

Manganese accumulation in hair and teeth as a biomarker of manganese exposure and neurotoxicity in rats

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Received: 13 September 2015 / Accepted: 3 March 2016 / Published online: 15 March 2016
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Abstract Manganese (Mn) is an essential trace element to humans. However, excessive Mn causes cognitive impairment resulting from injury to the central nervous system within the hippocampus. No ideal biomarker is currently available for evaluating Mn exposure and associated neurotoxicity in the body. Hence, this study used Mn levels in the serum (MnS), teeth (MnT), and hair (MnH) as biomarkers for evaluating the association between Mn exposure and cognitive impairment in Mn-treated rats. A total of 32 male Sprague–Dawley rats were randomly divided into four groups, received 0, 5, 10, and 20 mg/(kg day) of MnCl₂·4H₂O for 5 days a week for 18 weeks, respectively. Lifetime Mn cumulative dose (LMCD) was used to evaluate external Mn exposure. Hippocampus, serum, teeth, and hair specimens were collected from the rats for Mn determination by graphite furnace

atomic absorption spectrometry. Learning and memory functions were assessed using the Morris water maze test. Results showed that chronic Mn exposure increased the hippocampus (MnHip), MnS, MnT, and MnH levels, as well as impaired learning and memory function in rats. MnHip, MnT, and MnH levels were positively correlated with LMCD ($r=0.759$, $r=0.925$, and $r=0.908$, respectively; $p<0.05$), escape latency ($r=0.862$, $r=0.716$, and $r=0.814$, respectively; $p<0.05$), and the number of platform crossings ($r=-0.734$, $r=-0.514$, and $r=-0.566$, respectively; $p<0.05$). No association was observed between MnS levels and the number of platform crossings ($r=-0.286$, $p>0.05$). Thus, MnT and MnH detected long-term low-dose Mn exposure. These parameters can be reliable biomarkers for Mn exposure and associated neurotoxicity in Mn-treated rats.

Responsible editor: Philippe Garrigues

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Keywords Rats · Manganese neurotoxicity · Biomarker · Hippocampus · Hair · Teeth · Serum

Introduction

Manganese (Mn) is an essential element to humans. High Mn dosage can lead to excessive Mn accumulation in the body, resulting in damaged central nervous system (CNS) (Aschner et al. 2007). Previous studies have demonstrated Mn accumulation in the CNS, especially in the hippocampus (Blecharz-Klin et al. 2012), and its correlation with low cognitive performance and working memory in animals and humans (Blecharz-Klin et al. 2012; Riojas-Rodríguez et al. 2010; Schneider et al. 2009; Su et al. 2015; Zou et al. 2014). However, no ideal biomarker of Mn exposure and neurotoxicity has been established despite the confirmed cognitive impairment caused by excessive Mn exposure. Hence, identifying a reliable and ideal biomarker of Mn exposure and

neurotoxicity is crucial for assessing Mn-exposed populations via biological marker inspection.

Biomarkers of Mn should generally be able to characterize and distinguish Mn-exposed groups from non-Mn-exposed groups, as well as predict neurological impairment as a result of short- or long-term Mn exposure (Viana et al. 2014; Zheng et al. 2011). Urine and blood Mn levels were previously frequently detected and suggested for evaluating internal Mn exposure (Apostoli et al. 2000; Bader et al. 1999; Jarvisalo et al. 1992; Laohaudomchok et al. 2011). However, urine Mn is generally not associated with exposure to Mn by inhalation (Laohaudomchok et al. 2011; Smith et al. 2007). Blood Mn levels promptly decline probably because of their redistribution from the blood or rapid clearance. Most importantly, blood Mn is rarely associated with indices of neurologic impairment on neuropsychological tests in the body (Apostoli et al. 2000; Bader et al. 1999; Laohaudomchok et al. 2011; Smith et al. 2007; Sriram et al. 2012). Thus, blood and urine Mn levels are generally not considered as suitable biomarkers to assess Mn exposure, although these parameters can predict the possibility of elevated Mn exposure (Smith et al. 2007; Zheng et al. 2011).

Numerous studies have recently shown that heavy metals accumulate in the teeth and hair; the levels of heavy metals in these areas were proposed as exposure biomarkers for long-term cumulative exposure assessment (Arora et al. 2012; Barton 2011; Eastman et al. 2013; Gunier et al. 2013). Lead accumulation in the teeth and hair was detected and used as a biomarker to evaluate lead exposure and neurotoxicity to the body (Barton 2011). Similarly, Mn is also a heavy metal that presents neurotoxicity, causing neurotoxic damage in the body. Detected Mn levels in the teeth (MnT) and hair Mn (MnH) are usually used for determining occupational or environmental Mn exposure, and just few studies used MnT as a biomarker for estimating their associations with neurobehavioral effects in human (Arora et al. 2012; Eastman et al. 2013; Gunier et al. 2013, 2015; Menezes-Filho et al. 2009; Mora et al. 2015). However, few studies discuss and compare the advantage of different Mn biomarkers and Mn-associated neurotoxicity in a paper. There are no animal experiments to verify whether MnT or MnH could serve as reliable surrogates to assess Mn-associated neurotoxicity. Moreover, the individual-level relationship between external Mn exposure and MnT or MnH has not been reported. In addition, detailed investigations on the Mn level in the serum (MnS), MnT, and MnH as biomarkers of Mn exposure, as well as their associated neurotoxicity, have not been performed in animals.

This study aimed to investigate the relationships of the Mn level in the hippocampus (MnHip), MnT, MnH, MnS, and lifetime Mn cumulative dose (LMCD) with cognitive impairment in the individual level in rats. The rats in our animal experiment were chronically exposed to Mn, and we dynamically observed the effects of Mn on the rats with the changes of body weight. Moreover, we investigated whether MnT and

MnH can serve as reliable surrogates to assess Mn exposure. We specifically examined the feasibility of these two parameters as potential biomarkers for Mn exposure and associated neurotoxicity.

Materials and methods

Animals and treatment

Thirty-two specific pathogen-free male Sprague–Dawley rats were obtained from the Laboratory Animal Center, Guangxi Medical University (Guangxi, China; animal code SCXK 2009–0002). At the onset of the study, the rats were 3 to 4 weeks old, weighing 80 ± 10 g (mean \pm standard deviation). Upon arrival, the rats were housed in a room maintained at 22 ± 2 °C and 50–70 % relative humidity on a 12/12-h light/dark cycle. The rats were acclimated for 1 week prior to experimentation. The rats were provided with standard chow and sterilized drinking water for 24 h/day in SPF animal houses. The Guangxi Medical University Animal Care and Use Committee approved all animal procedures.

The rats were randomly distributed into the following four groups ($n=8$ per group): control (saline-treated), low-dose (5 mg Mn/kg body weight), medium-dose (10 mg Mn/kg body weight), and high-dose (20 mg Mn/kg body weight) groups. We weigh the rats daily before Mn treatment. The rats were intraperitoneally injected with Mn or saline at 1 mL/kg once daily for 5 days per week for 18 weeks. Dosage selection was in accordance with previous studies. The dose regimen for Mn exposure was chosen because it was associated with significantly altered biochemical parameters and increased Mn concentration in the brain tissues in rats. The rats were killed after 18 weeks of Mn treatment. The blood, hair, teeth, and brains of the rats were removed for Mn content analyses. All experiments were performed in accordance with the Regulations for Studies with Experimental Animals.

MWM test

The Morris water maze (MWM) test is widely used in rats as a test of cognitive function (Li et al. 2014). Hence, the MWM test was designed to assess the spatial learning and memory of rats. The rats were subjected to four MWM trials per day for 5 days on the 6th, 12th, and 18th weeks. Escape latencies and swim speed in the water maze were automatically recorded using an HVS image analyzing system (Huabei Zhenghua Biological Equipment Co. Ltd., Anhui, China) and a videotape. Similarly, during the probe trial, the number of visits to the platform and swimming speed were automatically recorded.

The calculation of lifetime Mn cumulative dose

LMCD, the total cumulative dose combined together by the first injected dose to the last dose, was to evaluate external manganese exposure.

Analysis of hippocampal, serum, teeth, and hair Mn levels

The rats were weighed and anesthetized by intraperitoneally injecting 10 % chloral hydrate (2.0 mL/kg). Blood was collected from the abdominal aorta. The whole blood was allowed to stand to obtain the serum. The obtained samples were stored at $-80\text{ }^{\circ}\text{C}$. The rats were sacrificed through decollation. The hippocampus was removed immediately and frozen at $-80\text{ }^{\circ}\text{C}$. All the teeth were removed, dried, and pulverized, then mixing up the teeth powder. The rat’s back hair was shaved by surgical scissors, then cutting and mixing up the hair. Serum samples (0.5 mL) were digested with 3 mL of oxidizing acid mixture containing 4:1 (v/v) suprapure nitric acid (65 %): perchloric acid via microwave digestion technique. Afterward, 1 % nitric acid was added until the final volume of the serum samples reached 10 mL. Similarly, hair (approximately 0.2 g, dry weight), teeth (approximately 0.1 g, dry weight), or hippocampus (approximately 50 mg, wet weight) were digested with 3 mL of oxidizing acid mixture containing 4:1 (v/v) suprapure nitric acid (65 %): perchloric acid via microwave digestion technique. Afterward, 1 % nitric acid was added until the final volume of each tissue sample reached 10 mL. The samples were centrifuged and then subjected to graphite furnace atomic absorption spectrometry (GFAAS) (AA-7000, Shimadzu Japan). The limit of detection was 0.1 mg/L. At the same time, reagent blanks were analyzed in every batch. All samples were detected in duplicates and a difference of 7 % or less was considered acceptable. Routine checks of precision and accuracy were proficient using Mn standard reference material from the National Research Center for Certified Reference Material of China. The samples were then numbered by our laboratory assistants, and the experiment operator was not informed of the groupings.

Statistical analysis

Data were analyzed using SPSS version 16.0 for Windows (SPSS Inc.). Group means were compared via one-way ANOVA followed by LSD post hoc tests. Pearson correlation was determined between MnHip and the number of platform crossings. Beyond that, Spearman’s rank correlation was obtained to determine the linear relationship for the others. The significance level of the data was set to $p < 0.05$.

Results

Lifetime Mn cumulative dose in rats

The LMCD, which is the total cumulative dose from the first to the last injected dose, was determined to evaluate Mn exposure, and the results are summarized in Table 1.

Effects of Mn on body weights of rats

The effects of Mn on the body weights of rats are summarized in Fig. 1. The body weights of the rats in the four groups did not significantly differ in the first 2 weeks of the experiment. During Mn treatment, the body weight gain of the rats in the Mn-treated groups decreased in a dose-dependent manner. From the fourth week, the high- and medium-dose groups yielded significantly lower body weights than the control and low-dose groups ($p < 0.05$).

Effects of Mn on learning and memory

The results of the MWM test, which included escape latency and number of platform crossings, are published in Food and Chemical Toxicology (Liang et al. 2015). During the trials, the escape latency increased as the Mn dose increased. By contrast, the number of platform crossings decreased as the Mn dose increased (Fig. 2). Additionally, Mn treatment did not significantly affect swimming speed (data not shown).

Mn levels in hippocampus, serum, teeth, and hair

The levels of MnHip, MnS, MnT, and MnH in the Mn-treated rats (Fig. 3) increased significantly in a dose-dependent manner ($r_{\text{hippocampus}} = 0.731$, $p < 0.01$; $r_{\text{serum}} = 0.893$, $p < 0.01$; $r_{\text{teeth}} = 0.927$, $p < 0.01$; $r_{\text{hair}} = 0.918$, $p < 0.01$, respectively). The levels of MnHip, MnS, MnT, and MnH were higher in the Mn-treated groups than those in the control group (MnH, $F = 13.82$, $p < 0.01$; MnS, $F = 60.59$, $p < 0.01$; MnT,

Table 1 Lifetime Mn cumulative dose in every group of rats

| Group | LMCD (mg) |
|----------|-------------------|
| 0 mg/kg | 0.00 ± 0.00 |
| 5 mg/kg | 176.42 ± 9.57* |
| 10 mg/kg | 361.77 ± 71.96*** |
| 20 mg/kg | 618.49 ± 36.33*** |

Data are mean ± SD, $n = 8$ for each group
 * $p < 0.05$, comparison with control group;
 ** $p < 0.05$, comparison with low-dose (5 mg/kg) group; *** $p < 0.05$, comparison with medium-dose (10 mg/kg) group

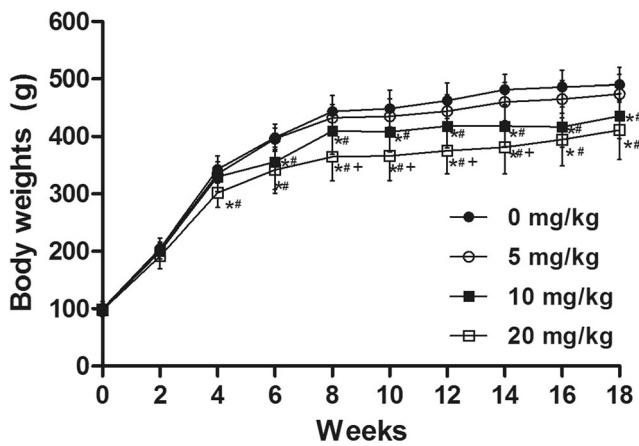


Fig. 1 Effect of Mn on the body weight of rats. Data are mean \pm SD; $n=8$ for each group. * $p<0.05$, comparison with control group; # $p<0.05$, comparison with low dose (5 mg/kg) group; + $p<0.05$, comparison with medium dose (10 mg/kg) group

$F=63.47$, $p<0.01$; MnH, $F=37.93$, $p<0.01$). Exposure to different doses of Mn (5, 10, and 20 mg/kg body weight) increased the levels of MnHip (2.05-, 2.6-, and 3.14-fold vs. control, respectively; $p<0.05$; Fig. 3a) and MnS levels (4.58-, 5.29-, and 7.7- vs. control, respectively; $p<0.05$; Fig. 3b). Similarly, MnH levels were moderately increased following Mn treatment (1.53-, 2.54-, and 3.34-fold vs. control, respectively; $p<0.05$; Fig. 3d). Compare to the increased fold of other tissues Mn levels, remarkably higher elevations

were observed in MnT levels (7.32-, 13.29-, and 25.78-fold vs. control, respectively; $p<0.05$; Fig. 3c).

Correlation analysis

The results of correlation analysis are shown in Table 2. The results of the MWM test showed a negative correlation between escape latency and number of platform crossings ($r=-0.635$, $p<0.05$). LMCD was positively correlated with escape latency ($r=0.657$, $p<0.05$) but negatively correlated with the number of platform crossings ($r=-0.464$, $p<0.05$). MnHip levels were positively correlated with LMCD and escape latency ($r=0.759$, $r=0.862$; $p<0.05$) but negatively correlated with the number of platform crossings ($r=-0.734$, $p<0.05$). MnS levels were positively associated with LMCD, escape latency, MnHip, and MnT ($r=0.849$, $r=0.510$, $r=0.630$, and $r=0.814$, respectively; $p<0.05$). However, no correlation was found between MnS levels and the number of platform crossings ($r=-0.286$, $p>0.05$). MnT levels were positively correlated with LMCD, escape latency, and MnHip levels ($r=0.925$, $r=0.716$, and $r=0.760$, respectively; $p<0.05$) but negatively correlated with the number of platform crossings ($r=-0.514$, $p<0.05$). MnH levels were positively correlated with LMCD, escape latency, MnHip, MnS, and MnT ($r=0.908$, $r=0.814$, $r=0.839$, $r=0.746$, and $r=0.925$, respectively; $p<0.05$) but negatively

Fig. 2 Effects of Mn on the learning and memory in rats. **a** Escape latencies in week 6. **b** Escape latencies in week 12. **c** Escape latencies in week 18. **d** Number of platform crossings on the probe trial. Data are mean \pm SEM; $n=8$ for each group. * $p<0.05$, comparison with control group; # $p<0.05$, comparison with low dose (5 mg/kg) group

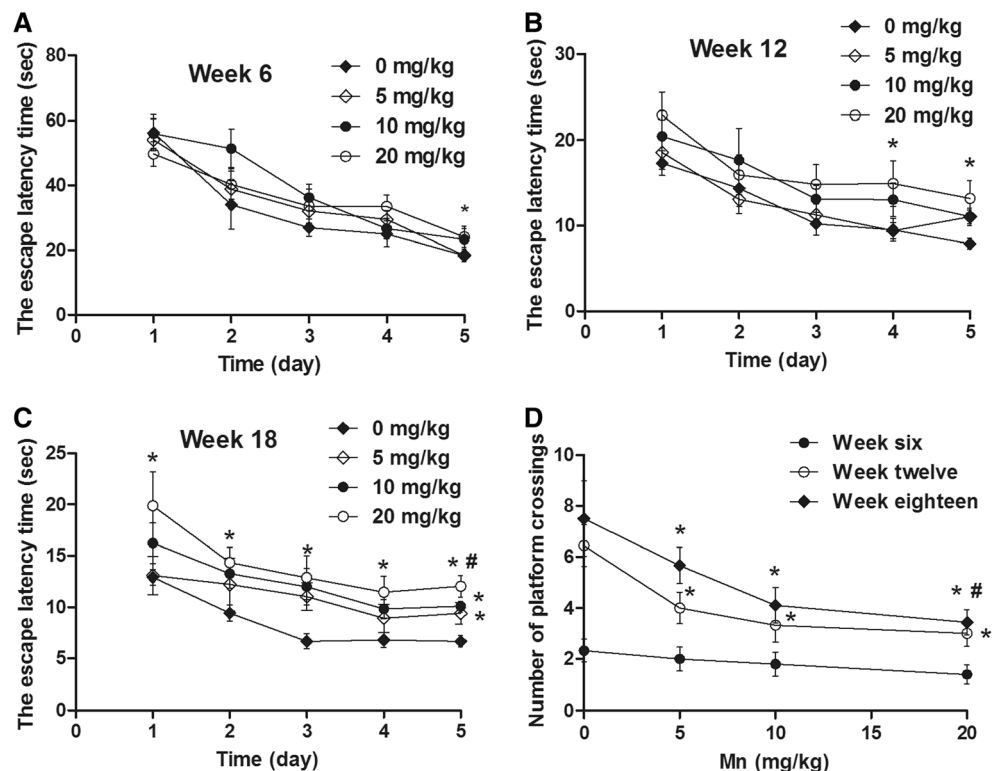
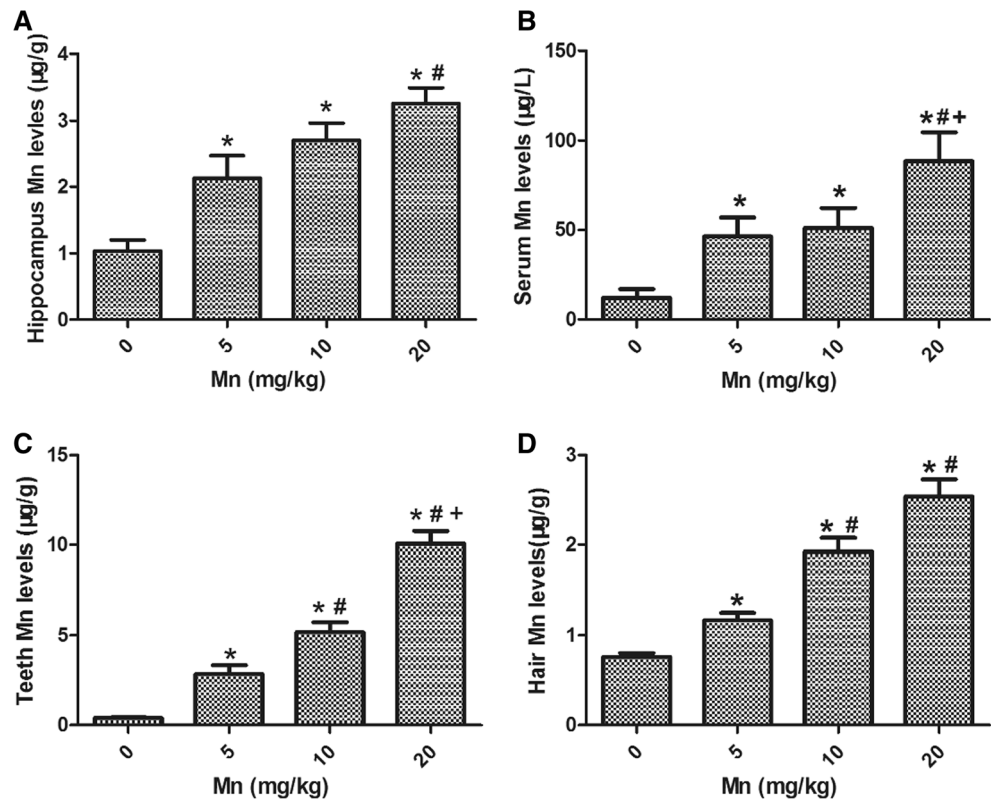


Fig. 3 Effects of Mn exposure on Mn accumulation in hippocampus (a), serum (b), teeth (c), and hair (d) of rats. Graphical representations are mean \pm SD ($n = 8/\text{group}$). * $p < 0.05$, comparison with control group; # $p < 0.05$, comparison with low dose (5 mg/kg) group; + $p < 0.05$, comparison with medium dose (10 mg/kg) group



correlated with the number of platform crossings ($r = -0.566$, $p < 0.05$).

Discussion

Three biomarkers, namely, MnS, MnT, and MnH, were fully measured for the first time to assess the association between Mn exposure and cognitive impairment in the individual level in Mn-treated rats. The results indicated that biological monitoring of MnT and MnH facilitated the assessment of Mn exposure and associated detrimental effects on cognitive function. These parameters may be potential biomarkers for Mn exposure and associated neurotoxicity in Mn-treated rats.

In this study, LMCD was used to evaluate external Mn exposure in rats. Mn treatment increased LMCD and MnHip in dose- and time-dependent manners. MnHip levels were positively correlated with LMCD. These results indicated that internal Mn dose, MnHip, could reflect external Mn exposure. Additionally, the hippocampus may be one of the primary regions where Mn accumulates. Former studies have shown that high MnHip levels may mediate Mn neurotoxicity, resulting in cognitive deficits in rats (Blecharz-Klin et al. 2012; Fitsanakis et al. 2009). Our results showed that MnHip levels and LMCD were significant negatively correlated with the number of platform crossings but positively correlated with escape latency. Mn may impair learning and memory in Mn-treated rats via accumulation in the hippocampus.

Therefore, MnHip levels may be a biomarker of Mn exposure and associated neurotoxicity in rats. No reference values have been established for the accurate detection of MnHip in humans because of ethical concerns. Hence, potential biomarkers that reflect the MnHip levels and Mn neurotoxicity in the body should be explored.

Three different biomarkers, namely, MnS, MnT, and MnH, used in this work are expected to reflect long-term Mn exposure, MnHip, and Mn neurotoxicity in rats. MnS increased along with Mn dosage. However, no significant correlation was found between MnS and the number of platform crossings, which indicated that cognitive impairment could not be tracked by MnS. Rodríguez-Agudelo observed that air Mn concentrations are not associated with Mn blood levels (Rodríguez-Agudelo et al. 2006). Moreover, serum manganese may be just served as a biomarker for assessment of recent exposure to airborne manganese (Lu et al. 2005). Indeed, previous studies have confirmed that MnS levels promptly decline probably because of the redistribution of Mn from the blood or rapid clearance (Sriram et al. 2012). Hence, MnS is not a promising or appropriate biomarker for evaluating chronic Mn exposure and neurotoxicity in the body.

Accumulation models and slow metabolism of Mn in the teeth can potentially reflect long-term retention of Mn (Hillson 1996) and perhaps emulate metal burden in the body more efficiently than biological fluids. In the present study, our findings showed that MnT levels increased with the Mn dosage and they were positively associated with LMCD and

Table 2 Pairwise correlation between Mn levels in various organs and index

| Index | Index | <i>r</i> | <i>p</i> value |
|--------------|------------------------------|----------|----------------|
| LMCD | Escape latency | 0.657 | <0.001* |
| | Number of platform crossings | −0.464 | 0.007* |
| MnHip levels | LMCD | 0.759 | <0.001* |
| | Escape latency | 0.862 | <0.001* |
| | Number of platform crossings | −0.734 | <0.001* |
| MnS levels | LMCD | 0.849 | <0.001* |
| | Escape latency | 0.510 | 0.003* |
| | Number of platform crossings | −0.286 | 0.112 |
| | MnHip levels | 0.630 | <0.001* |
| | MnT levels | 0.814 | <0.001* |
| MnT levels | LMCD | 0.925 | <0.001* |
| | Escape latency | 0.716 | <0.001* |
| | Number of platform crossings | −0.514 | 0.003* |
| | MnHip levels | 0.760 | <0.001* |
| MnH levels | LMCD | 0.908 | <0.001* |
| | Escape latency | 0.814 | <0.001* |
| | Number of platform crossings | −0.566 | 0.001* |
| | MnHip levels | 0.839 | <0.001* |
| | MnS levels | 0.746 | <0.001* |
| | MnT levels | 0.925 | <0.001* |

The relationships between tissue Mn levels, LMCD, and the parameters of learning and memory in MWM test. Spearman's rank correlation was determined when LMCD or the number of platform crossings was considered because these data were not normally distributed. Pearson correlation was obtained to determine the linear relationship when LMCD or the number of platform crossings was not involved

**p* < 0.05, significant

MnHip. MnT levels could reflect the external and internal Mn exposure doses or MnHip levels. Therefore, MnT levels could be a Mn exposure biomarker. Few studies have suggested that MnT levels may be a potential biomarker for Mn exposure in humans (Arora et al. 2012; Gunier et al. 2013) and used MnT to evaluate neurodevelopment in children (Mora et al. 2015, Gunier et al. 2015). However, there are no animal experiments to verify whether MnT could serve as reliable surrogates to assess Mn-associated neurotoxicity. Our results showed that MnT levels were positively correlated with escape latency but negatively correlated with the number of platform crossings. The confounding factors in the animal experiment were easily controlled. Therefore, we obtained clear evidence to show that MnT levels may be a promising biomarker for Mn exposure and neurotoxicity in Mn-treated rats. Meanwhile, teeth are hard to obtain from humans. Hence, a noninvasive method

of monitoring MnT levels is crucial to Mn-exposed populations.

Similar to teeth, the slow growth rate of hair can facilitate the continuous and chronological biomonitoring of Mn exposure. This parameter is advantageous over biological fluids, such as serum, which appear to predominantly reflect recent or short-term exposure. Our results indicated that MnH levels increased with the Mn dose, and they were positively associated with LMCD and MnHip. MnT levels reflected the external and internal Mn dose exposure, as well as MnHip levels. Thus, MnH may be beneficial in monitoring long-term exposure and may better reflect body and/or target organ burden. Several studies have examined hair as a biomarker of Mn exposure, but most of these studies only detected MnH levels and their association with Mn levels in air and drinking water (Haynes et al. 2010; Mora et al. 2014). Furthermore, the observed significant correlation between MnH and MnT levels, escape latency, and the number of platform crossings indicated that MnH could potentially reveal Mn neurotoxicity. This result was confirming and compatible with the previous studies in children, which is that poor neurobehavioral performance is significantly related, inversely, to hair Mn (Wright et al. 2006; Menezes-Filho et al. 2009). Correlation coefficients between the MnH levels and escape latency, number of platform crossings, and MnHip levels were higher than those between MnT and MnS. Additionally, external Mn contamination did not exist in this animal experiment because the rats were maintained at the SPF animal laboratory. Thus, MnH levels could be a sensitive and reliable biomarker for Mn exposure and neurotoxicity in Mn-treated rats. Compared with human studies, the veracity of hair Mn detection is highly sensitive to collection and wash methods because of external contamination. A hair cleaning methodology has been developed to support MnH as an exposure biomarker for Mn (Eastman et al. 2013). In addition, hair is more advantageous over other specimens because it can be obtained easily and noninvasively. Therefore, MnH may be a reliable biomarker for Mn exposure and neurotoxicity in the body.

Overall, we relatively comprehensively evaluated for the first time the advantages and disadvantages of MnS, MnT, and MnH as biomarkers of Mn exposure and neurotoxicity in rats. The MWM test data suggested that chronic Mn exposure caused cognitive deficits in Mn-treated rats. We investigated the relationship between MnH or MnT levels and LMCD, MnHip levels, and Mn-induced cognitive deficits in rats. Therefore, the results were reliable in revealing that MnH and MnT may reflect the external Mn dose exposure and MnHip levels. These parameters could be sensitive and reliable biomarkers for chronic Mn exposure and associated neurotoxicity in Mn-treated rats.

Acknowledgments This work was financially sponsored by the National Natural Science Foundation of China (Grant Nos. 81160339,

21467003, 81472962, and 21167004), China Postdoctoral Science Foundation (Grant No. 2014M562500XB), Guangxi Natural Science Foundation (Grant No. 2011GXNSFA018187), Scientific Research Foundation of the Education Department of Guangxi Province (Grant No. 201012MS049), and the Guangxi Science Fund for Distinguished Young Scholars (Grant No. 2012GXNSFFA060009).

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflicts of interest.

References

Apostoli P, Lucchini R, Fau - Alessio L, Alessio L (2000) Are current biomarkers suitable for the assessment of manganese exposure in individual workers? *Am J Ind Med* 37(3)(0271–3586 (Print)):283–290

Arora M, Bradman A, Austin C, Vedar M, Holland N, Eskenazi B, Smith DR (2012) Determining fetal manganese exposure from mantle dentine of deciduous teeth. *Environ Sci Technol* 46(9):5118–5125

Aschner M, Guilarte TR, Schneider JS, Zheng W (2007) Manganese: recent advances in understanding its transport and neurotoxicity. *Toxicol Appl Pharmacol* 221(2):131–147

Bader M, Dietz Mc Fau - Ihrig A, Ihrig A Fau - Triebig G, Triebig G (1999) Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries. *International archives of occupational and environmental health* 72(8)(0340–0131 (Print)):521–527

Barton HJ (2011) Advantages of the use of deciduous teeth, hair, and blood analysis for lead and cadmium bio-monitoring in children. A study of 6-year-old children from Krakow (Poland). *Biol Trace Elem Res* 143(2):637–658

Blecharz-Klin K, Piechal A, Joniec-Maciejak I, Pyrzanowska J, Widy-Tyszkiewicz E (2012) Effect of intranasal manganese administration on neurotransmission and spatial learning in rats. *Toxicol Appl Pharmacol* 265(1):1–9

Eastman RR, Jursa TP, Benedetti C, Lucchini RG, Smith DR (2013) Hair as a biomarker of environmental manganese exposure. *Environ Sci Technol* 47(3):1629–1637

Fitsanakis VA, Thompson KN, Deery SE, Milatovic D, Shihabi ZK, Erikson KM, Brown RW, Aschner M (2009) A chronic iron-deficient/high-manganese diet in rodents results in increased brain oxidative stress and behavioral deficits in the morris water maze. *Neurotox Res* 15(2):167–178

Gunier RB, Bradman A, Jerrett M, Smith DR, Harley KG, Austin C, Vedar M, Arora M, Eskenazi B (2013) Determinants of manganese in prenatal dentin of shed teeth from CHAMACOS children living in an agricultural community. *Environ Sci Technol* 47(19):11249–11257

Gunier RB, Arora M, Jerrett M, Bradman A, Harley KG, Mora AM, Koguta K, Hubbard A, Austin C, Hollanda N, Eskenazi B (2015) Manganese in teeth and neurodevelopment in young Mexican–American children. *Environ Res* 142:688–695

Haynes EN, Heckel P, Ryan P, Roda S, Leung Y-K, Sebastian K, Succop P (2010) Environmental manganese exposure in residents living near a ferromanganese refinery in Southeast Ohio: a pilot study. *Neurotoxicology* 31(5):468–474

Hillson S (1996) *Dental anthropology*: Cambridge University Press

Jarvisalo J, Olkinuora M, Fau - Kiilunen M, Kiilunen M Fau - Kivisto H, Kivisto H Fau - Ristola P, Ristola P Fau - Tossavainen A, Tossavainen A Fau - Aitio A, Aitio A (1992) Urinary and blood manganese in occupationally nonexposed populations and in manual metal arc welders of mild steel. *Int Arch Occup Environ Health*. 63(7)(0340–0131 (Print)): 495–501

Laohadomchok W, Lin X, Herrick RF, Fang SC, Cavallari JM, Christiani DC, Weisskopf MG (2011) Toenail, blood, and urine as biomarkers of manganese exposure. *J Occup Environ Med* 53(5):506–510

Li X-B, Zhang X, Ju J, Li Y, Yin L, Pu Y (2014) Alterations in neurobehaviors and inflammation in hippocampus of rats induced by oral administration of microcystin-LR. *Environ Sci Pollut Res* 21(21):12419–12425

Liang G, Qin H, Zhang L, Ma S, Huang X, Lv Y, Qing L, Li Q, Xiong Y, Huang Y et al (2015) Effects of chronic manganese exposure on the learning and memory of rats by observing the changes in the hippocampal cAMP signaling pathway. *Food Chem Toxicol* 83:261–267

Lu L, Zhang L, Li GJ, Guo W, Liang W, Zheng W (2005) Alteration of serum concentrations of manganese, iron, ferritin, and transferrin receptor following exposure to welding fumes among career welders. *Neurotoxicology* 26(2):257–265

Menezes-Filho JA, Paes CR, Pontes AM, Moreira JC, Sarcinelli PN, Mergler D (2009) High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxicology* 30(6):1207–1213

Mora AM, van Wendel de Joode B, Mergler D, Córdoba L, Cano C, Quesada R, Smith DR, Menezes-Filho JA, Lundh T, Lindh CH (2014) Blood and hair Manganese concentrations in pregnant women from the infants’ environmental health study (ISA) in Costa Rica. *Environ Sci Technol* 48(6):3467–3476

Mora AM, Arora M, Harley KG, Kogut K, Parra K, Hernández-Bonilla D, Gunier RB, Bradman A, Smith DR, Eskenazi B (2015) Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. *Environ Int* 84:39–54

Riojas-Rodríguez H, Solís-Vivanco R, Schilman A, Montes S, Rodríguez S, Ríos C, Rodríguez-Agudelo Y (2010) Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. *Environ Health Perspect* 118(10):1465–1470

Rodríguez-Agudelo Y, Riojas-Rodríguez H, Ríos C, Rosas I, Pedraza ES, Miranda J, Siebe C, Texcalac JL, Santos-Burgoa C (2006) Motor alterations associated with exposure to manganese in the environment in Mexico. *Sci Total Environ* 368(2):542–556

Schneider J, Decamp E, Clark K, Bouquio C, Syversen T, Guilarte T (2009) Effects of chronic manganese exposure on working memory in non-human primates. *Brain Res* 1258:86–95

Smith D, Gwiazda R, Bowler R, Roels H, Park R, Taicher C, Lucchini R (2007) Biomarkers of Mn exposure in humans. *Am J Ind Med* 50(11):801–811

Sriram K, Lin GX, Jefferson AM, Roberts JR, Andrews RN, Kashon ML, Antonini JM (2012) Manganese accumulation in nail clippings as a biomarker of welding fume exposure and neurotoxicity. *Toxicology* 291(1–3):73–82

Su C, Chen K, Zou Y, Shen Y, Xia B, Liang G, Lv Y, Wang F, Huang D, Yang X (2015) Chronic exposure to manganese sulfate leads to adverse dose-dependent effects on the neurobehavioral ability of rats. *Environ Toxicol*. doi:10.1002/tox.22161

Viana GF, de Carvalho CF, Nunes LS, Rodrigues JL, Ribeiro NS, de Almeida DA, Ferreira JR, Abreu N, Menezes-Filho JA (2014) Noninvasive biomarkers of manganese exposure and neuropsychological effects in environmentally exposed adults in Brazil. *Toxicol Lett* 231(2):169–178

Wright RO, Amarasingwardena C, Woolf AD, Jim R, Bellinger DC (2006) Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. *Neurotoxicology* 27(2):210–216

Zheng W, Fu SX, Dydak U, Cowan DM (2011) Biomarkers of manganese intoxication. *Neurotoxicology* 32(1):1–8

Zou Y, Qing L, Zeng X, Shen Y, Zhong Y, Liu J, Li Q, Chen K, Lv Y, Huang D et al (2014) Cognitive function and plasma BDNF levels among manganese-exposed smelters. *Occup Environ Med* 71(3): 189–194