J Occup Health 2005; 47: 445–449

Journal of Occupational Health

Oxidative DNA Damage in Relation to Neurotoxicity in the Brain of Mice Exposed to Arsenic at Environmentally Relevant Levels

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Abstract: Oxidative DNA Damage in Relation to Neurotoxicity in the Brain of Mice Exposed to Arsenic at Environmentally Relevant Levels: Fengyuan PIAO, *et al.* Department of Hygiene, Dalian Medical University, China—To clarify the association between oxidative DNA damage and the neurotoxicity of arsenic, the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as an index of oxidative DNA damage in the brain was examined in mice fed with drinking water containing 1 or 2 ppm arsenic, using an HPLC-electrochemical detector and immunohistochemical method. 8-OHdG levels were significantly increased in the brain of mice given arsenic and its immunoreactivity was distributed in the cerebral and cerebellar cortexes. Cerebral cortex neurons and Purkinje cells in the cerebellar cortex showed degenerative changes in accordance with the distribution of 8-OHdG immunoreactivity. The levels of arsenic in this study were lower than those reported in epidemiological studies. Thus, we conclude that environmentally relevant levels of arsenic induce pathological changes through oxidative DNA damage in the brain tissues *in vivo* and that cerebral and cerebellar cortex neurons seem to be the major targets of arsenic neurotoxicity.
(J Occup Health 2005; 47: 445–449)

Key words: Arsenic trioxide, 8-Hydroxy-2'-deoxyguanosine (8-OHdG), Cerebral cortex, Cerebellar cortex, Purkinje cell, Oxidative DNA damage

Arsenic is a common environmental contaminant widely distributed around the world. Human exposure to this metalloid comes from well water and contaminated soil, from fish and other sea organisms rich in methylated arsenic compounds, and from occupational exposure[1–3]. The risk of arsenic-induced human diseases is particularly high in developing countries such as Argentina, Bangladesh, India, Mexico, Thailand and China[4–7]. It is estimated that millions of people are exposed to health risks from arsenic in Bangladesh[8]. Epidemiological studies have demonstrated that arsenic causes neurotoxicity including impairments of learning and concentration[1] and deterioration in pattern memory and switching attention in humans[9]. In animals exposed to arsenic, delay in acquisition and extinction of an operant task[10], alterations in locomotor behavior and deficits in spatial learning paradigms[11, 12] have been observed. These epidemiological and experimental studies indicate that the cerebral and cerebellar cortexes may be affected by arsenic. However, the mechanisms by which arsenic exerts a toxic effect on the central nervous system are still unclear.

In recent years, evidence has accumulated that reactive oxygen species (ROS) are involved in neurological disorders[13]. It has been reported that arsenic exposure induces oxidative stress in the rat brain[14, 15] and cultured cells from the brain of the human fetus and newborn rats[16], and that arsenic-induced oxidative stress causes oxidative DNA damage in cultured cells[16–19]. These reports lead us to hypothesize that arsenic-induced ROS cause oxidative DNA damage and subsequent cell death in the brain, and are responsible for the pathogenesis of the neurobehavioral abnormalities. Therefore, an animal experiment on oxidative DNA damage and resulting pathological changes in the brain may contribute to the clarification of the mechanisms of arsenic neurotoxicity.

In the present study, we examined the formation and distribution of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a sensitive marker of oxidative DNA damage[20], in the

Received April 6, 2005; Accepted July 19, 2005
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brain of mice fed with drinking water containing 1 or 2 ppm of arsenic trioxide, using an HPLC-electrochemical detector (ECD) and an immunohistochemical method. Histological changes in the brain were also examined.

Materials and Methods

Animals and treatment

Thirty ICR mice (age 9 wk) weighing 26.2–30.9 g were purchased from Charles River Japan, Inc (Yokohama, Japan). These mice were randomly segregated into three groups of 10 each. One group received drinking water alone (control), the other two groups received 1 or 2 ppm arsenic trioxide (Wako Pure Chemical Industries, Osaka, Japan) through drinking water *ad libitum* for 40 d. The exposure duration was chosen with reference to a study by Chattopadhyay *et al*., in which 3 or 5 mg/*l* of sodium arsenite was given to rats for 20 d through drinking water and their neurobehavioral abnormality observed[16]. For immunostaining, the thoraxes of 4 mice in each group were opened and the tissue fixative (4% formalin) was injected via a needle inserted into the left ventricle of the heart[21]; then, the brain was removed and placed in fixative. For 8-OHdG measurement, the remaining 6 mice in each group were sacrificed by decapitation and the brain was taken out and stored at –80°C. The animal experiment was conducted in accordance with the in-house guidelines for the care and use of laboratory animals at Mie University.

Measurement of 8-OHdG formation in the brain

8-OHdG in brain DNA was measured by the method of Kawanishi *et al*.[22] with a little modification. Briefly, 200 mg of brain was scissored into small pieces, followed by homogenization in 0.25 M saccharose solution. DNA was extracted from the homogenate under an anaerobic condition. The 8-OHdG content in the brain was measured by using an HPLC-ECD as described previously[22]. Each brain was examined in duplicate.

Immunohistochemistry for 8-OHdG formation

8-OHdG immunoreactivity in the mouse brain was examined as described previously[23, 24]. Briefly, paraffin sections (6 μm thickness) were incubated with mouse monoclonal anti-8-OHdG antibody (4 μg/m*l*) (Japan Institute for the Control of Aging, Fukuroi, Japan) overnight at room temperature. Then, the sections were incubated with goat anti-mouse IgG-HRP (1:200). The immunostained sections were examined under a microscope. A histopathological study was performed after hematoxylin and eosin staining of paraffin sections as described previously[23].

Results

Arsenic-induced 8-OHdG formation and distribution in the brain of mice

The amounts of 8-OHdG in the brains of arsenic-exposed mice are shown in the Table. The level of 8-OHdG in the group given 2 ppm arsenic trioxide was significantly higher than that in controls ($p<0.05$). An increase in the 8-OHdG level in the group given 1 ppm arsenic trioxide was observed, though this increase was not statistically significant.

The distribution of 8-OHdG immunoreactivity in the brains of the mice is shown in Fig. 1. Weak 8-OHdG immunoreactivity was observed in cerebral and cerebellar cortexes in the mice given 1 ppm arsenic trioxide (data not shown). More intensive immunoreactivity was found in the cerebral cortex (a, c) and cerebellar cortex, particularly in Purkinje cells and granular cells (b, d) of the mice given 2 ppm arsenic trioxide. No immunoreactivity of 8-OHdG in brain tissues was observed in controls (e, f).

Histopathological changes in brain tissues of mice exposed to arsenic trioxide

Histopathological changes in cerebral and cerebellar cortexes are shown in Fig. 2. Nuclear vacuolation, neuritic loss and lysis of neurons were observed in the cerebral cortex of the mice given 2 ppm arsenic trioxide (a). These changes were also observed in Purkinje cells of the cerebellar cortex (b). No histopathological changes were observed in the cerebral and cerebellar cortexes of the controls (c, d). The morphological changes in the mice given arsenic were in accordance with the distribution of 8-OHdG immunoreactivity.

Discussion

In the present study, we demonstrated an increase of 8-OHdG in the brain of mice given arsenic at lower levels than those reported in previous epidemiological studies[1, 5]. 8-OHdG immunoreactivity and degenerative changes were observed in cerebral cortex neurons and Purkinje cells in the cerebellar cortex. It is noteworthy that the pathological changes were in accordance with the distribution of 8-OHdG immunoreactivity.

Arsenic can cross the blood-brain barrier and accumulates in the brain tissues[12]. It has been reported

Table 8-OHdG in brain tissue of mice administered arsenic trioxide and controls

Groups[a]	8-OHdG (per 10^5 dG) Mean (S.D.)	(Range)
2 ppm arsenic trioxide	2.27 (0.57) *	(1.61–3.04)
1 ppm arsenic trioxide	1.58 (0.45)	(1.10–2.33)
Controls	1.09 (0.37)	(0.62–1.65)

[a]Six mice in each. *p=0.011, compared with controls (Mann-Whitney U-test).

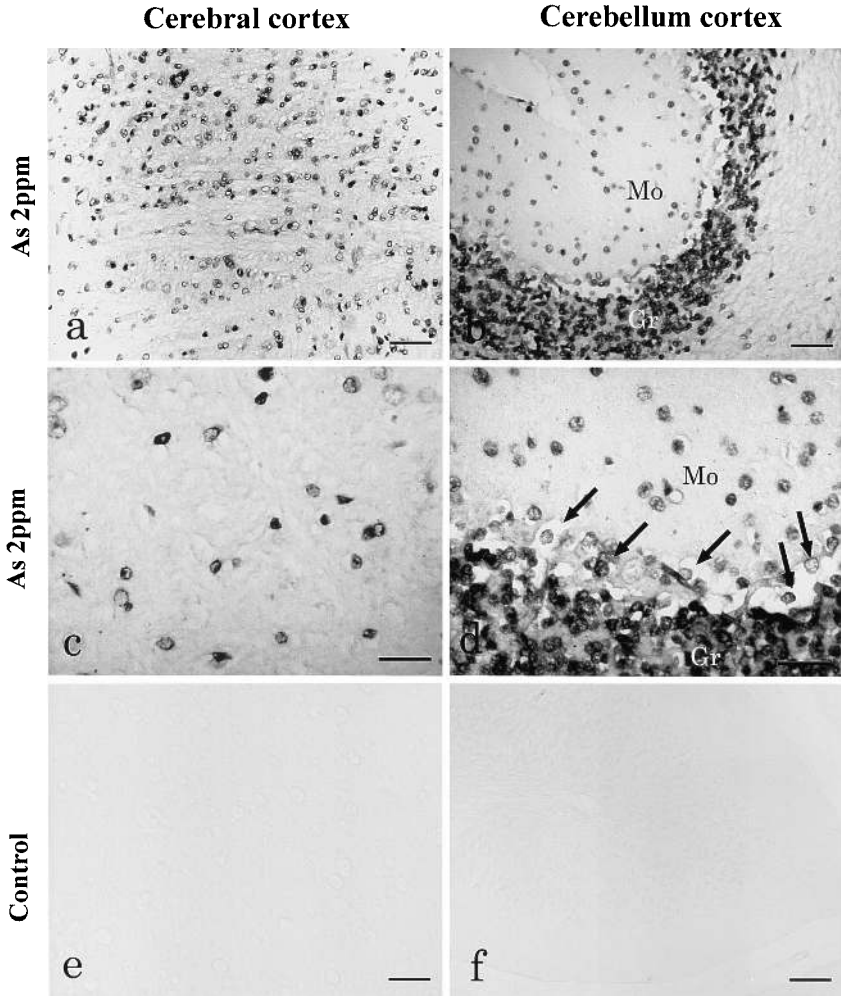

Fig. 1. 8-OHdG immunoreactivity in the brain of mice given arsenic trioxide. 8-OHdG formation was analyzed by immunohistochemistry with an HRP-conjugated antibody. 8-OHdG immunoreactivity is observed in the cerebral cortex (a, c) and cerebellar cortex, particularly in the Purkinje cells (arrows) and granular cells of the cerebellar cortex (b, d) of the mice given 2 ppm arsenic trioxide. No 8-OHdG immunoreactivity is observed (e, f) in the brains of controls. Mo=molecular layer; Gr=granular layer. Bar=100 μm (a, b, e, f) and 25 μm (c, d).

that arsenic generates ROS in nerve cells, resulting in oxidative DNA damage and abnormal apoptosis[16]. These earlier reports support our hypothesis that oxidative DNA damage in the brain resulting from arsenic-induced ROS is responsible for the pathogenesis of arsenic neurotoxicity. Glial cells have the ability to protect themselves from ROS[25], because these cells contain high concentrations of antioxidant enzymes and glutathione (GSH)[26]. In contrast, neurons are sensitive to oxidative stress, as they contain a comparatively low content of protective enzymes and free radical scavengers, e.g. catalase, glutathione peroxidase, GSH and vitamin E[1, 27]. In the present study, formation of 8-OHdG induced by arsenic was concentrated in the cerebral and cerebellar cortexes, which contain greater numbers of neurons than other parts of the brain[28]. Many studies have demonstrated impairments of learning, memory and motor coordination in humans[8] and experimental animals[10–12] exposed to arsenic compounds. As the cerebral cortex is responsible for cognitive functions[28] and the cerebellum is critical for posture and motor coordination[28], controlled by the Purkinje cells[29], oxidative DNA damage caused by arsenic in these parts of brain may explain the impairments of these central nervous system functions. Although accumulation of arsenic in brain has been reported, there is little

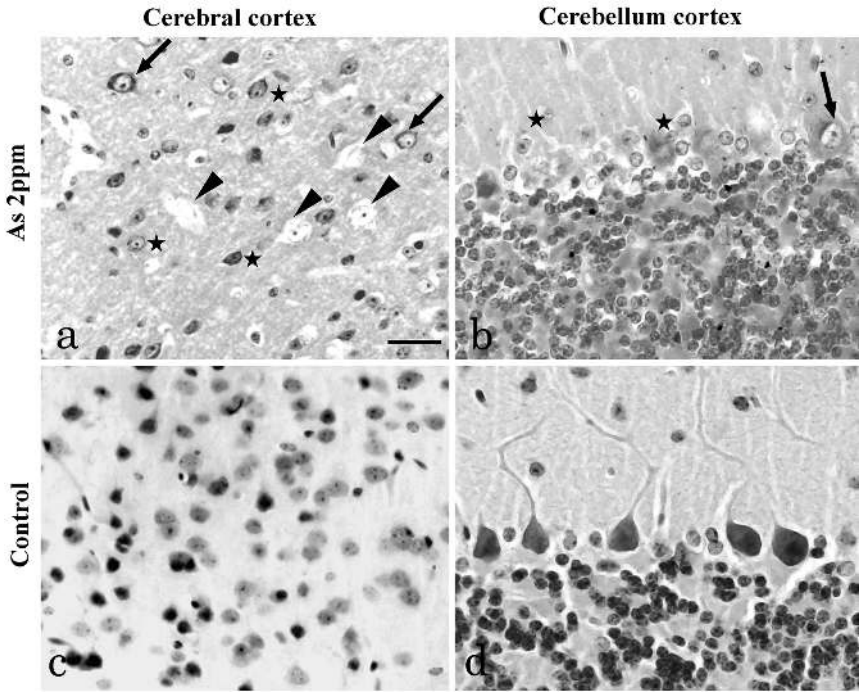

Fig. 2. Histopathological changes in the brain of mice given arsenic trioxide. Histopathological changes were examined by hematoxylin and eosin staining. Nuclear vacuolation (arrows), neuritic loss (star) and lysis of neurons (arrowheads) are observed in the cerebral cortex of the mice given 2 ppm arsenic trioxide (a). Neuritic loss (stars) and nuclei vacuolation (arrows) are also observed in the Purkinje cells (b). No pathological changes are found in the cerebral cortex (c) and cerebellar cortex of controls (d). Bar=25 μm.

information regarding the arsenic content in different parts of brain tissue[11, 30]. Therefore, it will be necessary to explore further the relation between the accumulation of arsenic in the cerebral and cerebellar cortexes, and oxidative DNA damage in these parts of the brain.

In summary, the present study showed 8-OHdG formation and pathological changes in cerebral cortex neurons and Purkinje cells in mice given arsenic. These results suggest that arsenic induces pathological changes through oxidative DNA damage in the brain *in vivo,* and that the cerebral and cerebellar cortex neurons seem to be major targets of arsenic neurotoxicity. Several authors have reported that exposures over long periods of time may occur in some occupational settings through inhalation and ingestion of dust[31–33], therefore neurobehavioral abnormality in workers exposed occupationally to arsenic should receive attention.

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