APOPTOSIS IN BRAIN CELLS OF OFFSPRING RATS EXPOSED TO HIGH FLUORIDE AND LOW IODINE

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SUMMARY: To assess brain cell apoptosis induced by high fluoride and/or low iodine in their offspring, 32 one-month old Wistar albino rats were divided randomly into four equal groups, each with six females and two males. The first group of rats served as the untreated controls; the second group received high fluoride (HiF) in their drinking water (100 mg NaF/L); the third group was placed on a low iodine (LI) diet (0.0855 mg I/kg); and the fourth group was exposed to the same concentrations of HiF and LI together. After the animal model was established, the rats were allowed to breed, and 36 offspring rats in each group were randomly selected for the experiments. The treatment for these second generation rats was the same as for their parents. At 0, 10, 30, 60, and 90 days after birth, these offspring rats were anesthetized and their brain cells prepared for flow cytometry. In comparison with the controls, the percent of brain cell apoptosis in the offspring rats in the three treated groups was obviously higher, especially in the HiF+LI group. With aging, brain cell apoptosis increased gradually in every group before the 30-day mark. These results indicate that cell apoptosis may play an important role in brain function affected by exposure to HiF, LI, and HiF+LI.

Keywords: Apoptosis; Brain cells; Flow cytometry; High fluoride intake; Iodine deficiency; Offspring rats.

INTRODUCTION

Apoptosis is the natural process of programmed cell death and is essential for the balance between proliferation, growth arrest, and cell death.[1] Apoptosis takes place continuously throughout the life of multicellular organisms. In response to specific signals instructing a cell to undergo apoptosis, a number of distinctive biochemical and morphological changes occur within the cell. Cell apoptosis is characterized by a series of biochemical and morphological changes, such as caspase family activation, nucleosomal DNA fragmentation and DNA ladder,[2] cell volume loss, chromatin condensation, cytoplasmic shrinking, and dilation of the endoplasmic reticulum.

In recent years, it has been reported that fluoride (F) can induce cell apoptosis in lung,[3,4] kidney,[5,6] liver,[7,8] and bone tissues.[9-11] The process of programmed cell death is not only pronounced in the periphery, but is also extensive in the central nervous system (CNS). Chen et al.[12] have reported that when they performed intraperitoneal injection of sodium fluoride (20 mg NaF/kg/day) in Sprague Dawley rats, flow cytometry (FCM) indicated that the percentages of apoptotic cells both in brain cortex and hippocampus were significantly higher ($P < 0.01$) in

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rats given this treatment (27.12 ± 3.08, 34.97 ± 5.46, respectively) than those in control group (4.63 ± 0.98, 5.35 ± 0.79). The same results were found for neuron apoptosis in rats under conditions of chronic fluorosis.[13] Moreover, at the same time degenerative changes and apoptosis in the process also occur. F in general is toxic to all types of living cells.[14] Another question that must be considered is whether F toxicity is enhanced by iodine (I) deficiency.[15] Epidemiological investigations reveal that the IQ of children in high F (HiF) and low I (LI) areas is 19-25% lower than the average IQ of children in control areas.[16]

In view of the results of our previous research on the effects of HiF and LI concentrations on various biochemical indexes including the histopathology of the brain, learning-memory in rat offspring,[17-19] and even DNA damage in brain and thyroid cells in adults,[20,21] we have been further impelled to study cell apoptosis in the brain cells of offspring rats exposed to HiF and LI by flow cytometry.

MATERIALS AND METHODS

Experimental protocol: As in our recent reports,[17-21] one-month old Wistar 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 our previous studies[17-21] was also employed here as shown in Table 1.

Table 1. NaF in the drinking water (mg F^-/L) and F and I levels in the diet (mg/kg) 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
Fluoride in drinking water	<0.6	45[a]	<0.6	45[a]

[a]From 100 mg NaF/L (as recorded in our three previous reports[17-21]).

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

Breeding of the offspring of iodine deficient rats: Three months after establishing the animal model, the females in each group were allowed to become pregnant by natural mating with their male group mates. The day of birth of their offspring was set as day 0. During and after nursing, the offspring rats 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, 30, 60, and 90, three males and three females were randomly selected from each litter of each group for further study.

Flow cytometry analysis of apoptosis: The rats were sacrificed by transcardial perfusion with 0.9% saline followed by 20% urethane (ethyl carbamate, $NH_2COOC_2H_5$) solution. The brains were then removed, washed three times with pH 7.4 phosphate buffered saline (PBS: NaCl 8.0 g, KCl 0.2 g, $Na_2HPO_4 \cdot 12H_2O$ 2.8 g, KH_2PO_4 0.2 g), and the brain cortex was dissected and fixed with 1 mL of 70% ethanol at 4°C for 24 hr. After washing twice with PBS, the brain cortexes were homogenized gently with the appropriate amount of PBS at 4°C. The cells were resuspended at approximately 10^6 per mL in PBS and then stained with 50 mg/mL propidium iodide (PI) for 30 min in the dark at 37°C. These samples were analyzed by FACS caliber flow cytometry (FCM, Becton Dickinson) with excitation set at 488 nm. In each sample, the cells with a lower DNA content than those of the G0/G1 phase were referred to as apoptotic cells.

RESULTS

In comparison with the controls, the percentages of brain cell apoptosis in offspring rats in the HiF group, the LI group, and the HiF+LI group was noticeably higher, especially in the HiF+LI group. With aging, the frequency of brain cell apoptosis increases gradually in every group before the 30-day mark (Table 2 and the Figure).

Table 2. Effect of high fluoride (HiF) and low iodine (LI) on brain cell apoptosis in offspring rats

(%, Mean ±SEM)

Days after birth	Controls	LI	HiF	HiF+LI
0	5.02±0.78	8.76±0.44*	6.58±0.55	8.88±0.01*
10	5.48±0.71	11.41±0.38	17.14±2.41*	17.35±2.01*
30	9.68±1.25	13.52±0.72*	16.71±4.91*	24.17±1.47**
60	10.85±1.24	18.46±0.01*	12.11±0.70	19.34±0.50**
90	10.99±3.03	11.24±0.72	12.93±0.67	32.55±2.31**
Mean values	8.43±0.77	12.51±0.56*	13.09±1.12*	21.65±2.09**

*p<0.05, **p<0.01.

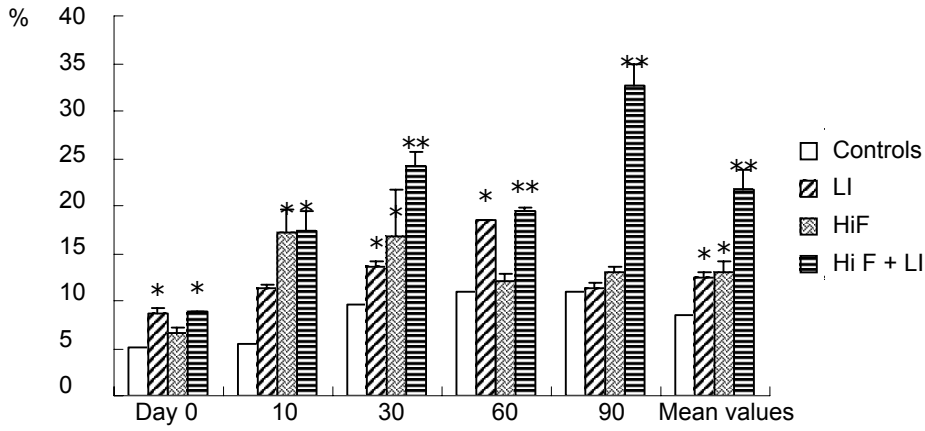

Figure. Effects of high fluoride and low iodine on brain cell apoptosis in offspring rats. *p<0.05; **p<0.01.

DISCUSSION

Apoptosis is a genetically regulated form of cell death, in which superfluous or abnormal cells are eliminated, thereby ensuring normal development of multicellular organisms and maintenance of tissue homeostasis. Fluoride (F), as an exogenous toxicant, induces cell apoptosis in various tissues. In our study, the percent of brain cell apoptosis in rat offspring exposed to high fluoride (HiF), low iodine (LI), and both treatments combined showed marked increase, especially in the HiF+LI group. With aging, brain cell apoptosis increased gradually in every group before the 30-day mark. After 90 days, the percentage of apoptosis in the HiF+LI group was almost three times that of the control group. This result may reflect different degrees of brain damage during the period of brain development.

One possible mechanism of cell apoptosis induced by F is as follows: (1) F is an effective activator of the signal transduction pathway regulated by G proteins and can probably induce a conformational change of G protein that regulate second messenger cAMP and Ca^{2+}, thereby ultimately leading to cell apoptosis.[22] (2) F is a chemically active ionized element. It can affect oxygen metabolism and induce the production of oxygen free radicals. At the same time, F in the body binds antioxidants (such as N-acetyl cysteine (NAC), glutathione (GSH) and so on) and other free-radical destroying enzymes, and it triggers oxidative stress and cell damage and even cell apoptosis.[23-25] (3) F can induce a change in the levels of expression of some apoptotic genes.[5,26] (4) As an archoplastic intoxicant, fluoride can induce DNA damage in different tissues, and thereby lead to cell apoptosis.[7,12,20,21,27] In our previous studies, the rate and degree of DNA damage in brain cells were generally higher in the HiF group.[20] Thus F may be an important factor in inducing cell apoptosis.

Various studies have shown that iodine deficiency induces cell apoptosis in the brain[28] and the thyroid gland.[29] In our study, the percent of brain cell apoptosis in the LI group was markedly higher at days 0, 10, 30, and 60 as compared with that of the control group. This supports the hypothesis that cell apoptosis is induced by

iodine deficiency. Potential mechanisms include the disturbance of thyroid hormones, caspase enzyme activity, protein kinase, and others.

Concerning the relation between F and iodine deficiency, we have reported previously that the effects of the interaction of both factors in tandem on rat brain function, cell structure, and oxidative DNA damage, are more obvious than of either HiF or LI levels alone. In the present study, the ratio of cell apoptosis in the HiF+LI group is higher than in all the other treatments, especially at days 30 and 90. Cell apoptosis may therefore play an important role in the regulation of cell quantities within the brain, and may be involved in the processes leading to decreased learning-memory abilities of children living in high F as well as iodine deficient areas, and especially in areas of both high F and low iodine.

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