EFFECTS OF HIGH FLUORIDE AND ARSENIC ON BRAIN BIOCHEMICAL INDEXES AND LEARNING-MEMORY IN RATS

Chunxiang Wu,[a,b] Xinli Gu,[c] Yaming Ge,[d] Jianhai Zhang,[a] Jundong Wang[a]

Shanxi, China

SUMMARY: Nine-six Wistar rats were randomly divided into four groups of 24 rats in each group (female:male = 1:1). Over a period up to 90 days, with one untreated group as controls, the other three groups were administered, respectively, high fluoride (100 mg NaF/L), high arsenic (50 mg As_2O_3/L), or both the same high fluoride and high arsenic concentrations in their drinking water in order to assess their effects on learning-memory ability and brain function. In comparison with the controls, learning-memory ability was depressed by high fluoride (HiF), high arsenic (HiAs), and their combination (HiF+HiAs). Brain protein contents decreased significantly in the HiF+HiAs group at days 10 and 30, and decreased cholinesterase (ChE) activities occurred in the HiF group at day 10, and in the HiF+HiAs group at days 10 and 90. Moreover, in the HiAs group, ChE activity was increased at day 30 and then decreased significantly at day 90. The total antioxidant capacity (T-AOC) in the brain was decreased in the three exposed groups. The hydrosulfide group (–SH) content of brains was decreased markedly only by HiAs. These results suggest that learning-memory ability and brain function in rats are affected by HiF and HiAs and that oxidative stress in the brain may be one of the causes of this damage.

Keywords**:** Arsenic and brain; Biochemical index; Brain protein; Fluoride and brain; Learning-memory; Rat brain.

INTRODUCTION

Fluoride (F) and inorganic arsenic (As) are often found in drinking water, and ingestion of drinking water containing high concentrations of F and As, primarily from natural contamination, is the main source of human environmental exposure worldwide.[1-3] Some reports show that F and As poisoning co-exist in certain areas. It is estimated, for example, that a resident of Aguascalientes City, Mexico, can ingest 0.233 mg F/kg bw/day and 0.678 µg As/kg bw/day from the local drinking water.[4] In the northern part of Sinkiang Municipality, in Kuitun City, China, 50,000 people suffer simultaneously from F and As poisoning.[5] In that region, wells in a 2500-km^2 area contain up to 21.5 mg F/L), and wells in 1200 km^2 of that area contain up to 0.88 mg As/L). In addition, in northern Guizhou Province, half the population with chronic exposure in coal-burning areas exhibit symptoms of F and As poisoning such as dental fluorosis, bone disease, osteoarthritis, numbness in limbs, pigmentation of skin, etc.[6]

Epidemiological studies have shown that excessive intake of F and As can affect the nervous system of humans and animals.[7] Life-long exposure of children to As is reported to produce significant hearing loss and lower scores on verbal and full IQ tests,[8] whereas adults exposed to high amounts of As have decreased verbal

[a]For Correspondence: Prof Jundong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, P.R. of China; E-mail: wangjd@sxau.edu.cn. [b]Taiyuan Farming and Veterinary College of Shanxi Agricultural University. [c]College of Animal Science and Technology, Shihezi University, Sinkiang, P.R. of China. [d]College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, 48 Xiyuan Road, Luoyang 471003, P.R. of China.

learning or memory, or they have impairment of concentration, new learning ability, and short-term memory.[9] Moreover, our previous studies suggest that learning and memory of newborn rats are diminished by exposure to high F.[10] Consequently, the aim of this research was to assess the action and mechanism of the effects of high F (HiF), high As (HiAs), and their combination on cerebral development and learning-memory ability in rats.

MATERIALS AND METHODS

Experimental protocol: Along with their standard diet, 96 one-month old Wistar albino rats, half males and half females, each weighing approximately 50 g, were obtained from the Experimental Animal Center of Shanxi Medical University for use in this study. The contents of fluoride and arsenic in the drinking water are listed in Table 1.

Table 1. Fluoride and arsenic levels in the drinking water of four groups of rats

	No. of animals	Fluoride and arsenic levels in drinking water	Diet
Controls	24	Distilled water	Standard
High fluoride (HiF)	24	100 mg NaF/L	Standard
High arsenic (HiAs)	24	50 mg As_2O_3/L	Standard
High F and High As (HiF+HiAs)	24	100 mg NaF plus 50mg As_2O_3/L	Standard

Animal test model: The 96 one-month old Wistar albino rats were randomly divided into four groups of 12 females and 12 males in each group and were maintained on the diets and water regimens shows in Table 1 under standard conditions of temperature (22–25°C), 12/12-hr light/dark cycle, ventilation, and hygiene.

Evaluation of learning and memory in young rats: Learning and memory were investigated in each group of rats for 30, 60, and 90 days by the same apparatus and "step-down test" procedure used in our previous study.[10]

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

RESULTS

The changes of brain weight in each group of rats are presented in Table 2.

Table 2. Brain weight (g) of the rats in each group (n=6; mean±SE)

Treatment days	Control	High fluoride (HiF)	High arsenic (HiAs)	High F and High As (HiF+HiAs)
10	1.1480±0.0205	1.1940±0.0749	1.1257±0.0461	1.1895±0.0287
30	1.3109±0.0090	1.1703±0.0267‡	1.1475±0.0438‡	1.1804±0.0397*
60	1.3318±0.0194	1.3511±0.0265	1.2459±0.0188*	1.2507±0.0248*
90	1.2710±0.0463	1.2934±0.0271	1.2771±0.0200	1.2468±0.0147

*$p<0.05$; ‡ $p<0.01$ (compared with the control group).

The results of first (EN1) and second (EN2) error numbers and sustaining time (ST)[12] are recorded in Table 3.

Table 3. Learning and memory ability of the rats in each group (n=6; mean±SE)

Treatment	days	Control	High fluoride (HiF)	High arsenic (HiAs)	High F and High As (HiF+HiAs)
EN1	30	0.833±0.307	1.833±0.365	2.000±0.577	3.167±0.857*
	60	1.167±0.307	1.833±0.276	1.500±0.537	3.833±1.033*
	90	1.000±0.000	1.333±0.372	1.500±0.236	4.000±1.049*
ST	30	96.63±37.84	11.24±0.865*	2.674±0.413*	14.57±2.236*
	60	103.4±45.73	57.42±11.45*	20.43±5.623*	18.37±2.357*
	90	127.3±53.44	15.32±5.863*	17.53±3.584*	13.97±3.584*
EN2	30	0.833±0.215	1.500±1.000	1.833±0.494	2.500±1.000*
	60	1.333±0.236	1.500±1.049	1.833±0.516	2.167±0.753
	90	1.500±0.425	1.333±0.516	2.167±0.365	2.333±0.865

*p<0.05 (compared with the control group).

Protein contents, activities of cholinesterase (ChE), total antioxidant capacity (T-AOC), and hydrosulfide group (–SH) contents in the rat brain tissue are recorded in Tables 4, 5, 6, and 7, respectively.

Table 4. Contents of protein (g/L) in rat brains of each group (n=6; mean±SE)

Treatment Days	Control	High fluoride (HiF)	High arsenic (HiAs)	High F and High As (HiF+HiAs)
10	4.6617±0.4411	4.6067±0.6149	4.3000±0.4394	4.0400±0.3424*
30	4.7967±0.4853	4.4350±0.2490	3.8583±0.1836	3.7583±0.2495*
60	3.9417±0.5283	3.2767±0.2092	3.1217±0.2543	3.3383±0.2152
90	4.3383±0.5262	3.1550±0.3631	3.5917±0.2954	3.5333±0.2874

*p<0.05 (compared with the control group).

Table 5. Activities of ChE (U/mg protein) in rat brains of each group (n=6; mean±SE)

Treatment days	Control	High fluoride (HiF)	High arsenic (HiAs)	High F and High As (HiF+HiAs)
10	0.444±0.072	0.183±0.0509*	0.346±0.0320	0.175±0.027*
30	0.247±0.056	0.319±0.062	0.488±0.2960*	0.286±0.088
60	0.519±0.063	0.355±0.080	0.555±0.0465	0.331±0.065
90	0.626±0.067	0.521±0.088	0.405±0.077*	0.217±0.028*

*p<0.05 (compared with the control group).

Table 6. Contents of –SH (mmol/g protein) in rat brains of each group (n=6; mean±SE)

Treatment Days	Control	High fluoride (HiF)	High arsenic (HiAs)	High F and High As (HiF+HiAs)
10	0.1428±0.0155	0.1281±0.0119	0.0885±0.0079*	0.1044±0.0188
30	0.1179±0.0069	0.0992±0.0075	0.0807±0.0115*	0.1005±0.0052
60	0.0944±0.0077	0.0933±0.0072	0.0892±0.0047	0.0898±0.0023
90	0.1273±0.0134	0.1131±0.0156	0.0835±0.0078*	0.0892±0.0047*

*p<0.05 (compared with the control group).

Table 7. Results of T-AOC (U/mg protein) in rat brains of each group (n=6; mean±SE)

Treatment days	Control	High fluoride (HiF)	High arsenic (HiAs)	High F and High As (HiF+HiAs)
10	1.7093±0.2814	0.9685±0.1135*	1.0073±0.2198*	0.9998±0.1829*
30	1.3728±0.4873	1.5148±0.1862	0.9876±0.1798*	1.3460±0.1372
60	1.3024±0.3459	1.1324±0.1705	1.0628±0.1019	1.2142±0.4372
90	1.8150±0.2417	1.6263±0.2032	1.2453±0.2420*	1.3606±0.2453*

*p<0.05 (compared with the control group).

DISCUSSION

Effect of high fluoride and high arsenic on brain development: In comparison with the controls, brain weights of rats in the three experimental groups decreased markedly after 30 days of treatment, and the difference was very obvious with the high fluoride (HiF) and high arsenic (HiAs) groups individually. This result suggests that brain development can be significantly affected by HiF, HiAs, and by HiF+HiAs.

Effect of high fluoride and high arsenic on learning-memory ability of rats: As found and discussed in our previous research,[10] learning-memory ability can reflect central nervous system function in animals. In the present study, error number (EN1) in the three experimental groups increased markedly compared with the control group, and error number after 24 hr (EN2) also increased by HiF, HiAs, and HiF+HiAs, but they increased markedly only in the HiF+HiAs group. The sustaining time (ST) in the three experimental groups was reduced significantly after treatment for 30, 60, and 90 days. These results indicate that learning-memory ability of the experimental rats decreased by exposure to HiF, HiAs, and their interaction.

Effect of high fluoride and high arsenic on brain protein and cholinesterase activity: Brain protein and cholinesterase (ChE) activity are important to maintain normal brain physiological function and learning-memory ability.[11] Decreased learning-memory ability is reflected by lower levels, lower recovery rates, and abnormal functioning in many kinds of neurotransmitters.[12] ChE is linked to cholinergic nerve function and plays a key role in deacetylating acetylcholine.[12] Increased and decreased ChE activities were found at different tissues of F-treated animals.[13-15] Decreased brain protein contents and changed ChE activity after exposure F were also investigated in our previous study.[10]

In the present work, the brain protein contents decreased significantly in rats treated for 10 and 30 days by the combination of HiF and HiAs. ChE activity deceased significantly in the HiF group at 10 days, in the HiAs group at 90 days, and in the HiF+HA group at days 10 and 90 compared to the controls. In addition, ChE activity in the HiAs group had a trend toward first increasing and later deceasing. These results indicate that the changes induced by F and As in ChE activity is complicated.

Effect of high fluoride and high arsenic on hydrosulfide group (–SH) content of brain: The hydrosulfide group (–SH) is a reductive base group and an important factor for cell protection. As^{3+} ions can combine with –SH groups, thereby

decreasing their content and thus affecting the activity of enzymes and other active matter containing –SH. Hu et al. observed that –SH content in the blood was decreased through chronic arsenic poisoning of mice.[16] In the present study, the content of –SH in the experimental groups was decreased, especially in the HiAs group. These results suggest a decreasing content of –SH groups followed by deactivation of affected enzymes may be one of the main pathways by which As injures brain tissue. However, HiF had no effect on the –SH content in the brain tissue during the entire experimental period.

Effect of high fluoride and high arsenic on T-AOC: Total antioxidant capacity (T-AOC) is a useful index of the combined action antioxidants in the body.[17] Many studies suggest that F and As can induce lipid peroxidation, increase free radical production, and reduce the total antioxidation capacity, respectively.[18-20] In the present study, T-AOC in the brain in the HiAs the HiF+HiAs group was deceased significantly, especially in experimental rats treated for 10, 30, and 90 days ($P<0.05$) compared with the control group. Decreased T-AOC also occurred in the brain tissue of the HiF group. This showed that increased lipid peroxidation was caused by HiF and HiAs, with the result that various forms of brain disturbances were produced.

However, it should also be noted that the oxidant/antioxidant system is regulated by thyroid hormones.[21,22] According to the literature, F may manipulate deiodinases directly as a TSH (thyroid stimulating hormone) analogue, since TSH levels directly correlate with MDA and SOD activity.[23,24] Likewise, As is known to influence deiodinase activity by interfering with selenium.[21,25] In our studies, we have also been investigating the thyroid status of rats treated with HiF and HiAs. This work will be discussed in our next paper.

In conclusion, excessive intakes of F and As or both together affect brain biochemical indexes and depress the learning-memory ability of rats. Oxidative stress, induced by HiF and HiAs, may be a key factor leading to a reduction in learning and memory ability.

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