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RESEARCH ARTICLE

HORMONAL AND HISTOLOGICAL CHANGES IN THE OVARIES OF RATS TRAITED BY VALPROIC ACID

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ABSTRACT

Objectives: The objective of this study was to investigate behavioral and histological abnormalities of valproic acid in non-pregnant adult mice. **Materials and methods**: 18 of the non-pregnant adult rats were divided into 3 groups; (1) distilled water group, (2) VPA 200 mg/kg group and (3) VPA 400 mg/kg group. The products were administered orally daily for 30 days. 24 hours after the end of the treatments, the animals were sacrificed, the blood and the ovaries collected for the hormonal assays (progesterone and estradiol) and the histological analyses. Serum progesterone and estradiol levels were determined using ELISA techniques. The histological analysis was made on the sections of the ovaries, under the electric microscope, after staining with hematoxylin eosin. **Results**: No change in the weight of the organs studied was observed. Exposure to VPA resulted in depletion of ovarian hormones (progesterone and oestradiol). Progesterone concentrations were 15.3 ± 21.3, 1.2 ± 1.6 and 17.4 ± 21.3 respectively for control animals, VPA 200 and 400 mg/kg. Histological analysis revealed the presence of subcostal hemorrhages and ovarian pseudocysts in animals treated with VPA. **Conclusion**: VPA, at the doses studied, causes a depletion of ovarian hormones and induces histological alterations of the ovaries.

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INTRODUCTION

Valproate acid (VPA) is a major antiepileptic,widely used for the treatment of epilepsy , but also other neuropsychiatric diseases such as bipolar disorder, migraine and mood disorders (Bowden, 2003; Owens and Nemeroff, 2003; Peterson and Naunton, 2005) . VPA has been associated with a number of adverse effects affecting multiple tissues and organs (Chang and al., 2016). Ornoy (2006) revealed that VPA is a teratogenic agent capable of inducing abnormalities of neurons, fallopian tubes, cardiac abnormalities, craniosynostosis and is responsible for skeletal malformations, such as adactyly and syndactyly.

In addition, mood swings, impaired cognition and behavior have been reported in patients treated with VPA (Senturk and al., 2007).From a reproductive perspective, polycystic ovary syndromes (PCOS) and hyperandrogenism have been associated with long-term treatment with sodium valproate (Isojarvi and al., 1993, 2005). On the other hand, conditions such as underlying epilepsy have also been reported to be linked to reproductive endocrine dysfunction (Morrell and Montouris, 2004; Herzog, 2008). Sodium valproate causes disorders of reproductive functions with endocrine disturbances. Isojarvi *et al*. (1998, 2001) reported cases of polycystic ovaries, hyperandrogenismor hyperinsulinemia in obese women treated with VPA.

In animals, some authors have shown that prolonged treatment with VPA caused a decrease in estradiol levels in female rats and an imbalance in the testosterone/estradiol ratio.(Sveberg Roste *et al*., 2002; Fisseha *et al*., 2010; Tauboll *et al*., 2003). Janneke *et al*. (2010) also demonstrated that there was a significant association between the exposure of mothers to VPA and the occurrence of cases of spina bifida, cardiac anomalies and polydactilia. The teratogenic effects of exposure by VPA have also been demonstrated in a recent study (Miguel *et al*, 2021). Several other studies have reported an over-representation of Polycystic Ovary Syndrome (PCOS) in women with epilepsy (Herzog at al, 2008) and a higher prevalence of PCOS in patients treated with VPA. The link between the increased risk of this PCOS-like condition in women with epilepsy and the underlying disease or antiepileptic treatment remains controversial (Fisseya and al., 2010). In this context, the present studie focused on determining the effect of prolonged treatment with VPA on reproductive hormone (progesterone and estradiol) and ovarian tissue in non-epileptic female rats.

MATERIAL AND METHODS

Animals and treatment: Wistar adult female rats, weighing between 135 and 180 grams, from the pet animal of the Faculty of Health Sciences of Marien University NGOUABI Brazzaville, were used. They were reared in polypropylene cages, at constanttemperature and humidity on a 12 hour light and darkness schedule.The rats were kept on standard pelleted rat diet and given tap water *ad libitum*. All experiments were conducted in accordance with Directive 2010/6106/EU for the protection of laboratory animals (Hartung, 2010).

Pharmacological treatments: A standard commercially available valproate mixture was used together with a placebo solution (distilled water).The rats were divided into 3 groups of 5 animals and treated orally daily for 30 days: distilled water (5 ml/kg), valproic acid 200 and 400 mg/kg. VPA is known to be readily absorbed from the digestive tract (Bruni *et al*., 1979). Drug delivery was calculated for on the basis of dosing of VPA used in humans (15-60mg/kg/day) (Axnaout and al., 1986; Badura and al., 1992). Thus, the dose administered corresponded to a third of the dose in humans.

Animal sacrifices, blood collection and histological analysis: Briefly, 24 hours after the end of the last treatment, the animals were anesthetized by inhalation of ether,between 08:00 a.m. and 10:00 a.m. in order to reduce the circadian effects on the concentrations of circulating hormones,and the ovaries were delicately removed. Blood samples (2 ml) were collected in EDTA tubes, then centrifuged at 3000 rpm for 10 minutes. The resulting plasma was aliquoted and stored at -20° C until hormonal assays. The removed organs were immediately weighed and then fixed by immersion in a 10% formalin solution. The specimens were routinely processed, embedded in paraffin, and 4 µm thick sections were cut and stained with hematoxylin and eosin for light microscopic evaluation. The slides were read by a pathologist, with an electric microscope, looking for abnormal structures.

Hormonal assays: Progesterone and estradiol levels were determined by ELISA (Enzym Linked Immunoassy) techniques, using commercial kits (Estradiol kit CYPRESS and progesterone kit CYPRESS), following the manufacturer's instructions.

Statistical analyzes: Results are expressed as mean ± standard error of the mean (sem). Statistical analysis was performed using *Student* 's *t test*, using *GraphPad Prism* software (San Diego, CA, USA). The significance level corresponds to $p < 0.05$.

RESULTS

Effects on ovary weight: Table I shows the weight of the ovaries of the animals. Although no significant difference was found after 30 days of treatment ($p>0.05$), a trend towards a decrease in the absolute weight of the ovaries was observed in animals having received VPA at a dose of 400mg/kg.

Effects on progesterone and estrogen levels: A significant decrease ($p<0.0001$) in hormone levels (progesterone and estradiol) was observed in treated animals compared to those of control group. Progesterone mean concentrations were 15.3 ± 21.3, 1.2 ± 1.6 and 17.4 ± 21.3 respectively for control animals, VPA 200 and 400 mg/kg.

Histological analysis: Histological evaluation of animal sections in the 200 and 400 mg/kg VPA groups revealed the presence of cystic follicles (FK), areas of acellular necrosis (Na), areas of inflammatory reaction (Ri), cystic microcavities (MCK), ovarian pseudocysts, and subcortical hemorrhages (H) (Figure 1).

DISCUSSION

The central nervous system is believed to play a critical role in modulating physiological and behavioral events associated with normal reproductive functions in humans and animals. In women, the hypothalamus regulates pituitary function in various ways and maintains ovarian cycles. Thus, several drugs acting on the nervous system, also have effects on the functions of reproduction (Stoker and al., 2001). In this study, we investigated the effects of valproic acid on reproductive hormones rate and ovarian tissue were studied in non-epileptic adult female rats, after oral treatment for 30 days. We used the dose of 200 mg/kg and 400 mg/kg. The results obtained show that valproic acid does not modify the absolute weight of the ovaries of the treated animals compared with the control group. In male rats, on the other hand, Roste and al., (2001) have reported atrophy of the gonads after exposure to VPA, at doses of 200 and 400 mg/kg for 90 days. Previous studies has shown reports of hormonal disturbances in epileptic subjects treated with VPA (Herzog *et al*, 1989a, 1989b). In this study, exposure to VPA (200 mg/kg) significantly reduced progesterone and estradiol concentrations. These results confirm those obtained and are still with the observations made in epileptic women (Aktas *et al*., 2010). Authors have also observed that VPA reduced the secretion of progesterone and estrogen by follicular cells isolated from the ovaries (Roste *et al*., 2002). The mechanism of this hormonal depletion has been described by Tauboll *et al*. (2003). VPA would affect the secretion of steroid hormones by inhibiting the conversion of testosterone into estrogen (Aktas *et al*., 2010; Roste *et al*., 2002). Furthermore, Tauboll *et al*. (2003) also observed that VPAaffects steroidogenesis in porcine ovaries.

Table I. Effects of VPA on ovary weight

Organ weight (g)	DE 10ml/kg	VPA200 mg/kg	VPA400 mg/kg
Right ovary	0.07 ± 0.05^{NS}	0.09 ± 0.04^{NS}	0.07 ± 0.04^{NS}
Left ovary	0.06 ± 0.04^{NS}	0.07 ± 0.04^{NS}	0.05 ± 0.03^{NS}

Results are expressed as mean ± standard error. (NS): not significant by t Student test (n=6 animals).
DE: distilled water; AVP: valproic acid.

Table II. Effects on hormonal parameters

Settings	DE 10ml/kg	VPA 200 mg/kg	VPA 400 mg/kg
Progesterone	15.3±21.3	$1.2 \pm 1.9^{***}$	17.4 ± 21.3^{NS}
Estradiol	184.8±4.5	$6.93 \pm 16.9^{***}$	$0 \pm 0.0^{***}$

Results are expressed as mean ± standard error. (***): p<0.0001. (NS): not significant by t Student test (n=6 animals).
DE: distilled water; VPA: valproic acid.

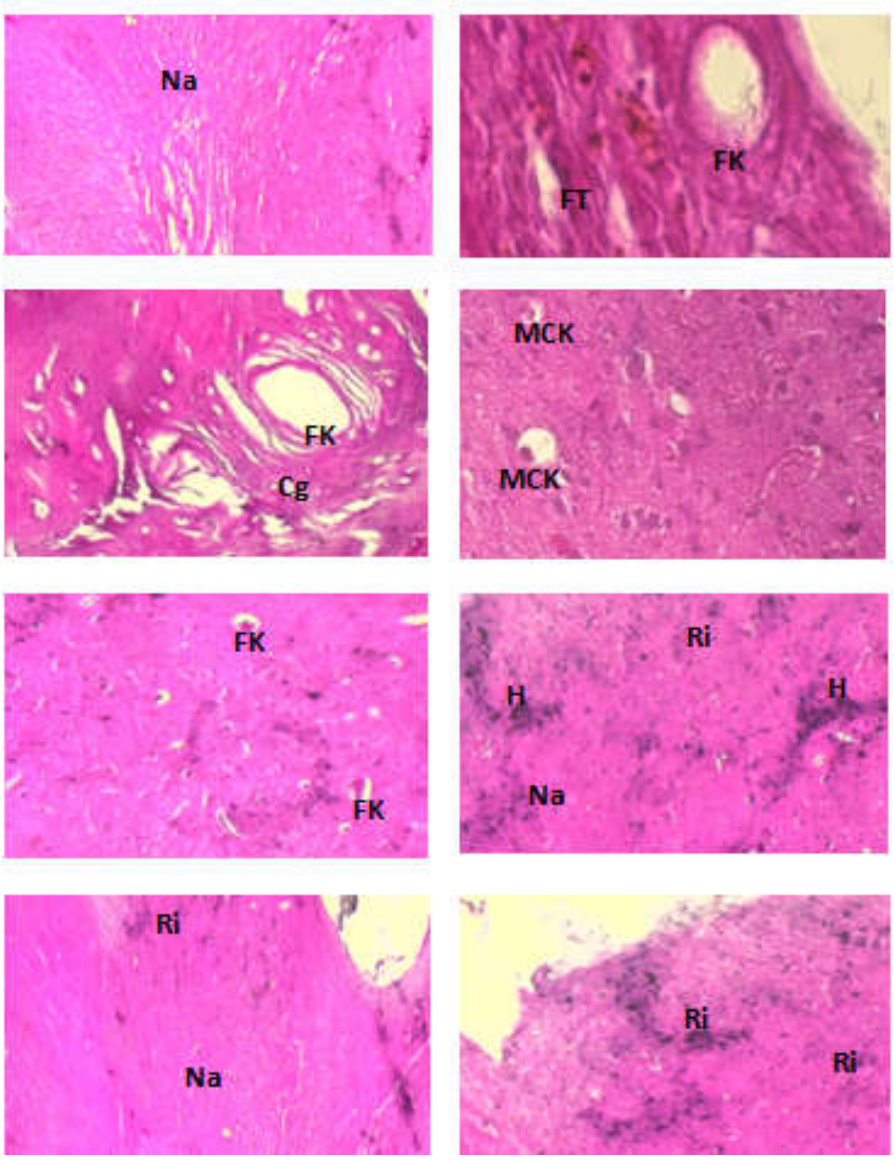

Figure 1 : Photomicrographs of the ovaries illustrating the presence of cystic follicles (FK), areas of acellular necrosis (Na), areas of inflammatory reaction (Ri), hemorrhagic (H), congestion (Cg) and cystic microcavities (MCK)

Thus, valproate has a significant influence on the secretion of reproductive hormones in non-epileptic animals of both gender. Histological analysis of sections of the ovaries shows a large number of ovarian pseudo-cysts in non-epileptic female rats exposed to VPA at doses of 200 and 400 mg/kg. Exposure to VPA also induced cell apoptosis in the ovaries, thereby delineating areas of tissue necrosis.

These observations support the hypothesis of a direct effect of VPA on the gonadal tissue (Roste *et al*., 2001). The effects of VPA on apoptosis and cell proliferation have already been studied in previous works (Tauboll and al., 2003). In the ovary, estrogens are essential for the survival and development of pre-ovulatory follicles, while androgens are apoptotic factors (Aktas and al., 2010).

Studies suggest the direct involvement of estrogen in apoptotic processes (Aktas and al., 2010). Theestrogens would also have beneficial effects on neuronal cells via caspase (Aktas and al., 2010) or by stimulating the anti-apoptotic expression of certain proteins such as Bcl-2 or Bcl-XL (Kipp and Ramírez, 2001). Thus, the hormonal depletion observed in animals exposed to VPA should be compared with the presence of areas of cellular necrosis.

Conclusion

The administration of VPA at doses of 200 and 400 mg/kg to non-epileptic female rats does not modify the absolute weight of the ovaries, leads to a depletion of ovarian hormones (progesterone and oestradiol). This exposure also induced the appearance of cystic follicles, areas of acellular necrosis, inflammatory and hemorrhagic reaction and cystic microcavities. In-depth studies would confirm these results, clarify their molecular mechanisms and to reveal the clinical implications of these findings when treating epileptic.

Contributors : LM Miguel, EMA Boungou-Malonga, DG N'jilo Tchatchouang, and C Lékana performed complete experimental research. LM Miguel and CR Dobhat-Doukakini performed data analysis and wrote a manuscrit. AA Abena, D Moukassa and E Mokondjimobe revised the manuscript. RB Bolanga and EG Nkounkou Matondo contributed to the study design, data analysis, and manuscript writing.

Compliance with ethics guidelines : All authors declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

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Conflict of interest : The authors declare no conflict of interest.

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