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Effects of Acute Manganese Chloride Exposure on Lipid Peroxidation and Alteration of Trace Metals in Rat Brain

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ABSTRACT

Although manganese (Mn) is an essential element, exposure to excessive levels of Mn and its accumulation in the brain can cause neurotoxicity and extrapyramidal syndrome. We have investigated the differences in the accumulated levels of Mn, the degree of lipid peroxidation, and its effects on the levels of trace elements (Fe, Cu, and Zn) in various regions in the brain of rats having undergone acute Mn exposure. The rats in the dose–effect group were injected intraperitoneally (ip) with $MnCl_2$ (25, 50, or 100 mg $MnCl_2$/kg) once a day for 24 h. The Mn significantly accumulated ($p<0.05$) in the frontal cortex, corpus callosum, hippocampus, striatum, hypothalamus, medulla, cerebellum, and spinal cord in each case. The rats in the time-course group were ip injected with $MnCl_2$ (50 mg $MnCl_2$/kg) and then monitored 12, 24, 48, and 72 h after exposure. The Mn accumulated in the frontal cortex, corpus callosum, hippocampus, striatum hypothalamus, medulla, cerebellum, and spinal cord after these periods of time, In both the dose–effect and time-course studies, we observed that the concentration of malondialdehyde, an end product of lipid peroxidation, increased significantly in the frontal cortex, hippocampus, striatum, hypothalamus, medulla,

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and cerebellum. However, no relationship between the concentrations of Mn in the brain and the extent of lipid peroxidation was observed. In addition, we found that there was a significant increase ($p<0.05$) in the level of Fe in the hippocampus, striatum, hypothalamus, medulla, and cerebellum, but the Cu and Zn levels had not changed significantly. These findings indicated that Mn induces an increase in the iron level, which provides direct evidence for Fe-mediated lipid peroxidation in the rats' brains; these phenomena might play important roles in the mechanisms of Mn-induced neurotoxicology.

Index Entries: Manganese chloride; lipid peroxidation; trace metals; brain.

INTRODUCTION

The essential trace element manganese (Mn) is necessary for brain growth and function *(1)*; it plays the role of a cofactor in many enzymes that are vital for normal functioning of the human central nervous system and other body organs. Exceedingly high concentrations of Mn in the brain can, however, cause neurotoxicity *(1)*. Presently, the mechanism of Mn toxicity remains unknown. Mn is used widely industrially, in applications such as the production of steel and batteries, and it is used as an additive in gasoline. Mn neurotoxicity has been reported in miners exposed to manganese dioxide by inhalation *(2)*, workers in dry-cell battery factories *(3)*, and in agricultural workers exposed to high levels of the metal. Exposure to a high concentration of Mn can produce irreversible syndromes and extrapyramidal dysfunctions, such as psychomotor excitement, rigidity, dystonia, and severe gait disturbances that often resemble those observed in sufferers of Parkinson's disease *(4)*. Mn has been implicated in disturbance of neurotransmitter metabolism *(5,6)* and in the induction of oxidative stress *(7)*.

Oxidative stress is a well-established general mechanism through which cell and tissue injury occurs *(8,9)*. Such cellular oxidative damage is caused primarily by free radicals and reactive oxygen species (ROS). Metabolism of oxygen by cells generates potentially deleterious ROS. Under normal conditions, the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination. A disturbance of the balance between the formation of active oxygen metabolites and the rate at which they are scavenged by enzymatic and nonenzymatic antioxidants is referred to as "oxidative stress" *(10)*. The prime targets of these ROS are the polyunsaturated fatty acids in cell membranes and, thus, they cause lipid peroxidation, which could lead to damage to the cell structure and function *(11)*. Oxidative stress has been suggested to play a role in some physiological conditions and in many disease processes, including metal toxicity *(10,12)*. Recently, there has been growing interest in the role played by lipid peroxidation in metal toxicity, with numerous studies undertaken using malondialdehyde (MDA) as biomarker of oxidative stress *(8,9)*.

A growing body of evidence has indicated that trace metals play important roles in a number of biological processes by activating or inhibiting enzymatic reactions, by competing with other elements and metalloproteins for binding sites, by affecting the permeability of cell membranes, or through other mechanisms *(13,14).* It is, therefore, reasonable to assume that these trace elements would exert some action, either directly or indirectly, on metal-induced pathological processes. Statistically significant differences from the normal distributions of Fe and Cu can occur in patients having various forms of cancer *(8,9).* Divalent ions of transition metals can promote lipid peroxidation in vitro, and much attention is being focused currently on the role of lipid peroxidation in the pathogenesis of metal toxicity *(15).* The mechanisms of pathogenesis might be mediated by the direct effects that certain trace elements (e.g., Fe or Cu) have on the formation of hydroxyl free radicals from hydrogen peroxide and superoxide via the Fenton and Haber–Weiss reactions or by the influence that trace elements have on the intracellular concentrations of Se, Fe, Cu, and Zn *(15,16).* As far as Mn toxicity is concerned, there have been investigations into the variations in the levels of trace elements in the brains and tissues of Mn-treated animals, but, to the best of our knowledge, the relationship between the changes in the levels of trace elements and oxidative stress in the brains of experimental animals has never been explored previously.

Our aim in this study was to determine the effects that acute manganese chloride exposure in vivo has on lipid peroxidation and on the levels of trace metals in the brains of Sprague–Dawley rats. We examined the concentrations of a range of trace metals (Mn, Fe, Cu, and Zn) and the degree of lipid peroxidation in various brain regions (frontal cortex, corpus callosum, hippocampus, striatum, hypothalamus, medulla, cerebellum, and spinal cord) after intraperitoneal administration of manganese chloride. We hypothesized that Mn-induced neurochemical changes are produced via lipid peroxidation or through an imbalance of certain trace metals that are involved in oxidative stress.

MATERIALS AND METHODS

Reagents

$MnCl_2 \cdot 4H_2O$ and 1,1,3,3-tetraethoxypropane (TEP), a standard for thiobarbituric acid-reactive substances, were purchased from Sigma Chemical Co. Thiobarbituric acid (TBA), Mn, Fe, Cu, and Zn standard solutions (1000 mg/L) were purchased from E. Merck.

Experimental Animals and Treatments

Male Sprague–Dawley rats (body weight: 320–385 g) were kept in stainless-steel mesh cages, housed under controlled conditions (22 ± 2°C, 50 ± 20% relative humidity, a 12-h light/dark cycle), and fed laboratory rat

chow and tap water ad libitum. The rats were divided into two groups: a dose–effect group and a time-course group. The dose–effect group was subdivided into control, low-dose (25 mg $MnCl_2$/kg), middle-dose (50 mg $MnCl_2$/kg), and high-dose (100 mg $MnCl_2$/kg) groups. $MnCl_2{\cdot}4H_2O$ dissolved in sterile saline was administrated to the rat (25, 50, or 100 mg $MnCl_2$/kg) though intraperitoneal injection in a single dose. The time-course groups (12, 24, 48, and 72 h exposure) were subdivided into control groups and test groups. The control rats were treated with a NaCl vehicle intraperitoneally and, at the same time, the test rats were administered intraperitoneally—at a dosage of 50 mg $MnCl_2$/kg—with $MnCl_2$ dissolved in the NaCl vehicle solution. Each group consisted of five rats. The rats were decapitated 24 h after injection and their brains were removed and dissected over ice to yield eight discrete regions: frontal cortex, corpus callosum, hippocampus, striatum, hypothalamus, medulla, cerebellum, and spinal cord. The dissected brain tissues were stored at –70°C prior to their analysis.

Graphite Furnace Atomic Absorption Spectroscopy for Mn, Fe, Cu, and Zn Analysis

The determination of metal levels was performed on nitric acid digests of tissues using a Perkin–Elmer 5100PC atomic absorption spectrophotometer. The limits of detection were as follows: Mn, 0.4 ppb; Fe, 0.7 ppb; Cu, 0.9 ppb; Zn, 0.4 ppb. All assays were performed in duplicate.

HPLC Assay for Malondialdehyde

Tissues were homogenized in cold KCl solution through sonication. Samples were analyzed for MDA—applying a procedure modified according to Wong et al. *(17)*—by performing the TBA reaction and using high-performance liquid chromatography (HPLC) (JASCO model 880PU equipped with C18 column and UV–Vis detector; Sigma, Tokyo) to separate the MDA–TBA adduct; TEP was used as the standard. Each assay was performed in duplicate.

Statistical Analysis

Data were analyzed using SPSS software. The analysis of variance (ANOVA) was used with Dunntte test post hoc ANOVA tests to determine specific group differences. The criterion for significance was set at $p < 0.05$.

RESULTS

After administration of the Mn compound, major behavioral changes were recorded during the subsequent exposure period. In the present study, no marked changes in motor activity, eating behavior, and weight loss were observed in rats after injection with $MnCl_2$. It might be because

of the short exposure period. Table 1 lists the dose–effect relationships for the Mn concentration in the various brain regions of the $MnCl_2$-treated rats. The mean Mn concentrations in the frontal cortex, corpus callosum, hippocampus, striatum, hypothalamus, medulla, cerebellum, and spinal cord increased significantly 24 h after a single ip injection of 25 mg $MnCl_2$/kg, and they were even higher when the dose was increased to 50 and 100 mg $MnCl_2$/kg. Table 2 provides a summary of the time courses of the Mn concentrations in the various brain regions of the rats injected with 50 mg $MnCl_2$/kg. After 12, 24, 48, and 72 h, the Mn concentrations were significantly increased in the frontal cortex, corpus callosum, hippocampus, striatum, hypothalamus, medulla, cerebellum, and spinal cord.

Table 3 lists the effects that the doses of $MnCl_2$ have on the induction of lipid peroxidation in the various regions of the brains of the treated rats. After injection with $MnCl_2$ (50 mg $MnCl_2$/kg), we observed that the degree of lipid peroxidation increased significantly in the frontal cortex, corpus callosum, hippocampus, hypothalamus, medulla, and cerebellum, and at 100 mg $MnCl_2$/kg, the levels in the frontal cortex, corpus callosum, striatum, hypothalamus, medulla, and cerebellum had all increased significantly. At 25 mg $MnCl_2$/kg, a significant increase of the lipid peroxidation level was found only in the frontal cortex, corpus callosum, and cerebellum. Table 4 summarizes the time course for the $MnCl_2$ induction of lipid peroxidation in the various regions of the rats' brains. After injection with $MnCl_2$ (50 mg $MnCl_2$/kg), the levels of lipid peroxidation in the hypothalamus and cerebellum after 12, 24, 48, and 72 h had all increased significantly. In the frontal cortex, the levels of lipid peroxidation increased significantly at 24, 48, and 72 h, and in the striatum, the degree of lipid peroxidation increased significantly 72 h after injection with $MnCl_2$. In contrast, the level of lipid peroxidation in the medulla and hippocampus increased significantly only at 24 h after injection with $MnCl_2$.

We detected no significant differences in the levels of Cu and Zn in the exposed group relative to those in the control group, but the effect on Fe was significant. Tables 5 and 6 present data for the effect that the dose and time, respectively, have on the rat brain levels of Fe after acute administration of $MnCl_2$. The Fe concentrations increased significantly in the hippocampus and striatum after injections with 25, 50, and 100 mg $MnCl_2$/kg and in the hypothalamus, medulla, and cerebellum after injection with 50 and 100 mg $MnCl_2$/kg (Table 5). The Fe concentrations increased significantly in the hippocampus and striatum 24, 48, and 72 h after injection with 50 mg $MnCl_2$/kg, in the medulla and cerebellum 24 h after injection, and in the hypothalamus 12, 24, 48, and 72 h after treatment.

DISCUSSION

Manganese has been linked to neurotoxicity as a consequence of its excessive accumulation in the brain. Experimental results have indicated

Table 1
Dose Effect of Mn Accumulation in Brain Regions of $MnCl_2$-Treated Rats

$MnCl_2$ dose (mg/kg)	Mn (ng/g wet tissue)							
	Frontal cortex	Corpus callosum	Hippocampus	Striatum	Hypothalamus	Medulla	Cerebellum	Spinal cord
0(vehicle)[A]	0.39 ± 0.01	0.27 ± 0.13	0.41 ± 0.16	0.38 ± 0.03	0.42 ± 0.04	0.50 ± 0.03	0.40 ± 0.06	0.37 ± 0.09
25[B]	0.63 ± 0.13	0.63 ± 0.11	0.69 ± 0.12	0.72 ± 0.08	1.01 ± 0.19	0.90 ± 0.08	0.69 ± 0.10	0.98 ± 0.11
50[C]	0.64 ± 0.12	0.89 ± 0.15	1.01 ± 0.43	1.20 ± 0.10	1.74 ± 0.26	1.49 ± 0.11	1.12 ± 0.04	1.92 ± 0.35
100[D]	0.75 ± 0.07	0.92 ± 0.19	1.39 ± 0.56	1.56 ± 0.21	1.69 ± 0.39	1.78 ± 0.24	1.93 ± 1.78	1.89 ± 0.35
F value	93.05	243.22	473.20	229.41	244.05	151.44	43.34	547.01
Post Hoc	A*B	A*B	A*B	A*B	A*B	A*B	A*B	A*B
	A*C	A*C	A*C	A*C	A*C	A*C	A*C	A*C
	A*D	A*D	A*D	A*D	A*D	A*D	A*D	A*D

* $p < 0.05$.

Table 2
Time Course of Mn Accumulation in Brain Regions of $MnCl_2$-Treated Rats

Treatment	Hours after injection	No. of Rats	Mn (ng/g wet tissue)							
			Frontal cortex	Corpus callosum	Hippocampus	Striatum	Hypothalamus	Medulla	Cerebellum	Spinal cord
Controls	12-72[A]	20	0.38 ± 0.06	0.30 ± 0.04	0.32 ± 0.06	0.38 ± 0.04	0.46 ± 0.03	0.46 ± 0.04	0.37 ± 0.08	0.40 ± 0.07
$MnCl_2$	12[B]	5	0.63 ± 0.04	0.74 ± 0.10	0.61 ± 0.09	1.10 ± 0.30	1.96 ± 0.43	0.96 ± 0.11	0.76 ± 0.22	1.20 ± 0.16
	24[C]	5	0.64 ± 0.12	0.89 ± 0.15	0.63 ± 0.09	1.20 ± 0.10	1.74 ± 0.26	1.50 ± 0.11	1.12 ± 0.04	1.92 ± 0.35
	48[D]	5	0.83 ± 0.17	0.85 ± 0.15	0.67 ± 0.15	1.01 ± 0.28	1.44 ± 0.13	1.28 ± 0.15	0.88 ± 0.13	1.22 ± 0.26
	72[E]	5	0.67 ± 0.19	0.90 ± 0.19	0.77 ± 0.16	1.39 ± 0.35	1.24 ± 0.19	1.35 ± 0.21	0.98 ± 0.03	0.93 ± 0.14
F value			85.30	219.53	311.40	133.30	199.20	213.95	209.53	1044.50
Post Hoc			A*B	A*B	A*B	A*B	A*B	A*B	A*B	A*B
			A*C	A*C	A*C	A*C	A*C	A*C	A*C	A*C
			A*D	A*D	A*D	A*D	A*D	A*D	A*D	A*D
			A*E	A*E	A*E	A*E	A*E	A*E	A*E	A*E

* $p<0.05$.

Table 3
Dose Effect of Lipid Peroxidation in Brain Regions of $MnCl_2$-Treated Rats

$MnCl_2$ dose (mg/kg)	MDA (μmol/g wet tissue)							
	Frontal cortex	Corpus callosum	Hippocampus	Striatum	Hypothalamus	Medulla	Cerebellum	Spinal cord
0(vehicle)[A]	0.22 ± 0.01	0.16 ± 0.03	0.06 ± 0.01	0.14 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.35 ± 0.09	0.34 ± 0.09
25[B]	0.25 ± 0.07	0.19 ± 0.06	0.05 ± 0.01	0.17 ± 0.07	0.05 ± 0.02	0.08 ± 0.01	0.54 ± 0.15	0.38 ± 0.11
50[C]	0.35 ± 0.04	0.19 ± 0.02	0.09 ± 0.01	0.14 ± 0.02	0.12 ± 0.03	0.13 ± 0.03	0.76 ± 0.10	0.38 ± 0.09
100[D]	0.46 ± 0.04	0.28 ± 0.12	0.06 ± 0.01	0.22 ± 0.05	0.16 ± 0.21	0.16 ± 0.06	0.83 ± 0.17	0.31 ± 0.09
F value	363.48	152.12	56.00	19.38	173.84	86.79	134.66	15.23
Post Hoc	A*B	A*B	A*C	A*D	A*C	A*C	A*B	
	A*C	A*C			A*D	A*D	A*C	
	A*D	A*D					A*D	

* $p < 0.05$.

Table 4
Time Course of Lipid Peroxidation in Brain Regions of $MnCl_2$-Treated Rats

Treatment	Hours after injection	No. of Rats	MDA (μmol/g wet tissue)							
			Frontal cortex	Corpus callosum	Hippocampus	Striatum	Hypothalamus	Medulla	Cerebellum	Spinal cord
Controls	12-72[A]	20	0.26 ± 0.05	0.18 ± 0.04	0.05 ± 0.01	0.15 ± 0.02	0.06 ± 0.01	0.08 ± 0.01	0.34 ± 0.07	0.27 ± 0.10
$MnCl_2$	12[B]	5	0.28 ± 0.06	0.18 ± 0.04	0.05 ± 0.002	0.16 ± 0.06	0.11 ± 0.03	0.07 ± 0.02	0.22 ± 0.08	0.22 ± 0.06
	24[C]	5	0.35 ± 0.04	0.19 ± 0.02	0.09 ± 0.01	0.14 ± 0.02	0.12 ± 0.04	0.13 ± 0.03	0.25 ± 0.30	0.28 ± 0.09
	48[D]	5	0.22 ± 0.01	0.18 ± 0.02	0.04 ± 0.01	0.16 ± 0.04	0.13 ± 0.07	0.07 ± 0.03	0.25 ± 0.02	0.20 ± 0.04
	72[E]	5	0.31 ± 0.08	0.18 ± 0.04	0.05 ± 0.01	0.26 ± 0.07	0.12 ± 0.09	0.05 ± 0.01	0.20 ± 0.02	0.25 ± 0.02
F value			21.13	0.99	47.86	26.63	19.96	33.41	17.56	7.72
Post Hoc			A*C		A*C	A*E	A*B	A*C	A*B	
			A*D				A*C		A*C	
			A*E				A*D		A*D	
							A*E		A*E	

* $p < 0.05$.

Table 5
Dose Effect of Fe Concentrations in Brain Regions of $MnCl_2$-Treated Rats

$MnCl_2$ dose (mg/kg)	Fe (ng/g wet tissue)							
	Frontal cortex	Corpus callosum	Hippocampus	Striatum	Hypothalamus	Medulla	Cerebellum	Spinal cord
0(vehicle)[A]	14.21 ± 0.70	9.06 ± 2.11	2.72 ± 0.14	1.88 ± 0.17	3.51 ± 0.34	4.17 ± 0.24	11.95 ± 2.92	11.83 ± 2.91
25[B]	15.00 ± 3.82	7.61 ± 1.24	5.75 ± 0.97	3.59 ± 0.38*	5.42 ± 1.68	7.53 ± 0.71	13.70 ± 4.03	13.09 ± 3.47
50[C]	13.73 ± 1.05	9.02 ± 1.08	7.67 ± 0.54	6.01 ± 0.53	10.85 ± 1.57	9.35 ± 0.67	16.96 ± 1.46	10.98 ± 1.28
100[D]	15.23 ± 0.86	7.38 ± 1.50	8.64 ± 0.77	8.42 ± 1.61	11.06 ± 2.17	10.28 ± 1.19	18.18 ± 3.00	9.68 ± 1.95
F value	7.82	6.84	74.44	272.68	109.66	132.07	27.41	31.63
Post Hoc			A*B	A*B	A*B	A*B	A*B	
			A*C	A*C	A*C	A*C	A*C	
			A*D	A*D	A*D	A*D	A*D	

* $p < 0.05$.

Table 6
Time Course of Fe Concentrations in Brain Regions of $MnCl_2$-Treated Rats

Treatment	Hours after injection	No. of Rats	Fe (ng/g wet tissue)							
			Frontal cortex	Corpus callosum	Hippocampus	Striatum	Hypothalamus	Medulla	Cerebellum	Spinal cord
Controls	12-72[A]	20	15.32 ± 1.80	8.45 ± 1.69	2.49 ± 0.22	1.88 ± 0.20	3.32 ± 0.62	3.83 ± 0.31	11.93 ± 2.13	11.05 ± 2.37
$MnCl_2$	12[B]	5	17.20 ± 4.11	7.93 ± 1.49	3.84 ± 0.62	5.48 ± 1.55	12.25 ± 2.74	3.07 ± 0.41	14.17 ± 2.83	9.96 ± 1.84
	24[C]	5	13.73 ± 1.05	9.02 ± 1.08	7.67 ± 0.54	6.01 ± 0.53	10.85 ± 1.57	9.35 ± 0.67	16.96 ± 1.46	10.98 ± 1.28
	48[D]	5	17.83 ± 2.48	9.70 ± 2.27	5.38 ± 0.82	5.54 ± 0.82	9.01 ± 0.85	7.73 ± 0.99	14.92 ± 2.97	10.16 ± 2.63
	72[E]	5	15.24 ± 1.53	10.08 ± 0.76	5.48 ± 0.99	4.75 ± 0.59	7.75 ± 1.18	6.50 ± 0.89	15.23 ± 1.31	9.61 ± 2.16
F value			9.91	6.04	95.16	81.26	50.12	119.35	10.69	4.03
Post Hoc				A*E	A*B	A*B	A*B	A*C	A*C	
					A*C	A*C	A*C	A*D	A*D	
					A*D	A*D	A*D	A*E	A*E	
					A*E	A*E	A*E			

* $p < 0.05$.

that Mn can accumulate to a greater extent in dopamine-rich regions *(18)* than in other regions. Our present study indicates, however, that under conditions of high-dose exposures (100 mg $MnCl_2$/kg, ip), the levels of Mn increase to a similar degree in various brain regions (two to four times higher). A slightly higher concentration of Mn accumulated in the cerebellum than in the striatum, relative to their control groups (480% in the cerebellum and 410% in the striatum at high dosage). This finding is in agreement with that of Yu et al. *(19),* who found that the cerebellum is the main Mn accumulation site in the brain. Interestingly, we found that the increases in the levels of Mn are similar (threefold to fourfold) in the medium- and high-dose groups; these observations are consistent with those of previous studies *(20,21).*

The different routes used to administer $MnCl_2$ to animals led to similar degrees of accumulation because, once absorbed and within the biological media, Mn is expected to bind to the same ligands and behave in an analogous pharmacokinetic manner. Thus, the physical and chemical properties of these salts will govern only their absorption and elimination properties *(22).* Therefore, in this study, we chose the ip route because of its practical simplicity and subsequent high adsorption rate. In addition, we used $MnCl_2$ because this form of Mn exhibits the highest rate of transportation into the brain *(23).* We observed significant increases in the levels of brain Mn in both dose-dependence and time-course studies. Samaragiassi and Mutti *(24)* demonstrated that Mn has a short half-life in blood after acute exposure through inhalation. This phenomenon probably arises from Mn being distributed from the blood into other tissues and organs. Thus, in the present study, we targeted the brain, not the blood.

In the current study, we determined that the degree of lipid peroxidation obviously increased in the brain regions of the rats—especially the striatum—after acute administration of a high dose of Mn. Some neurotoxic substances, including Mn and Fe, can damage the striatum, one of the dopaminergic neurons. This damage is caused by the generation of ROS and increased dopamine autooxidation. Sloot et al. *(25)* injected $MnCl_2$ into a single side of the striatum on the brain of a rat and proved that an increase occurred in the level of hydroxyl radicals—the main free radicals that cause oxidative stress in cells and tissues—followed by increases in the concentrations of lipid peroxidation products. In addition, the accumulation of Mn influences metals' homeostasis in the brain, such as that of Fe. A study by Lai et al. *(21)* indicated that the Fe level increased in the hypothalamus, cerebellum, hippocampus, pons, medulla, and striatum after chronic treatment. Accordingly, in this study, we also searched for changes in the Fe, Cu, and Zn levels in the rats' brains. Our results from both dose-dependence and time-course experiments clearly indicated that the levels of Fe increased in many regions of the brains (see Tables 5 and 6), but we witnessed no changes in the Cu and Zn levels. We note that in Sloot and coworkers' study *(25),* the Fe level was not observed to change; this result disagrees with our findings. Indeed, we observed that the concentration of

Fe, one of the important factors that cause the Fenton reaction to occur, was elevated in the striatum. Because the Fenton reaction is one of the most important major pathways to lipid peroxidation, our observation might be the key to explaining the increased degree of lipid peroxidation in the rats' brains (i.e., it results from the elevated Fe level, which increases the amount of lipid peroxidation occurring through the Fenton reaction). Moreover, Fe is a redox-active metal that plays an important catalytic role in the function of many enzymes. Furthermore, the Fe level must be strictly regulated to prevent ROS formation leading to cellular toxicity.

Another possibility is that the lipid peroxidation increase is caused by the Mn itself; Mn might undergo a redox reaction, react with catecholamines, such as dopamine, and then generate superoxide *(25,26).* Mn accumulates in cells, especially in the electron transport system of mitochondria, which is the major source of superoxide. In mitochondria, Mn^{2+} can be oxidized in vitro by superoxide to the powerful oxidizing agent Mn^{3+} *(27).* As such, Mn is likely to accumulate in dopaminergic neurons, the striatum, and the hippocampus. Moreover, recent studies in our laboratory *(28)* have provided evidence that after exposing rats to Mn for 30 d, the degrees of lipid peroxidation decreased in both their frontal cortex and cerebellum. This phenomenon is probably the result of Mn playing a different role when administered at a low level. Whether Mn behaves as an oxidant or antioxidant is not yet clearly understood, because many of the studies have used a variety of conditions, such as the exposure pathway, the form of Mn, and either in vivo or in vitro monitoring, that have led to various conclusions *(29–32).* In the current study, we used high doses and acute exposure of rats to Mn; these conditions resulted in a marked increase in the level of lipid peroxidation, probably because of the acceleration of Mn oxidation or its reaction with dopamine, and, thus, an increase in the occurrence of oxidative stress in tissues.

In summary, from a study of MDA, a biomarker of lipid peroxidation, our findings indicate that Mn exposure induces lipid peroxidation in various regions of the brain. Mn induces lipid peroxidation, and Fe seems to play a key role. We also have demonstrated a Mn-induced elevation of the level of Fe, which provides direct evidence that Fe-mediated lipid peroxidation occurs in these regions of the brain; it might play an important role in the mechanism of Mn-induced neurotoxicology. Notably, our present study provides evidence for oxidative stress in the brain after the administration of manganese.

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