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Sodium valproate effect on the structure of rat glandule thymus: Gender-related differences

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ABSTRACT

Sodium valproate (VPA) was shown to inhibit cell growth mechanisms such as cell cycle arrest, proliferation suppression, increase of apoptosis. Many aspects of the contribution of the VPA pharmacological mechanisms and their significance in gender-related processes have not been investigated. In our study, we have tested hypothesis that the influence of VPA on thymus weight and structure might be gender-related. The thymus of Wistar rats of both genders aged 8 weeks was investigated in the following groups ($n = 6$ each): controls, treated with VPA, castrated male and female treated with VPA, and the castrated control of both genders. The thymus weight, structural changes and area of cortical and medullar parts of the gland in slides stained with hematoxylin and eosin and immunohistochemically were assessed. A comparison of thymus weight of castrated male and of castrated VPA-treated male rats showed a significant thymus weight loss after VPA treatment (0.66 ± 0.04 g vs. 0.43 ± 0.03 g, $p < 0.05$). The treatment with VPA caused an about 6-fold (0.39 ± 0.12 vs. 0.07 ± 0.03) increase of Hassall's corpuscles (HCs) numbers per 1 mm^2 in male and more than 4-fold increase (0.46 ± 0.07 vs. 0.10 ± 0.04) in female rats. In castrated males and females, the HCs number was also increased, but this increase was statistically significant only in male animals vs. controls (0.46 ± 0.10 vs. 0.07 ± 0.03, $p < 0.001$ in males; 0.29 ± 0.13 vs. 0.10 ± 0.04, $p > 0.05$ in females). When castrated male and female rats were treated with VPA, further increase of HC numbers was found. In our study, VPA has inhibited the proliferative capacity of thymocytes by diminishing the thymus weight and inducing a differentiation of thymic medullar epithelial cells into HCs. The diminishing of the gl. thymus weight under the influence of VPA was significant in castrated male rats. The number of HCs increased in animals of both genders under the influence of VPA. Gender differences in HCs development were noted in castrated animals.

1. Introduction

Sodium valproate (VPA) is a medicine preparation which currently is used for the treatment of a wide variety of neurological disorders (Bowden, 2009). VPA increases the turnover of gamma-aminobutyric acid (GABA) (Loscher, 2002). The specificity of VPA for GABA A receptors suggests that this interaction may be an important mechanism through which VPA exerts its pharmacological effects (Owens and Nemeroff, 2003). The GABA A receptor subunits form a functional chloride channel (Bureu et al., 1997). They are expressed in rat kidney tubules and in other tissues (Li et al., 2012; Sarang et al., 2001; Zhang et al., 2013). VPA is recognized as a histone deacetylase (HDAC) inhibitor. HDAC inhibitors play a dual role in tumorigenesis: cell proliferation suppressive in the early stages and oncogenic in established tumor cells in mice models (Santoro et al., 2013). Recently, we have found the negative effect of VPA on the progression of urethane-induced lung tumors in the male but not in female mice model (Stakisaitis et al., 2014). VPA was shown to inhibit cell growth mechanisms such as cell cycle arrest, proliferation suppression, increase of apoptosis (Kwiecińska et al., 2012; Osuka et al., 2012; Sidana

Abbreviations: VPA, valproic acid, sodium salt; GABA, gamma-aminobutyric acid; HDAC, histone deacetylase; HCs, Hassall's corpuscles; HE, hematoxylin and eosin; HMW CK, high molecular weight cytokeratins.

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et al., 2012). VPA also affects immunocompetent cells in various pathologies such as valproate-suppressed proliferation in vitro of both HIV-infected and uninfected T cells (Lee et al., 2010), increases the suppressive function of CD4+ and CD25+ cells in experimental arthritis (Saouaf et al., 2009), reduces lymphoproliferation (Dowdell et al., 2009). Female MRL/lpr(−/−) mice treated with VPA for 8 weeks showed significant reductions in spleen and lymph node weight and cellularity compared to controls (Dowdell et al., 2009). VPA acts through the initial suppression of immune cell recruitment and inhibition of inflammatory cell pathways in NK cells (Alvarez-Breckenridge et al., 2012).

Many aspects of the contribution of the VPA pharmacological mechanisms and their significance in gender-related pathophysiological processes have not been investigated. Thymus involution is an age-related physiological phenomenon depending on gonadal hormones (Leposavić and Perisić, 2008). Reduction of thymus weight was induced after prenatal exposure to VPA in male but not in female newborn rats (Schneider et al., 2008).

In our study, we tested the hypothesis that the influence of VPA on thymus weight and structure might be gender-related. To exclude the influence of gonadal hormones on thymus involution, castrated male and female rats were studied also. Our hypothesis was based on the previous investigation in which VPA has been shown to enhance the urinary excretion of sodium and chloride ions in Wistar rats of both genders, but the 24-h chloriduretic response was found to be gender-related (Grikiniene et al., 2005). Chloride plays an important role in cell proliferation: the intracellular chloride concentration would be one of the critical messengers in cell growth/proliferation and differentiation processes (Hiraoka et al., 2010; Ohsawa et al., 2010; Shiozaki et al., 2006). We expected that changes might occur in the number of Hassall's corpuscles (HCs) because they represent the terminal stage of thymic medullar epithelial cell differentiation (White et al., 2010; Yano et al., 2008) and participate in the removal of apoptotic or the maturation of developing thymocytes (Blau and Veall, 1967; Senelar et al., 1976). Both thymic epithelial cells and thymocytes possess functional androgen receptors (Olsen et al., 2001).

The present study showed a significant thymus weight loss in castrated male VPA-treated rats and a significant decrease of the medullar area in castrated male-treated with VPA. Treatment with VPA increased cytokeratin-positive zones in the cortex of the thymus and caused a significant increase of HCs numbers in non-castrated and castrated rats of both genders.

2. Materials and methods

2.1. Experiment design

Wistar rats of both genders aged 8 weeks were investigated in the following groups ($n = 6$ each): controls, treated with VPA, and castrated–orchidectomized male and ovariectomized female treated with VPA, and the castrated control of both genders. VPA and castration effect on thymus in rats of both genders was assessed. The intragastric single daily 300 mg/kg dose of VPA (valproic acid sodium salt, Sigma-Aldrich) was given for 4 weeks. Treatment with VPA started in the prepubertal age (4 weeks) of rats. The study was approved by the Lithuanian Committee for Animal Care and Use (No. 0231; 09-07-2012). The VPA dose was chosen according to literature data on preclinical pharmacodynamic studies of VPA and our own data (Ahmad et al., 2005; Grikiniene et al., 2005; Haley et al., 2005).

Rats were castrated at the age of 28 ± 2 days (in the peripubertal period). The mean age of rats at the end of the experiment was 57 ± 3 days and did not differ significantly between the groups. The animals were housed in standard colony cages with free access to food (chow pellets) and tap water. The room temperature was 21 ± 1 °C. The rats were on a natural light-dark cycle. All experiments were performed according to the institutional guidelines for animal care in order to avoid any unnecessary distress to the animals and to reduce the number of animals used. The animals were housed in the described conditions and acclimated for at least 5 days before experiments with free access to tap water, in the same temperature and light conditions.

2.2. Tissue sampling, histology and immunohistochemistry

The study rats after anesthesia were decapitated, and the weight of rats and of thymus was assessed. The thymuses were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned in 3 μm sections and stained with hematoxylin and eosin (HE). For immunohistochemical examination, the 3 μm thick slices of paraffin-embedded thymus tissue were mounted on poly-L-lysine coated glass slides. After deparaffinization in xylene and rehydration, the sections were pre-treated with antigen-retrieval solution (0.01 mol/L of citrate buffer, pH 6) in a pressure-cooker and then incubated with cytokeratin monoclonal antibodies (clone 34βE12, dilution 1:50, Dako A/S, Denmark) for identification of high molecular weight cytokeratins (HMW CK). Antibodies detection using a commercially available kit EnVisionPlus-HRP, Dako, was performed following the protocols of the provider. Sections were counterstained in weak Mayer's hematoxylin, dehydrated, cleared, and mounted for light microscopy. The histological and immunohistochemical evaluation of the samples was performed with a cold light microscope OLYMPUS BX40F4 (Olympus Opticae co. LTD., Japan) under 4×, 10× and 40× magnification using CellSensDimention1.9 Digital Imaging Software for Research Applications (Olympus Corporation of the Americas, USA). According to Haley et al. (2005) and Elmore (2012) recommendations, we evaluated changes in the density and distribution of lymphocytes in the cortex and medulla, changes at the cortico-medullar junction, structural changes in the epithelial component of the thymus.

2.3. Morphometric analysis

Histomorphometric evaluation of the thymus parameters was performed on images obtained with an Olympus digital camera (Olympus U-CMAD3). To perform accurate morphometric analysis, histological sections were made from the middle portion of each thymus lobe. In 8 slides from every lobe (the total number of slides investigated for every case was 16) we measured the total area of the thymus lobe and the area of the medulla. The area of the cortex was calculated in every histological slide by the deduction value of the medullar part area from the total area of the lobe. The data are presented as the average value from 96 histological sections in every group. In eight slides from each case (48 sections in every group), we evaluated the presence of HCs and counted them. Data are presented as the average numbers per mm^2 of thymus medulla in every group.

2.4. Statistical analysis

The data were expressed as a mean ± SD. Using Student's t test, a comparison among the groups was made. A value of $p < 0.05$ was considered significant. We applied the STATISTICA for Windows software to perform the analysis of our data. Pearson's correlation coefficient was calculated to characterize relationships between rat body weight and gl. thymus weight.