[Translated by Shan Ying and published with the permission of the Chinese Journal of Pathology 1992;21(4):218-20.]

THE EFFECT OF FLUORINE ON THE DEVELOPING HUMAN BRAIN

Li Du,[a] Changwu Wan, Xumao Cao, Jialiu Liu

Guiyang, China

SUMMARY: Fifteen fetuses from an endemic fluorosis area that were aborted therapeutically at the 5th–8th month of gestation were compared with 16 aborted fetuses from a non-endemic area. Stereological study of the brains showed that the numerical density of the neuron volume and the undifferentiated neuroblasts as well as the nucleus-cytoplasm ratio of the neurons were increased. The mean volume of the neurons, however, was reduced. The numerical density of volume, the volume density, and the surface density of the mitochondria were significantly reduced. The results showed that chronic fluorosis in the course of intrauterine fetal life may produce certain harmful effects on the developing brain of the fetus.

Keywords: Brain damage; Computer data treatment; Electron microscopy; Fetal fluoride; Fluoride poisoning; Human fetuses; Image processing; Neuron development.

INTRODUCTION

It is known that fluoride can cross the placenta from the mother's blood to the developing fetus. However, the theory there is a direct link between fluoride effects and brain cell damage is still controversial due to lack of adequate evidence. In order to determine if there are any adverse effects on the developing human brain, especially starting from formation of the embryo, fetuses from an endemic fluorosis area at the 5th–8th month of gestation were compared with those from a non-endemic area.

MATERIALS AND ANALYTIC METHODS

PHASE I. FETUSES AND GROUPS: Upon carefully inducing abortion, fetuses were preserved with a 2% polysaccharide formaldehyde and 2% amyl aldehyde solution mixed into their aorta. The groups were sorted by living area of their female parents and the gestation month. In total, 31 samples were divided into two groups for comparison.

Group A. Endemic fluorosis area fetuses: Fifteen aborted fetuses at the 5–8th month of gestation were from an endemic fluorosis area in Guizhou Province, China. This is an area that has been exposed to environmental fluoride pollution for a long time. The selected mothers lived in the area without protection from the pollution. Everyday they ate food containing high levels of fluoride in their daily diet and drank water containing fluoride. All mothers were observed to have different levels of dental fluorosis. The fluoride in their urine samples was 6.51±3.92 ppm. No other fluorosis symptoms or clinically related diseases were noted.

Group B. Non-Endemic fluorosis area fetuses: Sixteen aborted fetuses at the 5th–8th month of gestation were from Guiyang, a non-endemic area in the same province of China. All their mothers were free from chronic fluorosis disease, in

[a]Department of Pathology, Guiyang Medical College, Guiyang 550001, PR China.

good healthy condition, and did not use any fluoride toothpaste. The fluoride in their urine samples was 1.40±1.06 ppm.

PHASE II. ANALYTICAL METHODS FOR DETERMINATION OF FLUORIDE IN BRAIN OF FETUSES:

i) The widely accepted method of ion selective electrode (ISE) was used to measure the levels of fluoride in the samples.

ii) The cortex, hippocampus and cerebellum were collected for hematoxylin and eosin (HE) and Nissl staining. By a image analysis instrument,[4] a computer-assisted stereological method was used to measure all the morphological indexes (Von Economo unit = 100 × 100 × 100 μm^3), including: 1) cortex delamination and differentiation of growth; and 2) numerical density, mean volume and volume density of neurons, as well as ratio of nucleus to cytoplasm, and the numerical density of undifferentiated neuroblasts.

iii) A scanning electron microscope (SEM) was used for imaging of fluorine and chemical analysis. Normally specimen cones with conductive coated samples is required for energy emission in 2.5% glutaric dialdehyde fluid. This transmission electron microscope (TEM) equipped with an energy dispersive spectrometer (EDS) provided 60 kV for activity acceleration. High resolution provided elemental analyses for areas as small as a few nanometers in diameter with detectable quantities down to 19,000 magnification. Larger micrographics double in size of that detected were prepared for computer calculation. Statistical analysis of the results was done with the exact alternate T-test, the rank sum test, and multiple regression analysis.

RESULTS

The fluoride level in fetus brains from the endemic fluorosis area was 0.28±0.14 μg/g which was higher than the levels in the non-endemic area at 0.19±0.06 μg/g ($p < 0.05$).

The lamination of the brain cortex was still incomplete in those fetuses of 5–6 months of gestation. Only three items, molecular density, neurons, and granular layers, could be distinguished at this early period. With more complete fetus formation at the 7–8 gestation month, the stereological study of dysmorphology also included data on hippocampus cells, cerebellum Purkinje organization, and cone cells.

Normal Purkinje cells from the non-endemic fluorosis area were observed in single or parallel lines and were well organized in the fetal cerebellum. Purkinje cells of fetuses from the endemic fluorosis area were abnormally disorganized and had a thicker granulated layer in the cerebellum. Other dysmorphology, including higher nucleus-cytoplasm ratio of brain cones, hippocampus cones, and Purkinje cone cells, supports the theory that fluoride has an adverse effect on brain development.

SEM analysis also found reduced neurons of brain cortex, decreased numerical density, volume density, and surface density in those fetuses from the endemic fluorosis area.

The following Tables 1–3 give the analytical results comparing data of the fetuses from endemic and non-endemic fluorosis areas.

Table 1. Numerical density of cortical neurons in fetal central forebrain

Group	Gestation month	Samples	Numerical density (unit/100 μm^2)			
			Granule cell	Cone cell	Undifferentiated neuroblasts	Interior cone cell
Non-endemic area	5	5	3286±1125	2344±237	226±81	
	6	4	2432±701	1490±730	137±80	
	7	3	2126±283	924±57	113±8	936±31
	8	4	1584±218	770±86	76±34	748±107
Endemic fluorosis area	5	4	3389±265	267 3±433	348±106	
	6	4	2864±200	1852±765	334±23 *	
	7	5	2524±762	1633±651	280±81*	1247±110*
	8	2	2356±664	1262±168†	232±51†	1104±142*

*p<0.05; †p＜0.01.

Table 2. Stereological comparison of hippocampal cortical neurons

Group	Gestation month	Samples	Cone cells		Undifferentiated neuroblasts
			Numerical density (unit/100 μm^2)	Mean volume (μm^3)	Numerical density (unit/100 μm^2)
Non-endemic area	5	5	2068±879	344±148	97±17
	6	4	1611±186	369±113	76±45
	7	3	1599±534	364±82	54±23
	8	4	1363±198	471±59	49±18
Endemic fluorosis area	5	4	2511±673	207±30	378±59†
	6	4	2074±352	219±53	283±70†
	7	5	1608±327	260±66	203±42 †
	8	2	1365±297	338±82	145±14†

*p＜0.05; †p＜0.01.

Table 3. Stereological comparison of Purkinje cells

Group	Gestation month	Samples	Numerical density (unit/100 μm^2)	Mean volume (μm^3)
Non-endemic area	5	5	273±18	2631±599
	6	4	250±30	2835±459
	7	3	221±27	3403±739
	8	4	146±20	4043±580
Endemic fluorosis area	5	4	387±71	1634±63*
	6	4	294±35	1699±191†
	7	5	241±33	1936±645*
	8	2	228±35*	2195±130*

*p<0.05; †p<0.01.

DISCUSSION

Fluoride is known to produce detrimental biochemical and functional changes in the developing human brain. Exposure may commence with fluoride in the maternal blood passing through the placenta to the fetus.[1-3] Fluoride can pass through the blood-brain barrier, and fluoride accumulated in brain tissue might interfere with the metabolism of brain phospholipids, which is related with the degeneration of neurons. The changes in brain phospholipid metabolism could be involved in the pathogenesis of chronic fluorosis. Our stereological study of the fetus brains showed a higher numerical density and nucleus-cytoplasm ratio of brain cortex, hippocampus cones, Purkinje cells, and undifferentiated neuroblasts. But the mean volume, numerical density, and surface density of mitochondria neurons were lower, compared with those from the non-endemic area. According to Rabinowich,[5] increased numerical density of volume of neurons and the undifferentiated neuroblasts are signs of poor morphology of nervous tissue cells. In addition, the increased ratio of nucleus-cytoplasm reflects cell proliferation and maturation, and protein synthesis is adversely affected. In fluorotic rats, RNA losses reduce production of ATP, resulting in metabolic abnormalities.[6] Taken together, these effects of excessive fluoride, in turn, may facilitate penetration of the blood brain barrier, interfere with RNA synthesis and enzymatic protein metabolism, and cause sluggish differentiation.

In summary, the passage of fluorine through the placenta of mothers with chronic fluorosis and its accumulation within the brain of the fetus impacts the developing central nervous system and stunts neuron development.

REFERENCES

1 Geeraert F. Kinetics of fluoride penetration in liver and brain. Fluoride 1986;19:108-12.
2 Guan ZZ, Yu YN, Liu JL, et al. Morphology of the brain of offspring of rats with chronic fluorosis. Zhonghua Bing Li Xue Za Zhi 1986;15:297-9.
3 He H, Chen ZS, Liu WQ. The influence of fluoride on the human embryo. Chinese Journal of Control of Endemic Diseases 1989;4:136-8.
4 Weibel ER. Stereological methods. Vol. 1. Practical methods for biological morphometry. London: Academic Press; 1979. p. 40-50.
5 Rabinowich TH. The cerebral cortex of the premature infant of the 8th month. In: Purpura DP, Schadé JP, editors. Growth and maturation of the brain. Progress in brain research, volume 4. New York: Elsevier; 1964; 39-92. p. 39-47.
6 Holland RI. Fluoride's inhibition of protein and DNA synthesis in cells *in vitro.* Acta Pharmacol Toxicol 1979;45:96-101.

Translation by: Shan Ying, Assistant editor, China Journal of Modern Medicine, Shanghai, and Biomedical Engineering Graduate from Tongji University, China.

Editing by: A Lum and A Chang, Honolulu, Hawaii; 2006. Email: changjak@lava.net
Albert W Burgstahler; 2008.