Removal of ^{54}Mn from the Mouse Body by Dilution with Stable Manganese and by Chelation with DTPA

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^{54}Mn is one of the activation products generated in nuclear reactors. This study was carried out to find a method appropriate for the removal of ^{54}Mn . Intraperitoneal administration of stable manganese effectively promoted the excretion of ^{54}Mn from the mouse body. The efficacy for removing ^{54}Mn was estimated to be 56, 67, 77 and 82% for manganese doses of 0.3, 1, 3 and 10 mg/kg, respectively. Oral administration of stable manganese was also effective for the removal of ^{54}Mn , but the efficacy was inferior to that obtained by intraperitoneal administration because of low gastrointestinal absorption of manganese. Ca-DTPA and Zn-DTPA promoted the excretion of ^{54}Mn when administered 1 h after administration of ^{54}Mn , but these chelating agents had little effect when administered after 3 h or more. Zn-DTPA was less effective than Ca-DTPA. These results demonstrate that dilution with stable manganese is more effective than chelation with DTPA for the removal of ^{54}Mn from the body.

Key Words : manganese-54, removal, manganese, DTPA

1. Introduction

Radioactive manganese, ^{54}Mn , is an activation product generated in nuclear reactors, and is found in the primary coolant of atomic power plants¹⁾. The yield of this nuclide is far less than that of fission products such as ^{90}Sr and ^{137}Cs , but food and environmental contamination with this nuclide has been caused by atmospheric nuclear tests²⁾⁻⁵⁾ and by leakage of the coolant from nuclear reactors⁶⁾. The International Commission on Radiological Protection assumes that 10% of the ingested ^{54}Mn is absorbed via the digestive tract, and 70% and 30% of the incorporated ^{54}Mn remain in the body with biological half-lives of 40 days and 4 days, respectively⁷⁾. As the incorporated ^{54}Mn irradiates tissues with 835 keV gamma rays, it should be removed from the body to reduce the risk of possible radiation injuries. The National Council on Radiation Protection and

Measurements has reported therapeutic procedures and drugs for external and internal contamination with several radionuclides⁸⁾, but no information is available concerning the removal of ^{54}Mn from the body.

Manganese is an essential trace element and its concentration in tissues is maintained homeostatically by regulating the absorption from diet and the excretion from the body⁹⁾. Thus, isotopic dilution with stable manganese is expected to be effective for removing ^{54}Mn from the body. On the other hand, a chelating agent, DTPA (diethylenetriaminepentaacetic acid), has been recommended as an eliminant for many radionuclides, e.g. americium, plutonium and zinc⁸⁾, but it has not been reported whether DTPA is available for the removal of ^{54}Mn . Therefore, dilution with stable manganese and chelation with DTPA were applied to mice to find a method appropriate for the removal of ^{54}Mn from the body.

2. Materials and Methods

2.1 Animals and reagents

Adult male mice of the ICR strain (12 weeks old) were housed in aluminum cages and maintained in a room with a temperature controlled at 23°C. They were allowed free access to a commercial diet (MF, Oriental Yeast Co. Ltd., Tokyo) and tap water. ^{54}Mn (MnCl_2 , 3.9 GBq/mg, NEN/DuPont U.S.A.) was diluted with physiological saline and adjusted to a concentration of 370 kBq/ml, pH 7.0–8.0. Stable manganese (MnCl_2) was dissolved in distilled water and adjusted to various concentrations of 0.03–20 mg-Mn/ml. Ca-DTPA and Zn-DTPA were diluted with distilled water to a concentration of 10 mg/ml.

2.2 Removal of ^{54}Mn by parenteral administration of stable manganese

Five groups of 5 mice each were administered intraperitoneally with ^{54}Mn (37 kBq/mouse), and their initial whole-body radioactivity was measured with a small animal counter (NaI scintillation counter). Twenty-four hours later, four of the groups were treated with a single intraperitoneal administration of stable manganese (0.3, 1.0, 3.0 or 10 mg/kg). The remaining group was used as a control without any treatment. Thereafter, whole-body retention of ^{54}Mn was measured for 14 days at suitable intervals. The efficacy for removing ^{54}Mn was estimated by the following equation.

$$\text{Removal of } ^{54}\text{Mn} = (R_c - R_t) / R_c$$

R_c : retention of ^{54}Mn in the control group

R_t : retention of ^{54}Mn in the treated group

From a practical viewpoint, stable manganese should be administered intravenously, but intraperitoneal administration was adopted in this study because of the difficulty of continuous intravenous injection in mice.

2.3 Excretion of stable manganese administered intraperitoneally

Four groups of mice were given an intraperitoneal administration of stable manganese which contained a trace amount of ^{54}Mn at the same doses as described in the above experiment. Excretion of the stable manganese was observed by measuring the whole-body retention of ^{54}Mn . The proportion of stable manganese which was excreted more rapidly than normal was estimated by extrapolating the slow component of the retention curves to the zero time.

2.4 Removal of ^{54}Mn by oral administration of stable manganese

Twenty-four hours after intraperitoneal administration of ^{54}Mn , the animals were given stable manganese orally (3, 10, 30 or 100 mg/kg). The efficacy for removing ^{54}Mn was estimated as described in 2.2.

2.5 Gastrointestinal absorption of stable manganese administered orally

Five other groups of mice were given an oral administration of carrier-free ^{54}Mn or ^{54}Mn -containing stable manganese at a dose of 3, 10, 30 or 100 mg/kg. Apparent absorption of the stable manganese was estimated by extrapolating the slow component of the retention curves to the zero time point.

2.6 Removal of ^{54}Mn by chelation with DTPA

Nine groups of mice were administered intravenously with ^{54}Mn . Eight of the groups were treated with an intraperitoneal administration of Ca-DTPA or Zn-DTPA (100 mg/kg) 1, 3, 6 or 24 h after administration of ^{54}Mn . The remaining group was used as a control without any treatment. Whole-body retention of ^{54}Mn was mea-

sured for 14 days to determine the efficacy for removing ^{54}Mn .

3. Results

3.1 Removal of ^{54}Mn by parenteral administration of stable manganese

Figure 1 shows the whole-body retention curves of ^{54}Mn in mice. Intraperitoneal administration of stable manganese markedly decreased the retention of ^{54}Mn , but the retention curves became parallel with that of the control mice within a few days after administration of stable manganese. The efficacy for removing ^{54}Mn was estimated to be 56, 67, 77 and 82% for manganese doses of 0.3,

Fig. 1 Effect of an intraperitoneal administration of stable manganese on the whole-body retention of ^{54}Mn in mice.

1, 3 and 10 mg/kg, respectively (Table 1).

3.2 Excretion of stable manganese administered intraperitoneally

Stable manganese containing a trace amount of ^{54}Mn was administered intraperitoneally to mice. A large proportion of the stable manganese was excreted within 24 h after administration. The proportion of stable manganese which was excreted more rapidly than the normal clearance rate was estimated to be 74–93% of dose (Table 1).

3.3 Removal of ^{54}Mn by oral administration of stable manganese

Oral administration of stable manganese also promoted the excretion of ^{54}Mn and the efficacy was estimated to be 14.0, 18.5, 38.1 and 55.0% for manganese doses of 3, 10, 30 and 100 mg/kg, respectively (Table 2). The efficacy of oral administration was inferior to that of intraperitoneal administration when compared by the dose of stable manganese.

3.4 Gastrointestinal absorption of stable manganese administered orally

Several amounts of stable manganese which contained ^{54}Mn as a tracer were administered orally to mice, and the apparent absorption was

Table 1 Removal of ^{54}Mn by an intraperitoneal administration of stable manganese, and excretion of stable manganese

Dose (mg/kg)	Retention on 14th day (%)	Removal of ^{54}Mn (%)	Excretion of stable Mn (%)	Exchangeable ^{54}Mn (%)
0	42.6±1.2	—	—	—
0.3	18.9±1.1	55.6±2.9*	73.6±1.3	75.5±4.2
1.0	13.9±1.4	67.4±3.4*	84.3±0.8	80.0±4.1
3.0	9.9±0.8	76.8±2.0*	89.8±0.3	85.5±2.2
10.0	7.6±0.9	82.2±2.2*	93.2±0.3	88.2±2.4

Mean±SD for five mice, *: Significant effect ($p<0.01$), Exchangeable ^{54}Mn = Removal of ^{54}Mn /Excretion of stable Mn

Table 2 Removal of ^{54}Mn by an oral administration of stable manganese, and gastrointestinal absorption of the stable manganese

Dose (mg/kg)	Retention on 7th day (%)	Removal of ^{54}Mn (%)	G. I. absorption of Mn		Mn uptake (mg/kg)
			Apparent (%)	True (%)	
0	64.9 $\pm$ 2.4	—	0.61 $\pm$ 0.12	0.61 $\pm$ 0.12	—
3	55.8 $\pm$ 1.8	14.0 $\pm$ 4.2*	0.50 $\pm$ 0.15	0.58 $\pm$ 0.18	0.017 $\pm$ 0.005
10	52.9 $\pm$ 3.3	18.5 $\pm$ 5.9*	0.18 $\pm$ 0.03	0.22 $\pm$ 0.04	0.022 $\pm$ 0.004
30	40.2 $\pm$ 1.7	38.1 $\pm$ 3.5*	0.15 $\pm$ 0.06	0.24 $\pm$ 0.10	0.072 $\pm$ 0.029
100	29.2 $\pm$ 3.4	55.0 $\pm$ 5.5*	0.11 $\pm$ 0.04	0.24 $\pm$ 0.09	0.244 $\pm$ 0.094

Mean $\pm$ SD for five mice, * : Significant effect ($p < 0.01$), True absorption = Apparent absorption / (1 - Removal of ^{54}Mn), Mn uptake = Dose $\times$ True absorption.

determined by extrapolating the slow component of the retention curves. Apparent absorption of manganese was very low; it was estimated to be 0.6% when ^{54}Mn was administered without carrier manganese, and was decreased further as the dose of stable manganese was increased (Table 2).

3.5 Removal of ^{54}Mn by chelation with DTPA

Ca-DTPA and Zn-DTPA slightly promoted the excretion of ^{54}Mn when administered 1 h after administration of ^{54}Mn . Three hours or more later, Ca-DTPA had a little effect on the excretion of ^{54}Mn , while Zn-DTPA had no effect (Table 3).

4. Discussion

Manganese metabolism and effect of manganese intake on its metabolism have been studied from nutritional and physiological viewpoints, but few studies have been carried out from the viewpoint of radiation protection. Although the effect of parenteral administration of manganese on its metabolism has already been reported by Cotzias and Greenough¹⁰⁾, Papavasiliou et al.¹¹⁾ and Bertinchamps et al.¹²⁾, these studies did not provide data regarding protection against internal radiation exposure to ^{54}Mn . In the present study, we clarified the quantitative relationship between the

Table 3 Removal of ^{54}Mn by an intraperitoneal administration of chelating agents

Time of treatment	Chelating agent	Whole-body retention on 14th day (%)	Removal of ^{54}Mn (%)
Control	—	45.5 $\pm$ 2.0	—
1 h later	Ca-DTPA	37.4 $\pm$ 0.8	17.8 $\pm$ 4.0*
	Zn-DTPA	40.7 $\pm$ 1.4	10.5 $\pm$ 5.0*
3 h later	Ca-DTPA	39.6 $\pm$ 1.8	13.0 $\pm$ 5.5*
	Zn-DTPA	45.7 $\pm$ 2.1	-0.4 $\pm$ 6.4
6 h later	Ca-DTPA	43.6 $\pm$ 3.1	4.2 $\pm$ 8.0
	Zn-DTPA	43.8 $\pm$ 1.6	3.7 $\pm$ 5.5
24 h later	Ca-DTPA	41.7 $\pm$ 2.7	8.4 $\pm$ 7.2*
	Zn-DTPA	46.7 $\pm$ 2.7	-2.6 $\pm$ 7.5

Mean $\pm$ SD for 5 mice, * : Significant effect ($p < 0.05$), † : Significantly higher effect than Zn-DTPA ($p < 0.05$)

dose of stable manganese and the efficacy for removing ^{54}Mn , and demonstrated that the parenteral dilution method was very effective for removing ^{54}Mn from the body.

In the dilution method, there are three important factors which determine the efficacy for removing radionuclides. The first factor is the dilution ratio, i.e. the proportion of the dose of an element to its amount in the body. For ^{54}Mn , the amount of manganese in the body is so little (0.2–0.3 mg/kg) that a high dilution ratio can be obtained by a relatively small dose of stable manganese^{7),9)}.

The second factor is the body's ability to excrete the element. Under ordinary conditions, manganese is excreted mainly via the bile to feces^{11),12)}. Manganese excretion also takes place via the pancreatic juice and intestinal mucosa as auxiliary routes^{12),13)}. In this study, 1.5 – 50 times as much manganese as the whole-body content was administered intraperitoneally to mice, but a large proportion of the stable manganese was excreted rapidly from the body (Table 1). This result suggests that these routes have a large capacity to excrete manganese.

The third factor is the exchangeability of radionuclides in the body, since radionuclides cannot be removed by the dilution method if they are firmly fixed to tissues in the body. We assumed that ⁵⁴Mn consists of two components in the body, exchangeable and unexchangeable ⁵⁴Mn. The proportion of exchangeable ⁵⁴Mn was estimated as follows.

$$\text{Exchangeable } ^{54}\text{Mn} = \frac{\text{Removal of } ^{54}\text{Mn}}{\text{Excretion of stable Mn}}$$

Although the percentage of exchangeable ⁵⁴Mn depended on the dose of stable manganese, high exchangeability of 76 – 88% was obtained (Table 1). This result suggests that most of the incorporated ⁵⁴Mn exists in some movable form in the body¹⁴⁾.

Oral administration of stable manganese was less effective than the parenteral administration when compared by the dose of stable manganese. The inferiority of oral administration was due to low gastrointestinal absorption of manganese. Gastrointestinal absorption of manganese has been reported to be 2 – 4% in humans and animals^{9),15),16)}, but it is affected by the age of animals, the level of dietary manganese and other factors⁹⁾. Therefore, gastrointestinal absorption of orally administered stable manganese was estimated and values of 0.61 – 0.11% were ob-

tained. However, these values might be underestimations, because the amount of manganese which was absorbed and then excreted within 24 h was not taken into consideration for the estimation of the apparent absorption. As the proportion of manganese excreted within 24 h was expected to correspond to the rate of removal of ⁵⁴Mn, true absorption was estimated by the following equation.

$$\text{True absorption} = \frac{\text{Apparent absorption}}{1 - \text{Removal of } ^{54}\text{Mn}}$$

As shown in Table 2, the true absorption was greater than the apparent absorption. The true absorption of manganese varied with the dose, but there were no further changes when the dose was increased above 10 mg/kg. Under manganese-deficient conditions, gastrointestinal absorption of manganese is closely related to the level of dietary manganese^{17),18)}, but the controlled absorption may not significantly contribute to the homeostasis of manganese under manganese-excess conditions.

The product of the dose of stable manganese and the corresponding value of the true absorption was regarded as the uptake of manganese (Table 2). Although the oral dilution method was less effective than the parenteral one when compared by the dose of stable manganese, the relationship between the uptake of manganese and the efficacy for removing ⁵⁴Mn showed good agreement between the oral and the parenteral administration groups (Fig. 2). Therefore, the inferiority of the oral dilution method is entirely due to low gastrointestinal absorption of manganese.

DTPA is a chelating agent recommended as an eliminant for many radionuclides, but it was less effective than stable manganese for the removal of ⁵⁴Mn. Moreover, DTPA had little effect when administered 3 h or more after administration of ⁵⁴Mn. Manganese concentration in the plasma is

Fig. 2 Relationship between the dose of stable manganese and the efficacy for removing ^{54}Mn .

Elimination factor =

$$\frac{\text{Retention of } ^{54}\text{Mn in the control mice}}{\text{Retention of } ^{54}\text{Mn in the treated mice}}$$

very low⁹⁾, and intravenously administered ^{54}Mn disappears rapidly from the blood into cells¹⁹⁾. DTPA cannot penetrate the cell membrane and is excreted rapidly via the kidneys²⁰⁾. Therefore, DTPA must be administered as soon as possible in order to form a chelate of ^{54}Mn -DTPA in the body.

DTPA forms a chelate with many kinds of metals, but the stability of DTPA chelates varies as follows: $\text{Zn-DTPA} > \text{Mn-DTPA} > \text{Ca-DTPA}$ ²¹⁾. Ca-DTPA dissociates its calcium and combines with ^{54}Mn to form a more stable chelate, whereas Zn-DTPA cannot combine with ^{54}Mn to the same level as Ca-DTPA because Zn-DTPA is more stable than Mn-DTPA. Therefore, Ca-DTPA is more effective than Zn-DTPA for removing ^{54}Mn from the body.

The present study demonstrated that dilution with stable manganese was more effective than chelation with DTPA. For therapeutic application, it is essential that the benefit of the treatment is greater than its risk. Manganese is less toxic to animals, and incidents of oral manganese poison-

ing are quite rare. Many animals tolerate manganese-supplemented diets up to 1000–2000 mg-Mn/kg without ill effects. Although oral administration of manganese may cause iron deficiency anemia, this side effect can be suppressed by a dietary supplement of iron⁹⁾. These observations support the safety of the dilution method. However, it has been reported that manganese has neurotoxicity when ingested or inhaled chronically⁹⁾. Therefore, toxicological studies are required before this treatment method can be applied to humans.

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要 旨

安定体マンガンおよび DTPA による体内 ^{54}Mn の除去

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^{54}Mn は原子炉内で生成する誘導放射性核種の一つであるが, ^{54}Mn の体外除去に関する研究はほとんど行われていない。本研究は, ^{54}Mn に適した除去方法を見いだすことを目的とした。安定体マンガンの腹腔内投与は, マウス体内からの ^{54}Mn の排泄を著しく促進した。その除去効果は, 0.3, 1, 3 および 10 mg-Mn/kg でそれぞれ, 56, 67, 77 および 82% であった。また, 腹腔内投与された安定体マンガンも速やかに排泄された。安定体マンガンを経口投与した場合にも ^{54}Mn の除去効果は認められたが, マンガンの消化管吸収率が低いため, その効果は腹腔内投与したものよりは低かった。Ca-DTPA および Zn-DTPA は, ^{54}Mn の投与 1 時間後に投与した場合には除去効果が認められたものの, 3 時間以上経過した場合にはほとんど効果が認められなかった。また, Zn-DTPA の ^{54}Mn 除去効果は Ca-DTPA よりも低かった。以上の結果から, 安定体マンガンによる希釈法は DTPA によるキレート法よりも ^{54}Mn 除去方法として有効であることが確認された。