Prenatal Mercuric Chloride Exposure Causes Developmental Deficits in Rat Cortex

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Introduction: Environmental pollution with heavy metals such as mercury is a major health problem. Growing studies on the field have shown the deleterious effects of mercury on human and nonhuman nervous system, especially in infants, however the effects of prenatal exposure to mercuric chloride on cortical development are not yet well understood. The aim of this study was to investigate the effect of prenatal exposure to mercuric chloride on morphological characteristics of brain cortex.

Methods: Mercuric chloride (2 mg/kg) or normal saline were injected (I.P.) to 36 Sprague – dawley rats in the 8th, 9th or 10th day of gestation. The embryos were surgically removed in the 15th day of gestation, and brain cortices were studied by histological techniques.

Results: Histological studies showed that embryos of mercuric chloride treated rats had cortical neuronal disarrangement with different orientations of nuclei, increased diameter of cortex, increased mitosis of cells, increased cell death, decreased cellular density and increased intracellular space.

Conclusion: These findings suggest some micro structural abnormalities in cortical regions after prenatal exposure to mercuric chloride. These structural abnormalities may underliesome neurologic disturbances following mercury intoxication.

Key Words:
Mercuric Chloride, Prenatal Period, Brain Cortex

1. Introduction

ntoxication with heavy metals such as lead, cadmium, and mercury is a major health problem (L. Jarup, 2003). Mercuric compounds may enter the human body through different routes. Some reports have shown the contamination of foods (e.g. sea foods) and environment with mercury compounds. Mercury is present in some commonly used materials such as amalgam and has being added as preservatives in vaccines and some cosmetic creams, some antiseptic and antifungal agents (N. Rustagi and R. Singh, 2010).

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• The authors have no any financial interest regarding the statements of this paper.
Mercury is shown to have deleterious effects on many organs in the body, such as kidneys, thyroid gland, and pancreas. It also affects respiratory, reproductive, immune, and nervous systems (T. W. Clarkson, 1997; W. Crinnion, 2000; M. Boscolo et al., 2009; S. Diez, 2009; K. Mahour and P. N. Saxena, 2009). Mercury can induce apoptosis and cell death in affected organs (G. Olivieri et al., 2000; I. S. Reus et al., 2003; R. K. Monroe and S. W. Halvorsen, 2006; V. Singh et al., 2007; M. B. Wolf and J. W. Baynes, 2007; A. Stacchiotti et al., 2009).

Neurologic and neurobehavioral disturbances caused by mercury compounds have gained more attention in recent decades. Some mercuric compounds can pass through placenta and blood brain barrier and affect the developing central nervous system of embryos (H. Satoh and T. Suzuki, 1983; T. Suzuki et al., 1984; R. Kishi et al., 1994; M. Sakamoto et al., 2004). The prenatal exposure to mercuric chloride has shown to have teratogenic effects (H. Satoh and T. Suzuki, 1983; M. Yoshida, 2002). It can cause structural abnormalities in the neural tube and spinal cord of embryos in rats (T. Rastegar et al., 2010). Studies have suggested a link between prenatal mercury exposure and neurobehavioral disturbances (R. Kishi et al., 1994; M. C. Newland et al., 1996).

Prenatal development of brain cortex is a very important process in mammals and any disturbances caused by insults or intoxications can have deleterious consequences later in life (H. Satoh, 2003). However, the effects of prenatal exposure of mercuric chloride on cortical development are not yet well understood. The aim of this study was to investigate the effects of prenatal mercuric chloride intoxication on morphological characteristics of brain cortex of rat.

2. Material and Methods

Thirty six adult female Sprague-dawley rats (weight 200-270 gr.) were used in this study. Following the formation of vaginal mating plaque (zero day of gestation), rats were selected, weighted and divided into six groups (n=6); three control groups (Cont-1, Cont-2 and Cont-3) and three experimental groups (Exp-1, Exp-2 and Exp-3). Rats maintained in 12 hours dark/light cycle with free access to food and water and 23-24 degree temperature. Numbers of three rats were kept in each clear polycarbonate cage with sawdust bedding. Experimental groups (Exp. 1-3) were injected intraperitoneally (I.P.) with 2mg/kg of mercuric chloride at 8th, 9th, and 10th days of gestation, respectively besides the, control groups (Cont-1-3) were injected with normal saline (1ml/kg, I.P.) on the same days, respectively.

On the 15th day of gestation, rats anesthetized with ketamine (100 mg/kg, I.P.) and xylazine (5mg/Kg, I.P.), and embryos were extracted from the uterus by making a flank section.

The embryos were fixed in bouins fixative (6-24 hours); following tissue passage procedures, paraffin embedded blocks were provided; Then 5 µm sagittal sections were prepared using a microtome (Leica) from the brains and mounted on gelatinized slides. The slides stained by Hematoxylin and Eosin method and studied by a light microscope (Olympus, Japan). For morphometric measurements an eye-piece graticule of Olympus microscope was used with graticule length of 100 units (1 unit was equal to 12.5 µm ×3.2 and 4 µm ×10 objective magnification).

![Figure 1. Comparison of telencephalic cortical diameter (A) and cell density (B) between mercuric chloride (EXP1-3) and matched control groups (Cont1-3). ANOVA test and LSD post hock analysis showed increased diameter of cortex and decreased cell density in treated groups (*P < 0.05), [n: 6 rat in each group].](image-url)
Afterwards the diameter of brain cortex between meninges layers and lateral ventricle wall and cortical cell density was measured. The Means were compared with each other by one way ANOVA test, using SPSS (ver.14) software, later the LSD post-hock analysis was done for revealing differences among groups; P values less than 0.05 were considered significant. And finally in microscopic analysis, cellular shapes and arrangement, and their nuclear orientation were studied.

3. Results

This study is the second part of a published project by the same writers therefore the animals are the same (T. Rastegar et al., 2002; T. Rastegar et al., 2010).

To assess the effects of prenatal exposure to Mercuric Chloride on morphometric characteristics of telencephalic cortex, the treated and control groups’ telencephalic cortex were compared with each other. Results showed a significant difference between the groups (Figure 1). Post hoc analysis showed an increase in the diameter of cortex in all mercuric chloride in treated group especially in Exp-2 group (9th day of gestation) \( p < 0.05 \). Also, the mean of cortical cell density between groups showed a significant difference \( p < 0.05 \); LSD post hoc analysis showed a decrease in cell density of telencephalic cortex in all experimental groups compared with the control group (Figure 1).

Lastly, Microscopic study of telencephalic cortex showed structural disorganization in mercuric chloride treated groups comparing with control groups. In control groups the nuclear orientations were regular and vertical to ventricular lumen; few mitotic cells and normal extracellular space were seen. But in experimental groups nuclei were irregular and positioned in different orientations, more mitotic cells were seen and extracellular space was increased (Figures 2-4).

4. Discussion

Results of the present study showed that mercuric chloride have opposing effects on developing brain cortex in rat. Prenatal exposure to mercuric chloride resulted in cortical disorganization, decrease in cell density with more mitotic cells, and growth in cortical diameter with increased extracellular space; this may indicate an apoptotic process in the telencephalic cortex.

Developing brain cortex is a very sensitive structure and is vulnerable to devastating effects of many environmental factors—biological and nutritional—exposure to heavy metals like mercury and lead (T. Schettler, 2001). Aberrant cortical development is associated with many neuropsychological disorders such as attention deficits, learning disabilities, autism disorders, mental retardation and schizophrenia (T. Schettler, 2001). Increasing prevalence of developmental brain disorders is a major concern and more studies are needed to reveal the causes and underlying pathophysiologic mechanisms.

Neurotoxic effects of mercury are shown in both in vitro and in vivo studies in human and animals and previous researches have shown the effects of intoxication with mercury compounds on behavioral, neurological and psychological functions. Mercury compounds can induce cerebellar degeneration, sensorimotor and gating disturbances, tremor, ataxia, and depression (M. Berlin et al., 1969; J. A. Hughes and Z. Annau, 1976; R. Kishi...
et al., 1994; T. Yamashita, et al, 1997; G. J. Myers and P. W. Davidson, 1998; S. Diez, 2009; A. Nahavadi and A. Shahbazi, 2010). The findings of the present study also indicate the teratogenic effects of mercuric chloride on rat embryos. In our previous study we showed that mercuric chloride can induce cell death and neural tube deficits in rat embryos (T. Rastegar et al., 2010), Here, we demonstrated a significant impairment in cortical organization of telencephalic cortex; and by the research we found that Cortical disorganization correlate with cognitive and neuropsychological deficits in other psychiatric disorders.

At the end this study would say that a cortical structural abnormality in subjects maternally exposed to mercury compounds, which could explain the neurobehavioral abnormality associated with them.

Decreasing in cell density and increasing of the number of apoptotic cells in telencephalic cortical regions indicate the presence of an apoptotic process attributed to this neurotoxic effect of mercuric chloride. It also shows that mercury can induce cell death by oxidative processes, mitochondrial dysfunction, and impairment of cell membrane integrity. However, finding the exact mechanisms of cell injury by mercuric chloride in cortical neuroblasts needs further studies.

In conclusion, the findings of this study showed the teratogenic effects of mercuric chloride on telencephalic cortex of maternally exposed rat embryos which may explain the neurobehavioral disturbances accompanied with mercury exposure. Furthermore, it suggests that, precautions should be taken in exposure of pregnant women and children to mercuric compounds.
References


