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Comparative Toxicokinetics of Manganese Chloride and Methylcyclopentadienyl Manganese Tricarbonyl (MMT) in Sprague-Dawley Rats

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Abstract

The toxicokinetics of manganese (Mn) was investigated in male and female rats either following a single intravenous (iv) or oral dose of $MnCl_2$ (6.0 mg Mn/kg), or following a single oral dose of methylcyclopentadienyl manganese tricarbonyl (MMT) (20 mg MMT/kg or 5.6 mg Mn/kg). The plasma concentrations of manganese were quantified by atomic absorption spectrophotometry (AAS). Upon iv administration of $MnCl_2$, manganese rapidly disappeared from blood with a terminal elimination $t_{1/2}$ of 1.83 h and CL_s of 0.43 L/h/kg. The plasma concentration-time profiles of manganese could be described by $C = 41.9e^{-4.24t} + 2.1e^{-0.44t}$. Following oral administration of $MnCl_2$, manganese rapidly entered the systemic circulation (T_{max} = 0.25 h). The absolute oral bioavailability was about 13%. Oral dose of MMT resulted in a delayed T_{max} (7.6 h), elevated C_{max} (0.93 μg/ml), and prolonged terminal $t_{1/2}$ (55.1 h). The rats receiving MMT had an apparent clearance (CL/F = 0.09 L/h·kg) about 37-fold less than did those who were dosed with $MnCl_2$. Accordingly, the area under the plasma concentration-time curves (AUC) of manganese in MMT-treated rats was about 37-fold greater than that in $MnCl_2$-treated rats. A gender-dependent difference in toxicokinetic profiles of plasma manganese was also observed. Female rats displayed a greater AUC than that of male rats. Although the apparent volume of distribution of manganese was similar in both sexes, the apparent clearance in males was about twice that observed in females. The results indicated that after oral administration, the MMT-derived manganese displayed higher and more prolonged plasma concentration-time profiles than $MnCl_2$-derived manganese. Thus, MMT-derived manganese appeared likely to accumulate in the body following repeated exposure.

Keywords

manganese; methylcyclopentadienyl manganese tricarbonyl (MMT); toxicokinetics; bioavailability; half-life; clearance; volume of distribution; Sprague-Dawley rats

Chronic manganese intoxication leads to adverse neurologic and psychological disorders (Barbeau *et al*., 1976; Chandra *et al*., 1979; Gorell *et al*., 1997; Mena *et al*., 1967). Health

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risks of exposure to manganese have been associated with organic manganese-containing pesticides, such as manganese ethylene-bis-dithiocarbamate (MANEB) (Ferraz *et al.*, 1988), inorganic manganese dust or vapor among steel manufacturing workers or welders (Roels *et al.*, 1987, Wang *et al.*, 1989), or a cocaine-based drug called Bazooka, which is contaminated with manganese carbonate (Ensing, 1985). Recently, several countries, including the United States, have replaced lead (Pb) in gasoline with the manganese-containing antiknock compound methylcyclopentadienyl manganese tricarbonyl (MMT). In the United States, MMT is produced by the Ethyl Corporation and marketed as HITEC 3000 or AK-33X. MMT contains 24.4–25.2% manganese (Frumken and Solomon, 1997; Zayed *et al.*, 1994). The combustion of MMT in the automobile with the expected increase in ambient manganese level has raised concerns about the health risks associated with environmental exposure to manganese.

Manganese-induced neurologic lesions are located in the globus pallidus and striatum of the basal ganglia. The pallidus and striatum display a marked decrease in myelinated nerve fibers, accompanied by depletion of striatal dopamine (Bonilla, 1980; Eriksson *et al.*, 1987; Mena *et al.*, 1967, Olanow *et al.*, 1996). These neuropathologic alterations have been observed among manganese-intoxicated experimental primates, which display the illustrative neurologic symptoms (Olanow *et al.*, 1996; Pentschew *et al.*, 1963). In rodents, the similar neurobiochemical alterations have also been demonstrated. For example, chronic exposure to manganese in rodents has been shown to alter brain dopaminergic neurotransmitters (Bonilla, 1980; Eriksson *et al.*, 1987; Gianutsos and Murray, 1982; Parenti *et al.*, 1986; Rodriguez *et al.*, 1998) and inhibits critical enzymes involved in energy production (Seth *et al*, 1977; Zheng *et al.*, 1998, 1999). Manganese in rat brain may interact with other essential metal ions and cause oxidative stress in targeted brain areas (Ali *et al.*, 1995; Sloot *et al.*, 1996). Some researchers have characterized behavioral and pathologic changes in rodents due to chronic manganese exposure (Chandra *et al*, 1979; Singh *et al.*, 1974). Local injection of manganese into rat brain also interferes with motor activity (Brouillet *et al.*, 1993; Ingersoll *et al.*, 1995). Moreover, following exposure to MMT, rodents show significant neuroexcitatory toxicity and altered normetanephrine levels (Fishman *et al.*, 1987; Komura and Sakamoto, 1994).

In light of the broad application of rodents as acceptable models for the mechanistic studies of manganese neurotoxicity, it is surprising that no toxicokinetic study of either inorganic or organic manganese has been conducted in rodents. Particularly sparse is the knowledge on toxicokinetic parameters such as half-lives, volume of distribution, and clearance in some routinely used animal species. The lack of these fundamental measures has rendered it difficult to establish a physiologically relevant dosing regimen for chronic investigation of manganese toxicity in rodents. This study was therefore undertaken to evaluate the plasma kinetics of manganese in Sprague-Dawley rats following intravenous or oral administration of $MnCl_2$ or following oral administration of MMT.

MATERIALS AND METHODS

Chemicals

Chemicals were obtained from the following sources: manganese chloride ($MnCl_2 \cdot 4H_2O$) and methylcyclopentadienyl manganese tricarbonyl (MMT) from Fluka (Milwaukee, WI); ether, Triton X-100, and ethylene diamine tetraacetic acid (EDTA) from Sigma (St. Louis, MO); manganese standard for atomic absorption spectrometry from Alfa Products (Danvers, MA). All reagents were of analytical grade or the highest available pharmaceutical grade.

Animals

Male Sprague-Dawley rats were purchased from Harlan Inc. (Indianapolis, IN) and were 210–230 g (2 months old) at the time of the experiments. The animals were housed in a temperature-controlled, 12:12 h light/dark room, and were allowed free access to tap water and food (Teklad 4% Mouse-Rat Diet, Teklad, Madison, WI). For the oral dosing study, animals were fasted for 12 h prior to administration of $MnCl_2$ or MMT.

Administration of manganese compounds

$MnCl_2$ was dissolved in sterile saline for iv or oral administration. For the iv dosing study, $MnCl_2$ was injected via the tail vein at a dose of 6.0 mg Mn/kg (1.0 ml/kg) over approximately 5 sec. For the oral dosing study, $MnCl_2$ was administered by a single gavage at the same dose level. This dose regimen was chosen because it was known to be associated with a significant reduction of succinic dehydrogenase and aconitase in rat brain (Seth *et al.*, 1977; Singh *et al.*, 1974; Zheng *et al.*, 1998).

MMT was dissolved in corn oil and administered orally by gavage to rats at a dose of 20 mg/kg (3.3 ml/kg in volume). This dose was equivalent to 5.6 mg Mn/kg.

Collection of blood samples

At the appropriate times, the rats were subjected to light anesthesia with ether. Blood samples (0.3– 0.5 ml) were collected from the orbital sinus through heparin-pretreated glass capillary tube and transferred to an Eppendorf tube. For the $MnCl_2$ study, the blood was collected prior to (as 0 h) and at 0.05, 0.17, 0.33, 0.5, 1, 2, 4, 8, and 12 h following either iv or oral administration. The blood was centrifuged at 5000 × *g* for 5 min, and the plasma was separated and stored at −20°C until analyzed. The plasma samples were usually analyzed for manganese content within 1 week.

For the MMT study, the procedure for sample collection was identical to that described above, except for the times of blood collection, which were prior to (as 0 h) and at 0.17, 0.5, 1, 2, 4, 8, 12, 24, 48, 120, 168, 288, 384, and 456 h following oral gavage.

Atomic absorption spectrophotometry (AAS) analysis

Manganese concentrations in plasma were determined by a flameless graphite furnace AAS. Aliquots (50 μl) of plasma samples were diluted (10–50 fold) with an appropriate volume of 8% Triton X-100 and 5% EDTA in distilled, deionized water prior to AAS. A Perkin-Elmer Model 3030 Zeeman AAS, equipped with an HGA-600 graphite furnace, was used for

quantification. The standard curves were established using freshly made manganese standards on the day of analysis. The detection limit for this method was 0.2 ng Mn/ml of assay solution (Zheng *et al.*, 1998, 1999).

Toxicokinetic analysis

Under normal conditions, the rats used in this experiment had baseline plasma manganese concentrations of 0.029 ± 0.011 μg/ml (mean ± SD, $n = 13$). To eliminate background interference, all plasma data were corrected for this baseline value prior to kinetics calculations.

For the iv dosing study, plasma concentration-time data were analyzed by a two-compartment model represented by the following equation:

$$C(t)=Ae^{-\alpha t}+Be^{-\beta t} \quad (1)$$

where C(t) is the plasma concentration at time "t"; and A and B are the preexponential coefficients. Calculation of the terminal phase elimination rate constant (β) was based on the values of the terminal data points of the individual curves, and the initial rate constant (α) was estimated by the method of residual (Gibaldi and Perrier, 1982). The total area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule and extrapolating to time infinity. The systemic or total body clearance (CL_s) was computed by dividing the iv dose by AUC. The total volume of distribution (V_β) was calculated from the relationship $V_\beta = CL_s/\beta$, and the apparent volume of the central compartment (V_c) determined by the equation $V_c = Dose_{iv}/(A + B)$. Each individual data set was further evaluated by a pharmacokinetic data analysis program PKAnalyst (MicroMath, Inc., Salt Lake City, UT). The model that best described the observed data was a two-compartment model with first-order elimination from the central compartment (Gibaldi and Perrier, 1982). PKAnalyst was used to generate the best-fit critical toxicokinetic parameters for each animal, including α, β, A, B, elimination rate constant (k_{10}), intercompartmental transfer rate constants (k_{12} and k_{21}), and AUC.

For the oral dosing study, the terminal elimination phases of most tested animals usually comprised 4–6 data points, which were inadequate for compartment model analysis. Thus, the plasma concentration-time data were analyzed by a noncompartmental method, rather than the compartment-based curve fitting approach. Values of C_{max} and T_{max} were obtained directly from plasma concentration-time profiles. The apparent first-order disposition rate constant (K_e) was estimated by linear least-squares regression of the data in the terminal phase. From these values, the half-lives were calculated ($t_{1/2} = 0.693/K_e$). The AUC and the total area under the first moment curve (AUMC) were calculated using the linear trapezoidal rule and extrapolating to time infinity. The mean residence time (MRT) and mean absorption or input time (MAT) were calculated as follows:

$$MRT_{iv}=\left[\frac{AUMC}{AUC}\right]_{iv} \quad (2)$$

$$\mathrm{MATpo}=\left[\frac{\mathrm{AUMC}}{\mathrm{AUC}}\right]_{\mathrm{po}}-\mathrm{MRT}_{\mathrm{iv}} \quad (3)$$

The absolute oral bioavailability (F) of manganese as $MnCl_2$ was estimated from the ratio of AUC_{po}/AUC_{iv}. The apparent body clearance (CL/F) was computed by dividing the oral dose by AUC ($CL/F = Dose_{po}/AUC_{po}$). The apparent volume of distribution (V_d/F) was calculated from the following relationship,

$$\mathrm{V_d/F}=\frac{\mathrm{CL/F}}{\mathrm{K_e}} \quad (4)$$

Statistics

All data are presented as mean ± SD. Statistical analysis for comparison of two means was performed using one-way ANOVA. In all cases, a probability level of $p < 0.05$ was considered as the criterion of significance.

RESULTS

Intravenous dose of $MnCl_2$

After an iv-bolus injection of $MnCl_2$ in Sprague-Dawley rats, the concentration-time profile of manganese in plasma followed a multiexponential equation:

$$\mathrm{C(t)}=41.94\mathrm{e}^{-4.24\mathrm{t}}+2.08\mathrm{e}^{-0.44\mathrm{t}}$$

In general, the two-compartment model with first-order elimination from the central compartment provided a good fit to the observed data (Fig. 1). Manganese was rapidly eliminated from the plasma with an initial faster phase between 0 and 3 h and a slower terminal phase between 3 and 12 h. Accordingly, the first-order initial disposition $t_{1/2\alpha}$ and the terminal elimination $t_{1/2\beta}$ were estimated to be 0.19 h and 1.8 h, respectively. By 12 h, manganese concentrations in plasma were restored to normal levels in all tested animals (Fig. 1). Although the total volume of distribution (V_β) of manganese was about 1.16 l/kg, the central volume of distribution (V_c) was only 0.14 l/kg, suggesting an extensive distribution of manganese to the peripheral compartment following iv injection of $MnCl_2$.

Oral dose of $MnCl_2$

Single oral gavage of $MnCl_2$ resulted in a rapid appearance of manganese in plasma (Fig. 2). The C_{max} (0.296 μg/ml) was achieved within 0.5 h of the oral dose. Thereafter, manganese concentrations declined and the terminal phase followed the first-order kinetics. The absolute oral bioavailability (F) of manganese following oral $MnCl_2$ was 13.2% at a dose of 6 mg/kg. Similar to iv injection, plasma manganese returned to normal levels 12 h after dosing. Oral dosing of $MnCl_2$ resulted in a significant increase in terminal $t_{1/2}$ compared to rats receiving iv injection (Table 1). By adjusting the apparent clearance (CL_s/F) and

apparent volume of distribution (V_d/F) with the oral bioavailability (F), the clearance in the oral dose group remained unchanged; however, the volume of distribution in the oral dosing group was significantly increased (2.5 fold) compared to the iv dosing group ($p < 0.05$).

Oral dose of MMT

Following oral administration of MMT, manganese appeared in plasma and attained a C_{max} between 2 and 12 h after dosing (Fig. 3). The elimination of manganese after T_{max} was monophasic, with an average elimination $t_{1/2}$ of 55 h. Although the absolute dose of manganese in the MMT dose formula (5.6 mg Mn/kg) was comparable to that of $MnCl_2$ (6 mg Mn/kg), the C_{max} (0.931 μg/ml, Table 2) following oral MMT was significantly higher (about three times) than that following oral $MnCl_2$ (0.296 μg/ml, Table 1). MMT-derived manganese was eliminated extremely slowly from the rats. The rats receiving MMT had an apparent oral clearance (CL/F = 0.089 l/h·kg, Table 2) of about 37-fold less than did those who received oral $MnCl_2$ (CL/F = 3.2 ± 0.98SD, l/h · kg). Accordingly, the AUC in the MMT rats was about 37-fold higher than that in $MnCl_2$ rats.

Gender differences of MMT toxicokinetics

A gender-related difference in the toxicokinetic properties of MMT-derived manganese was observed in this study with a single oral dose at the same dose level investigated (Fig. 3, Table 2). For example, the female rats had higher AUC and longer $t_{1/2}$ of plasma manganese than did the male rats (Fig. 4). Further analysis of the T_{max} in both sexes revealed that the T_{max} was longer in females (10 h) than in males (5.5 hr) following oral doing of MMT, although this difference did not achieve statistical significance ($p = 0.120$). Although the apparent volume of distribution (V_β/F) and C_{max} were not statistically significantly different between the two sexes, the elimination rate constant (K_e) was significantly greater in males than in females (Table 2). Moreover, the clearance in the female group was about 1.7-fold less than that in the male group ($p < 0.05$) (Fig. 4). Therefore, the longer $t_{1/2}$ and larger AUC of MMT-derived manganese in the female rats may be due to the slower elimination of MMT or MMT-derived manganese in this sex.

DISCUSSION

This report provides the first systemic study looking at the kinetic behavior of manganese in blood following administration of $MnCl_2$ or MMT in Sprague-Dawley rats. Following an iv injection of $MnCl_2$, the temporal pattern of manganese appeared to fit into a two-compartment model. Manganese was rapidly distributed from the central compartment to peripheral compartment. The rate of the initial distribution phase (α) was about 10 times more rapid than that (β, or K_e) in the terminal elimination phase. The central volume to which manganese ions were circulated (141 ml/kg) far exceeded the total rat blood volume, which is about 58 ml/kg (Hollinger, 1995). Hence, in addition to distribution in blood, manganese ions appeared to readily enter the initial well-perfused spaces, which probably include liver and kidneys. In a similar rat model, Klaassen (1974) demonstrated that manganese reached the highest concentrations in liver, followed by kidney and heart, at 2 h after an iv injection of $MnCl_2$. For a 5-day study period, over 99% of the dosed manganese was recovered in the feces, whereas less than 1% of the dose was excreted into the urine.

The biliary excretion appears to constitute the major pathway for removal of manganese from the body (Klaassen, 1976). Thus, the rapid elimination of manganese from the central compartment may be partly due to the fast hepatic clearance of manganese via the bile.

Given that hepatic clearance represents the majority of manganese systemic clearance, it is conceivable that the total body clearance of manganese would be close to the blood flow of the liver. In fact, by adjusting the body weight (i.e., 250 g), the total body clearance of manganese (1.8 ml/min) in the current study was about 9-fold less than (or 12% that of) the hepatic blood flow (15 ml/min) in rats (Hollinger, 1995). Assuming that the liver clears nearly all manganese ions present in hepatic blood flow, the hepatic extraction ratio (the ratio of hepatic clearance/hepatic blood flow) of manganese would seem unlikely to exceed 12% of manganese passing through the liver. Such a low hepatic extraction ratio is unexpected and might suggest a membrane-limited passive diffusion or transport of manganese ions by the liver. Further studies are needed to address this issue.

One of the perplexing questions in clinical monitoring of manganese toxicity is that blood levels of manganese usually poorly reflect the body burden of manganese and the ensuing disease status. There is a discrepancy between blood manganese and intracellularly distributed tissue manganese. This was apparently the case when our data were compared with those of others (Klaassen, 1974; Takeda *et al.*, 1995). In the current study, the terminal phase elimination $t_{1/2}$ following iv injection was about 1.8 h, and by 12 h, the plasma manganese returned to normal levels in all tested animals. Theoretically, such a short $t_{1/2}$ would not likely result in the accumulation of manganese in the body, assuming that manganese transport among tissues follows the mass balance. However, Takeda *et al.* (1995), by using autoradiography technique, reported that manganese persistently presented in various brain regions following radiotracer injection. The biologic $t_{1/2}$ of manganese in the basal ganglia, brain stem, and cerebral cortex was estimated to be between 51–74 days. Klaassen also showed that manganese levels in liver after an iv injection dropped by 90% during a 5-day period. However, brain manganese levels were actually slightly increased (Klaassen, 1974). In primates, the brain $t_{1/2}$ of manganese from inorganic manganese exposure was found to be as long as 53 days (Newland *et al.*, 1987). Comparison of our results with these earlier studies indicated that the brain manganese did not seem to decline in response to the blood manganese status. One of the possible explanations is that manganese may readily enter the brain by one-way transport mechanism at the blood-brain barrier and blood-CSF barrier in the choroid plexus (Aschner *et al.*, 1999). It is also possible that the intracellular binding and sequestration of manganese in the brain may prevent the metal from emigration to the extracellular space. The slow effluent brain manganese thus merges into the background level of plasma manganese. Under this condition, the observed plasma $t_{1/2}$ would not reflect the true brain burden of manganese. By the same token, this may explain the lack of correlation between blood manganese levels and total body burden of manganese, which is presumably associated with manganese toxicity seen in clinic.

Manganese was rapidly absorbed in the gastrointestinal tract after oral dosing. The absolute bioavailability of manganese in rats was about 3–4 times higher than that in humans (3–5%). As an essential element, human minimum daily dietary requirement for manganese ranges from about 2.5 to 5.0 mg/day. In an adult human, the normal blood concentration of

manganese lies between 8.6–16 ng/ml (Aschner *et al.*, 1999). In comparison, the rats, prior to manganese administration in this study, had average blood manganese concentration of about 28.6 ng/ml. Increased bioavailability in rats may explain the higher blood level of manganese in the rats than in humans.

The temporal pattern of plasma manganese following MMT oral administration could be characterized by the substantially delayed T_{max}, large AUC, and prolonged $t_{1/2}$. Concerning the absolute manganese dosage in the dose formula, the MMT dose in this study (20 mg/kg) contained 5.6 mg/kg of manganese, which was comparable to the dose used in $MnCl_2$ study (6 mg/kg). However, AUC in MMT-treated rats was 37-fold higher than that in $MnCl_2$-treated rats. Furthermore, MMT-derived manganese appeared in blood in a much slower rate than did $MnCl_2$-derived manganese (Fig. 2 vs. Fig. 3). The T_{max} in MMT-treated rats was about 30-fold longer than that in rats receiving oral dose of inorganic manganese (Fig. 4). These results suggest a relatively complete but much slower absorption process following MMT dose administration.

This exceptionally slow absorption of MMT by the gastrointestinal tract was unexpected for MMT's high lipophilicity. As MMT was dissolved in corn oil, the slow release of MMT from the oily vehicle may contribute, at least in part, to the prolonged absorption duration. However, other biologic processes may also be taken into account. For example, unlike the inorganic manganese as the dose formula, MMT-derived manganese mainly accumulates in the lung and produces acute pulmonary hemorrhage edema, which is considered as the primary cause of MMT-induced death in animals (Hanzlik *et al.*, 1980a, McGinley *et al.*, 1987). Whereas the lung serves as the target organ, the hepatic cytochrome P-450 metabolizing enzymes may play a critical role in toxicokinetics of MMT. The studies by Hanzlik *et al.*, (1980a,b) demonstrated that rats pretreated with phenobarbital yielded a remarkable protection against lung injury and associated lethality caused by orally administered MMT. The same treatment also doubled the rate of excretion of biliary metabolites of MMT. These results suggest that MMT molecules, upon being absorbed from the gastrointestinal tract, undergo extensive first-pass hepatic biotransformation. The question as to what extent MMT or the manganese released from it enters the blood stream remains unanswered. Yet, the hepatic manipulation of MMT appears likely to underlie the distinct T_{max} between MMT and $MnCl_2$ in this study. It is also possible that the delayed absorption of MMT may be due to a saturated absorption mechanism in the gastrointestinal wall.

The terminal elimination $t_{1/2}$ and the AUC of plasma manganese in MMT rats were about 12-fold and 37-fold, respectively, higher than those in rats orally administered with $MnCl_2$ (Fig. 4). Our data are consistent with the observation by Gianutsos *et al.* (1985), who reported that in adult mice, administration of MMT produced a long-lasting elevation of manganese concentrations in both blood and brain. In a rat model, MMT administration also caused MMT or its metabolites to be accumulated and retained in lung, liver, kidney, and brain (McGinley *et al.*, 1987; Komura and Sakamoto, 1994). The prolonged $t_{1/2}$ and elevated AUC in this study, thus, suggest an accumulation of manganese in the body after exposure to MMT.

Comparison of clearance data between $MnCl_2$ and MMT groups showed that MMT-derived manganese was cleared at a rate of about 4.8-fold slower than that in the $MnCl_2$-dosed group (Table 2). As MMT is highly lipophilic, the main route of elimination of MMT-derived manganese in rats could be different from that of $MnCl_2$-treated rats. Earlier studies have shown that after MMT oral ingestion 73% of the dose as manganese is eliminated in the first 24 h, of which 36% of MMT-derived manganese was present in the urine (Moore *et al.*, 1974). This ratio was much higher than that in $MnCl_2$-exposed animals, whose urinary elimination was less than 1% of the dose. In phenobarbital-pretreated rats, during the first 48 h after administration of MMT, 74–89% of the dose was eliminated in the urine as MMT or its metabolites, and fecal elimination amounted to only 2–4% of the dose (Hanzlik *et al.*, 1980b). It was subsequently postulated that the large fraction of administered MMT apparently remains intact as an organometallic complex while undergoing biotransformation and excretion. In contrast to the extensive biliary excretion following inorganic manganese administration (Klaassen, 1974), a 6-h bile collection period found only about 10–11% of the injected manganese dose in the bile (Hanzlik *et al.*, 1980a). In addition, Komura and Sakamoto (1994) have suggested that MMT has a higher absorption rate from the digestive tract than that of inorganic manganese, although the absolute bioavailability of MMT is still unknown. Thus, a combination of complete absorption, special biotransformation pathway, shifted elimination route, and large tissue retention of MMT may explain the long $t_{1/2}$ and large AUC observed in MMT-treated rats.

A distinct and significant gender-dependent difference in the toxicokinetic profiles of MMT-derived manganese was observed in the current study. Female rats had higher plasma concentrations of manganese and AUC than did the male rats (Fig. 3). Although the apparent volume of distribution (V_β/F) in males was similar to those in females following oral dosing of MMT (Table 2), the apparent body clearance (CL/F) of manganese in the males was about twice as much as in the females. The elimination rate constant (K_e) was greater in male than in female rats. Hence, a slower elimination of MMT in female rats may account for the higher plasma manganese concentrations in this sex. As the gender difference in drug metabolism has been demonstrated in many other cases, we postulate that the gender-dependent metabolism of MMT may underlie this observation. Whether this gender difference is also present in humans would be interesting to explore.

In summary, manganese toxicokinetics after iv dosing of $MnCl_2$ appeared to follow a two-compartment disposition model. Manganese in rats displayed a moderate volume of distribution and a relatively short $t_{1/2}$. The discrepancy between the plasma $t_{1/2}$ and tissue accumulation of manganese was discussed. Following oral dose administration, manganese was rapidly but poorly absorbed from the gastrointestinal tract. The percentage of the dose absorbed was about 13%. After oral dosing of MMT, the MMT-derived manganese entered the blood circulation at a slower rate, but to a much greater extent as compared to the oral dosing of inorganic manganese. The relatively complete absorption and slow elimination of MMT-derived manganese in MMT-treated rats contribute to its higher plasma concentration-time profiles. The elimination of MMT-derived manganese appears gender dependent.

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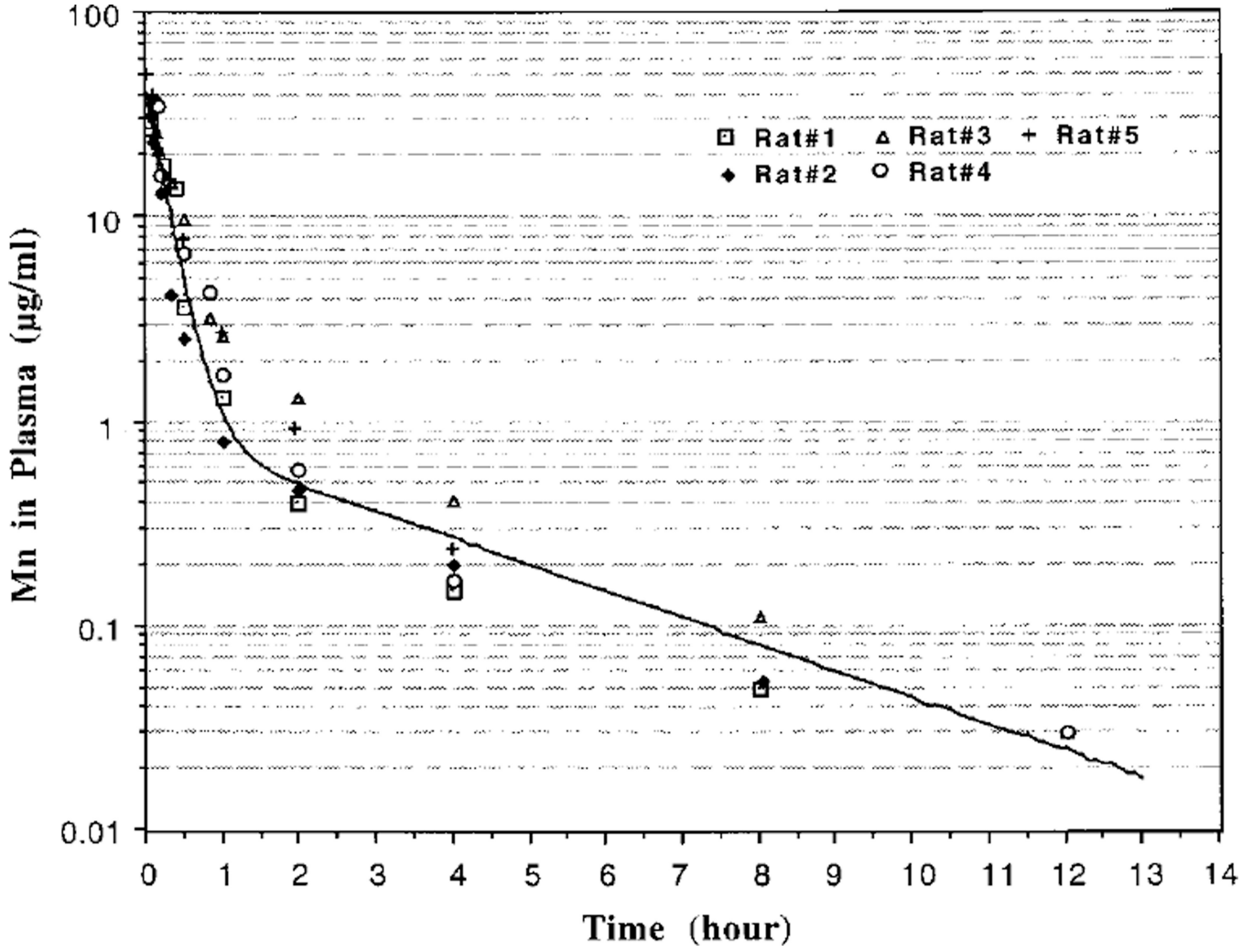

FIG. 1.

Plasma concentration-time profiles of manganese in male Sprague-Dawley rats following an iv administration of $MnCl_2$ (6.0 mg Mn/kg). Plasma concentration of manganese was determined by AAS. The line indicates the best fit of a two-compartment model with first-order elimination from the central compartment to the observed data.

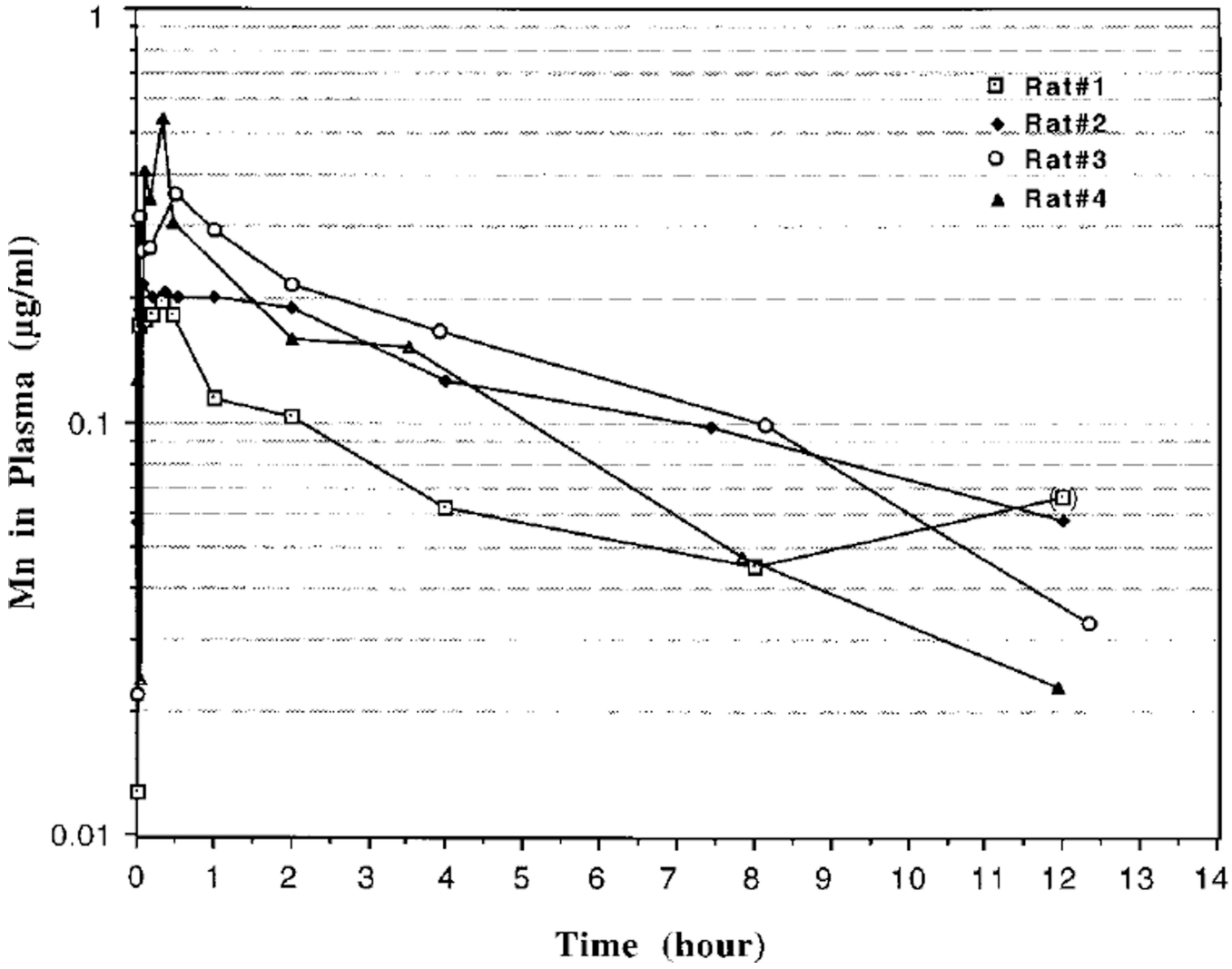

FIG. 2.

Plasma concentration-time profiles of manganese in male Sprague-Dawley rats following a single oral dose of $MnCl_2$ (6.0 mg Mn/kg by gavage). Plasma concentration of manganese was determined by AAS. The terminal-phase parameters were calculated from 1–8 h for rat 1, 1–12 h for rat 2 and 3, and 0.5–12 h for rat 4. The value in parenthesis was not used in data analysis.

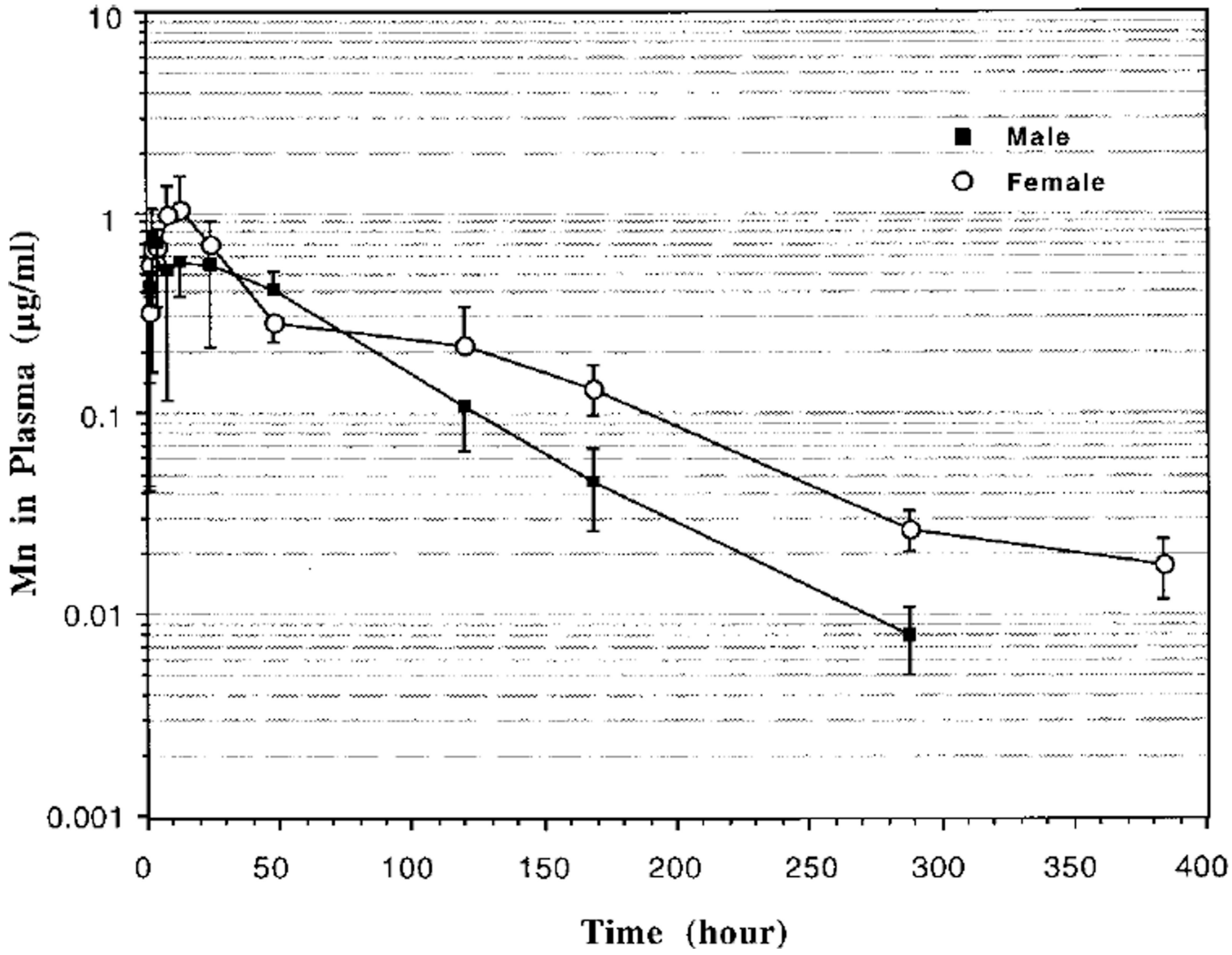

FIG. 3.

Plasma concentration-time profiles of manganese in male or female Sprague-Dawley rats following a single oral dose of 20 mg/kg MMT (5.6 mg Mn/kg) in corn oil by gavage. Plasma concentration of manganese was determined by AAS. Data represent mean ± SD, *n* = 4 of each sex.

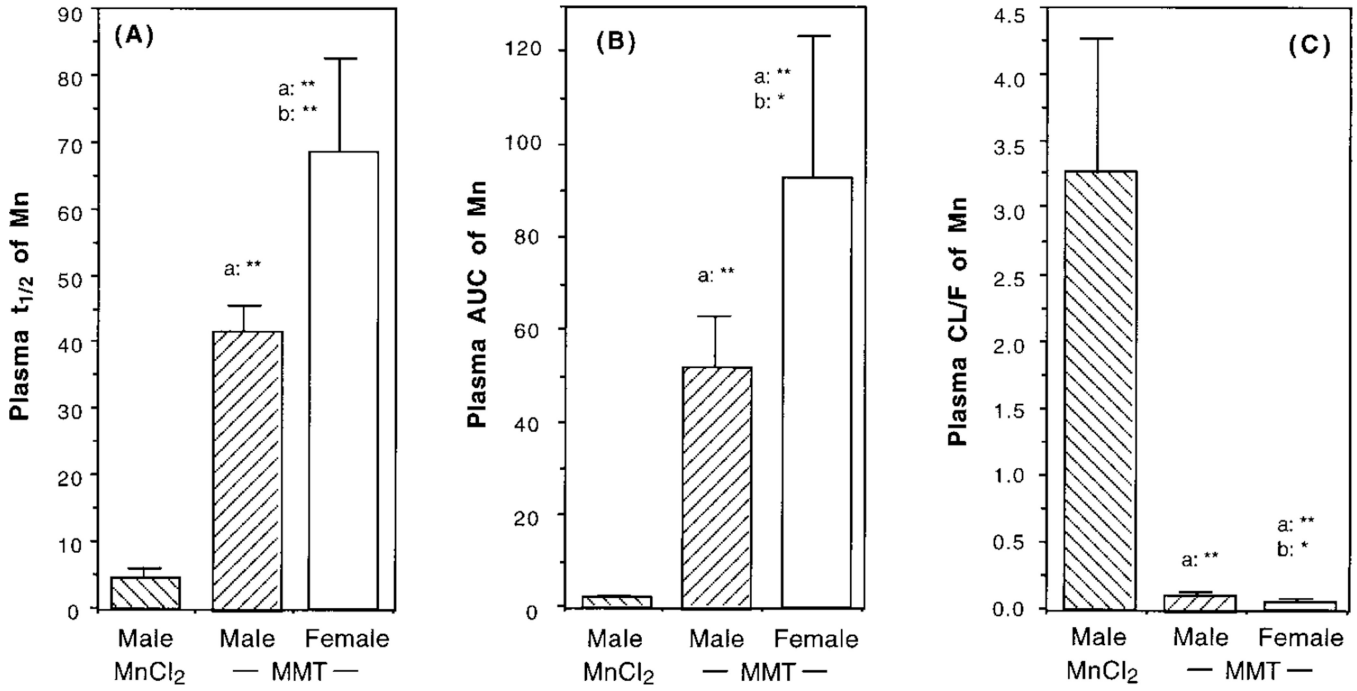

FIG. 4.

Gender differences in (A) $t_{1/2}$, (B) AUC, and (C) CL/F in Sprague-Dawley rats following a single oral dose of 20 mg/kg MMT (5.6 mg Mn/kg) in corn oil by gavage. Data represent mean ± SD (n = 4–5). a) The comparison was made between $MnCl_2$ group and MMT group. b) The comparison was made between the male and female rats in the MMT-treated group. *$p < 0.05$; **$p < 0.01$.

TABLE 1

Toxicokinetic Parameters of Mn in Sprague-Dawley Rats following Intravenous or Oral Administration of $MnCl_2$

	Intravenous dose	Oral dose
A (μg/ml)	41.9 ± 6.96	
B (μg/ml)	2.08 ± 2.18	
α (h^{-1})	4.24 ± 0.82	
K_e (β) (h^{-1})	0.44 ± 0.22	0.16 ± 0.04 *
$t_{1/2\alpha}$ (h)	0.17 ± 0.03	
$t_{1/2\beta}$ (h)	1.83 ± 0.63	4.56 ± 1.30 **
AUC (mM · h)	14.8 ± 3.60	1.95 ± 0.51 **
CL_s (L/h · kg)	0.43 ± 0.13	
V_β (L/kg)	1.16 ± 0.51	
V_c (L/kg)	0.14 ± 0.03	
MRT (h)	0.82 ± 0.23	
C_{max} (μg/ml)		0.30 ± 0.11
T_{max} (h)		0.25 ± 0.21
MAT (h)		2.72 ± 1.33
F (%)		13.19

Note. Rats (220 ± 10 g) were administered either an iv-bolus or oral dose of $MnCl_2$ (6.0 mg Mn/kg). Parameters were computed from the plasma concentration-time curves of each animal. Data represent the mean ± SD, $n = 5$ for iv dose, and $n = 4$ for oral dose studies.

* $p < 0.05$,

** $p < 0.01$ compared to values in the iv dose group.

TABLE 2

Toxicokinetic Parameters of Mn in Sprague-Dawley Rats following Oral Administration of MMT

	Male	Female	Combined[a]
$t_{1/2(e)}$ (h)	42.0 ± 3.35	68.4 ± 13.9**	55.2 ± 17.0
K_e (h^{-1})	0.02 ± 0.00	0.010 ± 0.002**	0.01 ± 0.00
AUC (mM · h)	51.8 ± 10.9	93.1 ± 30.7*	72.5 ± 30.7
C_{max} (μg/ml)	0.79 ± 0.36	1.07 ± 0.46	0.93 ± 0.41
T_{max} (h)	5.50 ± 4.43	10.0 ± 2.31	7.75 ± 4.06
CL/F (L/h · kg)	0.11 ± 0.03	0.07 ± 0.02*	0.09 ± 0.03
V_β/F (L/kg)	6.81 ± 1.73	6.25 ± 1.49	6.53 ± 1.53

Note. Rats (220 ± 10 g) were administered with oral dose of MMT (20 mg/kg, i.e., 5.6 mg Mn/kg). Parameters were computed from the plasma concentration-time curves of each animal. Data represent the mean ± SD, $n = 4$ for both sexes.

[a]The data from both male and female rats were combined to estimate mean ± SD, $n = 8$.

*$p < 0.05$;

**$p < 0.01$ compared to values in the male group.