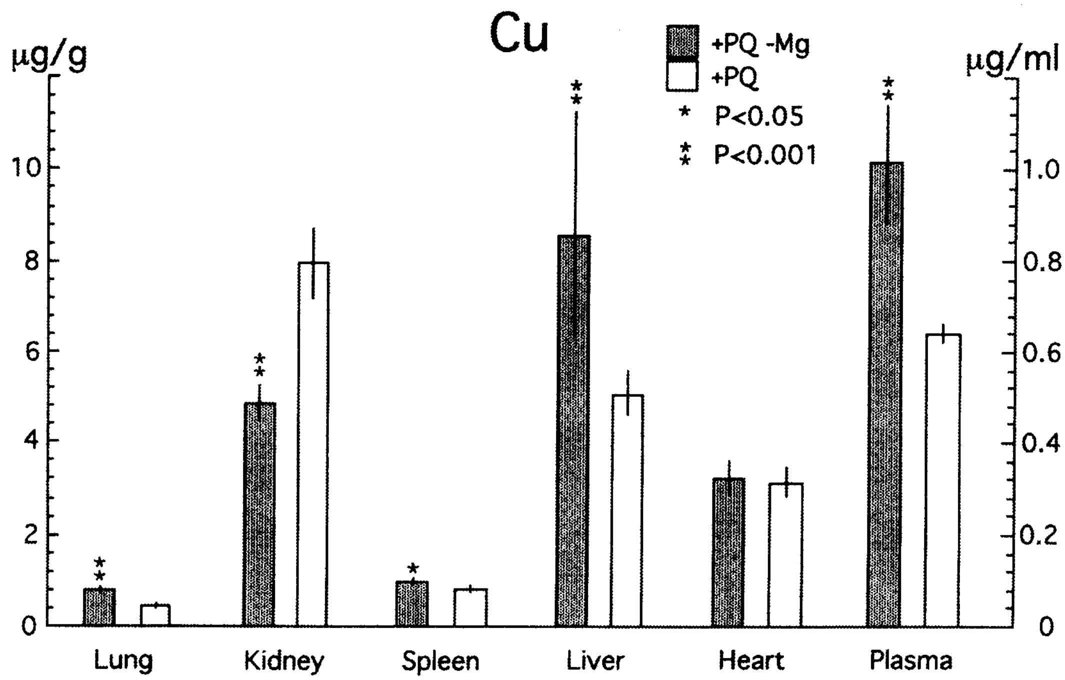

Fig. 3 . Cu levels in the organs and plasma of the Mg-restricted and control rats.

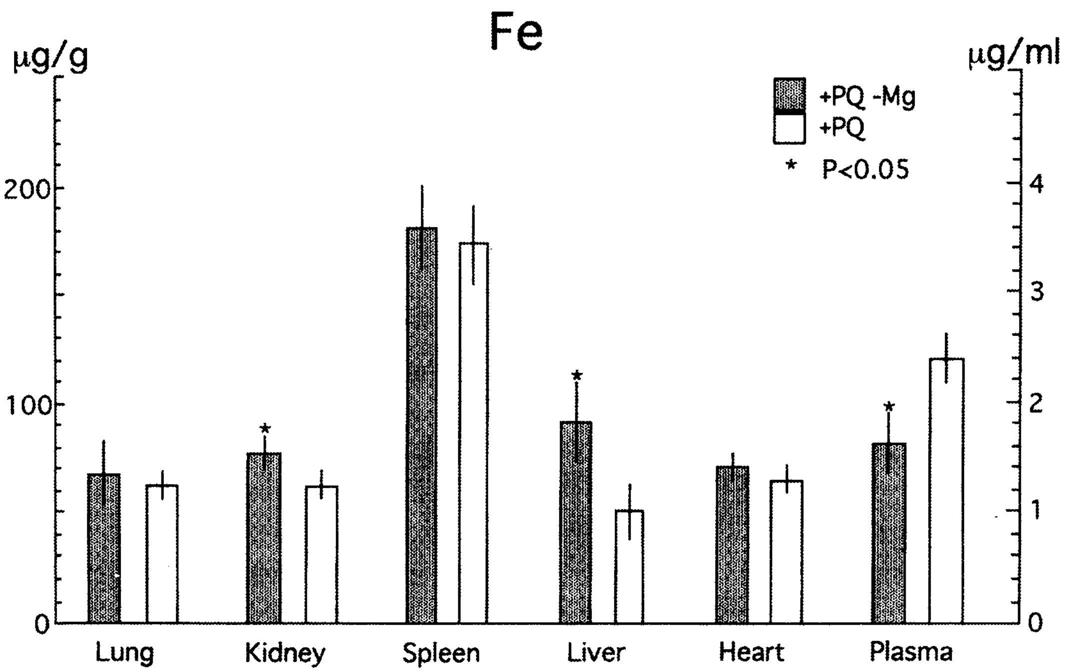

Fig. 4 . Fe levels in the organs and plasma of the Mg-restricted and control rats.

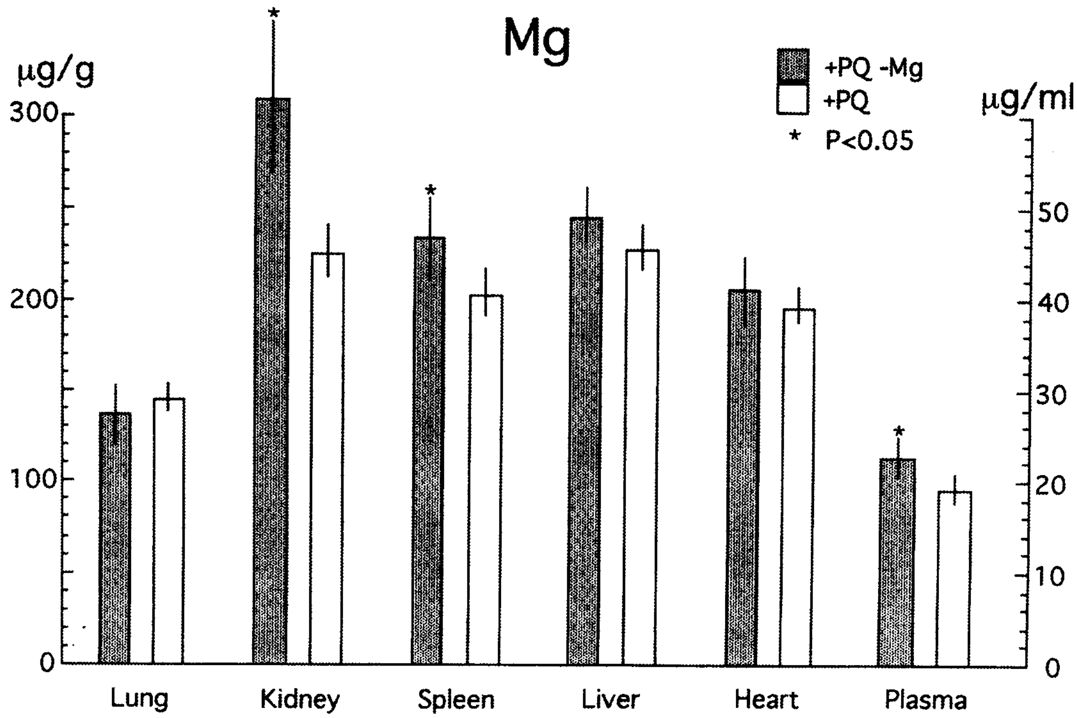

Fig. 5 . Mg levels in the organs and plasma of the Mg-restricted and control rats.

Results and discussion

The body and organ weights and the weight ratio of organ to body are shown in Figs. 1 and 2. The lungs of rats intoxicated with PQ usually show severe hemorrhages and congestion. In the present case, however, the lung of intoxicated rats showed only small hemorrhages and the weight ratio of the lung to the body increased only 1.2-fold as compared with that of the control rats. The surface of kidney showed granular degeneration and the kidney-to-body weight ratio increased 1.4-fold. Other organs of the restricted rats did not show any macroscopical differences from those of the control rats, but the liver-to-body weight ratio significantly decreased 0.8-fold in the Mg restricted group.

In the restricted rats, Cu concentrations increased 1.8-fold in the liver, 1.6-fold in plasma and 1.5-fold in the lung, and decreased 0.6-fold in the kidney as shown in Fig. 3. The increase in plasma Cu level might be due to the increase in ceruloplasmin (CP), because the 95 % of Cu are incorporated into CP in plasma. CP is an important extracellular antioxidant and free radical scavenger [9]. PQ elevated the CP activities in rats [6,10] and in monkeys [11]. Although CP is synthesized mainly in the liver, recent studies have identified the lung as another site of CP synthesis [12]. Therefore, the increase of Cu in the liver and lung may contribute partly to the increase of the synthesis of CP. The decrease of Cu in the kidney may be due to the consumption of Cu for the synthesis of CP.

Accumulation of Fe was observed in both liver and kidney of the Mg restricted rats as shown in Fig. 4.
On the contrary, plasma Fe level decreased 0.7-fold. It is of interest to note that the increase of plasma Cu accompanied the concomitant decrease of plasma Fe during 8 days of experiments.

The restriction of Mg in the diet was not reflected in tissue Mg levels as shown in Fig. 5. On the contrary, Mg levels were increased 1.3-fold in the kidney and 1.2-fold in the spleen and plasma.

The increase of transition metals may have a role of anti-oxidants, when they are conjugated onto its respective scavenger enzymes, such as Cu conjugated CP, Fe conjugated or Cu plus Zn conjugated superoxide dismutase, Fe conjugated catalase and Fe conjugated peroxidase. In the PQ intoxicated rats, these proteins may be synthesized more rapidly than in the control rats to prevent further degeneration due to oxidants. Cu and Fe, however, can become the center of hydoxyl radical production, when they are released from the conjugated proteins [2,3].

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific Research (10670385) from the Ministry of Education, Science, Sports and Culture of Japan.

References

1) Bus, J. S. and Gibson, J. E.: Paraquat; model for oxidant-initiated toxicity. Environ Health Persp, **55**, 37-46 (1984).
2) Kohen, R. and Chevion, M.: Transition metals potentiate paraquat toxicity. Free Rad Res Comms, **1**, 79-88 (1985).
3) Kohen, R. and Chevion, M.: Paraquat toxicity is enhanced by iron and reduced by desferrioxamine in laboratory mice. Biochem Pharmacol, **34**, 1843-1845 (1985).
4) Chevion, M., Korbashi, P., Katzhandler, J. and Saltman, P.: Zinc-a redox-inactive metal provides a novel approach for protection against metal-mediated free radical induced injury: study of paraquat toxicity in E. coli. In: Emerit, I. et al. (eds),Antioxidants in Therapy and Preventive Medicine, Plenum Press, New York, 1990, pp. 217-222.
5) Minakata, K., Suzuki, O., Saito, S. and Harada, N.: Dietary Mg and/or K restriction enhances paraquat toxicity in rats. Arch Toxicol, **72**, 450-453 (1998).
6) Divertie, M. B., Owen, C. A., Jr., Barham, S. S. and Ludwig, J. : Accumulation of radionuclide-labeled platelets and fibrinogen in paraquat-damaged rat lungs. Am Rev Respir Dis, **125**, 574-578 (1982).
7) Minakata, K., Suzuki, O. and Horio, F.: Quantification of copper in biological materials by use of electron spin resonance method. Clin Chem, in press.
8) Snedecor, G. W. and Cochran, W. G.: Statistical Methods, 6th ed., Iowa State Univ. Press, Ames, 1967, Chapter 4.
9) Atanasiu, R. L., Stea, D., Mateescu, M. A., Vergely, C., Dalloz, F., Briot, F., Maupoil, V., Nadeau, R. and Rochette, L.: Direct evidence of caeruloplasmin antioxidant properties. Mol Cell Biol, **189**, 127-135 (1998).
10) Beretta, C., Fadini, L., Montesissa, C., Pompa, G. and Stoppani, E.: Membrane lipid composition of subcellular fraction from lungs of rats treated withparaquat. Pharmacol Res Commun, **13**, 433-442 (1981).
11) Masaoka, T., Akahori, F., Arai, S. and Sakaguchi, K.: Effect of paraquat on plasmafibronectin, serum free hydroxyproline, serum ceruloplasmin and lung collagen contents in monkeys. J Toxicol Sci, **12**, 329-340 (1987).
12) Yang, F., Friedrichs, W. E., de Graffenried, L., Herbert, D. C., Weaker, F. J., Bowman, B. H. and Coalson, J. J.: Cellular expression of ceruloplasmin in baboon and mouse lung during development and inflammation. Am J Respir Cell Mol Biol,**14**, 161-169 (1996).