[Experimental Research]

Effects of fluoride on the ultrastructure of glandular epithelial cells of human fetuses

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[Abstract] Objective Ultrastructural changes in epithelial cells of livers, adrenal glands, and thyroid glands of human fetuses from a fluorosis-endemic area were observed to provide an experimental basis for investigating the mechanism by which fluoride causes cellular damage. **Methods** 10 human fetuses in a fluorosis-endemic area were collected, whose mothers all had dental fluorosis with urinary fluoride content of (4.37 ± 2.94) mg/L. 10 human fetuses in a non-fluorosis-endemic area were collected, whose mothers had no dental fluorosis with urinary fluoride content of (1.67 ± 0.82) mg/L. The fluoride electrode method was used to test the fluoride content in fetal bones. Tissues of livers, adrenal glands, and thyroid glands of the fetuses were taken for electron microscopic examinations. **Results** The fluoride content in bones of the fetuses in the endemic area was (2.77 ± 0.25) mg/kg; compared with the fluoride content in bones of fetuses in the non-endemic area of (2.50 ± 0.11) mg/kg, the differences are significant ($P < 0.01$). Electron microscopic examinations showed: the major changes of cell membranes were microvilli that were shortened, reduced in number or even vanished. Intercellular connections were loose and their structure was disordered. Myelin-like structures were formed in those with severe pathological changes. The major mitochondrial changes were: swollen mitochondria with increased volume, and even vanished and vacuolated cristae. The major pathological changes of endoplasmic reticulum were dilated and vesicular rough endoplasmic reticulum, and partially depleted nucleoproteins on the rough endoplasmic reticulum. The major pathological changes of cell nuclei were damaged and dilated, vesicular dual-layer structures of nuclear membranes. Huge inclusion bodies or particles with relatively high abnormal electron density appeared in some cytoplasm. **Conclusions** Fluoride damage to cell structures was multifaceted. Cell membranes, mitochondria, rough endoplasmic reticulum, and nuclear membranes could all be damaged at the time of fluorosis.

[Key words] Glandular epithelial cell; Human fetus; Fluoride; Ultrastructure
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As is well known, excessive fluoride can be accumulated in a fetus through the placenta, leading to damage of several types of tissues and cells, producing changes in subcellular structures, and even causing severe pathological changes[1-5,7]. Numerous scholars have proven that ultrastructural damage of fluoride mainly occurs in mitochondria. This article aims at reporting the effects of fluoride on the changes in the cell membrane, mitochondria, endoplasmic reticulum, cell nuclei, and other parts of the ultrastructure of human fetal glandular epithelial cells from the subcellular level, in order to provide the experimental basis for the research into the mechanism of fluoride on subcellular damage.

1 Materials and methods

1.1 Materials

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[About the author] YU Yanni, female, born 1955, associate professor, master's degree.

1.1.1 Fetuses: 10 fetuses from endemic areas and non-endemic areas, respectively. The ages of the fetuses were calculated to be between five and seven months according to the history of suspended menstruation of the pregnant women and fetal crown-rump lengths.

1.1.2 Pregnant women: Pregnant women in the endemic area came from a fluorosis-endemic area in Zhijin County, Guizhou Province; all had dental fluorosis and urinary fluoride content of (4.37 ± 2.94) mg/L. Pregnant women in the non-endemic area came from a non-fluorosis-endemic area in Guiyang City, without manifestations of fluorosis; urinary fluoride content was (1.67 ± 0.82) mg/L.

1.2 Methods

1.2.1 Fluoride content in fetal bones: The right femur of a fetus had soft tissues removed and was then dried, carbonized, and ashed, and was tested using the fluoride electrode method. Major reagents were: a 0.02 mol/L pH 5.0 buffer solution of trisodium citrate and sodium carbonate and a 1 μg/ml fluoride-ion standard solution. The instruments used were: PXJ-IB digital ion meter and CSP-F-1 fluoride ion-selective electrodes[8].

1.2.2 Ultrastructural observation of glandular epithelial cells: After induction of labor by water bag,

tissues such as fetal thyroid glands, adrenal glands, livers, and so on were taken immediately and fixed in 3% glutaraldehyde. After routine electron microscopy specimens had been prepared, they were observed under Hitachi 100cx transmission electron microscope.

2 Results

2.1 Fluoride content in fetal bones Compared with the fetuses in the non-endemic area, the fluoride content in bones of fetuses in the fluorosis-endemic area was significantly elevated. The differences were highly significant ($P < 0.01$), see Table 1.

Table 1 Fluoride content in fetal bones

Group	Number of cases	Fluoride in bones (mg/kg)
Non-endemic area	10	2.50 ± 0.11
Endemic area	10	2.77 ± 0.25

2.2 Electron microscopic observation findings Cell membranes: microvilli were shortened and reduced in number, or had even completely vanished, such as thyroid follicular epithelium (Figure 1). Intercellular connections were loose and their structure was disordered, such as adrenal cortical cells. Myelin-like structures were formed in cell membrane-like structures for those with severe pathological changes, such as adrenal cortical cells (Figure 2).

Mitochondria: varying degrees of swelling and increased volume were noted in the mitochondria of glandular epithelial cells; for those with severe pathological changes, the electron density was reduced, and the cristae vanished and were even vacuolated, for example hepatocytes and adrenal cortical cells (Figure 3).

Endoplasmic reticulum: the major pathological changes were that rough endoplasmic reticulum were dilated to become vesicular and the nucleoproteins on the rough endoplasmic reticulum had partially been depleted, for example hepatocytes (Figure 4), and it could be seen that the rough endoplasmic reticulum was arranged in a ring around mitochondria (Figure 5).

Cell nuclei: dual-layer structure of nuclear membrane was damaged, with vesicular dilation, and chromatin margination was seen in nuclei (Figure 6).

Inclusion materials in the cytoplasm: A huge inclusion body was seen in the liver cytoplasm. The inclusion materials inside were in multiple shapes such as awn-shaped, round, roundish and so on. The periphery was in a relatively intact membrane-like structure; particles with relatively higher abnormal electron density appeared in parts of the cytoplasm and were mostly in irregular round or roundish shapes (Figure 7).

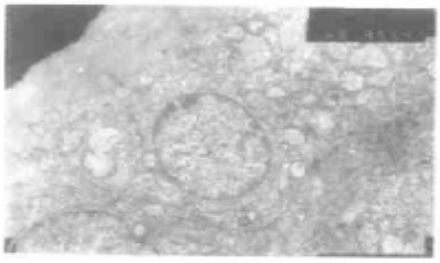

Figure 1 Microvilli in thyroid follicular epithelial cells were shortened and reduced in number, and even had vanished ×5800

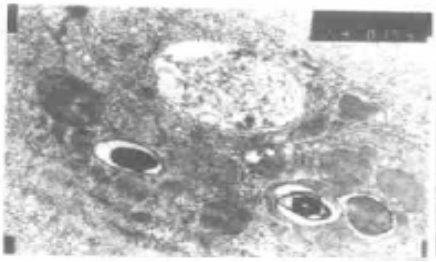

Figure 2 Myelin-like structures were formed in adrenal cortical cells ×1400

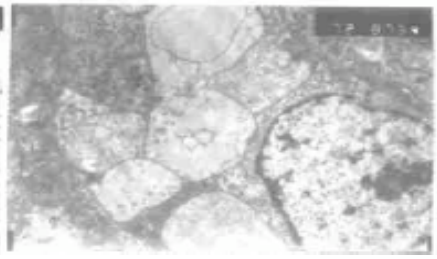

Figure 3 Mitochondria of hepatocytes were swollen with reduced electron density; cristae vanished and were vacuolated ×7200

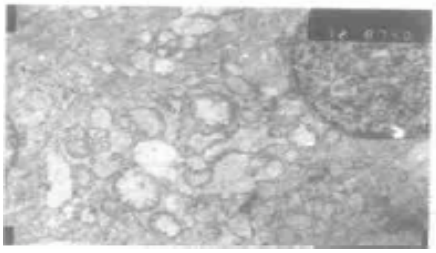

Figure 4 Dilated and vesicular rough endoplasmic reticulum of hepatocytes and partially depleted nucleoproteins on the rough endoplasmic reticulum ×7200

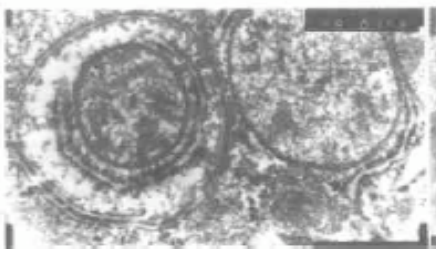

Figure 5 The rough endoplasmic reticulum was seen to be arranged in a ring around mitochondria in hepatocytes ×4800

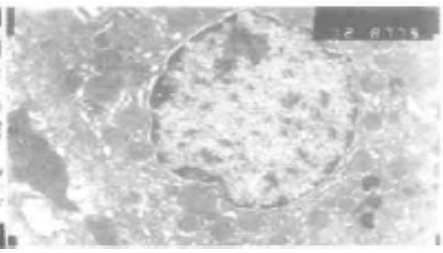

Figure 6 Cell nuclear membrane was dilated and vesicular, and chromatin margination was seen in nuclei ×7200

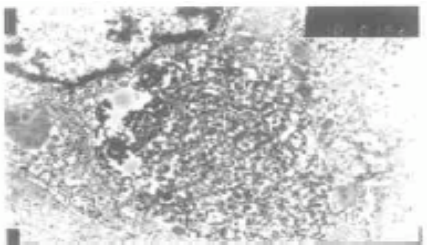

Photo 7 A huge inclusion body in cytoplasm of a hepatocyte ×10000

3 Discussion

Pregnant women in the fluorosis-endemic area were afflicted with dental fluorosis and their urinary fluoride was markedly elevated, indicating the accumulation of excessive fluoride in their bodies. The excessive fluoride could pass through the placenta barrier into fetuses, causing increased fluoride content in the fetuses, which was manifested as increased fluoride content in bones ($P < 0.01$).

The pathological changes in the cell ultrastructure are the changes at the subcellular level which occur relatively shortly after cells have been damaged. A mitochondrion is the cell organelle most sensitive to

damage. Manifestations are stromal swelling and crista-type swelling, with the former being more common. When mitochondria are significantly enlarged and appear as round or roundish, the stroma becomes lighter in color and even transparent; mitochondrial cristae become shorter, decrease in number and even disappear; this type of pathological change is classified under stromal swelling[6]. In these specimen materials, mitochondrial pathological changes of glandular epithelial cells in the fetuses with fluorosis were manifested as stromal swelling. For those with severe pathological changes, mitochondria were disintegrated into fragments and had even vanished. The rough endoplasmic reticulum is the main site where intracellular proteins are synthesized; when cells are damaged, the rough endoplasmic reticulum is manifested as the dilation of cisternae and the nucleoproteins on the rough endoplasmic reticulum fall off into the cytoplasm. The rough endoplasmic reticulum in the glandular epithelial cells of the fetuses in the fluorosis-endemic area in this study presented with varying degrees of cistern dilation. The nucleoproteins on the reticulum had partially fallen off into the cytoplasm, and even were markedly dilated and disintegrated into a bullous shape, which was more significant in the pathological changes of hepatocytes. This type of pathological change could indirectly demonstrate that protein synthesis of cells was affected. In cells with severe pathological changes, myelin-like structures were noted in cell membranes and the nuclear membranes manifested vesicular dilation, indicating that the cell membranes were structurally damaged.

Hence it can be inferred that fluoride causes multifaceted damage to cell structures. In the case of fluorosis, cell membranes, mitochondria, the rough endoplasmic reticulum, and nuclear membranes can all be damaged, thus affecting the information transmission of cell membranes, protein synthesis, cellular energy production and various other physiological functions.

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