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Short communication

CEREBELLUM ALTERATIONS IN OFFSPRING OF MERCURY TREATED RATS

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Summary

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This study was conducted to evaluate the effects of different doses of mercury on foetal cerebellum. Twenty adult female rats were divided in four groups. All animals became pregnant by natural mating. Three groups (T1, T2 and T3) were treated with different doses mercuric oxide (50, 100 and 500 μg/kg respectively) during the 10 terminal days of pregnancy. After parturition, the cerebellum was collected from the offspring of all rats. The weight of the neonates and mothers, and the number of foetuses were measured in all groups. Various histological parameters were determined using histomorphological and histomorphometric techniques. Results revealed a decrease in neonatal and maternal body weight compared to control. The thicknesses of the gray and white matter showed a decrease in all test groups. The number of cells in gray matter and white matter reduced in all test groups. The maternal body weight, the thickness of the gray and white matter in group T3, as well as the number of cells in gray and white matter of groups T2 and T3 were significantly lower ($P<0.05$) compared to controls. It was suggested that mercury exposure exhibited deleterious effects on the cerebellum during foetal life, which remained persistent during the post neonatal period.

Key words: cerebellum, mercury, offspring, rat

Ingestion of fish or grain contaminated with mercury resulted in epidemics of severe neurotoxicity and death in Japan in the 1950s and 1960s, and in Iraq in 1972 (Nierenberg *et al.*, 1998). Pollution of soil and water by natural phenomena such as volcanoes and industrial activities, pollution of marine food resources by mercury in the water and preservative compounds of vaccines are possible causes of mercury intoxication (Diez, 2009). Dental amalgam is also another source of mercury exposure for humans (Aronsson *et al.*, 1989) and it appears that MRI and microwave radiation emitted from mobile phones significantly release mercury from dental amalgam restoration (Mortazavi *et al.*, 2008). Fish is the main route of exposure to mercury, which mainly accumulate in large predators (Ramon *et al.*, 2009).

Studies have ascertained that mercury penetrates the placental barrier and accumulates in the foetus after exposure of pregnant animals to mercury (Yoshida,

2002). Some investigations showed that the concentrations of mercury of the umbilical cord blood and placental tissues were higher than those of maternal blood; also, the higher mercury accumulation and susceptibility to toxicity in the foetus than in the mother during the gestation period is well known. Therefore, mercury can be transferred to the foetus via the placenta and secreted to a newborn via milk (Yang *et al.*, 1997; Sakamoto *et al.*, 2002). After utilising the mercury, it crosses the blood brain barrier, which accounts for its accumulation in the CNS and a clinical picture dominated by neurological disturbances (Aschner & Aschner, 1990). The data suggest that the inorganic mercury accumulates in the brain after exposure (Charleston *et al.*, 1996). Robinson *et al.* (2010) suggest that mercury impacts within biological processes during gestational development may underlie teratogenic and neuro-developmental toxicity outcomes.

The sensitivity of the human central nervous system (CNS) to the toxic effects of mercury compounds is well documented. Similar neurotoxic effects have also been successfully demonstrated in mammalian animal models (Kim & Choi, 1995). Numerous studies have been performed to recognize the effects of different mercury compounds on CNS and cerebellar development, as well as the ultrastructural effects of this metal on CNS: mercuric chloride ($HgCl_2$) (Schiønning & Møller-Madsen, 1991; Monnet-Tschudi *et al.,* 1996), methylmercury chloride (CH_3HgCl) (Monnet-Tschudi *et al.*, 1996), mercury vapour (Schiønning *et al.*, 1993; Charleston *et al.,* 1996) and methylmercury (MeHg) (Clewell *et al.*, 1999). It has been demonstrated that the distribution of mercury in the cerebellum after mercury vapour exposure was similar to the distribution pattern after methylmercury exposure (Warfvinge, 2000).

The present study was conducted to evaluate the effects of different doses of mercuric oxide on cerebellar development in offspring of rats and to investigate histomorphometrical alterations in the cerebellum.

Twenty adult female Sprague Dawley rats (217.5±12.1 g; 4–5 months old) were housed in an air-conditioned room (22± 2 °C) and supplied with standard pellet food and tap water *ad libitum*. The animals were cared for and treated in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee.

Animals were divided into four equal groups, three experimental (T1, T2 and T3) and one control group (C). Female animals from the four groups in oestrus stage were caged with a male rat for mating. Mating was confirmed by vaginal plug observation. The body weight of all rats was measured before any experiment. Mercury was introduced in the three experimental groups by intraperitoneal injections of red mercuric oxide (Fluka, Switzerland) on days 10 to 20 of pregnancy. Each day, the animals were injected by doses of 50 µg/kg, 100 µg/kg and 500 µg/kg for groups T1, T2 and T3, respectively. Doses were determined as in previous studies (Schiønning & Møller-Madsen, 1991; Schiønning *et al.*, 1993; Charleston *et al.*, 1996; Clewell *et al.*, 1999).

After parturition, weights of the female rats were measured and weight loss was evaluated. All newborns were collected, their numbers and weights measured and the mean values were retained. Six neonates of each rat were selected, anesthetised with diethyl ether and killed by whole blood collection through a heart

puncture. The cerebellums were then isolated from the offspring of all rats.

All tissue samples were fixed in 5% buffered formalin fixative and subsequently embedded in paraffin. Sections (5 μm thickness) were stained with H&E and Green Masson's trichrome techniques (Luna, 1968). For histomorphological and histomorphometric studies, the sections were observed under a light microscope, and the average of the following parameters were evaluated in the cerebellums of control and tests groups: 1) thickness of gray matter (μm); 2) thickness of white matter (μm); 3) number of cells in the gray matter per mm^2; 4) number of cells in the white matter per mm^2; and 5) gray to white matter ratio.

Thicknesses of the gray matter and the white matter were measured by ocular micrometer and Olympus BX51 light microscope using Olysia software. The number of cells per mm^2 in both white and gray matter and the gray to white matter ratio were counted by ocular graticule and Olympus BX51 light microscope using Olysia software. To study each parameter, 10 random points of each section were observed. Analysis of morphometric data was carried out with Student's *t* test using SPSS software.

Fig. 1 demonstrated the maternal body weight of all groups after the experiment. After parturition, the weight of female rats from group T3 showed a significant decrease ($P<0.05$) compared to pre-experimental values (197.5±11.4 g vs 217.5± 12.1 g), indicating a significant weight loss in group T3 (by 19.6 g) during the experiment. The body weights of groups T1 (212.2±10.7 g) and T2 (205.4±12.5 g) showed only insignificant decrease after parturition. The body weight of control rats (217.5±12.1 g vs 217.9±12.5 g) was the same before and after the experiment.

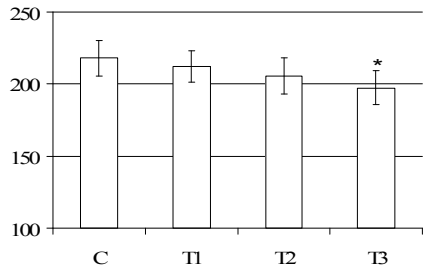

Fig. 1. Maternal body weight (g) of rats treated at 50, 100 and 500 μg/kg mercuric oxide (groups T1, T2, T3) and control rats (group C) after the experiment. The asterisk (*) indicates a significant difference at $P<0.05$ vs controls.

The neonatal body weight of the experimental and control groups is shown on Fig. 2. The mean body weights of the offspring from the test groups (3.55±0.54, 3.27±0.56 and 3.06±0.65 g in groups T1, T2 and T3, respectively) were insignificantly lower compared to the control group (3.92±0.49 g).

The number of neonates in the experimental groups (Fig. 3) was lower than that of controls (9.1±1.5, 8.5±1.4, 7.8±1.2 and 10.7±1.6 in groups T1, T2, T3 and controls, respectively). The reduction was significant in group T3 compared to that in untreated controls.

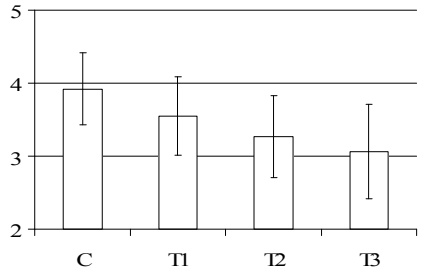

Fig. 2. Body weight of offspring (g) from rats treated at 50, 100 and 500 μg/kg mercuric oxide (groups T1, T2, T3) and control rats (group C) after birth.

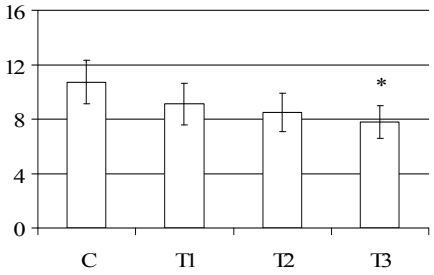

Fig. 3. Number of foetuses from rats treated at 50, 100 and 500 μg/kg mercuric oxide (groups T1, T2, T3) and control rats (group C). The asterisk (*) indicates a significant difference at P<0.05 vs controls.

Table 1 demonstrates different parameters of the cerebellum from the offspring of mothers treated with 3 different mercury doses (groups T1, T2 and T3) and control mothers (group C). The thickness of the gray matter showed a non-significant decrease in group T1 (531.2±24.2 μm), T2 (522.4±23.5 μm) and a significant decrease in group T3 (501.2±23 μm) compared to that of controls (561.1±24.8 μm) after birth. The thickness of the white matter showed a non-significant decrease in group T1 (36.7±3.2 μm), T2 (35.7±3.7 μm) and a significant decrease in group T3 (33.1±2.8 μm) vs control rats (40.2±3.6 μm) after birth. The gray to white matter ratio demonstrated non-significant reduction in groups T1 (14.75±0.82), T2 (14.61±0.79) and T3 (14.53±0.77).

The number of cells in the gray matter (Fig. 4) was slightly lower in group T1 (21131±1499) than in controls (23451± 1534), but groups T2 (20378±1439) and T3 (19661±1425) exhibited a significant (P<0.05) decrease. The number of cells in the white matter was insignificantly decreased in group T1 compared to control (12366±634 in group T1; 13324±691 in controls) but quite lower in groups T2 (11973±603) and T3 (11056±598) (P<0.05).

The maternal body weight of the test groups showed a loss after the trial. This weight loss, which is due to mercury, has been demonstrated previously in numerous studies (Newland & Reile, 1999; Davis *et al.*, 2001; Pan *et al.*, 2005). Body weight loss due to mercury exposure is a typical phenomenon in adult rats and is caused by anorexia (Pan *et al.*, 2005). The alterations in cycle and hormones in high concentrations of exposure to mercury were attributed to body loss and generalised toxicity (Davis *et al.*, 2001). The body weight of neonates has shown a decrease in the test groups compared to the control group. Higher umbilical cord blood concentrations of mercury were associated with reduced birth weight and, to a lesser extent, with reduced birth

Table 1. Thickness of gray (TGM) and white matter (TWM), and gray to white matter ratio (GWR) in control rats (group C) and rats treated at 50, 100 and 500 μg/kg mercuric oxide (groups T1, T2, T3). Values are presented as mean±SD

Groups	C	T1	T2	T3
TGM (μm)	561.10±24.80	531.20±24.20	522.40±23.50	501.20±23.00*
TWM (μm)	40.20±3.60	36.70±3.20	35.70±3.70	33.10±2.80*
GWR	15.03±0.78	14.75±0.82	14.61±0.79	14.53±0.77

The asterisk (*) indicates a significant difference at P<0.05 vs controls.

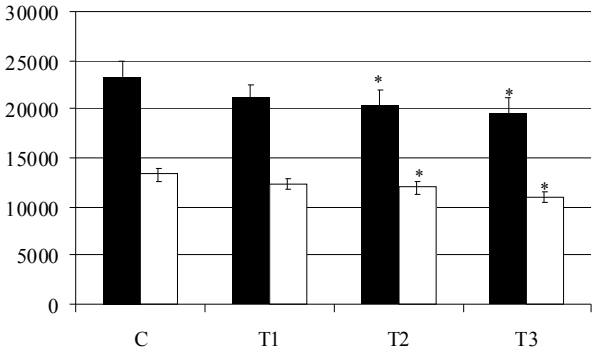

Fig. 4. Number of cells in gray (black bars) and white matter (white bars) in rats treated at 50, 100 and 500 μg/kg mercuric oxide (groups T1, T2, T3) and control rats (group C). The asterisk (*) indicates a significant difference at $P<0.05$ vs controls.

length (Newland & Reile, 1999), although there were no significant effects of mercury exposure on litter size or weight at birth. Higher mercury concentration has also been associated with an increased risk of being born small for gestational age in length (Ramon *et al.*, 2009).

The thicknesses of the gray and white matter were decreased in the experimental groups on account of mercury consumption. The gray to white matter ratio was decreased in the treated groups, due to a greater gray matter reduction in relation to the white matter. The number of cells in both the gray and the white matter was lower. Uchino *et al.* (1995) demonstrated that the cerebellar incoordination and cerebellar ataxia were clinically diagnosed with methyl mercury poisoning. Takahata and co-workers (2008) suggested that the nervous tissue in the case of mercury poisoning keeps high concentrations of mercury over a long period, whereas morphophysiologically is normally well retained. A study showed that mercuric chloride intoxication during the prenatal period can induce cell death and results in neural tube deficits in prenatal rats (Rastegar *et al.*, 2010). It has been shown that a mercuric compound at low concentrations was able to induce significant cellular toxicity in human neuron and foetal cells (Geier *et al.*, 2009). Mercury exposure may be responsible for the changes within the astrocyte and microglial populations (Charleston *et al.*, 1996). Issa and co-workers (2008) reported cytotoxic effects to human oligodendroglial cells.

The results of Brawer *et al.* (1998) indicated that mercuric chloride exposure initiated a constellation of changes in mitochondrial structure that typify the response of these cells to oxidative stress. It has been demonstrated that mercury induced generation of reactive oxygen species in the astrocytes and caused neurotoxic damage (Shanker *et al.*, 2004). Mercury within the motor neuron perikaryon therefore leads to increased oxidative damage to DNA and motor neurons damage (Pamphlett *et al.* 1998). These radicals contribute to increased neuronal death by oxidizing proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes (Hawkins & Davies, 2001). An investigation by Issa *et al.* (2003) indicates that $HgCl_2$ is toxic at low concentrations for oligodendroglial cells

and that this cell dies in an apoptotic manner when exposed to low concentrations of $HgCl_2$.

It has been suggested that methyl mercury accumulates in neuronal cytoplasm of cerebellar Purkinje cells, in addition cerebellar neurons accumulate more methyl mercury and at a faster rate than their companion astrocytes (Morken *et al.*, 2005). Eto (1997) has determined that the characteristic cerebellar alteration caused by methyl mercury poisoning was loss of granule cells with the presence of relatively well-preserved Purkinje cells. The lesions occurred in relatively deeper portions of the cerebellar hemisphere. One study (Schiønning & Møller-Madsen, 1991) has demonstrated that ultrastructurally, mercury deposits were exclusively located in lysosomes of neurons, astrocytes, endothelial cells and ependymal cells.

We conclude that the rat foetal cerebellum may be affected by exposure of pregnant mothers to mercury, which causes reduction in the thicknesses of cerebral white and gray matter, white and gray matter cell numbers, and the gray to white matter ratio. These alterations may remain after birth and affect the cerebral essential activities.

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