THE MECHANISM OF MANGANESE INDUCED LEARNING DISABILITY

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SUMMARY: To determine the mechanism by which manganese induces learning defect in human and animals, we carried out an experimental study using two months old female rats which were exposed daily 357 µg of manganese for 30 days. Manganese induced a significant increase in the cholesterol of blood and brain tissues. An obvious elevation in hippocampal cholesterol was associated with an impaired learning in rats exposed to manganese. Preventing the cholesterol biosynthesis with a drug which inhibits the biosynthesis of cholesterol, the manganese induced learning disability corrected completely. This correction led us to conclude that manganese by increasing hippocampal cholesterol levels impairs learning ability.

Key Words: Manganese, learning ability, cholesterol.

INTRODUCTION

It is well known that neuronal activity is very sensitive to changes in membrane cholesterol (2). Electrophysiological activity can be shut-off completely by massive incorporation of cholesterol and its extraction from the membrane of suppressed neurons can revive their activity to almost maximal capacity (14). The results of Kessler *et al.* (7) and Kessler and Yehuda (8) reporting a close relation between hippocampal cholesterol and learning supported the role of cholesterol in learning ability.

It has been shown that many factors influencing on hippocampal cholesterol content change learning capacity both in human and animals (4, 7, 8, 10, 15, 17, 18). Some enzymes which regulate the biosynthesis of cholesterol are manganese dependent. This manganese dependency results in hypercholesterolemia during high intake of manganese (6). Neurological and behavioral disturbances are also associated with hypermagnesemia in clinics (11). It has been shown that in children fed with infant formulas disable learning (13). Experimentally manganese induced impaired learning capacity supports the role of manganese in brain function. However the mechanism by which manganese - produces learning disability is unknown.

Taking into consideration the above mentioned reports, manganese by increasing cholesterol biosynthesis both in brain and peripheral tissues may disturb the learning ability. Owing to the lack of information about this topic in the literature. The effect of increased hippocampal cholesterol on manganese induced learning disability and its correction by an inhibitor of cholesterol biosynthesis were investigated experimentally in the present study.

MATERIAL METHODS

Two month old female albino rats receiving normal rat diet and tap water for 30 days were used in this experimental study. 10 animals which received no treatment except daily 1 ml distilled water intra-gastrically used as a control group whereas 10 animals in the second group were treated with 357 µg/kg of manganese as $MnCl_2 4H_2O$ in 1 ml of distilled water daily during this period. The rats of the third group received only 285.7 µg/kg mevinolin (a competitive inhibitor of 3-hydroxy-3 methylglutaryl coenzyme A reductase which is a rate limiting enzyme in cholesterol biosynthesis) in 1 ml of distilled water daily during experimental period. The coadministration of mevinolin with manganese were studied in rats of group four.

Daily food intake, water consumption and weekly changes in body weight were recorded. On day 14 and 29 of feeding; period all animals fasted for 16 hrs were exposed to a T-maze and given them a chance to get familiar with this maze and to find the food placed at the exit of the maze for two hours.

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24 hours later on 15th and 30th days (to allow for memory consolidation) the fasted animals were again placed into T-maze and time required to reach the exit and get the food was recorded. The length of reaching time was measured using a stop watch by the same observer who had no information about the treatment sort of rats. The mean of four records was accepted the index of learning ability or memory consolidation of each rat.

After the maze task all animals were anesthetized with urethane (1 g/kg) intraperitoneally and after taking blood samples. Their brain and liver blood were eliminated by perfusing with ice-cold saline, then they were removed for manganese and cholesterol analysis and were stored at -70°C until use, cholesterol in serum and tissue extract were measured by enzymatic method using Sclavo Cholest-Cinet kit. Weighed tissues were placed in acid washed test tubes, concentrated HNO_3 was added to all tubes and digested at 65°C for two hours in a water bath. After appropriate dilution with deionized water they were used for manganese determination by Atomic Absorption Spectrophotometry (Hitachi Z-8000) equipped with graphite furnace. Samples were dried 80-120°C for 30 seconds and charred at 500°C for 30 seconds then atomized at 2500°C for 10 seconds. Manganese content of the samples was determined by comparison with standards.

Student's test was used in the evaluation of the results.

RESULTS

During feeding period all animals displayed normal behavior and feeding habit. There was no difference between daily food intake and water consumption of rats in all groups.

The changes of manganese content in serum and tissues

In control rats the mean serum manganese level was 3.54±1.07 μg/L. Administration of Mn via gastric route for 30 days in group 2 significantly increased the serum manganese to 8.29±3.74 μg/L ($p<0.01$). In the animals of third group the mean manganese level decreased to 2.60±1.11 μg/L due to the treatment by 285.7 μg/kg of mevinolin for 30 days. However the addition of this drug on manganese treatment did not prevent the elevation of blood manganese level in the fourth group.

As seen in Table 1, oral manganese treatment caused a significant elevation in manganese content of liver, cerebellum, cerebral cortex and hippocampus. However the accumulation of manganese was obviously higher in hippocampus than the other brain regions. Inhibition of cholesterol biosynthesis by mevinolin caused a mild decline in liver manganese whereas the accumulation of this metal in cerebellum and hippocampus were not influenced by mevinolin treatment. The drug which inhibits the cholesterol biosynthesis did not prevent the accumulation of manganese given orally in the tissues of group 4.

The cholesterol levels of sera and tissues

The mean serum cholesterol level was found to be 65.91±8.28 mg/dl in control rats. Serum cholesterol levels increased significantly with manganese intake and decreased dramatically due to mevinolin treatment ($p<0.01$) but cholesterol lowering effect of mevinolin was not observed in sera of rats receiving both mevinolin and manganese (Table 2). The mean liver cholesterol was

Table 1: The levels of manganese in tissues and sera.

Oral treatment for 30 days	Serum (μg/dL)	Liver (μg/g wet wt)	Brain Cortex (μg/g wet wt)	Cerebellum (μg/g wet wt)	Hippocampus (μg/g wet wt)
Control	3.54±1.07	1.59±0.20	0.46±0.08	0.52±0.06	0.48±0.08
Manganese (357 μg/kg/d)	8.29±3.74	2.17±0.23	0.61±0.17	0.70±0.12	0.82±0.32
Mevinolin (285.7 μg/kg/d)	2.60±1.11	1.14±0.20	0.36±0.09	0.59±0.17	0.55±0.11
Manganese + Mevinolin	9.86±2.12	2.06±0.32	0.76±0.09	0.69±0.17	0.79±0.18

Table 2: The levels of cholesterol in tissues and sera.

Oral treatment for 30 days	Serum (mg/dL)	Liver (mg/g wet wt)	Brain Cortex (mg/g wet wt)	Cerebellum (mg/g wet wt)	Hippocampus (mg/g wet wt)
Control	65.91±8.28	2.80±0.20	18.28±2.53	23.19±5.24	25.40±3.38
Manganese (357 μg/kg/d)	78.41±8.52	3.27±0.37	20.28±2.25	29.48±3.30	29.14±5.13
Mevinolin (285.7 μg/kg/d)	58.25±8.10	2.58±0.30	15.38±1.65	23.42±2.73	25.41±3.38
Manganese + Mevinolin	72.45±8.05	2.29±0.26	15.17±0.97	22.36±0.68	26.69±1.71

found 2.8±0.2 mg/g wet wt in control rats. This value showed an insignificant decline by mevinolin and significant increase with oral manganese treatment ($p<0.01$). The manganese induced cholesterol increase in liver of fourth group was prevented completely by the treatment of mevinolin. The inhibiting of cholesterol synthesis with this drug also caused a decline in the cholesterol content of cerebral cortex, hippocampus and cerebellum. The significant cholesterol increase due to oral manganese intake in brain tissues were prevented completely by coadministered mevinolin.

The changes of learning ability

The time required to reach the exit and find the food was recorded as 35.1±14.7 second on day 15 and 28.7±11.4 second 30th day of experiment. The animals receiving oral manganese showed a marked delay in reaching for food both on day 15 and 30. The length of reaching time was 119.1±23.0 second and 113.3±25.7 second, respectively ($p<0.001$).

The mevinolin alone had no effect on the time required to reach for food. However it corrected completely the manganese induced delay which is accepted as learning disability and memory impairment in this experiment. The time required to reach the food returned to control values and was 36.6±9.6 second 15th day and 30.7±6.0 second on 30th day in rats receiving oral manganese plus mevinolin for 30 days ($p<0.001$).

There was a significant, positive correlation between manganese and cholesterol content of hippocampus as well as learning ability ($r= 0.80$, $p<0.01$).

DISCUSSION

Our previous studies showed that the diet rich in cholesterol impairs the electrophysiological activities of rat brain (16) as well as their learning ability (13).

In the present study, learning ability was found to be significantly affected in rats receiving oral manganese for 30 days. These findings were confirmed by the study of Murthy *et al.* (12) who showed impaired learning ability and memory consolidation in young rats receiving daily high manganese in drinking water. Early reports showing the relation between dementia in man and elevation in brain manganese levels (5,11) also agree with our results which suggest the critical role of manganese in brain functions Magour *et al.* (9) using the similar manganese dose and treatment period found no differences in food intake, water consumption and body weight gain between controls and manganese treated groups throughout the experiment as we did. They explained the defect in learning and memory consolidation in manganese treated rats by the inhibition of brain protein synthesis. However they did not measure the learning ability or memory and brain cholesterol content of their animals. In the present study the correction of manganese induced learning defect in rats treatment with the inhibitor of cholesterol biosynthesis clearly suggests that cholesterol elevation plays important role in the impairment of learning ability. This assumption was also strengthened by the significant correlation between cholesterol content of hippocampus and learning ability of rats. A positive significant correlation between hippocampal cholesterol and learning ability was confirmed by the report of

Kessler *et al.* (7) who showed significant inverse correlation between the cholesterol level of hippocampus and the learning performance of rat.

In this experiment, to imitate the clinical conditions the rats were exposed only five times of daily recommended manganese dose (by WHO). This dose is lower than that used in many experimental studies and learning defect was also observed with this lower dose in 30 days. When taking into consideration continuous exposure to manganese through air, water. In addition habituate of high consumption of food rich in manganese (such as tea, peanut, nut, wheat products). One can easily excess over the daily recommended dose of manganese and the findings which are inattributable to manganese toxicity may appear in clinic. Association with learning disability and high manganese levels in the hair of children fed infant formula (1, 3) supports clinical importance of manganese.

In this experiment as expected mevinolin significantly lowered the level of cholesterol in serum and liver. However it showed no influence on the cholesterol content of brain tissues. The mechanism of its manganese declining effect both in tissues and serum was unexplained and coadministration of mevinolin with manganese failed to decrease the tissues and serum manganese levels, but cholesterol lowering effect as well as its restoration of learning disability persisted.

As a conclusion the results of this experiment strongly suggested that high manganese intake impairs learning by increasing the biosynthesis of peripheral and brain cholesterol.

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