EFFECT OF METHYL MERCURY INDUCED FREE RADICAL STRESS ON NUCLEIC ACIDS AND PROTEIN: IMPLICATIONS ON COGNITIVE AND MOTOR FUNCTIONS

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ABSTRACT

Mercury pollution and acute neurotoxicity of mercury is well known. The recent reports suggest the adverse effect of low dose mercury, though the available literature is still silent on its mechanism. This study was therefore undertaken to probe the effect of low dose methyl mercury induced heavy metal toxicity on free radical stress and its impact on behaviour of male albino rats. Male albino rats were exposed to 1mg/kg body wt of methylmercury chloride for seven days, on day 8 they were tested for motor and memory functions. They were sacrificed later for biochemical estimations for rate of lipid peroxidation, nucleic acids, proteins in cerebrum, cerebellum and brain stem. There was an increase in the rate of lipid peroxidation showing methyl mercury induced free radical stress. The motor and memory functions demonstrated a clear decline, besides there was a lowering in the levels of nucleic acids and proteins as compared to controls. The results are important in view of recent reports that methyl mercury induced free radical stress results in early ageing and may serve as an initiating factor more specifically for neurodegenerative disorders like Alzheimer's disease and dementias. The current findings support the notion that incorporating dietary antioxidants like curcumin, ascorbic acid and α -tocopherol in routine diet from early age may help combat the risk of developing such disorders in ensuing years.

KEY WORDS

Low dose, methyl mercury, Nucleic acids, Free radical stress, Behaviour

INTRODUCTION

Mercury is a well-known neurotoxin. During the last decade, research towards low dose mercury toxicity has come to centre stage as various groups have demonstrated neurotoxic impact of low dose of mercury on fetus (1), children (2) and adults (3). Multiple pathways of Mercury through air (4), water (5), food (6), cosmetic products (7), and even vaccines (8) expose man to the toxicant. Populations with traditionally high dietary intake of food originating from fresh or marine environment have highest dietary exposure to mercury; for instance polar Eskimos (6). Vegetables which accumulate mercury include cabbage (*Brassica oleracea*), amaranthus (*Amaranthus oleraceus*) (9), water spinach (*Ipomoea aquatica* Forsk) (10), edible mushroom (*Boletus pinicola*) (11). Besides, it is an

occupational hazard for dental staff (12), chloralkali factory workers (13) and goldminers (14).

Mercury has been found to be a causative agent of various disorders including neurological, nephrological, immunological, cardiovascular, musculoskeletal, reproductive and even genetic. The available literature on the net has though suggestively tried to correlate the impact of low dose of mercury toxicity on various organ systems (15) but is largely silent on the mechanism of action. Organic mercury is considered to be more toxic as it can pass blood brain barrier. The present experiment was therefore designed to study the effect of low dose of methylmercury toxicity on motor and memory functions of rat, and its relationship to lipid peroxidation and its impact on nucleic acids (DNA and RNA) and protein.

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MATERIALS AND METHODS

The necessary permission from institutional ethics committee, JN Medical College, AMU was obtained prior to conducting

experiments for using male albino rats, experiment was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub. 82-23, rev. 1985). Male albino rats weighing 150 ± 20 gm from Central Animal House, JN Medical College were used throughout the study. Animals were housed in air-conditioned room and had free access to pellet diet Hindustan Lever Ltd., Mumbai, India and water ad libitum. Two groups comprising six animals each were used. Animals from Group I served as control while, Group II animals were given methyl mercury chloride (CH_3HgCl) 1 mg/kg body weight. Methyl mercury chloride was purchased from Fluka Co, USA. All other chemicals used were of analytical grade. All groups were treated once a day each for seven days intraperitoneally. On eighth day the animals were tested for Spontaneous Alteration Behaviour and Rota rod tests. The animals were sacrificed later on the same day by cervical dislocation and immediately the brains were taken out on ice and separated into cerebrum, cerebellum and brain stem. The harvested brain parts were later processed for the biochemical assays of lipid peroxidation, nucleic acids isolation, DNA, RNA and protein estimation.

Biochemical Assays

Brain parts- cerebellum, cerebrum and brain stem were separated and homogenized (10%w/v) in chilled 0.15 M KCl. Homogenate was used for the estimation of amount of malondialdehyde formed per 30 minutes during lipid peroxidation by the method of Utely et al (16). Nucleic acids were isolated by using method of Searchy and Macinnis (17). Different brain regions were weighed and homogenized in 5.0 ml of 0.5N perchloric acid. The homogenates were heated at 90°C in boiling water bath for 10 min., cooled and centrifuged at 3,000 rpm for 10 min. Supernatants were taken in graduated test tubes and the volume was maintained upto 5.0 ml. with 0.5 N perchloric acid. This extract was used in the estimation of DNA (18) and RNA (19). Protein level was also estimated in homogenate by Lowry et al (20).

Behavioural Experiments

Spontaneous Alteration Behaviour

The measure of spatial working memory was taken as percent alteration as previously used by King and Arendash (21). The test is particularly useful as an initial test of memory function in mice. A Y-shaped three-arm maze was selected, with each arm 25 cm long, 14 cm high, 5 cm wide and positioned at equal angles. Rats were placed at the end of one arm and allowed to move freely through the maze during a 5-minute session.

One alteration was counted when the rat successfully traversed all three arms of the Y-maze. The percentage alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two), multiplied by 100. Success in this test is indicated by a high rate of alternation in the control groups indicating that the animals can remember which arm was entered last. Data were analyzed to determine the number of arm entries without repetition.

Rota Rod Test

The rota rod was rotated at 10 rpm and the animal was placed at the rotating rod. The time was noticed till the animal was able balance itself on the rod. The maximum allocated time for observation was slotted at 300 seconds i.e., 5 minutes. The activity was repeated six times after a gap of 10 minute for each rat taken.

Statistical analysis

The level of significance between different groups was based on Student's t-test. The level of significance was chosen as $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

The effect of methyl mercury on the rate of lipid peroxidation (LPO), levels of DNA, RNA and protein in different regions of rat brain has been summarized in Table 1. Increase in the rate of lipid peroxidation (in all brain parts) was observed in methyl mercury treated rats as compared to controls. High TBARS (thiobarbituric acid reactive substances) in neuronal preparations of methyl mercury intoxicated rats indicate free radical stress on exposure to lower dose in our study. Although mercury induced increase in the rate of formation of Reactive Oxygen Species (ROS) has also been reported earlier at higher doses (22).

Nucleic acids (DNA /RNA) and proteins are molecules central to all life processes. Decrease in DNA as well as RNA content was observed in all brain parts of methyl mercury intoxicated rats, highest being in cerebellum (Table 1). This decrease in nucleic acid content may be attributed to free radical damage to DNA and inhibition of RNA synthesis by direct interaction of ROS. Though inhibition of DNA and RNA synthesis by mercury compounds has been reported earlier *in vitro* (23), to our knowledge this is the first study reporting lowering of DNA and RNA levels on short term low dose exposure of methyl mercury *in vivo*.

TABLE 1

Effect of Methyl mercury chloride (1 mg/kgb.wt i.p. for seven days) on lipid peroxidation (LPO), DNA, RNA and Protein in different regions of rat brain.

BRAIN PARTS	CONTROL #	ACTIVITY/ CONCENTRATION IN METHYL MERCURY INTOXICATED RATS #
Cerebrum		
LPO	3.90±0.025	4.92±0.013 (24.55%)*
DNA	5.27 ± 0.079	3.25±0.087(44.34%)*
RNA	4.47±0.009	3.13±0.026(29.97%)*
PROTEIN	112.4±0.23	86.02±0.281(23.46%)*
Cerebellum		
LPO	3.53±0.09	4.35±0.042 (23.22%)**
DNA	5.84±0.19	3.13±0.042(40.6%)*
RNA	4.51±0.076	3.14±0.0202 (30.37%)*
PROTEIN	115.5±0.343	86.1±0.258(25.45%)*
Brain stem		
LPO	2.84±0.013	3.42±0.012(20.42)**
DNA	4.79±0.09	2.9±0.027(39.45%)**
RNA	4.11±0.168	2.93±0.01(28.71%)*
PROTEIN	98.72±0.29	80.1±0.233(18.86%)*

Values are mean ± SE of six animals.

Figures in parentheses indicate change compared to controls.

* p < 0.05

** p < 0.01

LPO (n moles malondialdehyde/30 min)

DNA/RNA/PROTEIN Values expressed as mg/g fresh tissue, are mean of ± SE of six animals

In the present study, alongwith decrease in RNA, methyl mercury intoxication leads to a significant decrease in protein concentrations as well (refer to Table-1). This suggests that besides directly affecting proteins through oxyradical stress, methyl mercury also indirectly inhibits protein synthesis in brain on short term low dose exposure. There are various theories which attribute methyl mercury inhibition of protein synthesis to direct interaction of protein synthetic machinery (24), inactivation of RNA polymerase I by binding to mercury (25) or partially due to autoregulatory depression in tubulin synthesis (26).

A molecular lesion like the one seen when mercury vapour inhibits tubulin polymerization into microtubules has also been observed in 80% brains from patients with Alzheimer's disease (27). Thus, methyl mercury is also implicated in neurobehavioral dysfunction. We have also performed the spontaneous alteration behaviour and Rotarod tests for memory and motor function respectively in methyl mercury intoxicated rats (Tables 2 and 3). A decline in memory function by 75.98% was observed while 67% decrease was observed in motor function. The results obtained are in accordance with

TABLE 2

Effect of methyl mercury (1mg/kg body wt) on memory function of albino rats for seven days as assessed by Y-maze test

Test group	Alterations / 5 min #
Control	4.83 ± 0.16
Methyl mercury	1.16 ± 0.33* (75.98%)

Values given are mean ± SE of six animals.

Figures in parentheses indicate change compared to control

*p < 0.05.

TABLE 3

Effect of methyl mercury (1mg/kg body wt) on motor function of albino rats for seven days as assessed by rota rod test.

Test group	Error #
Control	2
Methyl mercury	6* (67%)

Values expressed are errors taken by rats on Rota rod at 10 rpm before completion of 300 seconds.

Values given are mean ± SE of six animals.

Figures in parentheses indicate change compared to control.

previous studies that report methyl mercury at higher doses impaired short term and spatial memory loss along with deficit in motor function (28).

Increased lipid peroxidation indicates an increase in ROS that may lead to oxidative damage to DNA, RNA and proteins in methyl mercury intoxicated rats. From the biochemical and behavioural studies it may be concluded that short term low dose mercury exposure exerts its toxicity in a manner similar to that found in early disease progression of prominent neurodegenerative disorders, ultimately leading to poor performance on tested behavioural indices corresponding to motor and memory functions. The results are important in view of recent reports that methyl mercury induced free radical stress results in early ageing and may serve as initiating factor more specifically for neurodegenerative disorders like Alzheimer disease and dementias (15). The current findings support the notion that incorporating dietary antioxidants like curcumin, ascorbic acid and α -tocopherol in routine diet from early age may help combat the risk of developing such disorders in ensuing years.

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