Journal of Neuroscience and Behavioural Health Vol. 3(7), pp. 87-90, July 2011
Available online http://www.academicjournals.org/JNBH
ISSN 2141-2286 ©2011 Academic Journals

Full Length Research Paper

Some effects of manganese dichloride administration on the body weight, Purkinje cell population, brain, and cerebellar weights of adult Wistar rats

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Accepted 20 June, 2011

Some effects of manganese dichloride an essential trace metal that is required for bone formation, brain development and metabolism of carbohydrate on the body weight, brain and cerebellar weights were investigated in adult Wistar rats. Twenty eight adult Wistar rats of both sexes weighing between 150 to 200 g were separated into four groups including controls, each containing seven rats. Treated rats in Groups A, B and C received $MnCl_2$ orally at a doses of 5, 15 and 25 mg/kg body weight respectively for 21 consecutive days. The rats in Group D (control) received volumes of distilled water equal to those in the treated groups. The rats were sacrificed by cervical dislocation on the 26th day of the treatment. The cerebellum of each rat was removed and-weighed before fixing in 10% formol saline for routine histological procedures. The body weights of the Wistar rats decreased significantly ($p < 0.05$) in the treated Groups B and C compared with the controls. The body weight of the treated Group A rats showed a nominal decrease that was not- statistically significant. The cerebellar Purkinje cell population decreased significantly ($p < 0.05$) for all treatment groups. Similarly, the total brain and cerebellar weight decreased significantly ($p < 0.05$) in Groups B and C compared with the controls; and Group A also demonstrated a statistically decrease in the total brain weights but not in the cerebellar weights. The observed decrease in cerebellar Purkinje cell population, the total brain and cerebellar weights suggest that brain functions may be adversely affected.

Key words: Manganese, brain, cerebellum, Purkinje cells, weight, decrease.

INTRODUCTION

Manganese is an essential trace metal that is involved in bone formation, brain development and in the metabolism of carbohydrates, cholesterol and amino acids (Keen, 1984). It is typically expected to enter the body either by ingestion or by inhalation. Mn^{2+} is thought to move by neuronal transport to the pallidum, thalamic nuclei and substantial nigra which are areas involved with motor control and movement (Aschner, 2005). At normal plasma level, Mn^{2+} enters the brain mainly across the capillary epithelium, at elevated level of Mn^{2+} in the blood, transport across the choroid plexus becomes more prominent (Aschner, 2005). Manganese exposure via the pulmonary route leads to more rapid absorption with higher efficiency and with greater transfer to the brain compared with other routes (Drown, 1986; Roels, 1997). Excessive exposure of humans to manganese by inhalation can result in a characteristic neurologic and psychological disorder called manganism (Lucchini, 1991). The brain is particularly susceptible to damage from high levels of manganese and accumulation associated with manganism is characterized by both central nervous system (CNS) abnormalities and neurobehavioral abnormalities (Santamaria et al., 2007). The cerebellum is the largest part of the hindbrain. It is made up of outer gray matter (cerebellar cortex) and an inner white matter (Singh, 1998). The cerebellar cortex is made up of neurons or cells arranged in three layers (Ganong, 2005). The molecular layer is the outermost layer, the granular layer is the innermost layer, while the Purkinje cell layer consisting of the Purkinje cells is

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Table 1. Mean ± SEM of the body weights of Wistar rats before and during the treatment.

Period (weeks)	Control group D (n = 7)	Treated group		
		Group A (n = 7)	Group B (n =7)	Group C (n = 7)
Zero	200.2 ± 26.1	152.4 ± 9.8	171.2 ± 10.9	215.40 ± 22.1
1st	219.8 ± 27.0	157.8 ± 9.7	173.0 ± 8.6	199.30 ± 19.7
2nd	234.1 ± 28.3	168.9 ± 11.2	160.4 ± 9.2	190.30 ± 17.0
3rd	282.0 ± 25.9	170.5 ± 9.1	153.1 ± 9.8*	179.90 ± 15.5*

*Values significantly different from corresponding control ($p < 0.05$).

sandwiched between the molecular and granular layers.

This paper focuses on the effect of manganese on the body weight, Purkinje cell population, brain and cerebellar weights in adults Wistar rats.

MATERIALS AND METHODS

Twenty eight adult Wistar rats of both sexes weighing between 150 and 200 g were used for this study. The rats were fed daily with normal rat chow purchased from Bovajay from mill (Ogbomoso, Nigeria) and water was given to the rats *ad libitum.* All the rats were routinely inspected, screened and confirmed to be healthy during the period of acclimatization. The animals were treated in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (1985).

The rats were then separated randomly into four groups with each group containing seven rats. Group D served as the control and the rats received distilled water as contained in experimental doses during the period. Groups A, B and C received oral doses of $MnCl_2$ dissolved in distilled water at 5, 15, and 25 mg/kg body weight respectively for 21 consecutive days. The rats were sacrificed by cervical dislocation on the 26th day of the treatment. The cerebellum of each rat was carefully dissected out after sacrifice -weighed and fixed in 10% formol saline for routine tissue processing by light microscopy. Serial sections of 7 µm thick were obtained using a rotatory microtome and the sections were subjected to histological staining following the methods of Carleton (1967).

The body weights of the rats were taken before and during the treatment in order to monitor changes in their weights.

Cerebellar Purkinje cells were counted using a microscope containing a graduated ocular micrometer inserted to its eye piece. The population of Purkinje cells was determined by counting the cells found along the length of the monolayer of Purkinje cells at several microscopic fields.

RESULTS

The body weights, brain and cerebellar weights were found to be smaller in the treated rats compared with the controls as shown in Table 1 and 2.

Results in Table 1 demonstrate that the body weights of manganese treated rats in Groups B and C were significantly reduced ($p < 0.05$) compared with controls after 3 weeks of treatment. The rat weights were assessed after the rats have been given adequate care.

Results shown in Table 2 indicate that the total brain weights at all doses were significantly reduced ($p < 0.05$). Similarly, data in Table 2 shows significantly reduced ($p < 0.05$) population of the cerebellar Purkinje cells in the manganese -treated rats.

DISCUSSION

Brain weight, cerebellar and body weights

The results obtained from the brain and body weights that were measured showed significant differences between the manganese- treated rats and controls. The effects seemed to be dose and duration dependent in relation to the body and cerebellar weights. For instance, body weights were not significantly different statistically until Week 3 of the treatment indicating a typical repeated dose requirement at these levels. Also the absence of statistical significance in the low dose group for both body and cerebellar weights indicates a dose-response relationship. Furthermore, the Purkinje cell population parameter appears to be the sensitive indicator of a dose-response relationship. Adverse effects from exposure to manganese have been shown in some studies. Atrophy of the cerebellar cortex was previously observed in a monkey subjected to inhalation of MnO_2 (VanBogaert and Dallemagne, 1945). Extensive loss of neurons in the cerebrum, brainstem and cerebellum had been reported following manganese administration (Pentschew et al., 1963). Body weight decreased significantly in pups that were exposed to high doses of manganese in drinking water from conception until post-natal day 30 (Pappas et al., 1997). Similarly, significant decrease in maternal weight had been reported by Kontur and Fetcher (1985) following exposure to manganese chloride in drinking water. Exposure of some Chilean miners to manganese concentrations in air has resulted in neurological disorders (Ansola et al., 1994). Brain atrophy occurred over the vertex and lateral aspects in a patient following manganese exposure who subsequently died - 14 years after the onset of the symptoms (Canavan and Drinker, 1934). Accumulation of manganese has occurred in critical areas of the brain following exposure to excess manganese which resulted in changes in brain chemistry and neuronal degeneration in mice and rats

Table 2. Mean ± SEM of brain, cerebellar and relative cerebellar weights number(s) of intact Purkinje cells per unit area in the Purkinje cell layer (/μm^2) mag × 40 at the end of treatment.

Rat groups (7 rats in each group)	Brain parameters(grams)		Relative cerebellar weight (%)	Mean ± SEM Purkinje cells number(s) per unit area in the Purkinje cell layer (/μm^2)
	Average total brain weight	Average cerebellar weight		
Control group D	1.58 ± 0.03	0.32 ± 0.01	20.4 ± 0.7	6.00 ± 2.00
Experiment group A	1.41 ± 0.06*	0.29 ± 0.01	20.4 ± 0.3	3.00 ± 1.00*
Experiment group B	1.42 ± 0.03*	0.24 ± 0.01*	16.9 ± 0.9*	3.00 ±1.00*
Experiment group C	1.49 ± 0.03*	0.20 ± 0.02*	13.2 ± 1.2*	2.00 ±1.00*

Values are expressed as Mean ± S.E.M of 7 rats in each group and analyzed by student's t-test.*Values significantly different from corresponding control ($p< 0.05$).

(Miller et al., 1975; Chandra and Shukla, 1978). As one focuses on the brain as a target organ for manganese, one notes that regulation of brain water content and therefore the volume, is critical for maintaining the inter cranial pressure within tolerable limits (Johanson, 1995). Brain volume is intimately related to water content. And the water content of cells depends directly on the total amount of osmotically active solutes (Johanson, 1995). As brain tissue swells or shrinks as in acute hyponatremia or hypernatremia, the activity of the cellular transporters is a potentially modified by up-or down-regulation (Johanson, 1995). Reduced brain and cerebellar weights in the manganese treated rats might be due to pathologic changes resulting from neurotoxic effect of manganese chloride that caused loss of cellular components and vacuolizations of the brain sections in rats that were treated with $MnCl_2$. This hypothesis is supported by degeneration and loss of cellular components in the cerebellar cortex of Wistar following quinine exposure where it was reported in H and E sections of the cerebellar cortex (Ajibade et al., 2006). Thus chronic exposure of adult Wistar rats to manganese may adversely affect the body weights, as well as total brain and cerebellar weights resulting from pathologic changes of the brain tissue over time, these changes may consequently lead to brain and cerebellar dysfunctions.

Population of Purkinje cells

Extensive cell death in the central nervous system has resulted from neuronal degeneration and neurotoxins have been implicated as prime candidates that induce neuronal degeneration (Waters, 1994).

There was a significant reduction ($p < 0.05$) in the population of the Purkinje cells in manganese-treated rats. The remarkable reduced population of the Purkinje cells in the treated rats might be due to neurotoxic - effect of manganese on the Purkinje cells. A fast Purkinje cell death is reported to be induced by mechanical lesion of the cerebellum (Rossi et al., 1995). Furthermore, degeneration and loss of Purkinje cells in the cerebellar cortex similar to findings in this study were observed in Wistar rats when they were treated with quinine dichloride (Ajibade et al., 2008) Cell degeneration occurs by necrosis and apoptosis. Necrosis affects extensive cell population which involves cytoplasmic swelling, while apoptosis is an organized form of self destruction that is characterized by cell shrinkage (Whyllie et al., 1980). Apoptotic cell death could be induced by cytotoxic drugs or physical (example, mechanical) stimulation (Cinthya and Rafael, 2004). While either of these mechanisms is possible, the Purkinje and Granule cells have been identified as the most important targets in the cerebellum for toxic substances (Fonnum and Lock, 2000), which suggests a common link between the two. We hypothesize that:

The marked reduction in the brain and cerebellar weights of the manganese –treated rats may have resulted from neuronal degeneration and loss which became evident in Purkinje cells that were enumerated during the study. There some Limitations in this study compared to other studies in addition to extrapolation to human health. For instance the agent here is $MnCl_2$, a fairly soluble form as opposed to a manganese metal oxide (MnO_X) common in human inhalation exposure. Also these were oral dosing regimens which may not reflect occupational exposure where inhalation is the primary route. Despite these limitations, this study strongly suggests that chronic exposure to manganese at sufficient doses may adversely affect

the body weight, brain and cerebellar weight with reduced Purkinje cell population -in Wistar rats, which may subsequently affect brain function. In addition the Purkinje cell population appears to be the most sensitive of these parameters in assessing possible adverse effect from manganese.

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