MANGANESE LEVELS IN TISSUES OF PARAQUAT DOSED AND/OR MAGNESIUM RESTRICTED RATS MEASURED BY AN ELECTRON SPIN RESONANCE METHOD

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Original

MANGANESE LEVELS IN TISSUES OF PARAQUAT DOSED AND/OR MAGNESIUM RESTRICTED RATS MEASURED BY AN ELECTRON SPIN RESONANCE METHOD

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ESR測定によるパラコート投与およびマグネシウム制限食投与ラットの組織中マンガン濃度

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Summary

The effects of drugs and nutritions on tissue manganese (Mn) levels have not been well studied, because of the quite low levels of Mn in mammalian tissues. Furthermore, Mn-complexes with chelators are so unstable that colorimetric assays of the Mn-complexes are interfered with by metal ions commonly present in tissues. To measure such trace amounts of Mn, we have used an electron spin resonance (ESR) method, since Mn^{2+} is paramagnetic. By this method, Mn^{2+} could be quantitated using 5 μl of its solution at 100 ppb with the error of 5%. The effects of paraquat dosing and/or magnesium (Mg) restriction in the diets for 8 days on tissue Mn and Mg levels were studied using osteogenic disorder Shionogi rats, a mutant strain which cannot synthesize vitamin C like humans. Tissue Mn levels of the animals were not affected by either dosing with paraquat at 125 ppm or Mg restriction at half of that recommended by American Institute of Nutrition-93. When both treatments were given together, however, Mn levels were significantly lowered in the liver, kidney and heart. Mn levels of rats dosed with paraquat at 250 ppm were more significantly lowered in all tissues studied.

Key words: Manganese; Electron spin resonance; ESR; Paraquat dosing; Magnesium restriction; Osteogenic disorder Shionogi rat

Introduction

The stability of complexes of bivalent transition metal ions is in the order of Pd > Cu > Ni > Co > Zn > Cd > Fe > Mn, irrespective of the nature of chelators [1]. This means that the binding of Mn with chelators is interfered with by other metals; this may be one of the reasons why sensitive colorimetric methods specific to Mn have not been reported yet. Most transition metals are paramagnetic, and some can be measured by electron spin resonance (ESR) even at room temperature, because they show specific *g* values as well as hyperfine structures in their ESR spectra [2,3]. For example, *g* values of Fe^{3+} and Cu^{2+} are 4.3 and 2.05, respectively. The *g* values of the 3rd and 4th lines of Mn^{2+} are 2.037 and 1.979, respectively, and the hyperfine splitting between them is 9.6 mT as shown in Figs. 1 and 2. Mn is well known to be an essential element in every species of all

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phyla; various Mn metallo-proteins/enzymes were discovered. One of the superoxide dismutases is an Mn-enzyme and detoxifies the superoxides produced from several drugs and poisons such as paraquat (PQ) [4,5] . It was reported that Mn deficiency was not only induced by Mn restriction itself, but was also accompanied by Mg deficiency in humans [6] and rats [7]. Therefore, in the present study, the effects of PQ dosing and/or Mg restriction on tissue Mn levels have been examined using an ESR method reported by us previously [2].

Experimental

Forty five male osteogenic disorder Shionogi (ODS) rats weighing 180± 10 g were purchased from Seiken Shizai K. K., Shizuoka. Animals were divided into 5 groups by changing the concentrations of PQ and Mg in the diet. Mineral mixture (M) was prepared according to American Institute of Nutrition-93. The concentration of Mg was either that recommended by the Institute to be designated as M-A (adequate) or half the concentration to be designated as Mg-R (restricted). The amounts of starch and sucrose were increased in accordance with the decrease of the minerals. The doses of PQ dichloride and Mg concentrations in the diets and the number of rats (n) were as follows. Group 1: no PQ, M-A and n=10 ; group 2: no PQ, Mg-R and n=5; group 3: 125 μg/g PQ, M-A and n=10; group 4: 125 μg/g PQ, Mg-R and n=10; and group 5: 250 μg/g PQ, M-A and n=10. The diets and distilled water were given freely. The rats were housed in individual cages in a temperature-controlled room (22℃) under 12 h light-dark cycle. They were sacrificed on day 8 by cardiac puncture under light nembutal anaesthesia. The livers, kidneys, lungs, hearts and spleens were separated, weighed and stored at – 80℃ until analysis.

A 0.2-g weight of each tissue was wet-ashed with conc. HNO_3 and used for quantitation of metals. A flame atomic absorption spectrometer (AA6200, Shimadzu, Kyoto) was used for the quantitation of Mg. ESR measurements were performed on a JEOL JES-FE2XG ESR spectrometer, (JEOL, Tokyo). Suitable microwave power and modulation width were 65 mW and 2 mT, respectively [2]. It took only less than 5 min for one measurement of an ESR spectrum. The standard Mn solution (0.1 – 1 ppm) in 1 M HNO_3 was prepared from the 1000 ppm Mn standard solution on the day of ESR measurements. The final concentration of HNO_3 of the wet-ashed tissue solution was also adjusted to about 1 M. A 5-μl aliquot of either standard Mn^{2+} solution or sample solution was put in a hematocrit capillary and measured at room temperature [2], and each peak height was compared with the standard ones for quantitation.

The standard Mn and Mg solutions were atomic absorption grade (Wako Pure Chemical Ind., Osaka), and other reagents were of analytical grade. The ultra-pure water having specific resistance of 18 MΩ cm was used. All glassware or plastics were soaked in conc. HNO_3 or 0.3 M HNO_3 overnight, respectively, and rinsed more than ten times with the ultra-pure water.

The significance of the differences was determined by Student's t-test [8], and P-values less than 0.05 were considered significant.

Results and discussion

The feed consumption of the rats could be regarded as an indicator of their intoxication. The consumption increased day by day in groups 1, 2 and 3; whereas it decreased day by day in groups 4 and 5 and finally became zero on day 7.

Tissue Mn concentrations were determined by comparing the ESR spectrum of Mn in tissues shown in Fig. 2 with that of the standard Mn shown in Fig. 1. The peak height of Mn in an ESR spectrum was not greatly affected by the concentrations of HNO_3 solution used as solvent; it changed by only 5% according to the different concentrations of HNO_3 from 0.6 to 1.2 M. Therefore, the Mn standard and the wet-ashed tissues were dissolved in 1 M HNO_3 solution. As shown in Figs. 1 and 2, the interference by other metals was not observed, though some metals in tissues were also paramagnetic.

The concentrations of Mg in tissues in groups 2 and 4 were not lowered as shown in Fig. 3, although these groups received the diets with a half concentration of Mg. On the contrary, the concentrations of Mg in the liver and kidney were even increased

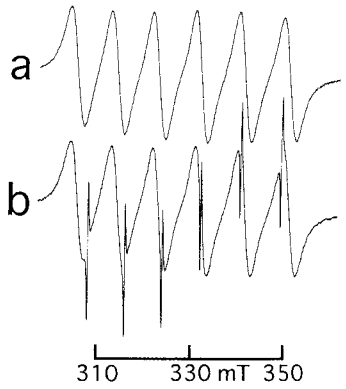

Fig. 1. ESR spectrum obtained from 5 μl of the 2 ppm Mn^{2+} standard solution (a) and signals of magnetic field calibrator (b). Gain setting was 2.5 x 10^2.

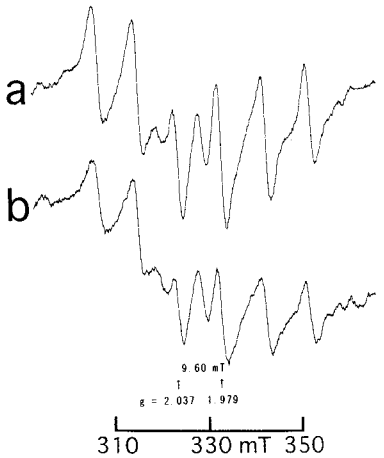

Fig. 2. ESR spectra obtained from 5 μl of wet-ashed solution made of 0.5 mg of the kidneys in group 1 (a) and in group 5 (b). Gain setting was 4 x 10^3.

in groups 4 and 5.

The Mn levels in tissues are shown in Fig. 4. They were lowered significantly in the liver, kidney and heart in group 4, and in all organs in group 5.

Mn can play dual roles in biological systems as a pro-oxidant when it is freely dissolved in cytoplasm and as an anti-oxidant when it is conjugated onto respective scavenger enzymes such as Mn superoxide dismutase. PQ reacts with oxygen to generate superoxide after its reduction within the cell. In the present study, Mn levels were found decreased by the administration of PQ in all five organs in group 5 (Fig. 4). This result suggests that synthesis of some Mn-proteins may be inhibited by PQ or these proteins may be denatured by PQ resulting in their more excretion than their synthesis.

Mg deficiency is known to induce peroxidation of tissues [9]. There were some reports describing that Mn deficiency was not induced by the loss of Mn itself, but was by the loss of Mg [6,7].

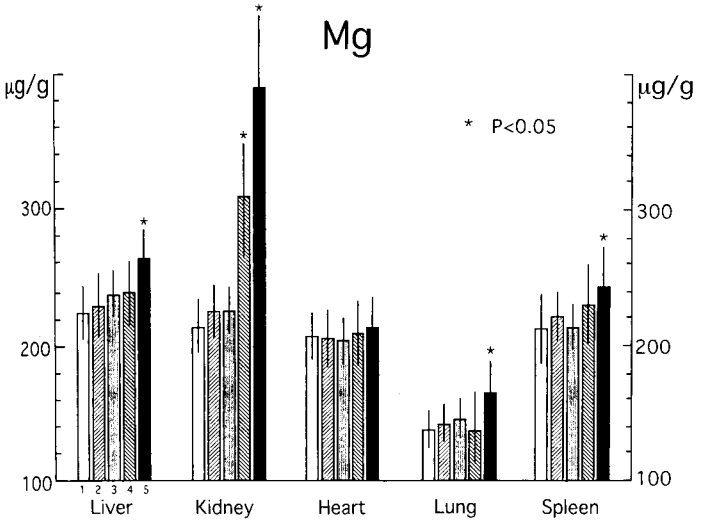

Fig. 3. Concentrations of Mg in tissues of ODS rats after the treatments described in the text. The columns of mean values with SD are shown; from left to right, groups 1 – 5. The values significantly different (P<0.05) from that in group 1 are indicated by *.

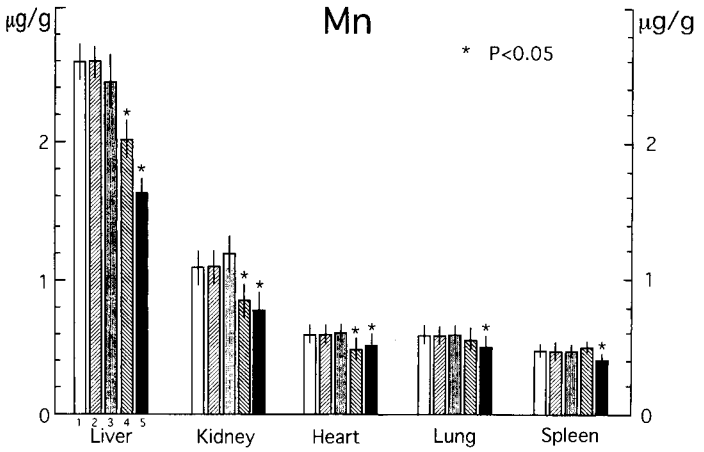

Fig. 4. Concentrations of Mn in tissues of ODS rats after the treatments. The explanations are the same as specified in Fig. 3.

In our study, Mg restriction for 8 days or dosing with PQ at 125 ppm only did not affect Mn levels as shown in groups 2 and 3 (Fig. 4). When both treatments were combined, Mn levels were lowered as shown in group 4. Mg and Mn may be replaced by each other in tissues. When poisons were introduced, the requirement of Mg was increased [9], and a part of Mn might be used in place of Mg when Mg was supplied insufficiently as in group 4. To explain the lowered Mn levels in the organs of groups 4 and 5, accumulation of Mn in other organs may be also considered as another possibility, although it has not been examined.

The present ESR method for analysis of Mn is simple, accurate and capable of measuring it not only in crude biological materials but also in waste water.

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