EFFECT OF FLUORIDE ON CENTRAL NERVOUS SYSTEM OF DEVELOPING RATS

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Abstract

Fluoride is one of the most important pollutant in water. In low concentration it is useful for teeth and bones but in higher concentration it causes fluorosis, the Permissible limit of fluoride intake is .... It has been reported that children of fluoride affected areas has less developed I.Q. the present investigation was to study the effect of fluoride on the central nervous system of the developing rat pups. The finding revealed that animals exposed to fluoride showed significant decrease in body weight followed by considerable decrease in the rate of feed and water consumption. Fluoride exposure resulted in marked accumulation of fluoride in the discrete regions of CNS of pups.

In the present investigation fluoride exposure significantly exacerbated the levels of LPO along with the decreased activities of antioxidant enzymes like SOD, CAT, GSH-Px, GST and GSH levels in discrete regions of CNS suggestive of oxidative stress.

KEY WORDS: SOD, CAT, GSH-Px, GST, GSH, LPO, IQ, CNS

INTRODUCTION

Introduction

Fluorine in the environment is therefore found as fluorides which together represent about 0.06–0.09 per cent of the earth’s crust. The average crustal abundance is 300 mg kg⁻¹ (Tebutt, 1983). Fluorides are found at significant levels in a wide variety of minerals, including fluorspar, rock phosphate, cryolite, apatite, mica, hornblende and others (Murray, 1986). Fluorite (CaF₂) is a common fluoride mineral of low solubility occurring in both igneous and sedimentary rocks. Fluoride is commonly associated with volcanic activity and fumarolic gases. Thermal waters, especially those of high pH, are also rich in fluoride (Edmunds and Smedley, 1996). Minerals of commercial importance include cryolite and rock phosphates. The fluoride salt cryolite is used for the production of aluminium (Murray, 1986) and as a pesticide (USEPA, 1996). Rock phosphates are converted into phosphate fertilizers by the removal of up to 4.2 per cent fluoride (Murray, 1986); the removed and purified fluoride (as fluorosilicates) is a source of fluoride that in some countries is added to drinking-water in order to protect against dental caries (Reeves, 1986, 1994).

Many fluoride minerals are known, but fluorite is most common. It is composed of calcium fluoride, with small impurities. The soft, colorful mineral is found worldwide and is common.

Fluoride has beneficial effects on teeth at low concentrations in drinking-water, but excessive exposure to fluoride in drinking-water, or in combination with exposure to fluoride from other sources, can give rise to a number of adverse effects. These range from mild dental fluorosis to crippling skeletal fluorosis as the level and period of exposure increases. Crippling skeletal fluorosis is a significant cause of morbidity in a number of regions of the world. Both national and international groups (USNRC, 1993; IPCS, 2002) have comprehensively reviewed available data on the metabolism and health effects of fluoride in both laboratory animals and humans. The
following is a summary of the conclusions that have been developed by these groups, particularly the IPCS working group on fluorides held in May 2001 (IPCS, 2002).

A number of sub-chronic and chronic studies have been carried out in laboratory animals in which relatively high doses of soluble fluoride were given in drinking-water. In some studies there is uncertainty regarding the actual dose because commercial laboratory animal rations contain variable amounts of fluoride. Dental fluorosis and a range of effects on bone were noted in several studies. A number of other adverse effects have also been reported, including increased hepatic cell size, nephrosis, myocardial mineralization and degeneration of the seminipherous tubules in testis.

A number of epidemiological studies have been carried out to examine other possible adverse outcomes as a consequence of exposure to fluoride, either from drinking-water or as a consequence of occupation. Studies on the association between exposure of mothers to fluoride in drinking-water and adverse pregnancy outcome have shown no increased risk of either spontaneous abortion or congenital malformations. No reasonable evidence of effects on the respiratory, haematopoietic, hepatic or renal systems have emerged from studies of occupationally exposed populations that could be attributed specifically to fluoride exposure. In addition, such studies have failed to produce convincing evidence of genotoxic effects.

The brain is prone to oxidative stress due to the presence of high levels of polyunsaturated fatty acids, relatively low antioxidant capacity, the presence of redox metal ions and high oxygen to fluoride stress.

Hence the present study is aimed to assess (i) fluoride intoxication to mother rats and the effect on the pups during development, (ii) the susceptibility of developing pups to oxidative stress.

Methodology

The pregnant rats were assorted into 2 groups and were given following treatment throughout the study. Group I (n=6), allowed to drink tap water orally and Group II (n=24) allowed to drink 100 ppm F water ad libitum orally during gestation and post gestation.

At the end of 21st postnatal day, pups from each group were randomly pooled, sacrificed and the discrete regions of CNS viz. cerebral cortex, medulla oblongata, cerebellum and spinal cord were separated and used for the determination of fluoride and oxidative stress markers. In addition, the average rate of feed and water consumption (per day) of all mother rats and body weight of all pups on autopsy day was recorded.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>Drinking water</td>
<td>Oral</td>
</tr>
<tr>
<td>Group II (Treatment I)</td>
<td>Drinking water with sodium fluoride @ 100mg/body weight</td>
<td>Oral</td>
</tr>
</tbody>
</table>

Results
Table 1 - Average feed / water consumption by the control and fluoride exposed pregnant (mother) rats

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Treatment</th>
<th>Feed consumption (g feed/day/animal)</th>
<th>Water consumption (ml water/animal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>21.46±0.79</td>
<td>25.84±0.56</td>
</tr>
<tr>
<td>2</td>
<td>Fluoride</td>
<td>17.55±0.46</td>
<td>22.38±0.55</td>
</tr>
</tbody>
</table>

Table 2- Oxidative stress markers in cerebral cortex of developing rat during fluoride exposure

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Group</th>
<th>Fluoride (µg of fluoride /g tissue)</th>
<th>LPO (in moles of MDA/mg tissue)</th>
<th>CAT (µ moles /min /mg protein)</th>
<th>SOD (µ moles /min /mg protein)</th>
<th>GSH-Px(µ moles /min /mg protein)</th>
<th>GST (in moles GSH–CDNB formed /mg protein/min)</th>
<th>GSH(mg of GSH/100g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.66±0.02</td>
<td>1.97±0.02</td>
<td>126.77±4.79</td>
<td>4.86±0.19</td>
<td>3.47±0.06</td>
<td>87.59±2.32</td>
<td>49.69±1.89</td>
</tr>
<tr>
<td>2</td>
<td>Fluoride</td>
<td>2.16±0.02</td>
<td>2.33±0.01</td>
<td>107.69±5.29</td>
<td>3.29±0.07</td>
<td>2.83±0.08</td>
<td>57.26±3.33</td>
<td>35.91±1.62</td>
</tr>
</tbody>
</table>
Table 3- Oxidative stress markers in Medulla oblongata of developing rat during fluoride exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluoride (µg of fluoride /g tissue)</th>
<th>LPO (in moles of MDA/mg tissue)</th>
<th>CAT (µ moles /min /mg protein)</th>
<th>SOD (µ moles /min /mg protein)</th>
<th>GSH-Px(µ moles /min /mg protein)</th>
<th>GST (in moles GSH – CDNB formed /mg protein/min)</th>
<th>GSH (mg of GSH/100g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.44±0.02</td>
<td>1.67±0.04</td>
<td>119.46±5.83</td>
<td>3.66±0.17</td>
<td>2.65±0.09</td>
<td>87.65±2.58</td>
<td>42.55±1.85</td>
</tr>
<tr>
<td>Fluoride</td>
<td>1.99±0.33</td>
<td>2.17±0.07</td>
<td>94.69±2.87</td>
<td>3.14±0.05</td>
<td>2.25±0.08</td>
<td>56.27±2.95</td>
<td>26.88±1.62</td>
</tr>
</tbody>
</table>

Table 4- Oxidative stress markers in Cerebellum of developing rat during fluoride exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluoride (µg of fluoride /g tissue)</th>
<th>LPO (in moles of MDA/mg tissue)</th>
<th>CAT (µ moles /min /mg protein)</th>
<th>SOD (µ moles /min /mg protein)</th>
<th>GSH-Px(µ moles /min /mg protein)</th>
<th>GST (in moles GSH – CDNB formed /mg protein/min)</th>
<th>GSH (mg of GSH/100g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.29±0.11</td>
<td>1.90±0.07</td>
<td>127.21±2.14</td>
<td>3.35±0.14</td>
<td>2.66±0.04</td>
<td>82.95±2.26</td>
<td>42.68±0.48</td>
</tr>
<tr>
<td>Fluoride</td>
<td>1.69±0.15</td>
<td>2.20±0.09</td>
<td>96.23±2.87</td>
<td>2.96±0.16</td>
<td>2.28±0.05</td>
<td>59.70±1.59</td>
<td>27.96±1.75</td>
</tr>
</tbody>
</table>

Table 5- Oxidative stress markers in Spinal cord of developing rat during fluoride exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluoride (µg of fluoride /g tissue)</th>
<th>LPO (in moles of MDA/mg tissue)</th>
<th>CAT (µ moles /min /mg protein)</th>
<th>SOD (µ moles /min /mg protein)</th>
<th>GSH-Px(µ moles /min /mg protein)</th>
<th>GST (in moles GSH – CDNB formed /mg protein/min)</th>
<th>GSH (mg of GSH/100g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.60±0.07</td>
<td>1.20±0.03</td>
<td>126.44±3.15</td>
<td>2.69±0.09</td>
<td>2.27±0.04</td>
<td>69.50±1.93</td>
<td>38.21±1.34</td>
</tr>
<tr>
<td>Fluoride</td>
<td>1.89±0.06</td>
<td>1.65±0.09</td>
<td>96.35±1.25</td>
<td>1.82±0.08</td>
<td>1.77±0.06</td>
<td>43.40±1.41</td>
<td>23.36±1.26</td>
</tr>
</tbody>
</table>

The SOD decreased in comparison to control it was found that the decrease was more in Spinal cord (32%) and Cerebral cortex (32%) followed by Medulla oblongata (14%) and Cerebellum (11%).

The CAT observation in comparison to control it was found that the decrease was more in Cerebellum (24%), Spinal cord (23%) Medulla oblongata (20%) and Cerebral cortex (15%).
The GSH observation in comparison to control it was found that the decrease was more in Spinal cord (38%), Medulla oblongata (36%) Cerebellum (34%) and Cerebral cortex (27%).

The GSH-Px observation in comparison to control it was found that the decrease was more in Spinal cord (22%), Cerebral cortex (18%) Medulla oblongata (15%) and Cerebellum (14%).

The GST was found decreased in comparison to control it was found that the decrease was more in Medulla oblongata (35%), Cerebral cortex (34%) Spinal cord (32%) and Cerebellum (28%).
Discussion

Fluoride is found in earth, water and food materials. The low concentration of fluoride is useful for health and in high concentration it is harmful. The present investigation was conducted to study the effect of fluoride on central nervous system of the developing pups through exposure to pregnant mother (rats).

It is known that fluoride can cross the placenta from the mother’s blood to the developing fetus. However, the theory there is a direct link between fluoride effects and brain cell damage is still controversial due to lack of adequate evidence. In order to determine if there are any adverse effects on the developing brain, especially starting from formation of the embryo due to fluoride exposure to pregnant mother.

Fluoride is known to produce detrimental biochemical and functional changes in the developing human brain. Exposure may commence with fluoride in the maternal blood passing through the placenta to the fetus. (Geeraert F 1986, Guan et al.1986, He H 1989). Fluoride can pass through the blood-brain barrier, and fluoride accumulated in brain tissue might interfere with the metabolism of brain phospholipids, which is related with the degeneration of neurons. The changes in brain phospholipid metabolism could be involved in the pathogenesis of chronic fluorosis. Our stereological study of the fetus brains showed a higher numerical density and nucleus-cytoplasm ratio of brain cortex, hippocampus cones, Purkinje cells, and undifferentiated neuroblasts.

High fluoride levels causes accumulation of large amounts of free radicals and peroxides by inhibiting superoxide dismutase and glutathione peroxidase activities causing cell damage in people living in areas endemic to fluorosis (Singh et al; 2013).

The animals exposed to fluoride showed significant decrease in body weight followed by considerable decrease in the rate of feed and water consumption. This could be attributed to atrophic gastritis and poor gastrointestinal absorption produced by fluoride ingestion, which may contribute to decreased food intake in experimental animals (Das et al.,1994). Results also indicate suppressed appetite and disturbed nutrient digestibility that can eventually lead to excessive breakdown of cellular macromolecules causing weight loss.
The weight loss, degeneration of organs, and altered antioxidant system, which may be prime factor in causing fluoride toxicity.

Fluoride exposure resulted in marked accumulation of fluoride in the discrete regions of CNS of pups. The accumulation of fluoride is natural due to its chronic exposure, as the exposure may commence in the maternal blood passing through the placenta to the fetus and continues during infancy through fluoride containing milk and drinking water. Moreover the immaturity of excretory or enzymatic systems in developing animals may favor the accumulation. The fluoride accumulation was found more in the cerebral cortex followed by Medulla oblongata, Cerebellum and then Spinal cord.

All the parts of the Central nervous system is very important and any stress makes the neurological changes. The medulla oblongata sends signals to the spinal cord and thalamus to control bodily functions. It is responsible for the respiration and circulation throughout the body, and it handles everything from breathing to vomiting. Several arteries like the anterior spinal artery and the posterior inferior cerebellar artery supply this part of the brain with a steady blood flow. It contains myelinated and unmyelinated nerve fibers, also called white and gray matter.

Considering the tasks the medulla oblongata is responsible for, it is easily the most important part of the brain. Any injury or disease affecting this part of the brain can result in paralysis of the opposite side of the body, loss of pain and temperature sensations, loss of the gag reflex and difficulty swallowing.

The medulla oblongata is also responsible for controlling arousal and sleep, and it controls movement. It is located at the base in the brain stem along with the midbrain and the pons. Other areas of the brain stem control other functions, like facial and cranial nerves, and help to transmit signals from the brain to other nerves in the body, primarily, the spinal cord.

The cerebellum is located behind the top part of the brain stem (where the spinal cord meets the brain) and is made of two hemispheres (halves). The cerebellum receives information from the sensory systems, the spinal cord, and other parts of the brain and then regulates motor movements. The cerebellum coordinates voluntary movements such as posture, balance, coordination, and speech, resulting in smooth and balanced muscular activity. It is also important for learning motor behaviors. It is a relatively small portion of the brain and about ten percent of the total weight, but it contains roughly half of the brain's neurons, specialized cells that transmit information via electrical signals.

The cerebellum is not unique to humans. Evolutionarily speaking, it is an older portion of the brain. It is present in animals that scientists believe existed before humans. Damage to the cerebellum, while not causing paralysis or intellectual impairment, might lead to a lack of balance, slower movements, and tremors (shaking). Complex physical tasks would become unsteady and halting.

The spinal cord is a white, soft and cord (rope) like substance running through the backbone. The internal structure of the spinal cord is much simpler and more uniform throughout its various parts than that of the brain. No matter where it is sectioned, it gives the same general appearance. The interior of the spinal cord looks gray because it is filled with neurons without having myelin sheath in their axons (unmyelinated axons).

In other words, the interior of the spinal cord is filled with some gray matter. Interestingly, the gray matter is so distributed that the interior looks almost as a butterfly whose essential form is the English capital letter "H". The outer of the spinal cord looks white as it is filled with some myelinated axons, in other words, the outer of the
spinal cord is filled with some white matter. The chief function of the gray part of the spinal cord is integrative in nature, whereas the chief function of the white part is communicative in nature.

There are thirty-one pairs of peripheral spinal nerves connected to the spinal cord. The sensory spinal nerves are connected to the cord at the back or dorsal part. In fact, there are two (left and right) dorsal roots through which sensory information enter into the spinal cord. The motor spinal nerves are connected to the cord at the front or ventral part. There are two (left and right) ventral roots through which motor information go out of the spinal cord. The sensory information go towards the brain in the two dorsal columns of neurons and motor information go downward of the spinal cord in the two ventral columns of neurons. There are also two sets of lateral (side) columns of neurons whose function is both sensory and motor in nature.

In the present investigation fluoride exposure significantly exacerbated the levels of LPO along with the decreased activities of antioxidant enzymes like SOD, CAT, GSH-Px, GST and GSH levels in discrete regions of CNS suggestive of oxidative stress. The results observed were in agreement with the previous findings (Chirumari et al., 2007, Inkielewicz I & Czanowski W, 2008, Gupta et al., 1993, Stadtman ER & Berlett BS, 1997, Vani ML & Reddy KP, 2000). The reactive oxygen species (ROS) formed exceeded the antioxidant capacity of a cell, which were highly toxic, react with proteins, enzymes and nucleic acids and may lead to the cell death via apoptosis or necrosis (Kannan K & Jain SK, 2000). Further, the oxidative stress not only increases free radical injury but also enhances excitotoxicity, since ROS, reactive nitrogen species and lipid peroxidation products can trigger the excitotoxic process documented both in animal and human studies (Blaylock, 2004).

The developing CNS while undergoing cell differentiation and migration, synaptogenesis and subsequent pruning of synaptic tree in response to ongoing neuronal activity may be selectively affected by exposure to fluoride. The significant accumulation of fluoride (P<0.001) in various parts of CNS, especially in cerebellum and spinal cord caused metabolic perturbations at subcellular level, which may be due to unprotected role of blood brain barrier. Cerebellum and spinal cord appear to be more susceptible to oxidative damage than cerebral cortex and medulla oblongata as they possess low endogenous levels of vitamin E, differences in iron content, oxygen consumption rate, cell types, functions etc. Further these areas are vulnerable to sensory function deficits, motor disturbances and adverse effects on cognitive functions, such as learning and memory, indicative of brain dysfunction on experimental fluorosis. Although there are differences among regions in response to fluoride exposure that cannot be explained through a simple model, a potential role of a number of elements as biomarkers in this kind of neuronal alterations cannot be excluded (Hillman D et al., 1979).

In conclusion, the results suggest that the fluoride intoxication to mother rats exert effects on pups through exacerbated oxidative stress and alter the cellular process. The developing animals no longer prevent the oxidative stress and are more prone to oxidative damage.

References


