HISTOLOGICAL CHANGES IN THE BRAIN OF YOUNG FLUORIDE-INTOXICATED RATS

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SUMMARY: Wistar albino rats were exposed to 30 or 100 ppm fluoride (as NaF) in drinking water during their fetal, weanling, and post-weaning stages until the age of ten weeks. Rats exposed to 30 ppm fluoride did not show any notable alterations in brain histology, whereas rats exposed to 100 ppm fluoride showed significant neurodegenerative changes in the hippocampus, amygdala, motor cortex, and cerebellum. Changes included decrease in size and number of neurons in all the regions, decrease in the number of Purkinje cells in the cerebellum, and signs of chromatolysis and gliosis in the motor cortex. These histological changes suggest a toxic effect of high-fluoride intake during the early developing stages of life on the growth, differentiation, and subcellular organization of brain cells in rats.

Keywords: Albino rats, Amygdala, Brain histology, Cerebellum, Fluoride intoxication, Hippocampus, Motor cortex, Neurodegenerative changes, Sodium fluoride.

INTRODUCTION

Long-term intake of high levels of fluoride in humans causes neurological complications such as paralysis of limbs, vertigo, spasticity in extremities, and impaired mental acuity.[1] Fluoride is also known to cross the blood-brain barrier.[2] Fluoride accumulation was observed in the brain of experimental animals exposed to chronic high-fluoride intake, and this accumulation increased as the drinking water fluoride levels increased.[3] Chronic fluoride toxicity is also known to cause altered neuronal and cerebrovascular integrity,[4] abnormal behaviour patterns,[3] and metabolic lesions in the brain,[5-7] in experimental animals.

In the present study, effects of chronic fluoride intoxication on brain cell structure were assessed by exposing rats to 30 or 100 ppm fluoride in drinking water at the early stages of life and then studying the histology of various brain regions.

MATERIALS AND METHODS

Adult (4-6 month-old) wistar albino rats were used in the study. The rats were fed with a standard pelleted diet (Hindustan Lever Ltd., India), and were given water *ad libitum*. The animals were maintained under proper temperature, ventilation, and hygienic conditions.

Pregnant rats were divided into three groups: control, 30F, and 100F. Control rats received drinking water with 0.5 ppm fluoride, while the 30F

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and 100F rats received 30 ppm and 100 ppm fluoride (from NaF), respectively, in their drinking water during the last (3^{rd}) week of pregnancy and throughout the lactation period. The litters were separated from the mother rats on weaning and were then exposed to the respective levels of fluoride (0.5 ppm, 30 ppm, and 100 ppm) in drinking water up to the age of 10 weeks. The ten-week-old rats of control (n = 15), 30F (n = 13), and 100F (n = 9) groups were sacrificed after light ether anaesthesia. They were perfused transcardially with 0.9% saline followed by 10% formalin, and the brains were removed.

The brains were fixed in 10% formalin, and 5-μm thick coronal sections of the hippocampus, amygdala, motor cortex, and cerebellum were taken. The sections were processed, and stained in 0.1% cresyl violet stain. The stained sections were observed under a binocular light microscope.

RESULTS AND DISCUSSION

No histological changes were seen in the brain regions of the 30F group when compared to the corresponding brain regions of the control group. The 100F group, however, showed significant changes in the histology of the various brain regions; the hippocampus, amygdala, motor cortex, and cerebellum showed neurodegenerative changes (Figures 1-6).

In the hippocampus, neurons were shrunken and darkly stained with a small nucleus, and there was a decrease in the cell number. Histological alterations were seen in cornu ammonis-1 (CA_1), cornu ammonis-4 (CA_4), and dentate gyrus sub regions of the hippocampus (Figures 1-3), but not in CA_2 and CA_3 sub regions. The hippocampus showed the most pronounced changes among the various brain regions of 100F rats. In rats exposed to long-term intake of high levels of fluoride in drinking water, Mullenix *et al*[3] reported fluoride accumulation in important regions of the brain, especially the hippocampus. The pattern of changes seen in this study are similar to the findings of Varner *et al*[4] who reported cellular abnormalities in the CA_1 and CA_4 areas of the hippocampus, and not in CA_2 and CA_3, in adult rats exposed to 1-ppm fluoride in drinking water for 52 weeks.

Neurodegenerative changes seen in the amygdala, motor cortex, and cerebellum were similar to those seen in the hippocampus (Figures 4-6). In the motor cortex, neurons in the superficial layers were the most affected, and there were also signs of chromatolysis and gliosis (Figure 5). Varner *et al*[4] observed chromatin clumping, enhanced protein staining, pyknosis, vacuolation, presence of ghost-like cells, and decreased neuronal density in adult rats exposed to chronic intake of 1-ppm fluoride in drinking water. In our work, the Purkinje cells in the cerebellum were the most affected cell population, and there was an increase in the number of granular cells but a decrease in the number of Purkinje cells and molecular cells (Figure 6).

Figure 1. Photomicrographs of CA_1 region of hippocampus in control (A) and 100 ppm fluoride-treated (B). Note the degenerated neurons (arrows) in B. Cresyl violet stain, scale bar = 25 µm.

Figure 2. Photomicrographs of CA_4 region of hippocampus in control (A) and 100 ppm fluoride-treated (B). Note the degenerated neurons (arrows) in B. Cresyl violet stain, scale bar = 25 µm.

Figure 3. Photomicrographs of dentate gyrus region of hippocampus in control (A) and 100 ppm fluoride-treated (B). Note the degenerated neurons (arrows) in B. Cresyl violet stain, scale bar = 25 µm.

Figure 4. Photomicrographs of amygdala region in control (A) and 100 ppm fluoride-treated (B). Note the degenerated neurons (arrows) in B. Cresyl violet stain, scale bar = 25 µm.

Figure 5. Photomicrographs of motor cortex in control (A) and 100 ppm fluoride-treated (B). Note the degenerated neurons (arrows) in B. Cresyl violet stain, scale bar = 25 µm.

Figure 6. Photomicrographs of cerebellum in control (A) and 100 ppm fluoride-treated (B). Note the degenerated neurons (arrows) in B. Cresyl violet stain, scale bar = 25 µm.

Earlier, Zhavoronkov[8] reported foci of demyelination in the cortex and subcortical areas, a decrease in the number of Purkinje cells in the cerebellum, swelling and irregular staining of the Nissl substance, and pyknosis of neurons in experimental animals subjected to fluoride intoxication.

In the present study, mother rats were exposed to high levels of fluoride in the last week of pregnancy and during lactation. This was done to ensure that the mother's body system became laden with fluoride so the litters would receive fluoride through the mother. Later, the litters were weaned and exposed directly to fluoride in drinking water. Thus, the young rats were subjected to chronic fluoride intoxication during the developing stages of life. Li *et al*[9] reported a lower intelligence quotient (I.Q.) in children living in fluoride-endemic areas and suggested that the effect of exposure to high level of fluoride on intelligence probably occurs at an early stage of development of the embryo and infant when differentiation of brain cells is occurring and development is most rapid. Earlier, Mullenix *et al*[3] observed abnormal behaviour patterns in rats exposed to intake of high levels of fluoride during prenatal, weanling, or adult stages. However, we did not investigate changes in the rat behaviour patterns that might have occurred in our study.

In conclusion, we find that chronic fluoride intoxication in the early stages of life caused marked neurodegenerative changes in the brain of rats. These changes may form the neural basis for impaired learning and memory, abnormal behaviour patterns, and disturbed overall body physiology.

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