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Full Length Research Paper

The effects of valproic acid on renal corpuscle of pregnant rats and protective role of folic acid and vitamin E

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We aimed to investigate the potential harmful effects of valproic acid (VPA), a widely used anticonvulsant in child delivery, and the protective effects of vitamin E (Vit E) and folic acid (FA) on kidney. Sodium valproate (400 mg/kg), folic acid (400 mg/kg) and vitamin E (250 mg/kg) were administered to rats on each of gestation days, 8 th, 9 th, and 10 th. The rats were sacrificed on the 20 th day of pregnancy. With thin sections of kidney biopsies, they were stained with uranyl acetate-lead citrate and examined under transmission electron microscope. The animals were divided into four groups randomly: control, VPA, VPA+FA and VPA+Vit E groups. In each group, drug procedure, surgical procedure and histological methods were performed. The histopathological findings of control group was normal. In VPA group, it showed degenerative changes especially in renal glomerular basal membrane and foot process. Both VPA+FA and VPA+Vit E groups exhibited similar ultrastructural changes and had almost the normal structure. Administration of single doses of SV (400 mg/kg) resulted in degenerative changes on kidney at ultrastructural level. Administration of FA and Vit E had a protective effect by preventing the degenerative changes to a certain degree. The aim of the present study is to examine histopathologic changes which may occur in a high risk experimental model after the administration of valproic acid. In addition, protective roles of the administration of folic acid and vitamin E are assessed.

Key words: Folic acid, kidney, rat, valproic acid, vitamin E.

INTRODUCTION

Valproic acid (VPA, 2-propylpentanoic acid or dipropylacetic acid) is a broad spectrum anticonvulsant which has proved useful especially in the treatment of primarily generalized epilepsies (Loscher and Nau, 1983). This drug is applied in the form of sodium valproate (SV). VPA and its derivatives are widely used in treatment of various seizure disorders and some psychiatric conditions. The majority of childbearing women exposed to VPA deliver healthy infants, however, the risk of congenital malfomation in the developing fetus is 2 or 3-fold higher (4-6%) than the risk in general population (2%) (Jamsheer et al., 2008; Yerby, 2003). First reports on possible teratogenic activity of VPA appeared in early 1980s (Dalens et al., 1980; Gomez, 1981).

Many animal studies were carried out in order to mimic the effects of VPA on the human embryo and elucidate the mechanism of its teratogenic action (Wagner et al., 2006; Arndt et al., 2005; Holmes et al., 2005; Emmanouil-Nikoloussi et al., 2004). In most animals the drug was teratogenic but the effective teratogenic doses differed widely. VPA induced malformations of multiple organs in mice, rats, and gerbils, renal and skeletal defects in rabbits, neural tube defects in mice and hamsters, craniofacial and appendicular skeletal defects in primates and behavioral deficits resembling autism in mice and rats (Ingram et al., 2000; Emmanouil-Nikoloussi et al., 2004; Rodier et al., 1996; Binkerd et al., 1988; Hendrickx et al., 1988).

In this study, the effects of sodium valproate in rat renal

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Abbreviations: SV, Sodium valproate; **VPA**, valproic acid; **FA**, folic acid; **Vit E**, vitamin E; **NTDs**, neural tube defects.

corpuscle and the possible protective roles of folic acid and vitamin E in the ultrastructural examination were investigated.

MATERIALS AND METHODS

Animals

The study was conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals. Twenty-four (24) adult female Wistar-Albino rats weighing 200 to 250 g, obtained from Experimental Research Institute of Dicle University (DÜSAM) were used. Rats were maintained on a 12 h light/dark cycle at 21 ± 1°C and 50 ± 10% humidity. The pregnant rats (n = 24) were randomly divided into four groups (18 pregnant rats in treatment groups, and 6 pregnant rats in control group). Each pregnant rat was put into an individual cage.

Experimental protocol

Control group (n=6): The first group of rats was used as control. This group was not given the drug. These rats were only fed with standard laboratory chow and tap water.

VPA group (n=6): Once per day on days 8^{th}, 9^{th} and 10^{th} of gestation, sodium valproate (Sodium valproate, Sigma P 4543) was administered subcutaneously into a loose fold of skin on the back leg at the dose of 400 mg/kg.

VPA + FA group (n=6): Once per day on days 8^{th}, 9^{th} and 10^{th} of gestation, sodium valproate was administered subcutaneously into a loose fold of skin on the back leg at the dose of 400 mg/kg and FA (folic acid, pteryoglutamic acid vit M, Sigma F 8798) was given (400 µg) ordinarily in drinking water per day during pregnancy.

VPA + Vit E group (n=6): Once per day on days 8^{th}, 9^{th} and 10^{th} of gestation, sodium valproate was administered subcutaneously into a loose fold of skin on the back leg at the dose of 400 mg/kg. One hour before the injection of VPA, vitamin E (α-tocopherol, Sigma T-3251) was administered by gastric intubation to dams at a dose of 250 mg/kg (Vorhees, 1987).

Surgical procedure and histopathological examination

The rats were sacrificed using ketamine (25 mg/kg) anasthesia, on the 20^{th} day of pregnancy. Kidney biopsies were fixed in 2.5% phosphate buffered glutaraldehyde. After postification with 1% osmic acid, they were dehydrated within acetone and semi-thin sections of tissue samples embedded in araldite were stained with toluidin blue. Then, the thin sections were stained with uranyl nitrate-lead and evaluated under Jeol 1010 transmission electron microscope and microphotographs were taken.

RESULTS AND DİSCUSSİON

No pathology was observed in the control group of rats kidneys. The basal lamina, foot processes and infiltration slits were observed in normal view (Figure 1). In the kidneys of the group of valproic acid, the irregularities on the basal lamina and deletion on the foot processes were observed (Figure 2). İn the group of valproic acid + folic acid, sections belonging to this group showed relatively normal ultrastructure when compared to VPA group.Glomerular basal lamina, foot processes and infiltration slits were observed in normal view (Figure 3). And in the group of valproic acid + vitamin E, glomerular basal lamina, foot processes and infiltration slifts view were found close to the control group (Figure 4).

VPA is in the market as an anticonvulsant since 1974, and is used in many countries because of its efficiency against several types of epilepsy and as a mood stabilizer. One of its main actions is the increase in the level of gamma amino butyric acid (GABA) in the brain. GABA is an important inhibitor of seizures, and reduction of GABA levels may potentiate seizures. For seizure control, the daily doses range between 300 mg to 2 g, aiming to achieve therapeutic plasma levels of 50 - 100 µg/mL. Lower doses are usually administered in the treatment of bipolar disorder-for manic patients, and against migraine (Ornoy, 2009).

The use of VPA during pregnancy is associated with a 1 - 2% incidence of neural tube defects. Although this term refers to all types of neural tube defects, including anencephaly/exencephaly, VPA is associated mainly with lumbosacral meningomyelocele (spina bifida aperta), the latter being 10–20 times the rate in the general population (Fried et al., 2004; Omtzigt et al., 1992; Lindhout et al., 1992).

In our study pathology was not observed in the first control group of rats kidneys (Figure1). In the kidneys of the group of valproic acid, the irregularities on the renal glomeruler basal membranes and deletion on the foot proces were observed (Figure 2).

SV is an antiepileptic drug known to induce hyperammonemia in humans. This hyperammonemia might result from a reduced detoxification of ammonium in the liver and/or from an accelerated renal ammoniagenesis (Rengel-Aranda et al., 1988). SV treat-ment has been indicated to produce hyperglycinaemia and hyperglycinurea. Hyperammonaemia may occur asymptomatically (Takeuchi et al., 1988). The main source of production is deamination of aminoacids taking place in different tissues (kidney, skeletal muscle and the colon) (Kvamme, 1983). Lenoir et al. (Lenoir et al., 1981) reported a case of severe myopathic syndrome and biochemical evidence suggestive of proximal tubular defect. Renal biopsy indicated giant mitochondria in the proximal tubular cells and abnormal round granular inclusions in the cytosol of tubular cells, podiocytes and interstitial cells which were recovered on withdrawal of valproic acid. Gossrau and Graf (Gossrau and Graf, 1989) reported a very severe damage to the tubules of the kidney in pregnant mice at a single dose of valproic acid (500 mg kg-1 i.p.) which persisted even after 48 h of drug administration. Warter et al. (1983) showed that an important source of ammonia production is located in renal tubule cells. Hulsman (1989) demonstrated that ammonia is metabolised in the ornithine cycle, beginning in the liver mitochondria. The initiating step (NH_3 + CO_2 + ATP) is

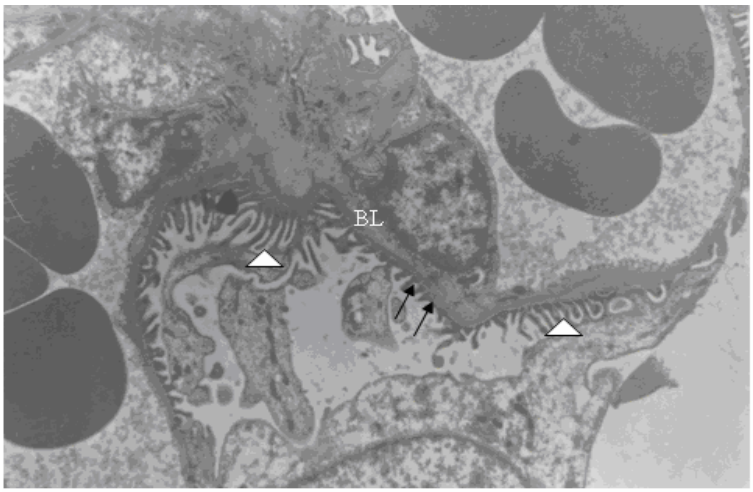

Figure 1. Electronmicrograph of control group rat kidney. Basal lamina (BL), foot processes (arrowheads) and infiltration slits (arrow) observed normal on rat kidney (uranyl acetate-lead citrate, X 3000).

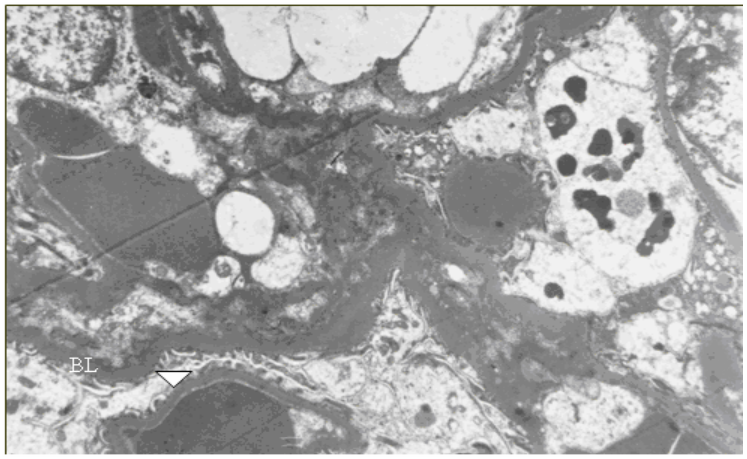

Figure 2. Electronmicrograph of valproic acid group rat kidney. The irregularities on the basal lamina (BL) and food deletion on the foot processes (arrowheads) observed (uranyl acetate-lead citrate, X 3000).

complex and is catalysed by carbamoyl phosphate transferase (CPT). When this enzyme is partially deficient hyperammonaemia occurs (Hjelm et al., 1986; Tripp et al., 1981). Furthermore, CPT may be inhibited with propionate, a degradation moiety of valproate. Although the exact biochemical mechanisms of SV toxicity to liver and kidney have not been well defined, several hypothesis havebeen proposed. The recent hypothesis suggests an involvement of lipid peroxidation (Olson et al., 1986; Buchi et al., 1984). The involvement of peroxidative injury in SV induced renal tubular disorder and hepatotoxicity is based on several lines of evidence. Valproate treatment decreased the rate of oxidized glutathione released into the bile (Olson et al., 1986); free radical scavengers like vitamin E and/or N-N'-diphenyl-p-phenylene-diamine supplements provided adequate protection against SV toxicity in hepatocyte cultures (Buchi et al., 1984).

Although these studies suggest that lipid peroxidation may play a role in SV toxicity, a detailed time course of biochemical changes in liver and kidney following subacute exposure has not been reported and may be critical to understanding the mechanism of SV toxicity. These experiments were designed to demonstrate the initiation of biochemical events and the role of lipid peroxidation in SV toxicity.

SV may undergo omega oxidation and delta-dehydrogenation (Dreifuss et al., 1987; Kassahun et al., 1994) producing unsaturated derivatives (2-n-propyl-4-pentenoic acid; 2-n-propyl-2 (E) pentenoic acid; 2 hydroxy, 3 hydroxy, and 4 hydroxy valproic acid) by the

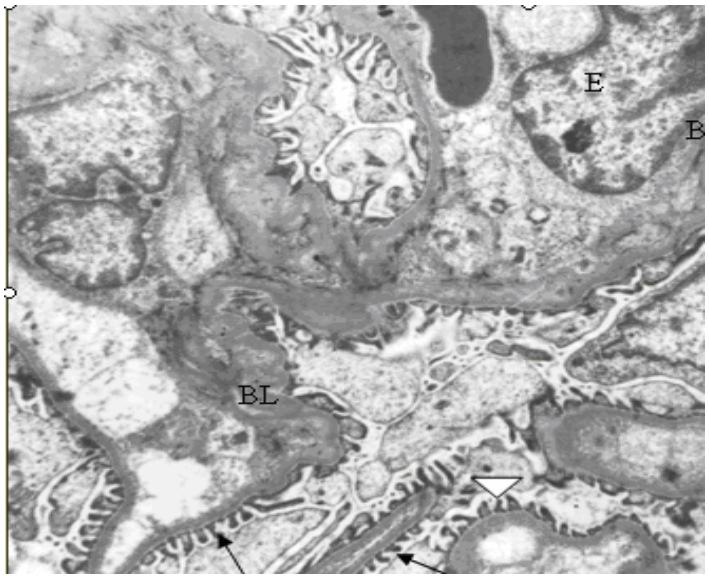

Figure 3. Electronmicrograph of valproic acid + folic acid group rat kidney. Regularity in basal lamina (BL), foot processes (arrowheads), infiltration slits (arrow) and endothelial cells (E) observed normal structure (uranyl acetate-lead citrate, X 3000).

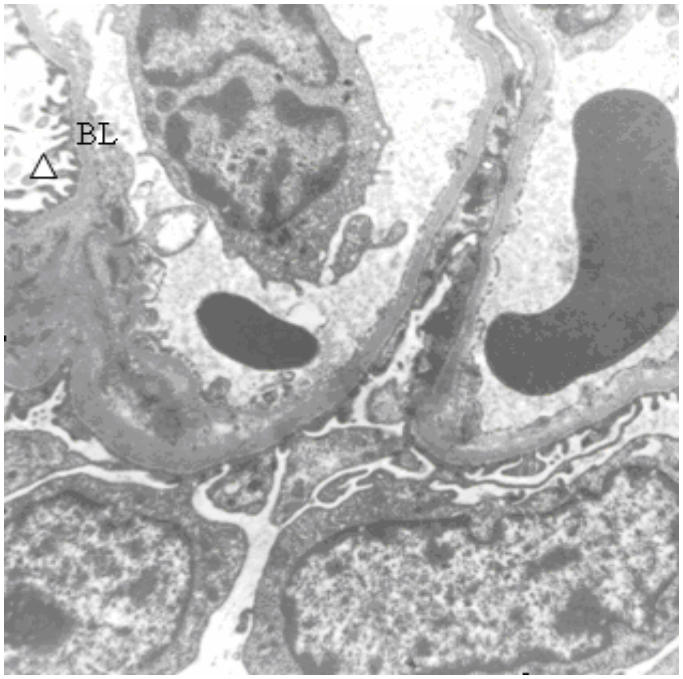

Figure 4. Electronmicrograph of valproic acid + vitamin E group rat kidney. Note basal lamina (BL) and foot processes (arrow heads) in normal view (uranyl acetate-lead citrate, X 3000).

inhibition of either mitochondrial or microsomal P-450 (Rittle et al., 1987). This may generate reactive oxygen species, to combine with polyunsaturated lipids and generate lipid hydroperoxides. SV, its omega oxidation products and delta dehydrogenation moieties are reported to be depressants of gluconeogenesis (Rogiers et al., 1985; Turnbull et al., 1986), and may inhibit the mitochondrial or microsomal reactions, a rate limiting factor.

Our findings in the present study, the group of SV+FA

(Figure 3), show that renal glomerular basal membranes and foot process were under normal view. Folic acid was studied in animals of one class of antimetabolites, those of pteroyglutamic acid, or folic acid as it is most commonly called, that led to interest in the role of this vitamin in human abnormal development, an interest that has extended over 50 years. In the first of such experimental work, the antimetabolites, when admini-stered to female mice and rats at the time of uterine implantation or earlier, caused early prenatal death, but not malformation; suggesting an all-or-none action (Kalter, 2003). Many antiepileptic drugs interfere with folic acid absorption (phenytoin, barbiturates, carbamazepine and lamotrigine) or metabolism (VPA) (Yerby, 2003). It is therefore recommended to treat women on antiepileptic drugs at preconception and in the first 2–3 months of pregnancy with folic acid that protects human and animals from neural tube defect (NTD). Although the use of folic acid supplementation has been shown to generally decrease the incidence of NTD in humans, there is disagreement as to the benefit of folic acid in reducing the rate of NTD or other anomalies following VPA or other antiepileptic drugs exposure, as several studies and case reports were unable to demonstrate any significant reduction of these anomalies by folic acid (Yerby, 1994; Yerby, 2003; Genton et al., 2006). In spite of the uncertainty of effectiveness, it is recommended to take 4-5 mg/day of folic acid prior to any planned pregnancy. Indeed, in a recent study, Pittschieler et al. (2008) found that periconceptual folic acid signi-ficantly reduced the rate of spontaneous abortions and premature delivery in women treated with VPA and carbamazepine. They did not study the rate of congenital anomalies. Other studies were inconclusive as to the beneficial effects of folic acid, even in reducing the rate of spontaneous abortions

Similar disagreement exists in experimental animal studies. It seems that the protective effect of folic acid on VPA induced NTD is strain dependent, as different results were obtained in different strains of mice. For example, administration of high doses of folinic acid to valproic acid-treated pregnant mice did not alter the production of exencephaly (Wegner and Nau, 1991; Wegner and Nau, 1992). On the other hand, pretreatment of pregnant CD-1 mice with high doses of folic acid and/or of pantothenic acid prior to the administration of valproic acid decreased the rate of NTD and almost normalized the levels of proteins involved in neurulation (Dawson et al., 2006). These conflicting results of human and animal studies imply that folic acid deficiency is not the major pathogenetic mechanism of VPA-induced teratogenicity.

Vitamin E is a beneficial antioxidant in pharmacological treatment of mitochondrial disorders (Bandyopadhyay and Dutta, 2005). In our findings in the group of SV+ Vit E, there was an appearance nearest to the control group (Figure 4). The antioxidant, vitamin E, has been shown to decrease the frequency of valproic acid-induced neural tube defects in mice, suggesting that ROS may play a role in the failure of the neural tube to develop properly (Al Deeb et al., 2002). In addition, vitamin E has provided significant protection against hydrophobic bile acid toxicity in an *in vivo* rat model (Sokol et al., 1998). Vitamin E decreased the rate of VPA induced anomalies and embryonic damage in Balb mice (Ehlers et al., 1996), pointing to the possibility that oxidative stress is involved in VPA induced embryonic damage.

Valproic acid seems to be a highly teratogenic antiepileptic drug. VPA treated women at childbearing age should use contraceptives and stop the medication before any planned pregnancy (Ornoy, 2009).

CONCLUSİON

In conclusion, our findings show that the usage of SV during gestational period might clinically create risk, but this risk can be reduced by FA administration. In addition, Vit E was found to have a similar protective effect as FA. This effect of Vit E may be due to its antioxidant property. We consider that administration of FA and Vit E in case of maternal SV administration during pregnancy may reduce degenerative changes seen in kidney.

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