EFFECTS OF HIGH FLUORIDE AND LOW IODINE ON BIOCHEMICAL INDEXES OF THE BRAIN AND LEARNING-MEMORY OF OFFSPRING RATS

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SUMMARY: Thirty-two Wistar rats were divided randomly into four groups of eight rats (female:male = 3:1). With one untreated group as controls, the other three groups were administered, respectively, high fluoride in their drinking water (100 mg NaF/L), low iodine (0.0855 mg/kg chow), or both high fluoride (100 mg NaF/L) and low iodine (0.0855 mg/kg) in order to assess the effects of the above three factors on the learning and memory ability of their 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 experiments. The treatment of these rats was the same as their parents. In comparison with control rats, the learning and memory ability of the offspring rats was depressed by high fluoride, low iodine, or the combination of high fluoride and low iodine. Brain protein was decreased by low iodine and even more by the combined interaction of high fluoride and low iodine. The activity of cholinesterase (ChE) in the brain was affected to some extent by high fluoride and low iodine but was especially affected by high fluoride and low iodine together.

Keywords: Brain protein; Cholinesterase; Iodine deficiency; Learning-memory; Offspring rats; Sodium fluoride.

INTRODUCTION

Clinical manifestations of fluorosis often occur in bones and teeth as a result of long-term intake of elevated levels of fluoride. Damage to hard tissues of animals caused by industrial fluoride pollution has been studied successively by our research group.[1-5] However, recent reports also point to an inverse relationship in China between early fluoride ingestion and intelligence, indicating a lower intelligence quotient (IQ) of 8 to 10 points in children living in villages with elevated fluoride intake from food or drinking water.[6-8]

In his review of these reports,[9] Spittle noted that the iodine status of the children had apparently not been determined; hence consideration must also be given to whether iodine deficiency was a factor. As he pointed out, striking differences were found in one study of the IQ of children (19–25 points lower) living in an iodine-deficient area of Xinjiang. Recently, Xiang *et al*[10] reported further findings on an association of fluoride in drinking water with lower intelligence in children, which was not related to any endemic iodine deficiency, or to elevated blood lead levels.[11]

The aim of this research was to assess indirectly through an animal offspring study the relationships that might exist between lower IQ of children and high

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fluoride, low iodine, and the combined effect of high fluoride and low iodine intake.

MATERIALS AND METHODS

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 experimental iodine-deficient diet was made from wheat, corn, and soybean grown in an iodine-deficient region—Weijiawan village of Anze County in Shanxi province. The normal diet was provided by the Experimental Animal Center of Shanxi Medical University. The contents of iodine and fluoride in the experimental feed and in the control feed are listed 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 (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
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 of the one-month old Wistar rats 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.

Feeding of first offspring rats: Three months after establishing the animal model, the female experimental animals were allowed to become pregnant by natural mating. The day of birth of the offspring rats was set as day 0. During and after nursing, they 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 day 10, 20, 30, 60, and 90, three male and three female offspring rats were randomly selected from each litter for study.

Evaluation of learning and memory in young rats: Learning and memory were investigated in each offspring group by a “step-down test” at day 30, 60, and 90. The apparatus consisted of five step-down chambers made of black plastic walls measuring 25×25×35 cm. The bottom chamber was equipped with copper bars that could be electrified to a charge of 36 volts, and a 10×10×10 cm rubber step mat was installed as a safe retreat to obviate the electric shock. To conduct the study, each rat was placed into the experimental chamber and allowed to adjust for 3 min. When the copper bar was suddenly electrified, the rat immediately jumped up to the rubber mat. Most rats repeatedly jumped down to the copper bar. When shocked, they quickly jumped back to the rubber mat. The number of shocks per rat over a five-minute period was recorded as the error number (EN1). Twenty-four hours later, with the copper bar electrified, the rat was placed on the rubber mat directly. The time from when the rat was put on the mat to when rat jumped down on the bar was recorded as sustaining time (ST). When the rat jumped on the bar and was shocked, it again jumped up to the rubber mat. The number of times the rat jumped from the mat to the bar during five minutes was recorded as the second-round error number (EN2). The above trials were carried out at 21:00~23:00, in quiet and dim conditions.

Assays of brain biochemistry: After the tests for learning and memory were completed, the experimental rats were killed by decapitation. The cerebra and thyroid were collected quickly and weighed. Then the left hemisphere of the cerebra was homogenized by 1:9 (W/V) in 0.9% saline at 0–4 °C. Protein contents of the brain tissue and activities of cholinesterase (ChE) were determined with reagent kits provided by the Nanjing Jiancheng Biological Institute.

RESULTS

Iodine levels in the urine of the parent rats: At the end of the adjustment period the mean iodine levels in the urine of the control rats and in the low iodine group (LI) were 1627.9 μg/L and 651.1 μg/L, respectively. Thus it is evident that the animal model had been established.

Development of offspring rats: Compared to the control group, the mean body weight was lower for the high fluoride and low iodine group (HFLI) at birth, and for the high fluoride (HF) and low iodine (LI) groups at 10 days (Table 2). Subsequently, the mean body weights of the three trial groups did not differ significantly from the mean body weight of the control group up to day 90.

Table 2. Change in body weight (g) of offspring rats (n=6, mean ± SD)

Day	Control	High fluoride (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
0	5.65±0.39	5.30±0.15	5.649±0.223	4.81± 0.13*
10	20.19±0.52	9.05±1.30†	16.53±0.78*	18.56±0.28
20	41.7 8±1.49	38.19±1.94	34.93±1.48	45.08±3.37
30	79.82±1.80	68.54±2.33	64.53±5.31	81.57±8.16
60	142.95±9.26	135.96±21.86	136.88±8.79	150.48±4.07
90	199.91±22.11	169.43±4.80*	198.91±8.92	202.10±12.73

*p<0.05, †p<0.01 (compared with the control group in all tables).

The changes in the ratio of brain weight to body weight in all groups are presented in Table 3.

Table 3. Ratio of brain weight to body weight (g/kg) in offspring rats (n=6; mean ± SD)

Day	Control	High fluoride (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
0	33.25±1.44	35.29±1.50	34.19±0.73	36.40±0.93
10	39.62±2.13	60.65±3.36†	46.81±2.34	41.78±0.64
20	25.51±0.71	28.31±1.88	29.74±1.27*	25.08±2.01
30	15.04±0.26	17.45±0.68†	17.55±1.13	15.34±1.45
60	8.80±0.54	10.47±1.19	9.12±0.53	8.49±0.27
90	6.75±0.58	8.01±0.19	6.33±0.17	7.12±0.36

*$p<0.05$, †$p<0.01$.

The ratios of thyroid weight to body weight are shown in Table 4.

Table 4. Ratio of thyroid weight to body weight (g/kg) in offspring rats (n=6; mean ±SD).

Day	Control	High fluoride (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
0	0.1380±0.011	0.1500±0.029	0.1366±0.019	0.1397±0.014
10	0.0971±0.011	0.1247±0.015	0.1310±0.014	0.1332±0.008*
20	0.1000±0.003	0.1259±0.006†	0.1172±0.009	0.1183±0.007†
30	0.0758±0.003	0.0836±0.005	0.1027±0.004†	0.0782±0.002
60	0.0678±0.005	0.0652±0.005	0.0805±0.009	0.0616±0.004
90	0.0588±0.004	0.0538±0.002	0.0573±0.002	0.0697±0.005

*$p<0.05$, †$p<0.01$.

Learning and memory effects in offspring rats: The results of first EN1) and second (EN2) error numbers and sustaining time (ST) are recorded in Table 5.

Table 5. Results of error number (EN) and sustaining time (ST, seconds) in learning and memory trials of offspring rats (n=6; mean ±SD)

	Day	Control	High fluoride (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
EN1	30	1.500±0.223	2.500±0.763	2.500±0.223*	3.333±0.614*
	60	1.333±0.210	1.500±0.341	2.333±0.421	3.666±0.988*
	90	1.333±0.333	1.666±0.210	2.833±0.542*	3.166±0.703*
ST	30	113.6±40.72	5.000±1.692*	11.73±3.011*	1.666±0.458*
	60	114.9±58.83	68.40±44.00	20.60±5.428	11.41±3.020
	90	118.4±57.77	16.75±6.120	14.86±5.839	3.933±2.761
EN2	30	0.000±0.000	0.666±0.210*	0.500±0.223*	2.333±0.494†
	60	0.333±0.210	0.166±0.166	1.333±0.494	2.500±0.921*
	90	0.166±0.166	0.666±0.210	1.333±0.954	1.500±0.670

*p<0.05, †p<0.01.

Brain biochemistry in offspring rats: Changes in brain protein content are shown in Table 6.

Table 6. Brain protein content (g/kg) of offspring rats (n=6; mean ±SD)

Day	Control	High fluoride (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
0	3.9772±0.5052	2.7802±0.3218	1.9260±0.2511 †	1.5175±0.2321 †
10	4.9255±0.3284	4.4631±0.1897	4.0342±0.7262	4.1044±0.2894
20	7.7946±0.5376	7.0975±0.2487	6.1470±0.0931*	4.8836±0.3662 †
30	9.2105±0.3257	9.0538±0.2296	9.1117±0.4984	5.3793±0.3714 †
60	5.7081±0.5033	5.6776±0.5077	4.5482±0.3117	5.7692±0.5444
90	5.0671±0.5644	5.0282±0.4650	5.0104±0.4110	6.0012±0.5017

*p<0.05, †p<0.01.

Cholinesterase (ChE) changes are listed in Table 7. Correlation coefficients (r) between ChE and EN1, between ChE and EN2, and between ChE and ST were –0.362 ($p<0.01$), –0.386 ($p<0.01$), and 0.369 ($p<0.01$).

Table 7. Change in ChE activity (μmol/mL) in the brain of offspring rats (n=6; mean ±SD)

Day	Control	High fluoride (HF)	Low iodine (LI)	High fluoride and low iodine (HFLI)
0	0.3111±0.0450	0.2516±0.0143	0.2797±0.1108	0.6435±0.0752*
10	0.4992±0.0962	0.2301±0.0235*	0.1644±0.0333*	0.8474±0.0967†
20	0.3683±0.0159	0.1952±0.0310†	0.3280±0.0334	0.4334±0.0678
30	0.5667±0.0383	0.4360±0.0377*	0.5040±0.0842	0.4500±0.0323*
60	0.7560±0.0971	0.9150±0.0746	0.4729±0.1082	0.2251±0.1056†
90	1.3399±0.0756	1.2091±0.1089	0.4900±0.0321†	0.3154±0.0214†

*$p<0.05$. †$p<0.01$.

DISCUSSION

Effect of high fluoride and low iodine on rat growth and development: Generally speaking, ingestion of high fluoride, low iodine, or the combination of high fluoride and low iodine showed no significant effect on body, brain, and thyroid weight gains in offspring rats up to day 90. However, the body weight at birth was affected by the interaction of high fluoride and low iodine. The influence of low iodine on brain protein in offspring rats was significant at day 0 and day 20, and an even greater reduction was apparent with high fluoride and low iodine. These results clearly suggest that the development of offspring rats was affected by the interaction of high fluoride and low iodine.

Effect of high fluoride and low iodine on cholinesterase activity: Cholinergic neurotransmitters of the central nervous system are necessary for learning and memory in animals. Acetylcholine is essential for memory tracking in neurotransmission and provides the physiological basis for long-term memory.[12] Decreased learning and memory ability is reflected by lower levels, lower recovery rates, and abnormal functioning in many kinds of neurotransmitters.[13] Owing to the role of cholinesterase (ChE) in decomposing acetylcholine, the activity of ChE is also linked to the functional condition of cholinergic nerve pathways.

Results reported by others indicate that low iodine can markedly decrease ChE activity in the brain cortex of young rats.[14] In our study, ChE activity in the brain increased less with time in the low iodine group than in the control group, and the difference was significant at day 10 and day 90. This result might be connected with the significant decrease in brain protein observed at day 0 and day 20, but the relationship between changes in brain protein and ChE activity is not known and clearly deserves further study.

As far as the effects of fluoride on ChE activity are concerned, the literature is somewhat limited, and the findings are varied. Wen[15] reported that blood ChE activity of residents in areas affected by fluorosis, was slightly decreased compared to that in residents of unaffected areas. Later, he determined ChE activity in rabbits and rats before and after they were exposed to fluoride, finding a decrease in ChE activity after fluoride exposure ($p<0.05$). These results indicated that ChE activity was depressed by high fluoride. However, Sun[16] and also Cheng *et al*[17] report that ChE activity in brain homogenates of rats drinking high-fluoride water increased markedly. Obviously, the effect of fluoride on ChE activity is not straightforward and needs further study.

In the present work, normal increases in brain ChE activity in offspring rats were depressed significantly at day 10, 20, and 30 in the high fluoride group compared to the control group. As already noted, the same trend was found in the low iodine group and the difference from the control group was significant at day 10 and day 90. Nevertheless, the brain ChE activity in the combined high fluoride and low iodine group increased markedly at day 0 and day 10, but then it decreased significantly from day 30 through day 90 compared with that of the control group, indicating that the change of the enzyme activity was complicated.

From these results, we can see that the learning and memory ability of the rats is related to the brain protein and ChE activity in offspring rats. The effect of high fluoride and low iodine on brain function in offspring rats is thus mainly observable in the early period of development and growth.

CONCLUSION

Under the conditions of these experiments, the brain protein content of offspring rats was significantly decreased at birth and at day 20 by low iodine, and it was reduced even more at birth and at day 20 and day 30 by the interaction of high fluoride and low iodine, but it was not affected by high fluoride only. ChE activity in the brain of offspring rats was diminished to differing extents by high fluoride or low iodine and especially by the combined interaction of high fluoride and low iodine. Over time, learning and memory were increasingly disturbed by exposure to high fluoride or to low iodine or together in combination.

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