

Determination of the Contents of Amino Acid and Monoamine Neurotransmitters in Fetal Brains from a Fluorosis Endemic Area

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Abstract: The contents of five types of amino-acid neurotransmitters and three types of monoamine neurotransmitters in the brains of fetuses aborted through induced labor in a chronic fluorosis-endemic area were determined. Findings revealed that the content of the excitatory amino acid, aspartic acid, was significantly lower than in the fetuses from the non-endemic area whereas the content of the inhibitory amino acid, taurine, was significantly higher; the content of the major spinal cord-inhibitory glycine was significantly reduced. Among the monoamine neurotransmitters, the content of norepinephrine was significantly reduced; the contents of 5-hydroxytryptamine in the frontal and the occipital lobes were elevated and the content of 5-hydroxytryptamine in the parietal lobe (precentral and postcentral gyri) was reduced.

Keywords: Fluorosis; Fetus; Brain Amino Acids; Norepinephrine; Serotonin

With progressively deepening studies on chronic fluorosis, the actions of fluoride on the central nervous system have become more of greater concern. Until now, however, there have been few reports on this aspect; only some epidemiological studies and animal experiments are available; moreover, there is very little evidence on fluoride's direct effects on the human brain. We have previously conducted stereological studies on fetal brains in fluorosis-endemic areas and there was evidence of neuronal growth delays and poor differentiation[1]. In recent years, the actions of central nervous system neurotransmitters have come to people's attention. In order to understand the effects of fluorosis on the growing brain, we have collected the brains from fetuses aborted through labor induction in a chronic fluorosis-endemic area, and determined the content of five types of amino-acid neurotransmitters and three types of monoamine neurotransmitters.

1. Materials and Methods

1.1 Collection of Fetuses

1.1.1 *Fetuses in the endemic area:* The fetuses were collected from Zhijin County, Guizhou Province, where the mean fluoride content in coal was 143 mg/kg and the mean amount of fluoride emitted during the combustion process was 128.6 mg/kg. The mean fluoride content in coal smoke-roasted corns, a staple food of local residents, was 13.9 mg/kg, while the mean fluoride content in drinking water was only 0.21 mg/L; hence it was a coal-burning endemic fluorosis area. In the 10 fetuses we collected, their mothers had long resided in

the fluorosis-endemic area, consumed high-fluoride food and were afflicted with dental fluorosis of varying degrees of severity; of these cases, there were 3 cases of [grade I], 5 cases of [grade II] and 2 cases of [grade III], but clinical symptoms and signs were absent and other diseases affecting bone metabolism were also absent.

1.1.2 *Fetuses in the control group:* Control fetuses were collected from Guiyang City, Guizhou Province, where the mean fluoride content in food was 4.7 mg/kg and the fluoride content in drinking water was 0.5 mg/L; hence it was a non-fluorosis-endemic area. The mothers of the ten fetuses were all healthy persons.

1.1.3 *Specimen treatment:* Artificial water-bag labor induction was applied and following labor induction, the three brain areas of the frontal, parietal and occipital lobes were immediately collected and immediately placed at -40°C for low-temperature storage.

1.1.4 *Fetal age:* The fetal age was determined on the basis of the mother's history of suspended menstruation and the fetal crown-rump length[2]; the ages of the fetuses collected from the fluorosis-endemic area and the control area in this experiment were 5–7 months.

1.2 Determination Methods

1.2.1 *Determination of urine and serum fluoride contents in pregnant women:*

Instruments and reagents: PXJ-IB digital ion meter and CSP-F-1 fluoride ion-selective electrodes; 1µg/ml F- standard solution prepared in 0.02M pH5.0 trisodium citrate-sodium nitrate buffer solution.

Specimen pretreatment and determination: Morning fasting venous blood was drawn from pregnant women and serum was separated; morning urine of pregnant women was also collected. The serum and urine fluoride contents were determined by using the fluoride ion-selective electrode method.

1.2.2 Determination of the fluoride content in fetal brain tissues and bone tissues:

Instruments and reagents: the ion meter and the reagents were the same as above; model 201 fluoride ion-selective electrodes were used.

Tissue pretreatment and determination: fresh brain tissues were weighed and were cut up in pieces, baked to dryness, carbonized and ashed; the muscles and the connective tissues were removed from the right femur and were then baked to dryness, carbonized and ashed. The fluoride contents in the aforementioned ashed brain tissues and bone tissues were determined by using the fluoride ion-selective electrode method.

1.2.3 Determination of the content of amino-acid neurotransmitters in brain tissues:

Instruments and reagents: polyamide films (Zhejiang Huangyan Chemical Experiment Factory), 7 cm x 7 cm; ultraviolet lamp for thin-layer chromatography; dual-wavelength thin-layer scanner. Standard mixed solutions of aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), γ -aminobutyric acid (GABA) and taurine (Tau) at concentrations of 2×10^{-4} M– 4×10^{-4} M.

Tissue pretreatment and determination Parietal tissues from the brain were collected, were homogenated with pre-cooled tri-distilled water at 1:4 (g/ml), then centrifuged, and their protein precipitates were

removed; they were subject to vacuum drying under reduced pressure, dansylated and subjected to thin-layer chromatography on a polyamide film; the film following thin-layer chromatography was scanned with a dual-wavelength thin-layer scanner and was then calculated[3].

1.2.4 Determination of the content of monoamine neurotransmitters in brain tissues at different sites:

Instruments and reagents: A high-performance liquid chromatography instrument (Waters Corporation, USA), including a 570 pump, a 460 electrochemical detector, a U6K sample injector, and an 810 chromatography workstation. Norepinephrine, epinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were purchased from Sigma Ltd.

Brain tissue pretreatment and determination: A certain amount of brain tissues were weighed to precision, were homogenated with 0.2N perchloric acid (1:5), and were centrifuged at 18000 r/min at 4°C for 40 minutes; 100 μ l of the supernatant was collected for sample injection. The operating voltage was 0.70V; the sensitivity was 0.2nAFs; the flow was 1.6ml/min.

2. Results

2.1 See Table 1 for serum and urine fluoride contents in pregnant women and the fluoride contents in fetal bone and brain tissues.

It can be seen from the table that the serum and urine fluoride contents in pregnant women from the fluorosis-endemic area were higher than those in the pregnant women from the non-fluorosis-endemic area, and the differences in the urine fluoride content between the

Table 1: Fluoride Contents in Pregnant Women and Fetuses

Group	No. of Cases	Pregnant Women		Fetuses	
		Urine (μ g/ml)	Serum (μ g/ml)	Bone (μ g/g)	Brain (μ g/g)
Non-endemic area	10	1.67 \pm 0.82	0.41 \pm 0.15	2.50 \pm 0.11	1.23 \pm 0.24
Endemic area	10	4.37 \pm 2.94	0.55 \pm 0.21	2.77 \pm 0.25	1.55 \pm 0.33
P value		<0.05	>0.05	<0.05	<0.05

The data in this table is $\bar{x} \pm$ SD

two groups were significant ($P < 0.05$). The fluoride contents in fetal bone and brain tissues in the fluorosis-endemic area were higher than those in the non-fluorosis-endemic area, and the between-group differences between the two types of tissues were also significant.

2.2 See Table 2 and Table 3 for the determination of the contents of amino-acid neurotransmitters in fetal brains.

It can be seen from Table 2 that the content of Asp in fetal brains in the fluorosis-endemic area was significantly lower than that in fetal brains from the non-fluorosis-endemic area but there were no significant differences in the content of Glu.

Group	Number of Cases	Asp	Glu
Non-endemic area	8	1762.89 \pm 496.12	4408.29 \pm 911.56
Endemic area	10	1009.31 \pm 225.25 ⁽¹⁾	4094.94 \pm 895.35

(1) $P < 0.01$

It can be seen from Table 3 that the content of Tau in fetal brains from the fluorosis-endemic area was significantly higher than in fetal brains from the non-fluorosis-endemic area, while the contents of Gly and GABA were significantly lower.

Group	Number of Cases	Tau	Gly	GABA
Non-endemic area	8	2506.10 \pm 342.21	3422.55 \pm 763.01	994.46 \pm 99.16
Endemic area	10	3653.09 \pm 468.00(1)	982.09 \pm 210.70(1)	779.51 \pm 214.78(2)

(1) $P < 0.01$ (2) $P < 0.05$; same for each of the following tables

2.3 See Table 4 and Table 5 for the determination of the content of monoamine neurotransmitters in fetal brain tissues at different body sites

3. Discussion

This study has determined the urine fluoride content during pregnancy in the mothers of 10 fetuses aborted through induced labor in a fluorosis-endemic area, and the findings were significantly higher than those in

Group	Number of Cases	NE			E		
		Frontal	Parietal	Occipital	Frontal	Parietal	Occipital
Non-endemic area	10	37.83 \pm 16.25	92.91 \pm 41.12	41.58 \pm 10.92	13.81 \pm 1.01	17.44 \pm 2.82	13.06 \pm 1.53
Endemic area	10	43.92 \pm 8.32	32.81 ⁽¹⁾ \pm 18.30	9.77 ⁽¹⁾ \pm 1.09	22.08 ⁽¹⁾ \pm 5.03	21.84 ⁽¹⁾ \pm 2.92	14.99 \pm 2.60

Group	Number of Cases	DA			DOPAC		
		Frontal	Parietal	Occipital	Frontal	Parietal	Occipital
Non-endemic area	10	3.53 \pm 1.70	5.28 \pm 2.79	4.25 \pm 1.66	6.35 \pm 1.98	8.01 \pm 4.36	0.94 \pm 0.36
Endemic area	10	15.66 ⁽¹⁾ \pm 12.14	7.34 \pm 3.92	4.07 \pm 2.48	15.91 ⁽²⁾ \pm 8.20	10.34 \pm 8.66	4.07 ⁽²⁾ \pm 2.82

pregnant women during the same period in a non-endemic area; moreover, these ten pregnant women were all afflicted with dental fluorosis, which demonstrated that these pregnant women were patients with chronic fluorosis and had accumulated excessive fluoride in their bodies.

Numerous animal experiments and epidemiological studies have demonstrated that when a pregnant woman takes in a higher quantity of fluoride, fluoride can enter the fetus through the placenta and can also accumulate in brain tissues through the blood-brain barrier [4]; our findings have also proved this. Thus, it can be considered that beginning from the fetal period, the children born to mothers with fluorosis start to come into contact with more fluoride than the fetuses from non-endemic areas, and moreover, such an excess of fluoride is accumulated in the brain and will produce certain effects on the brain.

The contents of the five types of amino acids in the brain as determined by us have demonstrated that the content of the excitatory amino acid, Asp, in fetal brains in the fluorosis-endemic area was significantly reduced compared to that in the fetuses from the non-fluorosis-endemic area; no significant between-group differences were found in the content of another type of excitatory amino acid, Glu. The findings with respect to the inhibitory amino acids revealed that Tau content was significantly increased in fetal brains from the fluorosis-endemic area, but the contents of the other two types of inhibitory neurotransmitters, namely Gly and GABA, were reduced in fetal brains from the fluorosis-endemic area; of these two, there was a very large reduction in the content of Gly.

A greater volume of data has demonstrated that norepinephrine (NE) in the brain enables an organism to be in a state of arousal and to be involved in the

regulation of the complex response systems, sleep and emotions[5]. The NE contents in the parietal and occipital parts of fetal brains in the fluorosis-endemic area were significantly reduced as compared to those in the control group, which suggests that this function of maintaining the central nervous system in a proper state of excitation has been weakened.

The parietal lobe of the brain has a somatosensory area which manages the somatosensory sensations such as pain, temperature, touch and pressure throughout the body as well as position sense and kinesthesia. Of these, the pain sensation is closely related to 5-hydroxytryptamine (5-HT). Many experiments have demonstrated that oversensitivity to pain sensation results when the 5-HT system is inhibited; the analgesic effects of morphine will become weakened. Data has also demonstrated that NE is involved in analgesia but its actions may be indirect. It can be seen from our experimental findings that the content of 5-HT at the parietal lobe of fetal brains in the fluorosis-endemic area was significantly reduced and the content of NE was also reduced; such findings were consistent with the findings obtained by YUAN Shude et al. concerning a reduction in the activities of the 5-HT system at the hypothalamus at the time of chronic fluorosis as well as patients' oversensitivity to pain sensation, which can explain its pathogenesis from the changes in the amount of neurotransmitters.

As opposed to the parietal lobe, the contents of 5-HT in the frontal and the occipital lobes in fetal brains from the fluorosis-endemic area were significantly higher than those in the fetuses in the control group. The actions of 5-HT are predominantly manifested as inhibitory actions in the central nervous system; thus its increase can weaken the functions related to the frontal and the occipital lobes. The frontal and occipital lobes include the

Table 6: Contents of 5-Hydroxytryptamine (5-HT) and its Metabolite in Fetal Brains (ng/g)

Group	No. of Cases	5-HT			5-HIAA		
		Frontal	Parietal	Occipital	Frontal	Parietal	Occipital
Non-endemic area	10	19.61 ± 7.13	450.60 ± 319.93	1.66 ± 0.43	24.36 ± 8.85	37.53 ± 20.14	177.96 ± 34.19
Endemic area	10	130.37 ⁽¹⁾ ± 52.30	123.34 ⁽¹⁾ ± 31.91	10.50 ⁽¹⁾ ± 2.17	56.21 ⁽¹⁾ ± 21.13	31.28 ± 18.95	23.36 ⁽¹⁾ ± 16.51

visual, auditory, speech and writing areas, and the visceral regulatory areas; more importantly, the prefrontal cortex is closely associated with higher mental functions such as memory. An enhancement in the inhibitory actions at these sites will produce adverse consequences.

Our findings on the content of dopamine (DA) in brains have demonstrated that the content of DA in the frontal lobe was significantly increased and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), was also significantly increased; the DA in brains was chiefly distributed in the frontal lobe and the areas of the limbic system; no differences were noted in the numerical values in the content of DA at the occipital lobe as compared to those in the control group; but the content of its metabolite, DOPAC, was significantly increased. Thus it can be deduced that its synthesis is still increased and this might lead to neuropsychiatric abnormalities.

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