Effects of Dietary Manganese Contents on $^{54}$Mn Metabolism in Mice

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(Received, October 23, 1995)
(Revision received, May 9, 1996)
(Accepted, May 16, 1996)

$^{54}$Mn/metabolism/elimination/manganese/diet

Several parameters of $^{54}$Mn metabolism were noted in mice maintained on diets with manganese contents of 80 to 8000 mg/kg. Excretion of $^{54}$Mn was promoted as the dietary manganese contents increased. Clearance of $^{54}$Mn from the liver, kidneys, pancreas, and spleen was markedly accelerated by feeding mice a high-manganese diet, but clearance from the muscles, femurs, and brain was relatively insensitive to the dietary managanese. Manganese concentrations in the tissues were regulated homeostatically up to the dietary manganese content of 2400 mg/kg, but marked accumulations of manganese occurred when mice were given 8000 mg/kg diet. No toxic symptoms were found up to the 2400 mg/kg diet, but consumption of the 8000 mg/kg diet was less than for other diets. These results suggest that an oral intake of excess manganese is effective for promoting the excretion of $^{54}$Mn from a body contaminated with this isotope.

INTRODUCTION

$^{54}$Mn is generated by the reaction of $^{54}$Fe (n, p)$^{54}$Mn in nuclear reactors and is found in the primary coolant of atomic power plants$^1$. It is also generated by nuclear explosions and has been detected in the fallout from atmospheric nuclear tests$^2,3)$. Because $^{54}$Mn emits 835 keV gamma-rays with a physical half-life of 312 days, it is considered a hazardous radionuclide.

$^{54}$Mn incorporated into humans is retained in the body with a biological half-life of 40 days$^4)$. Although incorporated $^{54}$Mn causes internal radiation exposure, the dose of this exposure can be reduced by promoting the excretion of $^{54}$Mn from the body. Manganese metabolism has been well studied$^5)$, but few studies have been done on the promotion of the excretion of $^{54}$Mn. In a previous study, we showed that an intraperitoneal injection of manganese chloride very effectively promoted the excretion of $^{54}$Mn from mice$^6)$. Oral intake of excess manganese also was expected to be effective for its excretion. We therefore investigated the excretion of $^{54}$Mn and several other parameters of its metabolism in mice maintained on diets with various contents of manganese.

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MATERIALS AND METHODS

Animals and Diets

One hundred adult male mice of the ICR strain (12 weeks old, 33–38 g) were divided into 20 groups of 5 mice each and housed in aluminum cages in a temperature-controlled room. They were maintained on a commercial diet (MF, Oriental Yeast Co., Ltd.) until the start of each experiment. The commercial diet contained 80 mg-Mn/kg. Experimental diets were prepared by adding manganese chloride solution to the powder of the commercial diet. The manganese contents of the experimental diets were adjusted to 240, 800, 2400, and 8000 mg/kg (dry basis). The mixtures were kneaded with distilled water and dried at 70°C in a drying oven.

Experiment 1. Excretion of ⁵⁴Mn

Five groups of mice were used to determine the effect of dietary manganese contents on the excretion of ⁵⁴Mn. Mice were injected intraperitoneally with a single dose of ⁵⁴Mn (⁵⁴MnCl₂, DuPont, 37 kBq/mouse), and their initial whole-body radioactivities were measured with a whole-body counter that had a 3-inch NaI (T1) crystal. Thereafter, four groups were maintained on the various experimental diets. The remaining group, used as the control, was fed the commercial diet. Whole-body retention of ⁵⁴Mn and food consumption were observed at suitable intervals for 21 days. The retention curves obtained were approximated by the sum of two exponential functions in order to estimate the efficacy of a high-manganese diet in eliminating ⁵⁴Mn from the body. Daily and total eliminations were determined by the following equations:

\[
\text{Daily elimination} (\% \text{ of control}) = \frac{(RC_t - RH_t/RH_{t-1})}{(RC_t/RC_{t-1})} \times 100
\]

\[
\text{Total elimination} (\% \text{ of control}) = \frac{(RC_t - RH_t)}{RC_t} \times 100
\]

where,

- \(RC\): whole-body retention of ⁵⁴Mn in mice fed the commercial diet
- \(RH\): whole-body retention of ⁵⁴Mn in mice fed the experimental diet
- \(t\): days after injection of ⁵⁴Mn

These values show the percentages of excess ⁵⁴Mn excreted by mice fed high-manganese diets as compared with control mice fed the commercial diet.

Experiment 2. Distribution of ⁵⁴Mn

Ten groups of mice were used to determine the effect of dietary manganese contents on the distribution of ⁵⁴Mn. Mice were given an intraperitoneal injection of ⁵⁴Mn as described above, then their initial whole-body radioactivities were measured. Five groups, used as controls, were maintained on the commercial diet. The mice in the other groups were fed a 2400 mg/kg diet. One, 2, 4, 7 and 14 days after the injection, mice were euthanized under ether anesthesia after their weights and whole-body retentions were measured. The liver, kidneys, pancreas, spleen, brain, muscles, and femurs were excised and weighed, after which their radioactivities were measured with a well type scintillation counter. The concentration ratio of ⁵⁴Mn in the tissues was calculated as.
Experiment 3. Manganese concentration in tissues

This experiment was done to clarify whether manganese concentrations in mouse tissues could be regulated homeostatically under various contents of dietary manganese. Five groups of mice that had been maintained on the various diets for 21 days were euthanized under ether anesthesia. The individual livers, kidneys, brains, muscles, and femurs were excised, weighed, and ashed at 450°C for 48 hours in an electric furnace. Residues were dissolved in 1N hydrochloric acid and the manganese concentrations were determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Experiment 1. Excretion of $^{54}$Mn

Fig. 1 shows the whole-body retention curves of $^{54}$Mn in mice maintained on diets with various manganese contents. Excretion of $^{54}$Mn was accelerated at increasing contents of dietary manganese. Mean whole-body retentions on the 21st day were 30.7%, 18.5%, 12.4%, 5.5%, and 2.5% for 80, 240, 800, 2400 and 8000 mg/kg diet groups, respectively. Table 1 gives the approximate equations of the retention curves and the biological half-lives of $^{54}$Mn in the fast- and slow components. The biological half-lives of both components were reduced and, moreover the percentage of the fast component, which had a shorter half-life, was markedly increased as

$$\text{Concentration ratio} = \frac{\text{Tissue activity/Tissue weight/F}^*}{\text{Initial whole-body activity/Body weight}}$$

*F: correction coefficient for the counting efficiency of the well counter which has a higher counting efficiency than that of the whole-body counter.
dietary manganese contents increased.

The daily and total eliminations shown in Fig. 2 were estimated from the approximate equations. These values are the percentages of excess excreted $^{54}$Mn, as compared to values for the controls fed the commercial diet. Daily eliminations on the first day respectively were estimated to be 3%, 11%, 24%, and 40% for the 240, 800, 2400, and 8000 mg/kg diets, but these values decreased rapidly with time. In contrast, total eliminations increased with the period of treatment, respectively reaching 24%, 49%, 74%, and 87% after 10 days.

Table 1. Approximate equations for the whole-body retention curves of $^{54}$Mn, biological half-lives of $^{54}$Mn, and daily consumption of the diet.

| Mn conc. (mg/kg diet) | Approximate equation* (% of dose) | Biol. half-life (day)** | Diet consumption** (g/day/mouse) |
|-----------------------|-----------------------------------|------------------------|----------------------------------|
| 80                    | $30.0 \exp^{-0.31t}+70.0 \exp^{-0.040t}$ | 2.3 ± 0.2              | 17.5 ± 1.0                       | 4.6 ± 0.4 |
| 240                   | $37.7 \exp^{-0.30t}+62.3 \exp^{-0.058t}$ | 2.3 ± 0.2              | 11.9 ± 0.3                       | 4.9 ± 0.6 |
| 800                   | $57.9 \exp^{-0.37t}+42.1 \exp^{-0.059t}$ | 1.9 ± 0.1              | 11.8 ± 0.4                       | 4.4 ± 0.5 |
| 2400                  | $74.7 \exp^{-0.52t}+25.3 \exp^{-0.073t}$ | 1.3 ± 0.1              | 9.5 ± 1.4                        | 4.3 ± 0.6 |
| 8000                  | $86.2 \exp^{-0.75t}+13.8 \exp^{-0.082t}$ | 0.9 ± 0.1              | 8.5 ± 0.7                        | 3.6 ± 0.4 |

* t: days after injection of $^{54}$Mn, ** Each value is the mean ± SD for 5 mice.

Methods for promoting the excretion of incorporated radionuclides can be classified in 4 groups: (1) combining the radionuclide with chelating agents, (2) diluting the radionuclide with its stable isotopes, (3) combining the radionuclide with adsorbents in the gut, (4) disturbing the...
metabolism with hormones. We focused on the dilution method for $^{54}$Mn because manganese is an essential trace element that is considered to be among the least toxic to animals\(^5\). In a previous study, we showed that an intraperitoneal injection of manganese chloride was very effective for eliminating $^{54}$Mn in mice, 45%, 60%, 71%, and 78% of the body burden respectively being eliminated by a single injection of a dose of 0.3, 1.0, 3.0, and 10 mg-Mn/kg-body weight\(^6\). Although daily elimination in our present experiment was lower than that obtained by intraperitoneal injection, total elimination was increased by prolonged treatment. Moreover, oral administration can load a $^{54}$Mn-contaminated person with manganese for a long period without pain. The oral intake of excess manganese therefore is considered to be a practical and effective method for promoting the excretion of $^{54}$Mn.

Daily consumption of the various diets ranged from 3 to 6 g/mouse in each group (Table 1). There were no significant differences among the contents of dietary manganese within the range of 80 to 2400 mg/kg, but consumption of the 8000 mg/kg diet was small as compared to the other diets. The respective approximate manganese intakes from the 80, 240, 800, 2400, and 8000 mg/kg diets were 10, 30, 100, 300, and 700 mg/kg-body weight/day.

Although manganese is considered to be one of the least toxic of the essential metals\(^5\), a very high content of dietary manganese is reported to induce anemia and lower weight\(^7,8\). These effects respectively are caused by the antagonism between manganese and iron at the absorption level\(^9,10\) and by decreased appetite\(^8\). It is not clear whether the high-manganese diets used in this study induced anemia, because no blood parameters were investigated. However, no toxic symptoms, such as changes in appearance and depressed appetite, were observed up to 2400 mg/kg-diet during the experimental period, indicative that the upper limit of daily manganese intake is 300 mg/kg-body weight.

Experiment 2. Distribution of $^{54}$Mn

Fig. 3 shows the concentration ratio of $^{54}$Mn in the tissues of mice maintained on a diet containing 80 or 2400 mg-Mn/kg. The liver, kidneys, and pancreas had relatively high $^{54}$Mn concentrations, whereas the muscles, femurs and brain had low ones. The diet rich in manganese promoted the clearance of $^{54}$Mn from the liver, kidneys, pancreas, and spleen. In the liver, in particular, the concentration ratio rapidly decreased to about one-tenth the value for the control (80 mg-Mn/kg diet) 4 days after injection. In contrast, the concentrations in the muscles, femurs, and brain were little affected by the dietary manganese contents.

Mahoney et al.\(^11\) reported that the retention of $^{54}$Mn in humans can be described by an exponential function having two components. The whole-body retention curves obtained in our experiment also were well approximated by the sum of two exponential functions. As shown in Fig. 3, the tissues can be roughly classified into two groups on the basis of the clearance pattern of $^{54}$Mn. One group consists of the liver, kidneys, pancreas, and spleen, all of which have high $^{54}$Mn concentrations, and are very sensitive to the dietary manganese content. The other group, comprised of the muscles, femurs, and brain, is low in $^{54}$Mn concentration and relatively insensitive to the manganese intake. The two exponential functions that describe the whole-body retention curve of $^{54}$Mn must reflect the kinetics of $^{54}$Mn in the two groups of tissues. Therefore the muscles, bone, and brain will be irradiated by $^{54}$Mn for a long time, even though the
Experiment 3 Manganese concentration in tissues

Manganese concentrations in various tissues of mice maintained on the special diets for 21 days are shown in Fig. 4. For the control mice maintained on the commercial diet, the manganese concentrations in the liver, kidneys, muscles, femurs, and brain respectively were 1.3, 1.6, 0.2, 2.7 and 0.5 mg/kg. In the muscles and brain, the amount of dietary manganese did not affect the manganese concentrations within the range of 80 to 2400 mg-Mn/kg-diet, but a significant rise in manganese concentration was observed for mice maintained on the 8000 mg/kg diet. Manganese concentrations in the liver, kidneys, and femurs were elevated as the dietary content of manganese increased, but marked accumulations of manganese were found only in the liver and kidneys of the mice fed the 8000 mg/kg diet.

The relatively constant concentrations of tissue manganese suggest that homeostatic mechanisms for manganese functioned effectively up to the manganese content of 2400 mg/kg in diet. The marked accumulation of manganese in the liver and kidneys of mice fed the 8000 mg/kg diet indicates a breakdown of manganese homeostasis, presumably because of saturation of manganese excretion capacity. Under ordinary conditions, bile is the main route of manganese excretion, excretion also taking place via the pancreatic juice the intestinal wall as auxiliary routes. These findings indicate that these routes have a remarkable capacity to excrete excess absorbed manganese, and support the validity of the dilution method as a means of eliminating $^{54}\text{Mn}$ from a body.

In conclusion, the excretion of $^{54}\text{Mn}$ was enhanced by increased amounts of dietary manganese over a wide range. Neither toxic symptoms nor marked accumulations of manganese
in tissues took place up to a dietary manganese content of 2400 mg/kg, which suggests that the upper limit of daily manganese intake is about 300 mg/kg-body weight. These results are evidence that an oral intake of manganese is effective for promoting the excretion of $^{54}\text{Mn}$ from persons contaminated with it. The proper dose for humans, however, has yet to be determined.

**Fig. 4.** Manganese concentrations in the tissues of mice maintained on diets with various manganese contents for 21 days. Vertical bars show the S. D. for 5 mice.

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