Bull. Environ. Contam. Toxicol. (1991) 46:921–928

Environmental Contamination and Toxicology

Short-Term Oral Administration of Several Manganese Compounds in Mice: Physiological and Behavioral Alterations Caused by Different Forms of Manganese

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Various compounds of manganese (Mn) are used industrially, e.g., in dyes and battery production. Manganese acetate (MnAc) salts occur in divalent and trivalent forms. Divalent MnAc is used mainly as a dye, pigment, or catalyst. The chief ingredients of Mn ore itself are manganese dioxide (MnO_2) and manganese carbonate ($MnCO_3$). In Kanazawa, Japan, air pollution from a Mn treatment factory was found to affect local junior high school students, who showed reduced respiratory function relative to their exposure to Mn (Nogawa et al., 1973). The use of Mn as an antiknock ingredient in automobile fuels has recently been introduced, placing the general population at risk of chronic low-level exposure. Joselow et al. (1978) have reported the elevation of blood Mn levels in children living in urban areas. There are few animal studies, on the effects of compounds other than manganese chloride ($MnCl_2$) (Rehnberg et al., 1981). In the case of environmental pollution by Mn, the differing physiological effects of the various chemical forms of Mn must be considered. The present study therefore examined the differences in the effects of several Mn compounds on the physiology and behavior of mice by short-term oral administration.

MATERIALS AND METHODS

Six-week-old male ddY mice weighing 28.2 ± 0.7 g were divided into 5 groups of 8 animals each. Groups 1-4 were fed diets containing 2 g Mn/kg in the form of $MnCl_2 \cdot 4H_2O$, $Mn(CH_3COO)_2 \cdot 4H_2O$, $MnCO_3$, or MnO_2 for 100 days. Standard laboratory mouse chow (130 mg Mn/kg, phytate (as mesoinositol-hexaphosphoric acid) 1.0%,

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crude fiber 3.4%, methionine 0.3%, and arginine 1.3%, Type F2, Sankyo Laboratories Service Co., Japan) was fed to the control mice group. All mice were allowed free access to food pellets and water, and body weight changes were recorded.

The mice were tested for spontaneous motor activity after an interval of 30 days. Activity was measured using an ANIMEX Activity Meter (Type SE, FARAD Electronics, Sweden). Two mice were placed in a plastic cage at night, and after 10 min of acclimatization, their activity was measured for 30 min.

Mice were decapitated at 24 hr after their last feed. Blood was collected by venesection and hematological parameters were measured using a Micro Cell Counter (TOA Electronics Ltd., Japan). Tissues samples were removed, weighed, and stored at -20 °C until analysis. Hair was washed by the method of Kumpulainen et al. (1982). In brief, it was rinsed with ethyl alcohol, washed with sodium lauryl sulfate solution, rinsed again with deionized water, and then dried. Tissues were digested by the wet ashing method and the resulting solutions were analyzed for Mn content by an atomic absorption spectrophotometer with a flame atomizer (Type 308, Hitachi Ltd., Japan).

Data were statistically analyzed using Student's t-test and analysis of variance.

RESULTS AND DISCUSSION

The intake rate decreased within the first month but then became constant in all groups (Fig. 1). Mean daily food intake per mouse and the standard deviation in each group were : 3.6 $\pm$ 0.9 g in the control group, 4.0 $\pm$ 0.7 g in the $MnCl_2$ group, 4.0 $\pm$ 0.8 g in the MnAc group, 3.7 $\pm$ 1.1 g in the $MnCO_3$ group, and 3.8 $\pm$ 0.7 g in the MnO_2 group. There was no significant difference in intake among the groups. The Mn concentration in most tissues in the Mn groups was about 2-3 times that of the controls (Table 1). Among the Mn groups, the MnAc and $MnCO_3$ groups had considerably higher Mn contents in the liver and kidney than the $MnCl_2$ and MnO_2 groups. The tissue Mn content varied significantly among the Mn groups (two-way analysis of variance, $p < 0.01$), although Mn intake did not vary. The concentration-related accumulation of Mn in human hair has been shown with varying concentrations of Mn in drinking water (3.6-14.6 μg/l, 81.6-252.6 μg/l, and 1800-2300 μg/l) (Kondakis et al., 1989). Although Mn ingested through drinking water usually comprises only a small percentage of the total intake (i.e., 2 to 5 mg/day), the concentration in water in some of these

Table 1. Manganese content of various tissues

	control	$Mn(CH_3COO)_2$	$MnCl_2$	$MnCO_3$	MnO_2
			(μg/g of tissue wet weight)[a]		
liver	2.80±0.39	7.43±1.34[c]	5.26±0.09[c]	7.15±2.23[c]	4.30±0.58[c]
kidney	2.84±0.23	6.11±0.36[c]	4.20±0.09[c]	6.08±1.57[c]	3.70±0.37[c]
pancreas	2.04±0.23	3.90±0.53[c]	3.80±1.10[c]	3.29±0.68[c]	2.75±1.01
prostate gland	1.18±0.21	3.01±0.52[c]	2.04±0.15[c]	2.34±0.25[c]	1.60±0.40[b]
spleen	0.40±0.06	0.85±0.41[b]	1.12±0.62	1.37±0.94	0.85±0.30
brain	0.97±0.25	1.51±0.23[b]	1.38±0.27	1.63±0.49	1.45±0.50[b]
hair	2.98±0.42	6.47±0.54[c]	3.53±0.25	3.24±0.33	4.13±0.85[b]
bone	1.66±0.32	4.57±0.30[c]	4 ±0.43[c]	2.88±0.14[c]	2.75±0.25[c]
muscle	0.37±0.04	0.90±0.14[c]	(±0.16[c]	0.80±0.08[c]	0.63±0.10[c]

a) Values represent the mean ± SD for 8 mice
Significantly different from the control, b) $p < 0.05$, c) $p < 0.01$.
(Data analyzed by Student's t-test.)

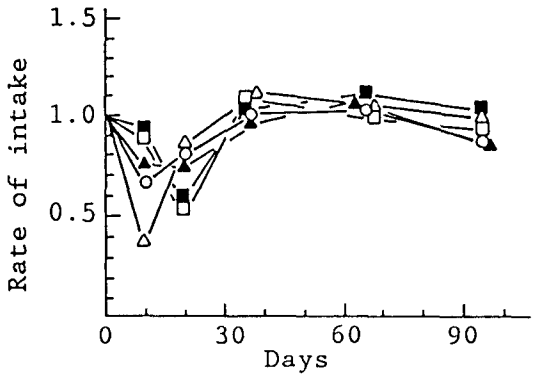

Figure 1. Daily food intake of mice

○ , control; □ , manganese chloride; ■ , manganese acetate; △, manganese carbonate; ▲, manganese dioxide. The rate of intake indicates the food intake of the mice in each group divided by the mean intake of all mice before the experimental period.

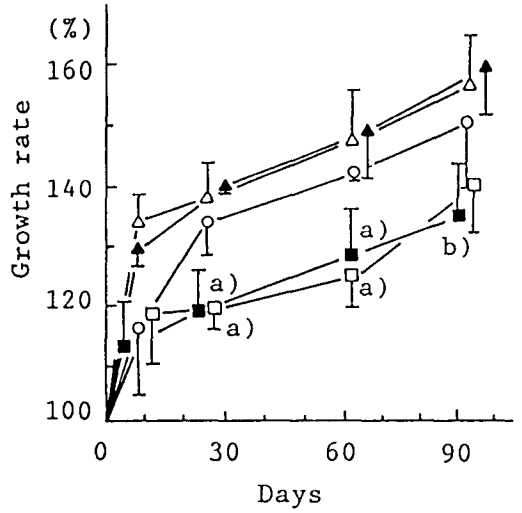

Figure 2. Growth rate of mice exposed to manganese

○ , control; □ , manganese chloride; ■ , manganese acetate; △, manganese carbonate; ▲, manganese dioxide. The growth rate indicates the body weight of the mice in each group divided by the mean body weight of all mice before the experimental period.
Significantly different from the control, a) $p < 0.05$, b) $p < 0.01$. (Data analyzed by Student's t-test.)
Error bars indicate the standard deviation of data from 8 mice.

Table 2. Hematology

	red blood cell count ($\times 10^4/mm^3$)[a]	white blood cell count ($\times 10^2/mm^3$)[a]	hemoglobin (g/dl)[a]	hematocrit (%)[a]
control	807± 95	59.3±18.0	13.6±1.0	45.4±4.6
MnAc	705± 87[b]	36.4±11.5[c]	12.9±1.4	41.1±6.4
$MnCl_2$	699±138	34.9± 2.6[c]	12.5±1.3	39.6±7.9
$MnCO_3$	682± 74[b]	43.6± 5.8	13.6±1.5	38.7±3.8[b]
MnO_2	760±163	40.6± 9.9[b]	12.3±3.7	40.2±8.1

a) Values represent the mean ± SD for 8 mice. Significantly different from the control, b) $p < 0.05$, c) $p < 0.01$. (Data analyzed by Student's t-test.)
MnAc : $Mn(CH_3COO)_2$

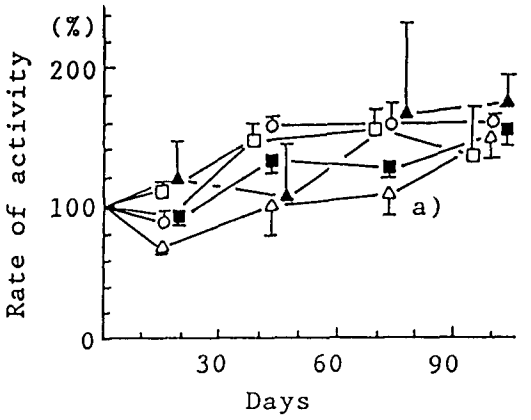

Figure 3. Spontaneous motor activity of mice exposed to manganese

○, control; □, manganese chloride; ■, manganese acetate; △, manganese carbonate; ▲, manganese dioxide. The rate of activity indicates the spontaneous motor activity of the mice in each group divided by the mean activity of all mice before the experimental period.
a) Significantly different from the control, $p < 0.01$. (Data analyzed by Student's t-test.)
Error bars indicate the standard deviation.

areas was approximately 2000 μg/l, substantially increasing the total intake. The Mn levels in the organs of female mice were increased 1.5 times by chronic dosing in drinking water (500 to 5000 mg Mn/l, 300 days) (Suzuki, 1974). In the present study, the Mn groups were fed daily an additional Mn dose of about 200 mg Mn/kg body weight. And the doses were observed

before the plateau level was reached on the biological response curve to increasing dietary Mn intake. Absorption determined by the isotopic dilution method varied from 2 to 29% in rats fed a total diet containing 1.5-100 mg Mn/kg, ad libitum without fasting (Weigand et al., 1986). Lee and Johnson (1989) suggested that the better Mn availability form a soy protein rather than a casein diet was due to neither the arginine nor phytic acid component of the soy diet. The present diet consisted of wheat flour, whitefish meal, and soybean protein. Mn was probably more rapidly absorbed from this diet than the other types of diets. MnAc and $MnCl_2$ are soluble in water and alcohol, while $MnCO_3$ and MnO_2 are virtually insoluble. Absorption of these latter two is therefore expected to be lower than that of the other soluble compounds. In this study however, the accumulation of Mn in tissues in the $MnCO_3$ group was similar to that of the MnAc group. The difference in accumulation between the $MnCO_3$ and MnO_2 groups was probably due to the differing absorptions of Mn^{2+} and Mn^{4+}.

Less weight gain was seen in the MnAc and $MnCl_2$ groups than the controls (Fig. 2). The $MnCO_3$ and MnO_2 groups, however, were similar to the controls in this variable. Chandra et al. (1979) observed normal growth rate in growing mice exposed to $MnCl_2$.

We also noted a decreased red blood cell count in the MnAc and $MnCl_2$ groups (Table 2). The white blood cell count was decreased in the MnAc, $MnCl_2$, and MnO_2 groups. Independent of the route of ^{54}Mn administration, transferrin has been identified as the major Mn-binding protein in plasma (Davidsson et al., 1989). Serum iron binds with transferrin in hemoglobin synthesis. Thus, the binding of transferrin by Mn effect hemoglobin synthesis, thereby reducing the red blood cell count. We did not analyze blood Mn levels as the concentration in blood is extremely low, rising only slightly even when dietary intake is greatly increased (Rehnberg et al., 1981).

The effects of Mn on spontaneous motor activity are shown in Fig. 3. In the control group, motor activity increased from days 15-45, thereafter remaining constant. The $MnCO_3$ group showed significantly less activity than the control mice. Recent work in the brains of growing rats, failed to reveal any histological changes despite extremely high levels of Mn. Thus, transient motor disturbances may be explained by the biochemical effects of Mn (Kristensson et al., 1986). Our own data on the exposure of mice to Mn for 12 months indicated that Mn content correlates with the level of biogenic amines in the brain (Komura et al.,

1986).

Reduced locomotor activity in the $MnCO_3$ and MnAc groups seemed to correlate with Mn content in the tissues. Weight gain differed between mice exposed to soluble Mn and those exposed to insoluble Mn, while hematological changes were manifold. MnAc seemed to have the greatest effect, followed by $MnCO_3$ and $MnCl_2$. A recent report suggested that the neurotoxicity of Mn may be related to its ability to increase catechol autoxidation in catecholaminergic neurons (Vescovi et al., 1989). In the present study, Mn^{4+} seemed to exert a less effect than Mn^{2+}. This could be explained by the different experimental conditions employed.

REFERENCES

Chandra SV, Shukla GS, Saxena DK (1979) Manganese-induced behavioral dysfunction and its neurochemical mechanism in growing mice. J Neurochem 33:1217-1221

Davidsson L, Lonnerdal B, Sandstrom B, Kunz C, Keen CL (1989) Identification of transferrin as the major plasma carrier protein for manganese introduced orally or intravenously or after in vitro addition in the rat. J Nutr 119:1461-1464

Joselow MM, Tobias E, Koehler R, Coleman S, Bogden J, Gause D (1978) Manganese pollution in the city environment and its relationship to traffic density. Am J Public Health 68:557-560

Komura J, Sakamoto M (1986) Effect of long-term exposure to manganese on catecholamines in mouse brain. Eisei Kagaku 32:p-26

Kondakis XG, Makris N, Leotsinidis M, Prinou M, Papapetropoulos T (1989) Possible health effects of high manganese concentration in drinking water. Arc Environ Health 44:175-178

Kristensson K, Eriksson H, Lundh B, Plantin L-O, Wachtmeister L, Azazi Mel, Morath C, Heilbronn E (1986) Effects of manganese chloride on the rat developing nervous system. Acta Pharmacol et Toxicol 59:345-348

Kumpulainen J, Salmela S, Vuori E, Lehto J (1982) Effects of various washing procedures on the chromium content of human scalp hair. Anal Chim Acta 138:361-364

Lee D-Y, Johnson PE (1989) ^{54}Mn absorption and excretion in rats fed soy protein and casein diets. Proc Soc Exp Biol Med 190:211-216

Nogawa K, Kobayashi E, Sakamoto M (1973) Epidemiological studies on disturbance of respiratory system caused by manganese air pollution (Report 1). Effects on respiratory system of junior high school students. Jpn J Public Health 20:315-325

Rehnberg GL, Hein JF, Carter SD, Linko RS, Laskey JW

(1981) Chronic ingestion of Mn_3O_4 by young rats: Tissue accumulation, distribution, and depletion. J Toxicol Environ Health 7:263-272

Suzuki Y (1974) Studies on excessive oral intake of manganese. Part 2 Minimum dose for manganese accumulation in mouse organ. Shikoku Ishi 30:32-45

Vescovi A, Gebbia M, Cappelletti G, Parati EA, Santagostino A (1989) Interaction of manganese with human brain glutathione-S-transferase. Toxicology 57:183-191

Weigand E, Kirchgessner K, Helbig U (1986) True absorption and endogenous fecal excretion of manganese in relation to its dietary supply in growing rats. Biol Trace Elem Res 10:265-279

Received August 6, 1990; accepted November 14, 1990.