The Prophylactic Effects of Folic Acid and Vitamin E against Valproic Acid During Fetal Thymus Development: an Ultrastructural Study

Los Efectos Profilácticos del Ácido Fólico y la Vitamina E contra el Ácido Valproico Durante el Desarrollo Fetal del Timo: un Estudio Ultraestructural


SUMMARY: To evaluate histopathologic differences in the thymus of Wistar Albino rat fetuses prenatally exposed to valproic acid (VPA), folic acid (FA) and vitamin E (Vit-E). VPA (400 mg/kg), FA (400 mcg/kg) and Vit-E (250 mg/kg) were administered to rats on each of gestation days 8, 9 and 10. The fetuses (n:24) were divided into four groups: control, VPA, VPA+Vit-E and VPA+FA groups. On the 20th day of gestation, all pregnant rats were sacrificed and the fetuses were extracted. Thin sections from thymus of live fetuses were stained with uranyl acetate-lead citrate and were examined under transmission electron microscope. The histopathological findings of control group was normal. In VPA group, it showed extensive degenerative changes by VPA were on all tissue compartments when compared to controls. In VPA-FA group, vacuoles, mitochondrial cristalysis and swelling were decreased in cytoplasm. In VPA-Vit-E group, lipid storage and vacuolization were observed. Mitochondrial cristalysis decreased. Our aim in the present study is to analyze histopathological changes which may occur in a high risk experimental model after giving of VPA. In addition, protective roles of the administration of FA and Vit-E are assessed.

KEY WORDS: Valproic acid; Fetal thymus; Vitamin E; Folic acid.

INTRODUCTION

The human thymus, primary lymphoid organ, is an organ consisting of two lobes located in the anterior mediastinum (Abo, 2001; Lamboez et al., 2002). Thymus organogenesis in vertebrates depends on interactions between cells of all three embryonic germ layer origins: endoderm-derived epithelium, neuroectoderm derived neural crest mesenchyme, and mesoderm derived hematopoietic cells and endothelial cells of blood vessels (Smith, 1965; Moore & Owen, 1967). Its function is critical during embryogenesis and in the early stages of life: during this period, its removal is lethal. However, soon after birth (a few days in mice, a few months in man), the thymus can be taken out with no detrimental effects for the organism. Its role is essential for the development of the T cell repertoire, though some T cells are of extra-thymic origin. T cell maturation is highly complex, and it is thought that the sequence of differentiation of thymocytes to T cells is controlled by the thymus microenvironment. The thymic microenvironment is made up by epithelial cells and cells of the mononuclear phagocytic system. Thymic epithelial cells show ultrastructural varieties. Electron microscopy enables an easy distinction between four cortical thymic epithelial cells (cTEC) and three medullary thymic epithelial cells (mTEC) in rats (Milic´evic´ & Milic´evic´, 1997). Precursor T cells originate mainly in the yolk sac at first and then in the liver (during fetal development), subsequently in the bone marrow (after birth), and later in other organs too (in aged individuals). They differentiate from hematopoietic stem cells (HSCs) in the bone marrow, where they undergo several phenotypic transformations to give rise to a number of different cell types, including the lymphoid lineage (Abo; Lamboez et al.; Blom et al., 1997; Lamontagne et al., 1998; Poccia et al., 1998).
Experimental procedure. Twenty-four Wistar rats were used in this study. They were randomly divided into four groups, each consisting of six rats (n=6): the control (n=6), VPA (n=6), VPA+FA (n=6), and VPA+Vit-E group (n=6).

Tissue preparation for electron microscope. On the 20th day of gestation, all pregnant rats were sacrificed and the fetuses were removed. Thymus were removed from fetuses. For electron microscopic examination, the pieces of thymic tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, postfixed with 1% osmium tetroxide and then dehydrated in graded alcohol series, and subsequently embedded in araldite CY212. Sections were cut with ultratome. Thin sections were stained with uranyl acetate and lead citrate, and examined using a Carl Zeiss EM 900 transmission electron microscope.

RESULTS

The ultrastructural examination of thymic tissue was carried out by electron microscope (Jeol TEM 1010). In the Control group sections, thymocytes and reticular fibers were observed in their normal appaerance. Nucleus was prominent,and small vacuoles were observed. Desmosomal complex were seen in normal appearance (Fig.1).

In the VPA group, electron microscopic observations have shown that the damage produced by VPA were in all tissue compartments. A great deal of swollen mitochondria were observed. The vacuoles that appeared in the cytoplasm of the cells had varying sizes. In thymus parenchyma, mast cells were observed. There is no change in the structure of Mast cells containing granules which were in normal size. Fat droplets were also observed in the cytoplasm. Condensation of chromatin in nuclei was evident. Widened perinuclear cisterna was observed. On the other hand, in some thymocytes, degeneration was seen (Figs. 2, 3).
In VPA+FA group, chromatin distribution of nuclei was better than VPA group. Vacuoles, mitochondrial cristalysis and swelling were decreased in the cytoplasm of thymocytes (Fig. 4).

In VPA+Vit E group, nuclear chromatin and perinuclear cisterna had normal appearance. Lipid storage and a small amount of vacuolization were observed. Mitochondrial cristalysis decreased (Fig. 5).

DISCUSSION

The thymic stromal compartment consists of several cell types that collectively enable the attraction, survival, expansion, migration, and differentiation of T-cell precursors. The thymic epithelial cells constitute the most abundant cell type of the thymic microenvironment and can be differentiated into morphologically, phenotypically, and functionally separate subpopulations of the postnatal thymus (Holländer et al., 2006). Thymus is divided into two distinct compartments; the outer cortex and the inner medulla. Both regions are densely populated with lymphocytes (or thymocytes while in the thymus). Most of the cortical lymphocytes are immature and unable to carry out immune functions.

Valproic acid formulations are FDA pregnancy category D drugs due to teratogenic effects seen in studies in experimental animals and human case reports. There is reference to the death of a newborn following therapeutic use of valproic acid in the mother during pregnancy, although no specific information is provided (Depakote Product Label Drugs FDA, 2009). In a study carried out by Arudchelvan et al. (2005), it was reported that, in thymic tissue samples, thymocyte had a marked nuclei, and there were vacuoles in cytoplasm. In our study, in addition to the points mentioned above, we observed that desmosomal structures were normal. In the same study, they also reported that thymus epithelial cells which were exposed to radiated proliferated 3rd day of recovery period and volume of vacuol within their cytoplasm. Graf et al. reported the effects...
of the steady-state valproic acid concentrations on the mouse thymus. Those showed that cortical and medullary morphology varied considerably in the thymus. In extreme cases, the cortical zone was either reduced in size or the medulla showed a cortex-like structure or vice versa (inverted type of thymus). The thymic cortical reticular cells showed increased aminopeptidase M and alkaline phosphatase reaction (Graf et al.). In our findings, in the group valproic acid, we observed that damage occurred in most thymic compartments, there was an increase in the number of vacuole and swelling of mitochondria. In addition, there were fat droplets in some places in cytoplasm, the nuclei was markedly evident, chromatin had a condensed appearance and there were expansions in perinuclear cisternae. However, mast cells had their normal histological structure. Folic acid (the anion form) are forms of a water-soluble B vitamin. Folic acid is necessary for cell development, for the metabolism of specific biochemical reactions in the body, such as the conversion of homocysteine to methionine; and for the metabolism of specific anticonvulsant drugs (Berg, 1999). Therefore, valproic acid use causes folic acid deficiency. Thus it causes neural tube defects, thymic atrophy, loss of thymic cellularity (Carl; Weber & Dib; Lewis et al.). The production of thymic hormones and differentiation of T lymphocytes are reduced. In the present study, chromatin distribution of nuclei was better, and cytoplasmic vacuoles and mitochondrial swelling was reduced in the VPA+FA group. We thought that folic acid decreased the effects of maternal valproic acid on the thymus.

Vitamin E is a fat-soluble vitamin that exists in eight different forms. Each form has its own biological activity, which is the measure of potency or functional use in the body (Traber & Packer, 1995). Alpha-tocopherol (a-tocopherol) is the name of the most active form of vitamin E in humans. It is also a powerful biological antioxidant. Antioxidants such as vitamin E act to protect cells against the effects of free radicals, which are potentially damaging by-products of energy metabolism. Free radicals can damage cells and may contribute to the development of cardiovascular disease and cancer. Studies are underway to determine whether vitamin E, through its ability to limit production of free radicals, might help prevent or delay the development of those chronic diseases. Vitamin E has also been shown to play a role in immune function, in DNA repair, and other metabolic processes. Vitamin E supplementation has various beneficial effects on the immune system. It plays an important role in the differentiation of immature T cells and increases the positive selection by thymic epithelial cells in thymus (Traber, 1999; Farrel & Roberts, 1994). In our study, the decreased cellular immunity with VPA is markedly improved by the intake of vitamin E, and vitamin E also induces the recovery of thymic atrophy. In conclusion, we are of the opinion that valproic acid should not be advised during pregnancy since its use has risks. If its use is a must, it should be given together with folic acid or vitamin E, since those two drugs decrease the harmful effects of VPA on the thymus tissue.

Fig. 4. Electron micrograph of Valproic acid + Folic acid group. The VPA damage was improved by folic acid. (Uranyl acetate-lead citrate X3000).

Fig. 5. Electron micrograph of Valproic acid + Vit E group. The recovery of thymus with Vit E. 1. Small thymic lymphocytes, 2. Medium-sized thymic lymphocytes, 3. Nucleus of epithelial reticular cell. (Uranyl acetate-lead citrate X3000).
REFERENCES


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