EFFECTS OF HIGH FLUORIDE AND LOW IODINE ON BRAIN HISTOPATHOLOGY IN OFFSPRING RATS

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SUMMARY: Thirty-two Wistar rats were divided randomly into four groups of six females and two males each. The experimental groups were exposed to high fluoride drinking water (45 mg $F^/L$ from 100 mg NaF/L), low dietary iodine (0.0855 mg/kg), or both together in order to assess the effects of these three factors on the structure of the brain of the offspring rats. After the animal model was established, offspring rats were bred, and thirty-six rats from each group (female:male = 1:1) were used for the study. The treatment of the offspring rats was the same as that of their parents. In comparison with the control rats, the nuclei of many nerve cells were pyknosed and absent, the Nissl substance also showed various degrees of decrease, and the dendrites were elongated. The results indicate that the histopathological changes in the brain were initially due to lipid peroxidation caused by the interaction of high fluoride and low iodine. These changes in brain histopathology apparently occurred mainly during the period of embryonic development and in the early stage of brain development after birth.

Keywords: High fluoride intake; Histopathological changes; Iodine deficiency; Offspring rats; Rat brain.

INTRODUCTION

The last decade of the twentieth century was named the decade of the brain by the international scientific community, and the beginning of the twenty-first century is regarded as the dawn of neuroscience. Thus it has become especially important to study the effects of environment on brain function. It is well known, for example, that low human dietary iodine intake levels can adversely affect the intelligence quotient (IQ). Moreover, in recent years, epidemiological investigations have revealed that elevated fluoride intake also has adverse effects on IQ. For example, Lu and Zhao found the IQ of children living in high fluoride areas of Tianjin, Guizhou, and other provinces of China was lower by 8–12% than in children living in low fluoride areas.^{1,2} In a related investigation, exposure to high fluoride concentrations were found to extend the response time to questions and to diminish imaginative capacities that, in turn, negatively influenced the reading and writing abilities of children.³

An earlier epidemiological investigation also revealed that high fluoride and low iodine concentrations have even stronger adverse effects on the IQ of children.⁴ In fact, not only high fluoride levels but also iodine deficiency exist in various areas of China, and in some areas they coexist.^{5,6} Thus, not only studying the effects of both high fluoride and low iodine intake, but also investigating their interactive effects on the central nervous system should be helpful in understand-

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ing the differences between these conditions in various epidemiological investigations.

On the basis of results of our research on the effects of high fluoride and low iodine concentrations on biochemical indexes of the brain and learning-memory in offspring rats,^{7,8} we were prompted to study the effects of both treatments on histopathological changes in their brains.

MATERIALS AND METHODS

Experimental materials: As in our two recent reports,^{7,8} one-month old Wister albino rats, each weighing approximately 50 g, were obtained from the Experimental Animal Center of Shanxi Medical University for use in this study.

The same iodine-deficient feed and high-fluoride water reported in those studies^{7,8} were also employed here as shown in the Table.

Table. Fluoride in the drinking water (mg F⁻/L) and fluoride and iodine levels in the diet (mg/kg) of the rats

	Control	High fluoride	Low iodine	High fluoride and low
		(HiF)	(LI)	iodine (HiF+LI)
lodine in diet	0.3543	0.3543	0.0855	0.0855
Fluoride in diet	25.57	25.57	26.01	26.01
Fluoride in drinking water	<0.6	45 ^a	<0.6	45 ^a

^aFrom 100 mg NaF/L, as should have been recorded in our two previous reports.^{7,8}

Establishment of test animal model: Thirty-two one-month-old Wistar albino rats (female:male = 3:1) were randomly divided into four groups of six females and two males each and were maintained on the diets and water shown in the Table under standard temperature (22–25 °C), ventilation, and hygienic conditions.

Breeding of iodine-deficient offspring rats: Three months after establishing the animal model, the female experimental animals were allowed to become pregnant by natural mating. The day of the birth of their offspring was set as day 0. During and after nursing, the pups were raised under the same conditions as their parents. After one month, the offspring rats were separated according to sex. At day 0 and then at days 10, 20, 30, 60, and 90, three male and three female offspring rats were randomly selected from each litter for further study.

Morphological observation of brain tissue: The offspring rats were perfused transcardially with 0.9% saline followed by 20% urethane solution, and the brains were removed. (Note: The 0-day rats were killed by decapitation and the brains were removed immediately.) The right hemispheres of the brains were fixed in 10% formalin for 72 hr and embedded in paraffin. Five-micrometer thick sections were processed and stained with hematoxylin and observed under a light microscope.

RESULTS

Histopathological changes in varying degrees were observed in the brains of the offspring rats of the treated groups. To reduce the number of figures, only the results for day 0 and day 20 are presented here.

Control group: These brains showed normal features under microscope observation (Figures 1 and 5).



Figure 1. Structure of brain cortex of a 0-day-old offspring rat in the control group (×660).



Figure 2. Histopathological changes in the brain cortex of a 0-day-old offspring rat in the low iodine group (×660).



Figure 3. Histopathological change in the brain cortex of a 0-day-old offspring rat in the fluoride-treated group (×660).



Figure 4. Histopathological changes in the brain cortex of a 0-day-old offspring rat in the high fluoride and low iodine group (×660).



Figure 5. Structure of brain cortex of a 20day-old offspring rat in the control group (×660).



Figure 6. Histopathological changes in the brain cortex of a 20-day-old offspring rat in the low iodine group(×660).



Figure 7. Histopathological changes in the brain cortex of a 20-day-old offspring rat in the high fluoride group (×660).



Figure 8. Histopathological changes in the brain cortex of a 20-day-old offspring rat in the high fluoride and low iodine group (×660).

Low iodine (LI) group: The brains in this group did not show any changes in the structure of nerve cells compared to the control group. There were no changes in most nuclei or the Nissl substance of the neurons in the brains at day 0 or day 20, but they exhibited many pyknosed neurons and various degrees of pathology. The nuclei of some nerve cells were pyknotic or absent (Figures 2 and 6).

High fluoride (HiF) group: The neuron structures in this group revealed some degree of alteration, and tissue delamination was less distinct. Many neurons were shrunken, pyknotic, and darkly stained with small nuclei, and there was a decrease in their overall cell number. The Nissl substance also showed various degrees of pathology, and the dendrites were elongated or absent. In some neurons spheroid bodies were present in the neuroplasm (Figures 3 and 7).

High fluoride plus low iodine (HiF+LI) group: Neurotoxic changes in the brain of offspring rats were most apparent in this group. Most neurons displayed many irregularities in their structure and distribution compared to those of the control groups. Some neurons exhibited chromatolysis and were hyperatrophied. The nuclei of many nerve cells were pyknosed or absent. The Nissl substance also showed prodigious degrees of decrease and was even absent in some cases. Moreover, the dendrites were elongated, especially in the juvenile period (Figures 4 and 8).

DISCUSSION

Effects of high fluoride and low iodine: In these experiments, the histopathological changes in the brains of the HiF+LI group were evident in the early stages of development. As already noted, many nerve cell nuclei were pyknosed or absent, the Nissl substance showed various degrees of decrease, and the dendrites were stretched. These histopathological changes are in accord with the results of Shivarajashankara et al⁹ and of Shashi¹⁰ for fluoride alone. But the changes we observed in the brains caused by HiF+LI were more serious than those from HiF or LI alone, thus indicating that HiF and LI interact synergistically to a considerable degree.

Relationship between neurotoxic effects and lipid peroxidation: Fluoride has long been known to affect various parts of the rat brain.¹¹ Some reports suggest

that lipid peroxidation caused by fluoride may be one of the important factors in the mechanism of neurotoxic effects of fluoride. In our previous research,⁸ all the changes in MDA and SOD caused by the HiF treatment were not so severe as those resulting from the LI treatment. However, the change in lipid peroxidation caused by the combined HiF+LI treatment was highly significant. In this connection it is worth noting that phospholipids containing unsaturated fatty acids are abundant in the brain and that they are particularly sensitive and vulnerable to peroxidation.¹²

It is also known that cell membranes are a main target of lipid peroxidation. Damage to cell membranes results in cell structure destruction. The products of lipid peroxidation have adverse effects on the DNA and chromosomes.¹³⁻¹⁵ In this paper, the disappearance of the nucleoli, pyknosis of the nucleus, and aberration of chromosomes were induced, probably by the result of lipid peroxidation.⁸ More research is required to determine whether or not this phenomenon is apoptosis.

Relationship between the histopathological changes in the brain and IQ: Many studies have shown that deficiency in iodine intake (LI) can reduce IQ, but in recent years it has been found that elevated fluoride intake (HiF) also significantly decreases IQ.¹⁶ Our previous research shows that the combination of HiF and LI has a greater negative effect on learning-memory of offspring rats than treatment with either LI or HiF alone.⁷ It is our belief that histopathological changes in the brain are the histological basis of changes in brain function, especially in the hippocampus. Since the cerebral cortex and the hippocampus are the key tissues related to learning and memory, it is highly plausible that there is a direct relationship between IQ and histopathological changes of brain. These changes may form the neural basis for impaired learning and memory, abnormal behavior patterns, and distributed overall body physiology.⁹

In our experiments, we found that the histopathological changes in the brain occurred most notably in the early stages of development from day 0 to day 20. This is in accord with changes in SOD activity and MDA⁸ and protein levels,⁷ which were mainly affected before day 30. This early period is therefore the most important stage of brain development. In agreement with this view, Li et al¹⁷ reported a lower IQ in children living in fluoride-endemic areas and suggested that the effects of exposure to HiF intelligence probably occurs at an early stage of embryo and infant development, when differentiation of brain cells is occurring and development is most rapid.

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REFERENCES

- 1 Lu Y, Sun ZR, Wu LN, Wang X, Lu W, Liu SS. Effect of high fluoride water on intelligence in children. Fluoride 2000;33:74-8.
- 2 Zhao LB, Liang GH, Zhang DN, Wu XR. Effect of a high fluoride water supply on children's intelligence. Fluoride1996;29:190-2.

- 3 Calderon J, Blenda M, Navarro M, Carrizales L, Ortiz MD, Diaz-Barriga F. Influence of fluoride exposure on reaction time and visuospatial organization in children [abstract]. Epidemiology 2000;11 (4):S153.
- 4 Xu YL, Lu CHSH, Zhang XN. Effect of fluoride on children's intelligence. Endem Dis Bull 1994;9(2):83-49. [in Chinese].
- 5 Wang JP, Li J, Ai HT, Lin Q, Yang Y, Zhang L, et al. Investigation and analysis of fluorosis and IDD co-existing in areas of XinJiang. Endem Dis Bull 1997;12:57-9. [in Chinese].
- 6 An D, Duan RX, Li P, Wang SHQ. Geographical distribution differences of IDD and endemic fluorosis and their influenced factors in Guizhou province. Chin J Endemiol 997;16:86-8. [in Chinese].
- 7 Wang JD, Ge YM, Ning HM, Wang SL. Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. Fluoride 2004;37:201-8.
- 8 Wang JD, Ge YM, Ning HM, Wang SL. Effect of high fluoride and low Iodine on oxidative stress and antioxidant defense of brain in offspring rats. Fluoride 2004;37:264-70.
- 9 Shivarajashankara YM, Shivashankara AR, Gopalakrishna B, Muddanna RS, Hanumanth RS. Histological changes in the brain of young fluoride-intoxicated rats. Fluoride 2002;35:12-21.
- 10 Shashi A. Histopathological investigation of fluoride-induced neurotoxicity in rabbits. Fluoride 2003;36:95-105.
- 11 Singh A, Jolly SS. Endemic fluorosis with particular reference to fluorotic radiculo-myelopathy. Quart J Med 1961;30:357-72.
- 12 Sureshkumar JS. Venugopal PM. Effect of diabetes on level lipid peroxides and glycolipids in rat brain J. Metabolism 1993;42:1435-9.
- 13 Schulte-Frohlinde D. Mechanism of radiation-induced strand break formation in DNA and polynucleotides. Adv Space Res1986;6:89-96.
- 14 Yves C. Oxidative stress and Alzheimer disease. Am J Clin Nutr 2000;71:621s-9s.
- 15 Blaylock RL. Excitotoxicity: a possible central mechanism in fluoride neurotoxicity. Fluoride 2004;37:301-14.
- 16 Spittle B. Editorial: Fluoride and intelligence. Fluoride 2000;33:49-51.
- 17 Li XS, Zhi JL, Cao RO. Effect of fluoride exposure on intelligence in children. Fluoride 1995;28:189-92.