THE BRAIN DAMAGE IN FETAL ALCOHOL SYNDROME
(EXPERIMENTAL AND PATHOLOGICAL STUDY)

The research compares clinical and experimental studies of the effects of antenatal alcohol exposure on the central nervous system development. The experimental model, involving Vistar rats, was used to analyze the effects of one-month alcohol intake before and during pregnancy. To conduct light-optical analysis we used brain of one-month-old rat offsprings.

Pathological studies of brain in infants with fetal alcohol syndrome (three lethal cases) revealed focuses of tissue rarefaction and dystrophic degeneration in the neurons along with a decreased amount of basket cells in the cerebellum. Such findings are in agreement with morphological analyses of brain, conducted on experimental animals.

Moreover, employing heterogeneous solid-phase immunoassays, in experimental animals during pregnancy we analyzed the level of the transforming growth factor β1 (TGF-β1).

Keywords: Alcohol, fetal alcohol syndrome, growth factor


Introduction

Complicated course of pregnancy and delivery strongly affect infant CNS and mental development in comparison with endogenous and exogenous factors during the postnatal period. Among factors, which negatively affect normal course of pregnancy and cause a variety of deviations in fetus and infant development, along with somatic and infectious diseases of a pregnant woman, a significant adverse effect is caused by toxic exposure, in particular by alcohol consumption during pregnancy.

Fetal alcohol syndrome (FAS) is a pattern of mental and physical defects, which result from alcohol consumption during pregnancy (CDC, 2002). Currently, this diagnosis is based on three facial characteristics (smooth philtrum, thin upper lip and small eye openings), growth deficiency, underweight, structural and functional changes of the central nervous system and alcohol consumption during pregnancy (Astley and Clarren, 1995).

The majority of children with FAS syndrome are characterized by physical and mental retardation; they have sensory processing disorder and behave in a hyperactive manner. According to many reviews, FAS is one of the most significant reasons of mental retardation (Mirkes, 2003).

The aim of the research was to evaluate the impact of fetal alcohol exposure on cytomorphological features of the central nervous system in experimental animals and compare the obtained data with pathological studies of autopsied brain, obtained from newborn children who died due to FAS (three lethal cases).

Materials and methods

The research was conducted during fall-winter season. The subjects were 26 Vistar rats 1-2 months old that weighed 280-300 g. Rats were kept at room temperature, food was provided ad libitum at all times. They were divided into two groups; group 1 was the main group including 13 animals, which had access to 15% ethanol solution instead of water during one month before and during pregnancy, and group 2 was the control group, comprised of 13 intact rats. To conduct light optical analysis, we used brain from
offspring rats at the age of one month old (11 animals from each group). Brain tissues were treated with 5% formalin in the volume ratio 1:10. Upon fixation, segments of brain tissue were cut out of convexal surface of the sensorimotor cortex, hypothalamus, and cerebellum and embedded in paraffin. Staining was carried out using hematoxylin eosin, according to van Gieson's and Nissl's methods.

In parallel we analyzed the level of the transforming growth factor β1 (TGF-β1) during pregnancy (9-12 days of pregnancy) in rats. TGF-β1 plays an important role in growth regulation, tissue differentiation, apoptosis, modulating of the immune system, morphological development of an infant (Oyvin, 1960). We used heterogeneous solid-phase immunoassays (test system Bender MedSystems, Austria) and the photometer STATFAX. In the statistical analysis, we used Student’s t-test (CDC, 2002).

Results and discussion

Defects in eyes, ears, skull development were visualized by eye. In the group 1 distinct changes were observed, such as cortical focuses of tissue rarefaction of different intensity and extension (Figure 1). Although most focuses of tissue rarefaction were located in the medial cortex, occasional focuses of tissue rarefaction were observed in all the cortical layers. We also observed dystrophic changes in the neurons, including mainly chromatolysis in medial cortex and pycnotic changes in the upper layers.

FIGURE 1. MORPHOLOGICAL CHARACTERISTICS OF THE CEREBRAL HEMISPHERES SURFACE (rays indicate on tissue rarefaction)

FIGURE 2. MORPHOLOGICAL CHARACTERISTICS OF NUCLEI IN THE HYPOTHALAMUS (rays indicate on complete absence of neurocrine granules)
In nuclei in the hypothalamus (Figure 2), we also observed focuses of tissue rarefaction and cell dystrophic changes. Chromatolysis and pycnotic changes, as well as a decrease (and sometimes a complete absence) of neuroendocrine granules, were dominated.

In the cerebellum (Figure 3) we observed thinning in gyri. In the granular layer of the cerebellar cortex we showed bands of tissue rarefaction and a drastic reduction in the number of basket cells. The remained cells were unevenly located at different layers. Chromatolysis and pycnotic changes dominated.

Histological analysis of brain tissues in the control group of animals did not show any substantial changes. In the cerebral cortex we could observe clear stratification of neurons, there was also mild perivascular edema in the form of the enlargement of Vickhow-Robin space, most likely due to acute hypoxia caused by euthanasia of animals. In nuclei in the hypothalamus neurons were located unequally, in the cytoplasm we could see light secretion granules, which was a sign of the neurocrine function. The granular layer of the cerebellum was distinctively seen, the basket neurons (Purkinje cells) were located at the border of the granular layer and the white matter. The cytoplasm was roundly shaped; nucleus and nucleolus, as well as cytoplasmic borders were distinctively seen.

The obtained data we compared with three clinical pathological cases of infant lethal outcomes, caused by FAS. Autopsy showed acute venous congestion in the brain and all
internal organs. In samples of brain tissues (cerebral hemispheres surface, hypothalamus, the cerebellum) we revealed focal lesions and dystrophic changes in neurons, along with a decreased amount of basket cells in the cerebellum (Figures 4-5). In the control group M = 129.7 ng/ml, t = 2.68, p < 0.02. It is known, that TGF-β1 is one of the key players in the cell growth regulation, cell differentiation and apoptosis, such processes right after cell fertilization.

Many authors report that the accumulation of this transforming factor in blood (Ogura, Takakura, Yoshida, Nishiikawa, 1998) may be connected with the blocking of TGF-β1 receptors of growing cells by alcohol, which results in the deterioration of cell growth and apoptosis.

It is also known that alcohol induces an early transformation of radial astroglia into astrocytes, which leads to an impaired migration of neurons within the brain (Miller and Robertson, 1993).

**Conclusion**

Based on our experimental findings we report a severe impact of alcohol on the brain cells of alcohol-fed rat offsprings. In particular, we observed focuses of tissue rarefaction and dystrophic changes in neurons in the form of chromatolysis and pycnotic changes in cortex, hypothalamus, and the cerebellum. Besides, we showed a decrease (and sometimes a complete absence) of neuroendocrine granules. These findings are in agreement with morphological parameters of cerebral cortex of died infants with FAS. Observed elevated levels of TGF-β1 in pregnant alcohol-fed female rats points out on the receptors deficiency in both female rats and their offspring. Therefore, the revealed data on the expression of the growth factor TGF-β1 are in agreement with morphological changes in the brain of experimental animals and can be extrapolated on the similar processes in the mammalian and human brain development when exposure to alcohol.

**Acknowledgements**

Bazarnyy Vladimir Victorovich, professor of the Department of Clinical Laboratory Diagnostics and Bacteriology, Ural State Medical University, Yekaterinburg, Russia.

Kleyn Alexei Veniaminovich, Central Research Laboratory of Ural State Medical University, Yekaterinburg, Russia

**References**


