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Effect of subchronic exposure to arsenic on levels of essential trace elements in mice brain and its gender difference

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Abstract The interactions of toxic metals with essential metals may result in disturbances in the homeostasis of essential elements. However, there are few reports about toxic effect of arsenic (As) on the levels of essential trace elements in the central nervous system. To investigate whether subchronic exposure to As disturbs levels of main essential trace elements in the brain of mice and whether the gender difference in the response to As are altered, the concentrations of As, Iron (Fe), copper (Cu), selenium (Se), zinc (Zn) and Chromium (Cr) in the cerebrum and cerebellum of mice exposed to As subchronically were examined by inductively coupled plasma-mass spectrometry (ICP-MS). The gender difference in the changed levels of these essential trace elements was also statistically analyzed. The concentration of As was significantly higher in the cerebrum or cerebellum of mice exposed to As than that in control group ($P < 0.05$). It indicates that As can accumulate in brain of mice after subchronic exposure. The concentrations of Fe, Se and Cr in the cerebrum or cerebellum were significantly lower in mice exposed to As than those in control group ($P < 0.05$). On the contrary, the concentration of Cu in the cerebrum or cerebellum was significantly higher in mice exposed to As ($P < 0.05$). Our results indicate that subchronic exposure to As may decrease the levels of Fe, Se and Cr or increase the level of Cu in the brain of mice. Moreover, the significant gender difference was found relative to the effect of As on concentration of Se in cerebrum and concentrations of Cu and Se in cerebellum of mice. Therefore, more experiments are required to further understand mechanisms whereby As interacts with essential elements in brain and induces the gender difference.

Keywords Subchronic exposure to arsenic · Essential element · Brain · Gender difference

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Introduction

Arsenic (As) is a metalloid found in water, soil, and air from natural and anthropogenic sources (Hughes 2002),

As occurs in both organic and inorganic forms in nature. Human populations in many countries, such as India, Bangladesh, Thailand, Taiwan and China, are chronically exposed to arsenic through the consumption of As-contaminated groundwater, resulting in a variety of health effects, including dermatological, cardiovascular, neurological, and neuro developmental disorders, as well as cancers (Miyazaki et al. 2005) Epidemiological studies have demonstrated that As causes neurotoxicity including impairments of learning and concentration and deterioration in pattern memory (Bienert et al. 2008). In animals exposed to As, delay in acquisition and extinction of an operant task (Briére et al. 2005), alterations in locomotor behavior (Bruder et al. 2007) were observed. Chaudhuri et al. discovered that even if the As concentration fell into the provisional guideline issued by World Health Organization (WHO), As could impair the oxidation–reduction equilibrium, enhance the peroxidation level of cephalopin, decrease the glutathione concentration and render the brain tissue vulnerable to radical attack resulting in abnormal apoptosis of neural cells. These epidemiological and experimental studies indicate that central nervous system may be major targets of As neurotoxicity. However, the mechanism of As-induced neurotoxicity is unclear to date. Cui and Okayasu reported that As was dose-dependently accumulated in various organs of rats, such as the spleen, lung and kidney live so on after receiving a dose of 0, 1, 10 and 100 ppm of As in drinking water daily for 4- and 16-weeks (Cui and Okayasu 2008). Hughes et al. reported that As accumulated in the kidney and liver of rats exposed chronically to arsenate in drinking water (Hughes et al. 2003). It was shown in animal experiments that As could pass through blood–brain barrier and invade the brain parenchyma, and there was a noticeable correlation between the extent of As exposure and the concentration of As in the brain of guinea pigs and rats. It indicated that As may be also accumulated in the brain when it is exposed chronically.

The brain is a specialized organ that metabolizes and accumulates metals as part of its normal functioning (Zecca et al. 2004; Bartzokis et al. 2007). Iron (Fe), copper (Cu), selenium (Se) and zinc (Zn) function as cofactors in essential metalloproteins and are required for oxidative phosphorylation, neurotransmitter biosynthesis, modulation of neurotransmission, antioxidant defense, nitric oxide metabolism, oxygen transport, and synthesis of proteins, DNA, and RNA (Popescu et al. 2009; Todorich et al. 2009; Madsen and Gitlin 2007) Fe, Cu, and Zn are essential for myelin synthesis, structure, and maintenance with oligodendrocytes being the main Fe repository cells in the brain (Connor and Menzies 1996). Chromium (Cr) plays an important role in glucose utilization and is required for the release of energy from glucose to be utilized by neural cells (Nudler et al. 2009) In a rich metal environment, loss of function of metalloproteins and loss of defense against oxidative stress caused by metabolic disturbance of one or more metals (Takeda 2001) could also be responsible for injury in central nervous systems. Therefore, an adequate trace element homeostasis may be pivotal for proper brain tissue function. It has been known that toxic elements can compete or interfere with essential elements (Molin et al. 2008). The interactions of toxic metals with essential metals may result in disturbances in the homeostasis of essential elements, in fact, being one mechanism of their toxicity (Liu et al. 1992). Some studies showed that As could interfere with the transport and metabolism of many essential trace minerals (Wang et al. 2006). However, there are few reports about adverse effect of subchronic exposure to As on the levels of essential trace elements in the central nervous system.

In the present study, the concentrations of As and Cu, Fe, Se, Zn, and Cr as major essential elements were determined in the cerebrum and cerebellum of mice exposed to arsenic trioxide subchronically by inductively coupled plasma-mass spectrometry (ICP-MS). The gender difference in the distribution of As in mice brains and its effect on levels of these essential trace elements were also analyzed by statistical method. The aim of this study was to investigate whether subchronic exposure to As disturbs levels of these essential trace elements in the brains of mice and there is gender difference in distribution of As and its effect on the levels of essential trace elements.

Materials and methods

Chemicals

Arsenic trioxide (As_2O_3), HNO_3 (ultra-pure) and H_2O_2 (ultra-pure) were purchased from Sigma Chemical Company (St. Louis, USA). As standard solution,

Cu standard solution, Fe standard solution, Se standard solution, Zn standard solution and Cr standard solution obtained from Development of national standard materials center.

Animals and treatment

Forty mature, healthy Kunming mice (40 male and 40 female) were provided by Experimental Animal Center, Dalian Medical University, China). These mice were randomly segregated into four groups (each 20). One group received drinking water alone (control), the other three groups received 1, 2, or 4 mg/L (ppm) arsenic trioxide (Wako Pure Chemical Industries, Osaka, Japan) respectively, through drinking water ad libitum for 60 days. They were caged under a 12 h dark–light cycle in standard conditions of temperature (18–22 °C) and humidity (50 %). After the last administration, all the animals were weighed. Animals were decapitated and their cerebrum and cerebellum were removed and stored at −80 °C until use. The animal experiment was performed in accordance with the Animal Guideline of Dalian Medical University and in agreement with the Ethical Committee of Dalian Medical University.

Treatment of test samples

Cerebral tissue sample (0.250 g, wet weight) and cerebellar tissue sample (0.200 g, wet weight) was taken from each mice brain by microbalance (20 samples for each group, 10 samples for male mice and 10 samples for female mice). These samples were put into Teflon (100 mL) beaker and added 5 mL HNO_3 and 2 mL H_2O_2 to this vessel. After soaking for 30 min, digestion took place in microwave digestion furnace system (MLS1200PYRO, Italy Milestone Corporation). According to the procedure in Table 1, samples were digested. Then the digested samples were cooled to room temperature, and constant volume samples were measured on the machine. Ultrapure water was used for final sample dilution and the analysis was performed via ICP-MS utilizing collision reaction cell methods coupled with ion–molecule chemistry, a reliable new method for interference reduction.

Table 1 Digestion procedure of microwave digestion furnace

Procedure (n)	1	2	3	4	5	6
Power (W)	250	0	250	450	450	550
Time (min)	1	7	7	6	5	4

Analysis of heavy metals

As and Fe, Cu, Zn, Se, and Cr were analyzed by ICP-MS (ICP-MS, 7500CE, Agilent Technologies). The ICP-MS measurements were performed using the following conditions (Table 2). He (4.5 mL/L) was used as reaction gas. By this reaction gas mode, the concentration of As and Cu, Fe, Se, Zn and Cr were satisfactory measured without interference by ICP-MS. Ge (1 mg/L, $m/z = 72$) was used as internal standard in this mode instead of common Y or In. The calibration range of As and five essential trace elements were as follows: As was from 0 to 20 μg/L, Cu was from 0 to 300 μg/L, Fe was from 0 to 350 μg/L, Se was from 0 to 20 μg/L, Zn was from 0 to 300 μg/L and Cr was from 0 to 35 μg/L. The detection limits for the measured elements were as follows: As (0.3 μg/L), Se (0.2 μg/L), Cr (0.15 μg/L), Fe (0.5 μg/L), Cu (0.4 μg/L), Zn (0.9 μg/L).

Table 2 Operating conditions for ICP-MS measurements

Name	Settings
RF power (W)	1,300
Photomultiplier tube (W)	450
Atomization chamber	Quartz dual-channel, 2 °C
Sampling depth (mm)	6.8
Sample flow rate (mL/min)	0.5
Carrier gas flow rate (L/min)	1.0
Lens 1 voltage (V)	1
Lens 2 voltage (V)	−19
Quadrupole focusing voltage (V)	8
Collision cell entrance voltage (V)	−36
Collision cell outlet voltage (V)	−40
Resolution (V)	12.5
Outer gas flow rate (L/min)	16.0
Intermediate gas flow rate (L/min)	1.0
Nebulizer gas flow rate (L/min)	0.8
Isotopes monitored (m/z)	^{75}As, ^{64}Cu, ^{56}Fe, ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, ^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, ^{70}Zn, and ^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr

Statistical analysis

Values are expressed as mean ± SD for 20 rats in each group, significant differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Scheffe's test for multiple comparison, and gender difference was tested by two-way analysis of variance (ANONA). All test data was converted and manipulated by using Statistical Package for Social Sciences 17.0 (SPSS 17.0) computer package. P value < 0.05 was considered statistical significance.

Results

Concentrations of As in the cerebral and cerebellar tissues of mice

The concentration of As in brains of mice is shown in Table 3. The concentration of As in the cerebrum of mice was 3.47 ng/g in the control group. The concentration of As in the cerebrum of mice was 13.35, 22.22 and 29.79 ng/g in the groups received 1, 2 and 4 ppm As_2O_3, respectively. There were significant differences in concentration of As between the three experimental groups and control group ($P < 0.05$) and the concentrations of As in the experimental groups increased in a dose–response manner. The concentration of As in the cerebellum of mice was 4.53 ng/g in the control group and 14.05, 20.74 and 29.97 ng/g in the groups received 1, 2 and 4 ppm As_2O_3, respectively. The concentration of As in the experimental groups significantly increased in a dose–response manner ($P < 0.09$). The significant difference in As concentration was not found between the cerebral and cerebellar tissues.

Effect of As on concentrations of five essential trace elements in the cerebrum of mice

Table 4 shows the concentrations of Fe, Cu, Zn, Se and Cr in the cerebrum of mice. The concentrations of Fe, Se and Cr in cerebellum were significantly lower in the groups received As than those in control group ($P < 0.05$). Especially, the concentrations of these essential trace elements in cerebellum of mice received 4 ppm As_2O_3 were the lowest among the four groups. On the contrary, the concentration of Cu in cerebellum was significantly higher in the group received 2 or 4 ppm As_2O_3 than that in control group ($P < 0.05$). There were no significant differences in concentration of Zn between the three experimental groups and control group ($P > 0.05$).

Effect of As on concentrations of five essential trace elements in the cerebellum of mice

The concentrations of Fe, Cu, Zn, Se and Cr in the cerebellum of mice are shown in Table 5. The concentrations of Fe, Se and Cr in cerebellum were significantly lower in the groups received As than those in control group ($P < 0.05$). On the contrary, the concentration of Cu in cerebellum was significantly

Table 3 Concentration of As in brain of mice in the four groups

Groups	Treatment	No. of mice	Concentrations of As in brain (ng/g)	
			Cerebrum	Cerebellum
Control	–	20	3.47 ± 0.23	4.53 ± 0.63
1 ppm	As_2O_3	20	13.35 ± 0.34[a]	14.05 ± 0.68[a]
2 ppm	As_2O_3	20	22.22 ± 0.67[a,b]	20.74 ± 0.87[a,b]
4 ppm	As_2O_3	20	29.79 ± 0.57[a,b,c]	29.97 ± 1.98[a,b,c]
			F values: 0.044	

F value indicating comparison between concentration of As in the cerebral and cerebellar tissues by two-way analysis of variance (ANOVA)

*F: $P < 0.05$

[a] $P < 0.05$, compared with control group by the Scheffe's test

[b] $P < 0.05$, compared with 1 ppm As_2O_3 group by the Scheffe's test

[c] $P < 0.05$, compared with 2 ppm As_2O_3 group by the Scheffe's test

Table 4 Concentrations of five essential trace elements in cerebrum of mice exposed to As

Groups	Treatment	Concentrations of five essential trace elements (ng/g)				
		Fe	Cu	Zn	Se	Cr
Controls	–	28,630 ± 550	3,360 ± 40	13,140 ± 240	120.91 ± 4.22	143.50 ± 4.39
1 ppm	As_2O_3	23,300 ± 680[a]	3,200 ± 40	13,235 ± 290	115.24 ± 4.28[a]	123.74 ± 3.60[a]
2 ppm	As_2O_3	23,030 ± 360[a,b]	3,490 ± 50[a,b]	13,280 ± 320	109.70 ± 3.71[a,b]	113.63 ± 6.28[a,b]
4 ppm	As_2O_3	23,040 ± 580[a,b]	3,640 ± 80[a,b]	12,860 ± 360	102.29 ± 2.13[a,b,c]	107.92 ± 4.71[a,b,c]

[a] $P < 0.05$, compared with control group by the Scheffe's test

[b] $P < 0.05$, compared with 1 ppm As_2O_3 group by the Scheffe's test

[c] $P < 0.05$, compared with 2 ppm As_2O_3 group by the Scheffe's test

higher in the group received 2 or 4 ppm As_2O_3 than that in control group ($P < 0.05$). There were no significant differences in concentration of Zn between the three experimental groups and control group ($P > 0.05$).

Gender difference in distribution of As and its effect on concentrations of the five trace elements in brains of mice

Gender difference in distribution of As in brain of mice were analyzed by the two-way ANOVA. Distribution of As did not show the gender difference in the cerebrum and cerebellum of mice ($P > 0.05$) (Table 6). The differences in effect of As on concentrations of Fe, Cu, Zn, Se and Cr in brains between male and female mice were also analyzed by the two-way ANOVA (Table 7). There was significant gender difference in effect of As on concentration of Se in cerebrum and cerebellum of mice. Moreover, effect of As on concentration of Cu also showed significant gender difference in cerebellum of mice.

Discussion

Arsenic (As) is a common and conspicuous toxicant. Its toxicosis is becoming a global problem of public health (Piao et al. 2011). Some experimental studies showed that residual As concentration was elevated in tissues of various organs, including the kidneys, liver, muscle, and spleen, in the rodents and dogs dosed with arsenate or arsenite (Vahter and Norin 1980; Yamauchi and Yamamura 1985). These results indicated As has characteristics of accumulation in tissues. It has been documented that As can cause neurotoxicity (Rodríguez et al. 2003). Our previous study also showed that As exposure induces toxicity to central nervous system (Piao et al. 2005; Wang et al. 2009). However, there are few reports on accumulation of As in cerebrum and cerebellum after subchronic exposure. In the present study, the concentration of As was significantly higher in the cerebral and cerebellar tissues of mice exposed As than that in control group ($P < 0.05$) and increased in a dose–response manner. It indicates that As can also accumulate in cerebrum and cerebellum after subchronic exposure. It is well

Table 5 Concentrations of five essential trace elements in cerebellum of mice exposed to As

Groups	Treatment	Concentrations of five essential trace elements (ng/g)				
		Fe	Cu	Zn	Se	Cr
Controls	–	37,175 ± 760	3,850 ± 70	10,520 ± 150	128.19 ± 2.26	147.47 ± 3.48
1 ppm	As_2O_3	32,950 ± 750[a]	3,930 ± 70[a]	10,405 ± 280	121.62 ± 4.92[a]	112.24 ± 4.02[a]
2 ppm	As_2O_3	29,395 ± 520[a,b]	4,065 ± 80[a,b]	10,705 ± 160	115.77 ± 7.27[a,b]	101.05 ± 6.02[a,b]
4 ppm	As_2O_3	23,700 ± 800[a,b]	3,975 ± 90[a]	10,360 ± 220	104.69 ± 4.98[a,b,c]	95.76 ± 8.18[a,b,c]

[a] $P < 0.05$, compared with control group by the Scheffe's test

[b] $P < 0.05$, compared with 1 ppm As_2O_3 group by the Scheffe's test

[c] $P < 0.05$, compared with 2 ppm As_2O_3 group by the Scheffe's test

Table 6 Concentration of As in brain tissue of male and female mice (ng/g)

Group	Cerebrum		Cerebellum	
	Male	Female	Male	Female
Control	3.27 ± 0.27	3.66 ± 0.18	4.49 ± 0.38	4.57 ± 0.87
1 ppm	12.81 ± 0.36	13.88 ± 0.31	13.85 ± 0.56	14.24 ± 0.79
2 ppm	20.72 ± 0.46	23.71 ± 0.47	19.63 ± 0.56	21.84 ± 0.58
4 ppm	31.98 ± 0.17	27.59 ± 0.97	32.13 ± 0.39	27.81 ± 0.86
F values	0.000		0.088	

F value indicating comparison between the cerebral and cerebellar tissues in male and female mice by two-way analysis of variance (ANOVA)

*F: $P < 0.05$

Table 7 Gender differences in concentrations of the five trace elements in brains of mice exposed to As (ng/g)

Group	Cerebrum		Cerebellum	
	Male	Female	Male	Female
Fe concentration				
Control	29,280 ± 430	27,970 ± 970	39,180 ± 440	35170 ± 480
1 ppm	23,390 ± 710	23,200 ± 640	35,560 ± 960	30330 ± 530
2 ppm	22,140 ± 440	23,910 ± 470	29,770 ± 860	29020 ± 480
4 ppm	22,070 ± 390	22,820 ± 760	27,390 ± 470	20000 ± 430
F values	0.150		9.811	
Cu concentration				
Control	3,630 ± 60	3,090 ± 20	4,000 ± 50	3700 ± 80
1 ppm	3,240 ± 40	3,140 ± 40	4,040 ± 80	4000 ± 50
2 ppm	3,710 ± 50	3,260 ± 40	4,090 ± 80	4040 ± 80
4 ppm	3,720 ± 70	3,560 ± 60	4,170 ± 70	3780 ± 80
F values	8.426		11.042*	
Zn concentration				
Control	13,790 ± 350	12,480 ± 120	10,650 ± 100	10390 ± 200
1 ppm	13,470 ± 370	13,000 ± 200	10,810 ± 170	10000 ± 300
2 ppm	13,440 ± 380	13,120 ± 250	11,140 ± 126	10270 ± 360
4 ppm	13,020 ± 330	12,690 ± 280	10,430 ± 320	10290 ± 110
F values	6.590		7.752	
Se concentration				
Control	123.54 ± 3.25	118.28 ± 2.18	128.98 ± 1.51	127.39 ± 3.00
1 ppm	119.03 ± 3.10	111.44 ± 2.46	121.48 ± 5.56	121.75 ± 4.28
2 ppm	115.36 ± 0.59	104.04 ± 3.37	116.05 ± 3.95	115.49 ± 4.59
4 ppm	104.35 ± 1.74	100.23 ± 2.53	106.52 ± 4.39	102.86 ± 2.57
F values	19.803*		17.200*	
Cr concentration				
Control	137.29 ± 5.21	149.70 ± 3.56	145.71 ± 0.86	149.13 ± 2.76
1 ppm	126.10 ± 4.07	121.38 ± 3.12	99.26 ± 2.78	125.22 ± 2.33
2 ppm	110.96 ± 4.07	116.29 ± 2.47	92.79 ± 1.44	109.61 ± 2.59
4 ppm	104.35 ± 4.35	111.49 ± 5.06	90.57 ± 1.45	100.95 ± 2.19
F values	1.978		8.701	

F value indicating comparison in concentrations of the five elements in brains between male and female mice by two-way analysis of variance (ANOVA)

*F: $P < 0.05$

known that trace elements are involved as essential part of many physiological functions in the brain's biochemistry that deficiency or excess of these elements results in nervous system disorders (Madsen and Gitlin 2007). It was reported that the toxic elements can interfere with essential trace elements and the interactions between toxic metals and essential trace metals may result in disturbances in the homeostasis of essential elements (Liu et al. 1992). Therefore, we are interested in whether As exposure disrupts levels of essential trace elements in the brain.

In the present study, the concentrations of Cu, Fe, Se, Zn, and Cr as major essential trace element in the cerebrum and cerebellum were determined in the mice exposed to As subchronically by ICP-MS. The concentration of Fe in the cerebrum and cerebellum was significantly lower in mice exposed to As than that in control group ($P < 0.05$). Our results indicate that subchronic exposure to As may decrease level of Fe in the brain. Because the reports about the effects of As on level of Fe in tissues are very limited, the mechanism that As exposure disrupts the balance of Fe in tissues is poorly understood. Fe is mainly absorbed from the duodenum, where it is known that Fe^{2+} is more absorbed than Fe^{3+}. A divalent cation transporter (DCT1) is responsible for the uptake of Fe^{2+} from the lumen into the mucosa. Most dietary non-heme iron is in the form of Fe^{3+} complexes and these must be reduced to yield Fe^{2+} before iron can be transported by DCT1. It is the ferrireductase present in the brush border of the duodenum that converts Fe^{3+} into Fe^{2+}. This enzyme is responsible for transferrin-independent iron transport (Inman et al. 1994). Paul et al. reported that chronic administration of As caused significant reduction of ferrireductase activity. These literatures indicate that As may reduce conversion of Fe^{3+} to ferrous form through repressing ferrireductase activity and ultimately reduces the absorption of iron (Paul et al. 2002). Whether the decreased concentration of Fe in the brains of mice exposed to As is also associated with the As-induced inhibition of ferrireductase activity is needed to study further.

Cu is an integral component of various cuproenzymes, including cytochrome C oxidase, lysyl oxidase, superoxide dismutase (SOD), dopamine β-oxidase, tyrosinase and ceruloplasmin (Zheng and Monnot 2012). The biological functions of Cu include electron-transfer catalysis by means of its two accessible oxidation states (Georgopoulos et al. 2001). Copper plays a fundamental role in the biochemistry of the human nervous system. There is study showed that high dietary As caused a marked accumulation of copper in the kidney of rats (Wang et al. 2006). Wang et al. also reported that As feeding elevated ($P < 0.05$) liver and kidney copper concentration. In the present study, the concentration of Cu in the cerebrum and cerebellum significantly increased in mice exposed to As, being accordant to the above findings. Our results indicate that subchronic exposure to As may increase level of Cu in the brain. The cause of the increased brain copper on administration of As is unknown. Cu homeostasis is regulated mainly by biliary excretion (Wang et al. 2006) and the high dietary As could decrease biliary excretion of copper. It is needed to research further whether the increased brain copper is associated with the decreased biliary excretion of copper or As-induced alteration of the brain handling of copper through an as yet unknown mechanism.

Selenium (Se) is an essential trace element and is involved in various enzymes activities and in the oxidation–reduction processes. Se was recorded to improve mood, increased brain's energy and cognitive function, and clarity (Schweizer et al. 2004). Moreover, Se has been found to play a protective role against dopaminergic neurotoxicity. Therefore, Se deficiency in brain may induce disturbance of some functions in central nervous system. The effect of As on level of Se in the body has been extensively studied. Many experimental studies have shown an interaction between Se and As (Zeng et al. 2005). Epidemiological investigation showed that patients of chronic arsenicosis had lower Se levels than the non-affected controls in a Taiwan population (Yang et al. 2002). Molin et al. reported that compared to controls, Se concentration significantly decreased in the brains of mice treated intraperitoneally daily with 1 mg/kg As_2O_3 on days 5 and 7, respectively. In the present study, the concentration of Se in the cerebrum and cerebellum was also significantly lower in mice exposed to As than that in control group ($P < 0.05$), being consistent with the above literatures. These results indicate that subchronic exposure to As may decrease level of Se in the brain. Although it is generally accepted that uptake of one of these tow elements causes release, redistribution, or elimination of the other element by urinary, biliary, and/or expiratory routes (Goyer 1997), the precise mechanism at the cellular level is still unknown. Because of

their similar physical and chemical properties, As and Se compounds can be biologically antagonistic to each other. For example, both Se^{4+} and As^{3+} have the same electronic structure. This similarity of electronic structure results in selenite (Se^{4+}) absorption being markedly depressed by arsenite (As^{3+}) on tissue or organelle membrane as shown in a chick model (Zeng et al. 2005). In addition, earlier work demonstrated that As markedly increased the excretion of Se into the gastrointestinal tract when both arsenite and selenite were injected at subacute doses (Zeng et al. 2005). Further study demonstrated that As greatly increased the amount of Se excreted in rat bile. More recently, it has been demonstrated that the in vivo antagonism between arsenite and selenite forms seleno-bis (*S*-glutathionyl) arsinium ion as As–Se compound [$(GS)_2AsSe$], which is subsequently excreted in bile (Zeng et al. 2005). The latter could be exported from the hepatocytes to bile by ATP-driven glutathione S-conjugate export pumps (Zeng et al. 2005).

Because Cr is required for the release of energy from glucose, Cr deficiency could affect adversely glucose utilization and energy metabolism of neural cells. Aguilar et al. (1997) reported that co-administration of As and Cr reduced significantly the Cr level in mice. In the present study, the concentration of Cr in the cerebrum and cerebellum was significantly lower in mice exposed to As than that in control group ($P < 0.05$), being consistent with the result from above literature. Our results indicate that subchronic exposure to As may decrease level of Cr in the brain. Because the reports about the effects of As on level of Cr in tissues are scarce, the mechanism that As interacts with Cr in nerve tissue is still unclear. It is required to research further.

Zinc is the second most abundant trace element in the body and powerfully influences cell division and differentiation. Approximately 90 % of the total brain zinc is bound in zinc proteins. Constant Zn level is essential to maintain homeostasis within the brain and prevent the development of neurological disorders. In the present study, no significant difference was shown in Zn concentration of mice brains between the experimental and control groups. Molin et al. reported also that Zn concentration in brains of mice remained unaffected by the As_2O_3 treatment (Molin et al. 2008), being accordant with our results.

There is increasing evidence that health effects of As is manifested differently in males and females (Vahter et al. 2007). A number of studies have indicated that men are more susceptible to the arsenic-related skin effects than women (Lindberg et al. 2008). Epidemiological studies (Vahter et al. 2002) showed that there seem to be higher risks for arsenic-induced bladder and kidney cancer in women compared to men (Vahter et al. 2002). Certain gender differences in the biotransformation of arsenic by methylation have been also reported (Vahter et al. 2007), and women had higher arsenic methylation efficiency than men (Lindberg et al. 2008). However, there are few reports about gender difference in distribution of As and its effect on levels of essential trace elements in brain. In the present study, the gender difference in distribution of As was not shown in the cerebrum or cerebellum of mice. However, the significant difference was observed in effect of As on concentration of Se in cerebrum or concentrations of Cu and Se in cerebellum of mice. Our results indicate that there may be gender difference in the disturbed levels of essential trace elements in the mice brains by subchronic exposure to As.

In conclusion, in the present study, the concentration of As was significantly higher in the cerebral and cerebellar tissues of mice exposed As than that in control group ($P < 0.05$). It indicates that As can accumulate in brain of mice after subchronic exposure. The concentrations of Fe, Se and Cr in the cerebrum and cerebellum was significantly lower in mice exposed to As than that in control group ($P < 0.05$). On the contrary, the concentration of Cu in the cerebrum and cerebellum significantly increased in mice exposed to As. Our results indicate that subchronic exposure to As may decrease the levels of Fe, Se and Cr or increase the level of Cu in the brain of mice. Moreover, the significant gender difference was shown in effect of As on concentration of Se in cerebrum or concentrations of Cu and Se in cerebellum of mice. Therefore, more experiments are required to demonstrate further whether the observed changes in trace element levels correlated closely with the As-induced damages of central nerve system. Meanwhile, it is also necessary to further understand mechanisms that As interacts with essential elements in brain and induces the gender difference.

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