EFFECTS OF HIGH FLUORIDE AND LOW IODINE ON OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE OF THE BRAIN IN OFFSPRING RATS

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SUMMARY: Thirty-two Wistar rats were divided randomly into four groups of eight rats each (female:male = 3:1). With one untreated group as a control group, the other three groups were administered, respectively, high fluoride in their drinking water (100 mg F/L from NaF), low iodine in their chow (0.0855 mg/kg), or both the high fluoride and low iodine together, in order to assess the effects of the three treatments on oxidative stress in the brain of offspring rats. After the animal model was established, the rats were allowed to breed, and 36 offspring rats in each group (female:male = 1:1) were randomly selected for the experiment. These rats were given the same treatment for the next 90 days as their parents. Superoxide dismutase (SOD) activity and the malondialdehyde (MDA) content in the brain of the combined high fluoride and low iodine group were significantly higher during and at the end of the 90-day period than in the control group, but the SOD/MDA ratio in this high fluoride and low iodine group was consistently lower than in the control group. These results suggest that brain stress from high fluoride and low iodine is one of the causes of reduction in learning and memory in offspring rats.

Keywords: Antioxidant defense; Biochemical indexes; High fluoride; Iodine deficiency; Offspring rats; Oxidative stress; Rat brain.

INTRODUCTION

In recent years, reports dealing with the biochemistry of fluoride indicate that superoxide free radicals and lipid peroxidation play an important role in fluoride intoxication.[1-4] Although some findings differ,[5] various epidemiological investigations in China have reported a relationship between fluoride and intelligence showing an intelligence quotient (IQ) lowering of 8 to 10 points in children living in villages with high fluoride in food or drinking water.[6-9] Thus, it is important to study oxidative processes in brain stress and antioxidant brain defense in experimental offspring rats to explore the mechanism of lower IQ in high fluoride areas.

It is well known that lower IQ is strongly linked to iodine deficiency. The mechanism of lower IQ associated with iodine deficiency is related to certain neurotransmitters, enzymes, growth metabolism, and the development of the central nervous system.[10-13] There appear to be few reports indicating that low iodine is directly related to oxidative stress. The present study, therefore, aimed to examine oxidative stress and antioxidant defense in the brain as they might relate to our recent investigation showing reduction of learning-memory in offspring rats of mothers administered high fluoride or low iodine or a combination of high fluoride and low iodine.[14]

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MATERIALS AND METHODS

Experimental materials: As in our recent report,[14] one-month old Wistar albino rats, each weighing approximately 50 g, were obtained from the Experimental Animal Center at Shanxi Medical University for use in this investigation.

The same iodine-deficient feed and high-fluoride water reported in that study[14] were also employed here as shown in Table 1.

Table 1. Fluoride and iodine levels in diet (mg/kg) and fluoride in the drinking water (mg/L) of the rats

	Control	High fluoride (HiF)	Low iodine (LI)	High fluoride and low iodine (HiF+LI)
Iodine in diet	0.3543	0.3543	0.0855	0.0855
Fluoride in diet	25.57	25.57	26.01	26.01
Added fluoride in drinking water	<0.6	100	<0.6	100

Establishment of 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 Table 1 under standard temperature (22–25 ºC), ventilation, and hygienic conditions.

Breeding of filial generation of iodine-deficient rats: Three months after establishing the animal model, the female experimental rats 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 study.

Assay of lipid superoxides and antioxidant enzymes in brain: At day 0 and then at days 10, 20, 30, 60, and 90, the experimental rats were sacrificed by cervical dislocation, and the cerebra were quickly collected and cut into the left and right hemispheres. The left hemisphere of the cerebra was weighed and then homogenized with 1:9 (w/v) 0.9% saline solution at 0–4 °C. Superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, and catalase (CAT) activity in the brain tissue were determined with the reagent kit provided by the Nanjing Jianchen Biological Institute.

RESULTS

MDA content and SOD activity of the brain tissue: The total MDA content and SOD activity in the brain homogenates from the offspring rats according to their

treatment are listed in Tables 2 and 3, respectively. Table 4 shows the ratio of brain SOD activity to MDA content in the offspring rats, and Table 5 records the CAT activity.

Table 2. MDA in brain homogenates from offspring rats (nmol/mg protein; mean±SD)

Day	Control	High fluoride (HiF)	Low iodine (LI)	High fluoride and low iodine (HiF+LI)
0	3.28±0.47	3.07±0.48	7.52±0.76†	4.70±0.79
10	3.54±0.34	4.91±0.33*	5.25±0.54*	4.71±0.41
20	2.33±0.15	2.49±0.07	2.43±0.13	6.25±0.75†
30	3.02±0.26	3.22±0.28	4.63±0.36†	6.69±0.39†
60	3.43±0.52	3.43±0.64	3.36±0.29	5.11±0.56*
90	5.72±0.57	6.23±0.48	6.99±0.62	6.53±0.54
Mean value	3.55±1.14	3.89±1.40	5.03±1.98*	5.66±0.92†

*p<0.05. †p<0.01.

Table 3. SOD activity in brain homogenates from offspring rats (U/mg protein; mean±SD)

Day	Control	High fluoride (HiF)	Low iodine (LI)	High fluoride and low iodine (HiF+LI)
0	56.52±12.69	64.55±5.06	56.25±4.31	103.98±12.25*
10	76.62±6.26	78.66±2.67	46.45±3.56†	97.16±6.19*
20	50.56±3.66	55.97±2.84	77.07±2.30†	87.00±5.52†
30	54.44±2.58	51.31±1.08	45.56±6.56*	88.31±5.17†
60	82.41±8.05	81.56±7.25	82.29±7.56	83.50±7.67
90	87.05±8.21	85.49±7.72	83.88±7.48	78.56±7.81
Mean value	67.93±15.90	69.59±14.30	65.25±17.88	89.75± 9.28*

*p<0.05. †p<0.01.

Table 4. SOD/MDA ratio in brain homogenates from offspring rats (mean±SD)

Day	Control	High fluoride (HiF)	Low iodine (LI)	High fluoride and low iodine (HiF+LI)
0	23.93	21.51	8.42	22.60
10	23.03	16.25	9.31†	20.87
20	21.69	22.44	32.08	16.53
30	18.64	16.62	10.48†	13.21†
60	25.70	27.31	24.58	16.63*
90	15.44	13.68	12.07†	11.98†
Mean value	21.40±3.76	19.63±5.02	16.15±9.79	16.97±4.15

*p<0.05. †p<0.01.

Table 5. CAT activity in brain homogenates from offspring rats (U/mL; mean±SD)

Day	Control	High fluoride (HiF)	Low iodine (LI)	High fluoride and low iodine (HiF+LI)
0	0.29±0.07	0.50±0.06	0.99±0.23*	0.28±0.28
10	0.47±0.05	0.49±0.05	0.32±0.02*	0.26±0.05*
20	0.25±0.03	0.26±0.03	0.07±0.03†	0.15±0.05*
30	0.25±0.02	0.26±0.02	0.28±0.03	0.18±0.04
60	0.37±0.03	0.52±0.06*	0.31±0.06	0.39±0.06
90	0.48±0.08	0.42±0.03	0.34±0.07	0.28±0.04*
Mean value	0.35±0.10	0.40±0.11	0.38±0.31	0.25±0.08

*p<0.05; †p<0.01.

DISCUSSION

Effect of high fluoride and low iodine on brain MDA: Malondialdehyde (MDA) is considered an index of lipid peroxidation as demonstrated by many earlier studies reporting increased MDA levels in the erythrocytes of humans with fluorosis[3] and in erythrocytes, liver, kidney, and ovary of experimental animals.[2,15,16] Because it is rich in polyunsaturated lipids, and is dependent on aerobic metabolism, brain tissue is highly vulnerable to oxidative damage. This has been shown by studies revealing increased MDA levels in the brains of fluoride-intoxicated rats.[2,17-19] In our present study, the brain MDA level increased significantly in the high fluoride (HiF) group at day 10, in the low iodine (LI) group at days 0, 10, and 30, and in high fluoride and low iodine (HiF+LI) group from day 20 to day 60, compared with that of the control group. In particular, the MDA level in the high fluoride plus low iodine (HiF+LI) group was also higher at days 20, 30, and 60 than in the HiF group and in the LI group. The mean value of the entire 90-day period was slightly higher in the HiF group, but was significantly higher in the LI group and in the HiF+LI group. The above findings indicate that low iodine and the interaction of both high fluoride and low iodine can result in a more significant change in lipid peroxidation than high fluoride alone.

Effect of high fluoride and low iodine on antioxidant enzymes of the brain: From past reports in the fluoride literature, the change in antioxidant enzymes from their single determination at a given time is complicated. GSH-Px activity decreased in the erythrocytes of adult humans[20] and experimental animals[21] with fluorosis but increased in the erythrocytes of children with endemic skeletal fluorosis[2] and in the erythrocytes of experimental animals.[3] SOD activity decreased in the erythrocytes of children with endemic skeletal fluorosis,[2] and in fluoride-intoxicated rats.[3] It also decreased in the liver, kidney, and ovary of mice,[15,16] as well as in the brain and gastrocnemius muscle of mice,[22] and in earthworms,[23] though in a few reports SOD activity remained unchanged.[20,21] In studies of low and excessive iodine intake, changes in the activities of these enzymes also differed. Li *et al*[24] reported decreased SOD in the serum and thyroid of rats, but Fang *et al*[25] found increased SOD and GSH-Px activity in the rat thyroid.

It should be noted, however, that most of these results are from single determinations and reflect only the state at some point of toxic duration. In the present study, our dynamic observations showed that these indexes did not change significantly in the HiF group during the entire 90-day period compared with the control. On the other hand, significant changes with time were observed in the LI group and in the HiF+LI group. Thus the SOD activity in the LI group was significantly lower at day 10 and then increased markedly by day 20, only to fall again at day 30 before rising to the control level at days 60 and 90. Likewise, catalase activity in the LI group and in the HiF+LI group also decreased significantly at days 10 and 20 before increasing by day 60. Clearly, these changes would not be known if the determinations were made only once at the end of experiment.

Relationship between activity of antioxidant enzymes and lipid peroxidation: In previous fluoride studies, when lipid peroxidation was found to increase and antioxidant enzyme activity to decrease, the results were easy to explain. It was because of an increase in superoxide radicals and lipid peroxides and a decrease in the activity of some antioxidant enzymes that fluoride toxicity was manifested. If both lipid peroxides and the activity of some antioxidant enzymes were increased at the same time, the increased activity of antioxidant enzymes is explained as an adaptive response in the form of an increased antioxidant defense which counteracts oxidative stress. In the present study, the early increase in total SOD activity in the HiF+LI group paralleled the increased MDA content in the same group. Therefore, we agree with the opinion that changes in the activity of these enzymes could be an adaptive reaction to changes in the corresponding lipid peroxides. However, the fact that the ratios of SOD activity and MDA content in the HiF+LI group was lower than in the control group showed that the increase in lipid peroxidation caused by high fluoride and low iodine generally resulted in a decrease in the antioxidant ability, with the result that various types of lesions were likely to have been produced.

In conclusion, oxidative stress induced by high fluoride and low iodine should be considered as one of the pathways that lead to reduction in the learning and memory of offspring rats.

ACKNOWLEDGEMENT

This research was sponsored by the China National Natural Science Foundation (Grant No. 30170681).

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