GESDAV

Journal of Environmental and Occupational Science

available at www.scopemed.org

Original Research

Behavioral response of chicks exposed to manganese by drinking water and mobilization of body manganese burden by EDTA

Muna H.I. Al-Zubaidy, Fouad K. Mohammad

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Iraq

Received: June 22, 2012

Accepted: September 08, 2012

Published: October 24, 2012

DOI : 10.5455/jeos.20120908035809

Corresponding Author:
Fouad K. Mohammad,
College of Veterinary Medicine,
University of Mosul, Mosul, Iraq
fouadmohammad@yahoo.com

Keywords: Manganese, EDTA, Neurotoxicity, Open-field activity, Tonic immobility

Abstract

Manganese (Mn) is a neurotoxicant in various animal species including the chicks. The present study examines the effect of Mn on the behavioral response of chicks, plasma and tissue Mn levels and the effect of the metal chelating agent disodium EDTA in reducing high levels of the metal in the plasma and some vital organs. Day old chicks were provided with either deionized water (control group) or with Mn at 1 g/L of deionized water as the drinking water for 14 days. On treatment days 7 and 14, all chicks were individually monitored for 3-min open-field activity and then they were subjected to tonic immobility test. On days 7 and 14, the concentrations of Mn in the plasma, whole brain, liver and kidney were determined by atomic absorption spectrometry. Seven or eight day-old chicks were treated intramuscularly (i.m.) with either saline at 5 ml/kg (control), Mn at 20 mg/kg followed 30 min later with saline, or with Mn at 20 mg/kg followed 30 min later with EDTA at 50 mg/kg. The concentrations of Mn in the plasma, whole brain, liver and kidney were determined two hours after the Mn injection by atomic absorption spectrometry. Mn increased the general locomotor activity of the chicks in the open-field arena (lines crossed) and decreased the duration of tonic immobility response of the chicks on treatment day 14 in comparison with the control group. The administration of Mn in the drinking water at 1 g/L increased Mn concentrations in the plasma, whole brain, liver and kidney of the chicks on days 7 and 14 in comparison with the control values. Higher levels of Mn were attained on day 14 compared with those of day 7. Injection of Mn at 20 mg/kg significantly increased the metal concentrations in the plasma, whole brain, liver and kidney of the chicks. The highest concentration of Mn appeared in the kidney followed by the liver, whole brain and the plasma. Injection of EDTA at 50 mg/kg significantly reduced Mn concentrations in the plasma and whole brain when compared with those of the saline-treated group. The data suggest that Mn is well absorbed and distributed into the tissues after exposure of young chicks via the drinking water or i.m. Mn changed the behavioral performance of the chicks and EDTA effectively reduced acute Mn overload in the body. These results further support and expand previous studies concerning the experimental use of young chicks as a potential model of acute Mn neurotoxicity.

INTRODUCTION

Manganese (Mn) has been reported to induce neurotoxicosis in man [1,2] as an industrial poison and in laboratory animals under experimental conditions [3–6]. The toxicokinetics and tissue distribution of Mn are used as biomarkers for the assessment of potential toxic effects of the metal and its antidotal therapy, since Mn is differential distributed into various organs of the body including different regions of the central nervous system, (CNS) [4,7–10]. Recent studies have reproduced and characterized Mn neurotoxicity in young chicks [5,6,11]. Single doses of Mn as low as 5, 10 or 20 mg/kg, i.m. were associated with reductions in general locomotor activity and behavioral depression in 7–14 days old broiler chicks [6].The toxicokinetics and

tissue distribution of high doses of Mn have also been reported in chicks [6,11]. Mn administration at the dose rates of 10, 20, 50 and 100 mg/kg, intramuscularly (i.m.) resulted in high levels of the metal in the plasma, brain, liver and kidney [6]. The elimination half-life of Mn after an i.m. injection of chicks at 20 mg/kg was 3.02 h with steady state volume of distribution 24.34 L/kg and total body clearances of 4.78 L/h/kg with a 3.12 h elimination half-life from the brain [11]. Based on the chick studies [5,6,11], the potential avian model of Mn neurotoxicosis appears to be an added model to the already existing animal models of Mn neurotoxicosis [3,4]. The present study further examines a different aspect of such a model, including behavioral performance of chicks following exposure to Mn in the drinking water, measuring Mn levels in the plasma and tissues and the use of the metal chelating agent disodium EDTA (disodium ethylenediaminetetraacetate dihydrate) in reducing the toxic levels of Mn in the plasma and some vital organs. These are important aspects of any potential model of Mn neurotoxicity addressing the hypothesis that exposure to high level of Mn results in increased Mn concentration in the CNS affecting the animal behavioral responses, and the metal tissue burden would be vulnerable to modulation by the chelator EDTA. We attempted to monitor the behavioral response of the chicks following exposure to Mn via the drinking water subacutely (14 days) since the effects of injection form of Mn was reported to cause acute behavioral changes in chicks within 30 min at 5-20 mg/kg, i.m. [6]. The second part of this study aimed at reducing high Mn body burden within two hours with EDTA. This can only be achieved by injecting Mn systemically as oral intake by the drinking water needed days to achieve almost similar levels.

MATERIALS AND METHODS

One-day-old broiler chicks of both sexes, obtained from a local hatchery, were housed at a temperature of 32°C to 35°C, constant lighting with wood shavings as a floor litter. Water and feed were available ad libitum. The Scientific Committee of the College of Veterinary Medicine at the University of Mosul has reviewed and approved the protocol of the study in chicks. All the experiments complied with institutional regulations addressing animal use, and proper attention and care were given to the chicks used in this study.

Effect of Mn on open-field activity and tonic immobility response

Day old chicks (12/group) were provided with either deionized water (control group) or with Mn ($MnCl_2.4H_2O$, Avishkar, India) at 1 g/L of deionized water [4] as the drinking water for 14 days. On treatment days 7 and 14, the control or Mn-treated chicks were individually monitored for 3-min open-field activity (latency to move from the center of the open-field arena, number of lines crossed by both feet, number of escape jumps, frequency of defecations, pecking and distress calls) as described before [12,13]. After the open-field activity test, each chick was subjected to tonic immobility test [13,14].

Determination of Mn in the plasma and tissues

On treatment days 7 and 14, blood samples (1-2 ml) were collected from chicks of the control and Mn-treated groups (6/group) by jugular vein bleeding into heparinized test tubes [15]. Thereafter, the chicks were euthanized by cervical dislocation to obtain the whole brain, liver and kidneys. Plasma was separated from erythrocytes by centrifugation of blood samples at 3000 rpm (Chalice Medical Ltd., U.K.) for 15 minutes. Plasma and tissue samples were stored at –18ºC pending Mn determination within 48 hours. The plasma and tissue samples were digested in 65% nitric acid with 24-hour incubation at 70ºC [16]. The concentration of Mn was determined using atomic absorption spectrometry (Novaa 350, Germany) with UV-visible lamp and air-acetylene burner.

Effect of disodium EDTA on plasma and tissue Mn overload

Eighteen, 7 or 8 day-old chicks not treated with Mn previously, were randomly divided into three groups of six birds each. The three groups were treated as follows: Group 1 (control), saline solution at 5 ml/kg, i.m. followed 30 min later with saline solution at 5 ml/kg, i.m. Group 2, Mn at 20 mg/kg, i.m. followed 30 min later with saline solution at 5 ml/kg, i.m. Group 3, Mn at 20 mg/kg, i.m. followed 30 min later with disodium EDTA at 50 mg/kg, i.m. The injectable solutions of Mn and EDTA were prepared by separately dissolving either Mn chloride or disodium EDTA (Fluka, Germany) in deionized distilled water. The dose rate of Mn at 20 mg/5 ml/kg, i.m. was based on our previous study in young chicks [11], whereas that of EDTA (50 mg/5ml/kg, i.m.) was based on a preliminary experiment in chicks. Plasma and tissue samples were collected two hours after the injection of Mn for the determination of Mn concentration as described earlier.

Continuous data were statistically analyzed by one way analysis of variance followed by the least significant difference test [17]. Non-parametric data (ranked or discontinuous) of the open-field test were subjected to Mann-Whitney-U-test [17]. The level of significance

was at $p < 0.05$.

RESULTS

Effect of Mn on open-field activity and tonic immobility response

The 3-minute open-field activity patterns and tonic immobility performance of chicks treated with Mn at 1 g/L in the drinking water are shown in table 1. Generally, Mn did not produce overt signs of toxicosis. However, it increased the general locomotor activity of the chicks in the open-field arena as seen by a significant elevation in the numbers of lines crossed on day 14 when compared to the control group (Table 1). The metal also decreased the duration of tonic immobility response of the chicks on day 14 in comparison with the control group (Table 1).

Determination of Mn in the plasma and tissues

The administration of Mn in the drinking water at 1 g/L significantly increased the Mn levels in the plasma, whole brain, and kidney of the chicks on day 7 and in all the tissues on day 14 in comparison with respective control values (Figure 1). Higher levels of Mn were attained in the brain and liver of the chicks on day 14 compared with those of day 7 (Figure 1).

Effect of disodium EDTA on plasma and tissue Mn overload

Injection of Mn at the dose rate of 20 mg/kg, i.m. significantly increased the metal concentrations in the plasma, whole brain, liver and kidney of the chicks when compared with those of the control group (Table 2). The highest concentration of Mn appeared in the kidney followed by the liver, whole brain and the plasma (Table 2).

Table 1. Effects of manganese (1 g Mn/L drinking water) on 3-minute open-field activity and tonic immobility test in 7- and 14-day old chicks

Variable	Age (days)			
	7		14	
	Control	Mn	Control	Mn
Latency to move (seconds)	8.3 ± 2.8	15.5 ± 7.8	15.5 ± 9.3	4.8 ± 1.9
Lines crossed	18.7 ± 3.9	27.7 ± 5.9	7.0 ± 2.8	17.8 ± 2.4*
Escape jumps	2.5 ± 1.1	0.5 ± 0.4	0.8 ± 0.8	0.8 ± 0.8
Distress calls (scores)	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Pecking (scores)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Defecations	0.5 ± 0.2	1.3 ± 0.4	0.7 ± 0.2	0.7 ± 0.2
Tonic immobility (seconds)	25.5 ± 10.7	6.5 ± 2.8	118.0 ± 22.2	10.5 ± 3.8*

Values are mean ± SE of 6 chicks/group. * Significantly different from the respective control value, $p < 0.05$.

Table 2. Effect of disodium EDTA (50 mg/kg, i.m.) on manganese (Mn) concentrations in the plasma, whole brain, liver and kidney of chicks injected with Mn at 20 mg/kg, i.m.

Treatment	Plasma (µg/ml)	Whole brain (µg/g)	Liver (µg/g)	Kidney (µg/g)
Saline (control)	0.35 ± 0.01	0.39 ± 0.02	1.1 ± 0.09	0.97 ± 0.06
Mn + saline	0.59 ± 0.01*	0.60 ± 0.02*	14.4 ± 1.0*	43.8 ± 4.2*
Mn + EDTA	0.37 ± 0.05[a]	0.45 ± 0.04[a]	13.3 ± 2.6*	32.3 ± 7.2*

Values are mean ± SE of 6 chicks/group.

*Significantly different from the respective control value, $p < 0.05$.

[a]Significantly different from the second group (Mn + saline), $p < 0.05$.

EDTA was injected 30 minutes after the Mn injection. Blood and tissues were collected two hours after the Mn injection.

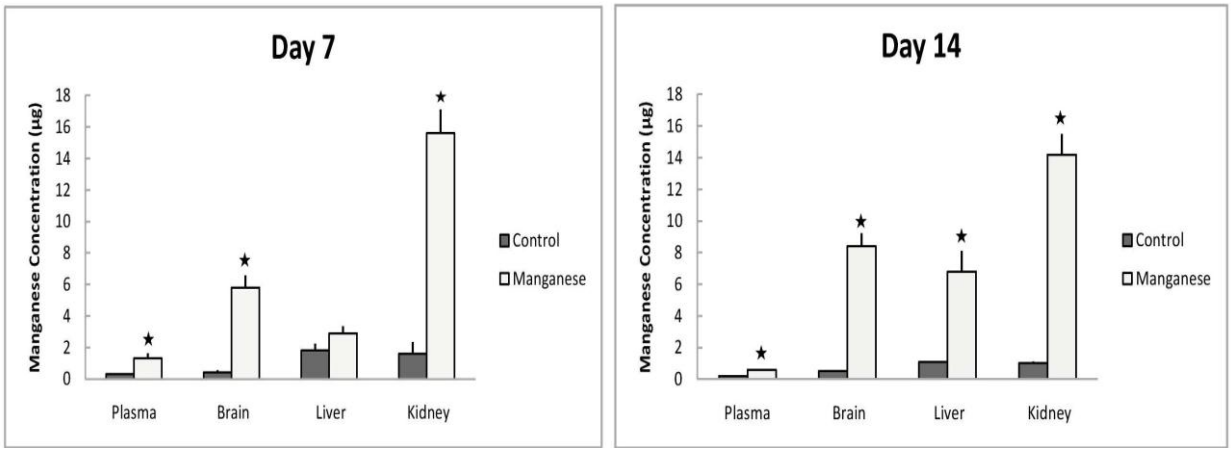

Figure 1. Plasma (µg/ml) and tissue (µg/g) manganese contents after exposure via the drinking water to the metal for 7 and 14 days in chicks. Values are mean ± SE of 6 chicks/group. * Significantly different from the respective control value, $p < 0.05$. Manganese treatment started at the age of one day.

Injection of disodium EDTA at 50 mg/kg, i.m. significantly reduced Mn concentrations in the plasma and whole brain when compared with those of the second group treated with saline solution (Table 2). The EDTA-decreases in Mn concentrations in the liver and kidney, however, did not attain the statistical significance difference (Table 2).

DISCUSSION

Limited information is available on the neurotoxicological profile of Mn in the chicken. The behavioral patterns of acute single dose injections of Mn in young chicks were found to be reduced general locomotor activity and central nervous system depression [5,6]. In the present study, we attempted a different method of Mn overload which was waterborne exposure via the drinking water and then examining the behavioral performance at ages of 7 and 14. Interestingly, this method of oral exposure resulted in changes in the open-field activity (lines crossed) that could be characterized as hyperactivity. The decrease in the duration of tonic immobility in Mn-exposed chicks further support the notion of stimulant action of the metal on the brain of the chicks [6,13,14]. Waterborne Mn overload was reported to induce locomotor hyperactivity in rats [4].The results of the present study when compared to our earlier findings [5,6] suggest that the route of administration and acute vs. subacute forms of exposure to Mn might produce different neurotoxic behavioral changes [4,8,10,18].

As found in the present study, Mn administration was reported to increase blood and tissue levels of the metal in various animal species [4,8–10] including the chicks [6,11]. Increased Mn burden in the tissues correlates with toxic effects seen in various organ systems [1,4,6,7,10,18]. The variations in Mn concentrations in the tissues could be attributed to the distribution, metabolic and excretory patterns of Mn [8,19]. Routes of exposure play also an important role in tissue Mn burden [4,8,10,18,19]. It is therefore pertinent in this context to suggest that waterborne exposure to Mn for 14 days could result in an accumulative form of Mn body burden when compared to the acute single dose injection of the metal. The plasma elimination half-life of Mn in chicks after an i.m. injection at 20 mg/kg was 3.02 h with high steady state volume of distribution 24.34 L/kg and 3.12 h elimination half-life from the brain [11]. This dosage of Mn was reported to alter the general activity of the chicks in the open-field behavioral test accompanied with depressant actions [6]. The significant appearance of Mn in the whole brain of the chicks further support the potential use of chick as an animal model of Mn neurotoxicity [6,11].

Disodium EDTA is a metal chelator used to reduce high body burden of metals [20,21]. In the present study, EDTA significantly reduced Mn concentrations in the plasma and whole brain (Table 1) within 1.5 hour after the administration. This is in accordance with the pharmacological effect of EDTA which differentially binds excess concentrations of metals [20,21]. Further, the reduction of brain Mn by the EDTA injection supports the potential experimental uses of this chelating agent to modulate neurotoxicity of Mn in chicks and possibly other species. EDTA also reduced Mn burden in the liver and kidney, though non-significantly. This lack of significant reduction of liver and kidney levels of Mn by EDTA could be attributed to the fact that chelators are not equally effective in reducing tissue metal burden or preventing the damages inflicted [20–22]. Furthermore, we used only a single injection of EDTA, and probably higher doses or

repeated treatments might have been more effective in reducing Mn burden in the liver and kidney. Monitoring urinary excretion of Mn might have been useful too, a measure which was not done in the present study. Generally, blood or tissue Mn burden is considered as a biomarker of the metal exposure and potential toxicity [23–25] which was clearly demonstrated in the present study. Further studies are also needed to examine the functional aspects of the liver and kidney following Mn overload with concurrent EDTA therapy.

The present data suggest that Mn is well absorbed and distributed into the tissues after exposure of young chicks via the drinking water or i.m. Mn changed the behavioral performance of the chicks and EDTA effectively reduced acute Mn overload in the body. These results further support and expand previous studies concerning the experimental use of young chicks as a potential model of acute Mn neurotoxicity.

ACKNOWLEDGMENTS

This report represents a portion of a dissertation to be submitted by the first author to the University of Mosul, Iraq as partial fulfillment of the requirements of PhD degree in Veterinary Toxicology. This study was supported by the College of Veterinary Medicine, University of Mosul, Iraq.

REFERENCES

1. Normandin L, Panisset M, Zayed J. Manganese neurotoxicity: behavioral, pathological, and biochemical effects following various routes of exposure. Rev Environ Health 2002;17:189–217.
2. Bowler RM, Gocheva V, Harris M, et al. Prospective study on neurotoxic effects in manganese-exposed bridge construction welders. Neurotoxicology 2011;32:596–605.
3. Newland MC. Animal models of manganese's neurotoxicity. Neurotoxicology 1999;20:415–432.
4. Fordahl S, Cooney P, Qiu Y, et al. Waterborne manganese exposure alters plasma, brain, and liver metabolites accompanied by changes in stereotypic behaviors. Neurotoxicol Teratol 2012;34: 27–36.
5. Mohammad FK, Faris GA-M. Behavioral effects of acute manganese chloride administration in chickens. Biol. Trace Elem Res 2006;110:265–274.
6. Al-Zubaidy MHI, Mohammad FK. Potential chick model of acute manganese neurotoxicity. Submitted to Arch. Indus. Hyg. Toxicol.
7. Normandin L, Beaupre LA, Salehi F, et al. Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. Neurotoxicology 2004;25:433–441.
8. Zheng W, Kim H, Zhao Q. Comparative toxicokinetics of manganese chloride and methylcyclopentadienyl manganese tricarbonyl (MMT) in Sprague-Dawley rats. Toxicol Sci 2000;54:295–301.
9. Erikson KM, John CE, Jones SR et al. Manganese accumulation in striatum of mice exposed to toxic doses is dependent upon a functional dopamine transporter. Environ Toxicol Pharmacol 2005; 20:390–394.
10. Andersen ME, Dorman DC, Clewell HJ, et al. Multidose-route, multi-species pharmacokinetic models for manganese and their use in risk assessment. J Toxicol Environ Health A 2010;73:217–234.
11. Al-Zubaidy M, Mohammad FK. Single intramuscular dose toxicokinetics of manganese in broiler chicks. Vet World 2012;5:560-564.
12. Al-Baggou' BKh, Al-Dewachi OS, Said MO, et al. Behavioral performance, serum glucose level and differential leukocyte count in local domestic chicks treated with xylazine. Iraqi J Vet Sci 1999;12:223–232.
13. Mohammad FK, Mousa YJ, Hasan MM. Acute toxicity and neurobehavioral effects of diphenhydramine in chicks. J Poult Sci 2012;49:51–56.
14. Hennig CW, Fazio JK, Hughes CA, et al. Duration of tonic immobility in chickens as a function of alpha adrenergic receptor stimulation and blockade. Pharmacol Biochem Behav 1984;20:731–738.
15. Stevens RWC, Ridgway GJ. A technique for bleeding chickens from the jugular vein. Poult Sci 1966;45:204–205.
16. Lamphere DN, Dorn CR, Reddy CS, et al. Reduced cadmium body burden in cadmium-exposed calves fed supplemental zinc. Environ Res 1984;33:119–129.
17. Petrie A, Watson P. Statistics for veterinary and animal science. 2nd ed. Oxford: Blackwell Publishing Ltd, 2006.
18. Zheng W, Fu SX, Dydak U, et al. Biomarkers of manganese intoxication. Neurotoxicology 2011;32:1–8.
19. Klaassen CD. Biliary excretion of manganese in rats, rabbits, and dogs. Toxicol Appl Pharmacol 1974;29:458–468.
20. Schonwald S. Manganese. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams and Wilkins, 2004;1433–1434.
21. Crossgrove J, Zheng W. Manganese toxicity upon overexposure . NMR Biomed 2004; 17:544–553.
22. Jiang Y, Mo X, Du F. Effective treatment of manganese-induced occupational parkinsonism with p-amino salicylic acid: A case of 17-year follow-up study. J Occup

Environ Med 2006;48:644–649.

23. Salehi F, Krewski D, Mergler D, et al. Bioaccumulation and locomotor effects of manganese phosphate/sulfate mixture in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. Toxicol Appl Pharmacol 2003;191:264–271.
24. Hoet P, Vanmarcke E, Geens T, et al. Manganese in plasma: a promising biomarker of exposure to Mn in welders. A pilot study. Toxicol Lett 2011 (in press).
25. Zheng W, Fu SX, Dydak U. et al. Biomarkers of manganese intoxication. Neurotoxicology 2011;32:1–8.