

Effect of Occupational Manganese Exposure on Uric Acid Levels in Human Urine*

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Abstract

Objective To investigate the effect of long-term and low-level occupational Mn exposure on the level of uric acid (UA) in human urine.

Methods In this study, 65 volunteers were recruited, who were working on welding and foundry work in an plant in Gansu province, China. Additionally, 29 control samples were collected from individuals who did not have any history of excessive Mn exposure. An improved high performance liquid chromatography system equipped with a diode-array detector (HPLC-DAD) method was developed to determine the UA level in human urine. A Spectra AA 220 Atomic Absorption Spectrophotometer (AAS) was used to measure the Mn level in the urine.

Results The analytical method was validated for concentrations ranging from 3.82–45.84 µg/mL with acceptable accuracy, precision, and recovery. Overall, the UA levels of Mn exposure samples were significantly lower than that of control samples ($P < 0.05$).

Conclusion The practical method developed here is suitable for both routine monitoring of UA level in human urine and metabolism research. Long-term and low-level occupational Mn exposure may lead to a lower UA level in urine, and UA might be an indicator of the early stage of manganism.

Key words: High-performance liquid chromatography; Uric acid; Occupational Mn exposure

Biomed Environ Sci, 2011; 24(3):222-227

doi:10.3967/0895-3988.2011.03.003

ISSN:0895-3988

www.besjournal.com(full text)

CN: 11-2816/Q

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INTRODUCTION

Mn is an essential trace element in the human body that plays an important role in the metabolism of protein and lipids and is a cofactor for enzymes^[1-2]. It has been shown that Mn is essential for the development and functioning of the brain^[3]. Some experimental models and human brain studies have suggested that Mn exposure is associated with oxidative stress^[4].

Nevertheless, excessive Mn exposure will induce its accumulation in the human body and influence the central nervous system^[5]. Pioneering studies of Mn toxicity have suggested that excessive Mn could result in neurotoxicity among workers engaged in battery assembly, ferroalloy production, iron and steel foundry work, and welding^[6]. Clinical symptoms of manganism include gait abnormalities, postural instability and micrographia^[7]. However, afflicted individuals always remain relatively asymptomatic

*This research was financially supported by the Hundred Talents Program of the Chinese Academy of Sciences (CAS) in 2007 and the National Natural Sciences Foundation of China (NSFC No.20775083).

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Received: June 24, 2010;

Accepted: April 25, 2011

during the chronic process of manganism^[8] until their condition is advanced; therefore, it is likely that there is ample opportunity to conduct monitoring before the onset of this disease. Although it is difficult to diagnose chronic manganism during its early stages, it is still very important for prevention and treatment. Therefore, it is necessary to identify sensitive and specific diagnostic markers^[9].

Uric acid (2, 6, 8-trihydroxypurine, UA) is the final product of catabolization of the purine nucleosides in humans. Experimental studies have demonstrated that the antioxidant activity of UA might protect against aging, oxidative stress, and oxidative cell injury^[10-11]. During studies of disease, excessive levels of UA have been shown to be an important predictor of prognosis in patients in all stages of gout, cardiovascular disease and kidney disease^[12-14]. Recently, there has been a great deal of interest in the role of UA as a neurotransmitter because some studies have indicated that gout prevents Parkinson's disease and high levels of UA can prevent neurodegenerative diseases^[15-16]. Moreover, both Mn and UA have antioxidant activity in the organism^[17]. Experimental studies have suggested that Mn increases the production of free radicals measured as increased prolactin and lipid peroxides, reduced glutathione, metallothionein^[18] and other specific biomarkers^[19]. Therefore, it is important to measure the UA levels of individuals that are exposed to Mn.

This study was conducted to determine if long-term and low-level manganese exposure influences the level of urinary UA. To measure the UA levels, an improved method and rapid sample pre-treatment process were developed.

MATERIALS AND METHODS

Chemicals and Reagents

Uric acid (purity>99%) was purchased from Sigma Company (St. Louis, MO, USA). All other chemicals were of analytical reagent and obtained from the Tianjin Chemical Reagent Company (Tianjin, China). All solutions and samples were filtered through a Millipore filter (0.45 µm pore size) prior to use. The concentration of Mn in urine was determined using a Spectra AA 220 Atomic Absorption Spectrophotometer (Varian, USA)

Chromatography The HPLC instrument employed in this study was an Agilent 1 200 system equipped with a diode-array detector (DAD). The detection wavelength was set at 294 nm with a reference

wavelength of 360 nm. A Sino-Chrom ODS-AP analytical column (RP-C18 column 250 mm × 4.6 mm i.d. 5 µm particle size) (Dalian Elite Analytical Instruments Co., Dalian, China) was used for separation. An Agilent Eclipse XDB-C18 guard column (12.5 mm × 4.6 mm i.d., 5 µm particle size) was connected to the analytical column. A G1328B sample injector with a 20 µL quantitative loop was used to introduce the samples. All chromatographic operations were conducted at 23 °C. Elution was conducted using 3:97 (v/v) methanol and deionized water at a flow rate of 0.7 mL/min. The injection volume was 20 µL.

Preparation of Standard Solution and Urine Samples

UA (70 mg) was dissolved in 100 mL deionized water and the pH was adjusted to 7.0 by adding sodium hydroxide solution (0.2 mol/L). The stock solution was stored at 4 °C and brought to room temperature (25 °C) before use. A calibration curve was obtained within the concentration range of 3.82-45.84 µg/mL.

A total of 65 employees of an AI factory in Gansu province, China were recruited as the Mn exposure group. Additionally, a control group of 29 individuals composed of office workers who had no history of occupational Mn exposure or exposure to other neurotoxins were investigated. The general characteristics of the subjects are shown in Table 1.

Table 1. General Subjects Characteristics

		Mn Exposure (n=65)	Control (n=29)
Age/year		37 ± 8 (24-57)	42 ± 14 (19-71)
Gender	F	33/65	13/29
	M	32/65	16/29
Range of Content/ µg/mL	C _{Mn}	1.19×10 ⁻³ ± 0.87×10 ⁻³ (0.013×10 ⁻³ -3.585×10 ⁻³) ^a	0.08-2.67 × 10 ⁻³ ^[18]
	C _{UA}	264.21 ± 153.30 (52.26-695.24)	477.29 ± 194.36 (112.41-896.43)

Note. ^aThe minimum and maximum levels of the corresponding samples.

The morning urine was collected in aseptic bottles and stored at -20 °C in the medical laboratory of the hospital until analysis. Diet was not controlled prior to collection. The samples were thawed before analysis, which was conducted at 4 °C. Solid particles at the bottom of the bottles were dissolved by placing the samples in an ultrasonic bath at 150 W

for 15 min. Each sample was then diluted 25 fold after adjusting the pH value to 7.0 using 0.2 mol/L sodium hydroxide solution or acetic acid.

Statistical Analysis

The chromatograms were collected and recorded using the Agilent Chemstation software. The UA in the urine was identified by comparison with the UA standard. All data were statistically analyzed by an independent samples *T*-test, multiple linear regression and one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test for multiple comparisons between groups found to differ significantly. All analyses were conducted using the SPSS software (11.5 version). The results are given as mean±SD. A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Calibration Curve

The corresponding concentrations of UA standard for linearity calibration were diluted from the aforementioned stock solution. The regression equation for UA was $y = 98.613x - 13.893$, $r = 0.9998$ (3.82–45.84 µg/mL), $n = 3$, where x is the concentration of UA, and y is the peak area. There was an excellent correlation between peak area and the concentration of UA within the range of 3.82 to 45.84 µg/mL, with a correlation coefficient of 0.9998. The limit of quantification (LOQ) determined at a single-to-noise ratio of 10 was 0.12 µg/mL. The chromatograms of UA in the urine sample and in the standard are shown in Figure 1.

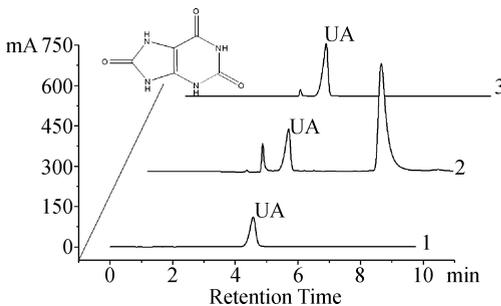


Figure 1. Chromatograms of UA detected by HPLC-DAD at 234 and 294 nm. 1: Standard solution of UA at 294 nm; 2, 3: Urinary sample at 234 and 294 nm, respectively.

Methodological Validation

The accuracy of the proposed method was

assessed by examining the recovery of UA added to urine samples of known concentration at three different levels (7.64, 15.28, and 22.92 µg/mL; $n = 5$ /level). The standards added to the urine were recovered satisfactorily (96.5%, 108.9%, and 105.2%, respectively). The relative standard deviation (R.S.D.) of inter-day and intra-day precision were 2.53% and 4.86% ($n = 5$), respectively.

The standard solution of UA (7.64 µg/mL) was stored at 4 °C during the study period, and was analyzed five times during 22 days with a R.S.D. of 3.24%. However, no UA was detected in the same solution stored at room temperature after ten days. These findings indicate that analytes should be stored at less than 4 °C prior to analysis.

Effect of Mn on UA Level in Urine

The relationship between Mn and UA in the Mn exposure group was analyzed by multiple linear regression. As shown in Figure 2A, increased levels of UA were correlated with increased Mn levels in the urine ($r = 0.750$). When compared with the control group, the UA level decreased significantly when the Mn level was greater than 1.00 µg/L. As shown in Figure 2B, the mean UA level (0.22 ± 0.02 , $n = 13$ when the level of Mn was between 2.01 and 3.60 µg/L) was significantly lower than that of the control (0.48 ± 0.03 , $n = 29$) and the other groups (0.37 ± 0.03 , $n = 36$; 0.44 ± 0.02 , $n = 8$; 0.37 ± 0.02 , $n = 8$).

Effect of Gender on UA in Mn Exposure Group

A significant difference was observed in the UA levels of the Mn exposure and control group when the Mn level was between 2.01 and 3.60 µg/L. The data describing the UA level in urine in this range were grouped by gender and compared with the control by an independent samples *T*-test. The UA levels for the male (0.22 ± 0.02 , $n = 5$) and female (0.22 ± 0.03 , $n = 8$) employees were lower than those of the control group (0.51 ± 0.04 , $n = 21$; 0.39 ± 0.09 , $n = 8$). As shown in Figure 3, the UA level for males was significantly lower than that of the control.

Effect of Age on UA in Mn Exposure Group

The UA levels in urine were grouped by age in years when the level of Mn was between 2.01 and 3.60 µg/L. As shown in Figure 4, multiple comparisons analysis revealed that the Mn level in urine increased as the working age increased. Moreover, the UA levels (0.22 ± 0.02 , 0.23 ± 0.04 , and 0.19 ± 0.04) tended to decrease as the level of Mn (2.279 ± 0.089 , 2.594 ± 0.230 , and 2.842 ± 0.381) increased.

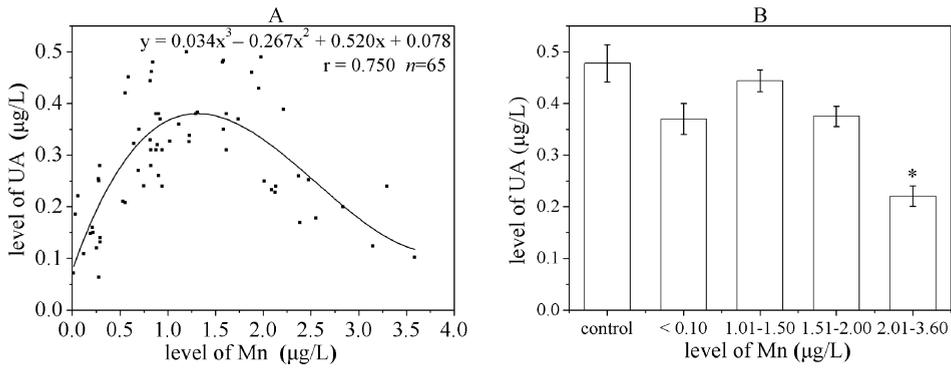


Figure 2. UA level in urine of Mn exposure group. (A) The fitting of multiple linear regressions between the levels of Mn and UA. (B) UA levels in the control and Mn exposure groups. Bars represent mean±SD, *P<0.05 vs. control.

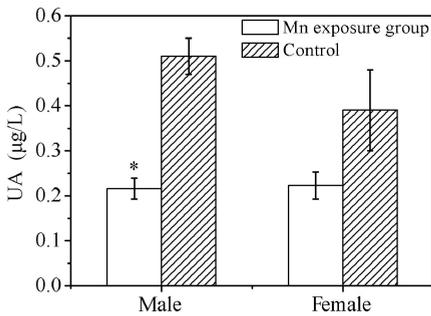


Figure 3. UA level of Mn exposure grouped by gender when the Mn level was between 2.01 and 3.60 µg/L. Bars represent mean±SD, *P<0.05 vs. control.

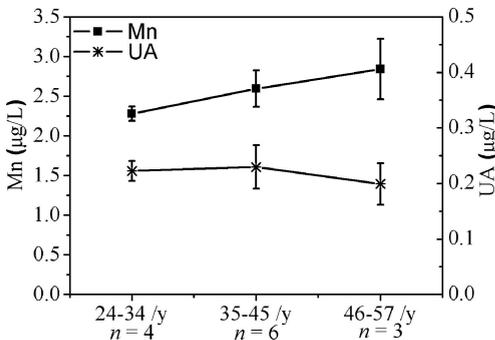


Figure 4. The levels of Mn and UA of Mn exposure grouped by age in years when the Mn level was between 2.01 and 3.60 µg/L. Bars represent mean±SD.

DISCUSSION

Mn is an essential metal found in many different biological tissues that is necessary for normal functioning of a variety of physiological process. However, Mn has been known to be a neurotoxicant for at least 150 years^[20]. Indeed, there is ample

literature available regarding the negative effects of Mn exposure on behavioral outcomes, and it has been suggested that alterations in dopamine biology may drive the effects associated with Mn neurotoxicity^[21]. As shown in previous studies^[22-23], Mn neurotoxicity has a significant relationship with neurotoxic alterations, neurotransmitters and modulator metabolism. In addition, uric acid plays a protective role in disease, including neurodegenerative disease and damage mediated by peroxynitrite^[24]. Moreover, uric acid protects cultured rat hippocampal neurons against cell death induced by insults relevant to the pathogenesis of cerebral ischemia^[25].

In this study, we found that low levels of UA appeared to be associated with long-term occupational Mn exposure. The level of UA was significantly lower in the Mn exposure group than the control. The fitting curve of multiple linear regressions showed that the levels of UA and Mn were closely related. Additionally, when the Mn levels in the urine were greater than 2.00 µg/L, the UA level was significantly lower than that of the control. Although excess Mn intake may result in neurological damage and many clinical effects of Mn toxicity are Parkinson-like in nature, some early diagnoses are difficult to perform without clinical manifestation. Workers who worked in an aluminum factory were recruited for this study because they are known to have long-term Mn exposure without clinical manifestation of neurological damage. All of the employees had worked in the aluminum factory since they were nearly twenty years old. Thus, the age based analyses can reflect the effects of Mn exposure for different lengths of time.

Some researchers have reported that the physiological symptoms of Mn exposure were identified based on plasma lipid peroxidation and

serum prolactin^[26-27]. Additionally, a relationship between the levels of UA and Mn has also been reported based on antioxidant activity by Trostchansky^[17], who believed Mn (III) porphyrins inhibited peroxynitrite flux-mediated LDL oxidation when plasma UA concentrations were between 150 and 500 $\mu\text{mol/L}$. The results of the present study showed a lower level of urinary UA under Mn exposure and were coincident with the results of a previous study that found high levels of UA can prevent neurodegenerative diseases^[15-16]. In other words, excessive Mn could lead to neural damage, which resulted in low levels of urinary UA. Based on the result of this study, people subject to long-term and low-level exposure to Mn have a lower level of urinary UA than those who are not.

Samples from the Mn exposure group were also analyzed according to gender and age. As shown in Figure 3, the UA level for males was significantly lower than that of the control when the Mn level was greater than 2.00 $\mu\text{g/L}$ ($P < 0.05$). Elevated Mn levels also had a greater influence on men than women. In the present study, there were no reports of gender-associated decreases in UA levels in the Mn exposure group. As shown in Figure 4, the Mn level increased and the UA level decreased as the Mn exposure time increased; however, no significant differences were observed among the three groups. Nevertheless, this tendency indicates that Mn levels increase as the Mn exposure increases, and UA level decreases with increasing Mn levels. Therefore, excessive Mn exposure has a greater influence on men than women, and time of exposure had the greatest effect on UA levels in urine in individuals exposed to Mn.

Collection of urine samples is considered to be noninvasive when compared with blood samples, and UA can indicate abnormal metabolism in the organism being studied. The results of the present study indicate that urinary UA is not only the metabolic product of purines or anti-oxidants, but may also be an indicator of occupational health problems and has some potential to be an early diagnosis parameter. However, the results of this study are based on a preliminary investigation. Accordingly, additional studies including animal experiments and investigations of other parameters are warranted.

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Supplement

Unfortunately, professor WANG ZhenQuan died from unexpected accidents, recently. We thank him for his commitment and help in this research. So, in memory of professor WANG ZhenQuan.