The Protective Role of Folic Acid and Vitamin E Against Toxical Effects of Valproic Acid on Liver Tissue During Period of Gestation

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SUMMARY

Valproic acid is an anticonvulsan drug used in epilepsy. The histopathological changes of valproic acid on liver and the protective effect of vitamin E and also folic acid were observed. 24 adult female Wistar Albino rats were used. The first control, the second valproic acid group that was given 300 mg/kg on 8.9. and 10. days of gestation, the third valproic acid +vitamin E group. Vitamin E was given 250mg/kg via nasogastric intubation before one hour administration of valproic acid on 8.9.10.days of gestation. The fourth valproic acid+ folic acid group; via valproic acid, folic acid was given 400 microgram ordinarily in drinking water per day. In the liver sections of valproic acid group, perivenullar dilatation, swelling of Kuppfer cells, microvesicular steatosis and degeneration were observed. In the second group there was moderate degeneration in hepatocytes,necrotic areas in some places, mononuclear cell infiltration. In valproic acid +vitamin E group normal-like appearance of the structure of Remark cell lines were observed. Under these source of results, we viewed antioxidants decreased the hepatotoxicity on liver tissue by using folic acid and vitamin E .

Key Words: *Liver, Valproic Acid, Folic Acid, Vitamin E, Hepatotoxicity*

Gebelik Sürecinde Valproik Asit'in Karaciğer Dokusundaki Toksik Etkilerine Karşı Folik Asit ve Vitamin E'nin Koruyucu Rolü

ÖZET

Valproik asit epilepside kullanılan antikonvülzan bir ilaçtır. Valproik Asit'in karaciğer üzerinde yaptığı histopatolojik değişiklikler ile folik asit ve vitamin E'nin koruyucu etkileri incelendi. 24 erişkin dişi Wistar Albino tipi sıçan kullanıldı. Birinci grup kontrol, ikinci grup gebeliğin 8.9. ve 10. günlerinde 300mg/kg dozunda valproik asit verilen grup, üçüncü grup valproik asit+vitamin E grubuydu. Vitamin E, valproik asit uygulamasından bir saat önce 250mg/kg dozunda nazogastrik intübasyonla yine gebeliğin 8.9.10.günlerinde verildi. Dördüncü valproik asit+folik asit grubuna valproik asit yanında, içme suyuna düzenli olarak her gün 400 mikrogram dozunda folik asit katıldı. Valproik asit grubunun karaciğer kesitlerinde perivenüller dilatasyon, Kuppfer hücre şişmesi, mikroveziküler steatozis ve dejenerasyon izlendi. Üçüncü grubun hepatositlerinde orta derecede dejenerasyon, bazı yerlerde nekrotik alanlar ve mononükleer hücre infiltrasyonu görüldü. Dördüncü grupta Remark hücre kordonlarının görünümü normale yakındı. Bu bulguların ışığı altında folik asit ve vitamin E kullanarak antioksidanların hepatotoksisiteyi azalttığını izledik.

Anahtar Kelimeler: *Karaciğer, Valproik Asit, Folik Asit, Vitamin E, Hepatotoksisite*

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INTRODUCTION

Valproic acid (VPA), an eight carbon branched chain fatty acid with a broad spectrum of anticonvulsant activity, is used in the treatment of epilepsy. (1,2) This structure may enable VPA to interact with cell membranes, which may account in part for both its therapeutic and adverse effects (3).

As early as 1979 and 1980, the first case reports of valproate (VPA) induced hepatic failure were published (4,5). The increased clinical use of valproate has been accompanied by reports of hepatic dysfunction and renal tubular defects (6,7).

Valproic acid, introduced as an anticonvulsant in Europe in 1968 and in the United States in 1978, and given to 200.000 new patients annually, has been associated rarely with fulminant fatal hepatitis (8,9).

Valproate toxicity is thought to be idiosyncratic and metebolic without an immunologic basis (8,10).

Valproic acid, a frequently used drug for the treatment of epilepsy, has been used worldwide (I) However, valproic acid therapy is responsible for a number of fatal cases of hepatic failure (11, 12). Hence the mechanism by which valproic acid causes hepatotoxicity is poorly understood (13).

VPA, a widely used anticonvulsant drug, induced the deterioration of trace metal homeostasis, including zinc (Zn) deficiency in experimental animals (14,15).

As clinical usage of VPA increased, reports of its hepatotoxicity began to appear. This toxicity ranges from mildly increased aminotranspherase enzymes in 15 to 30 percent of patients to liver failures (16) and death in some patients (17).

Two types of serious side-effects are associated with the use of VPA: hepatotoxicity and teratogenicity (7). This teratogenitic effects includes neural tube defects in human and experimental animals (18,19,20). The toxicity is apparently due to metabolites of VPA generated by w- oxidation, (21) which is one of three major metabolic pathways of VPA biotransformation (22,23). This is a cytochrome P-450 mediated reactin occuring in the microsomes and as such may be induced or inhibited by various agents (3). It normally accounts for 10 to 15 percent of VPA metabolism (22,23) but within the hepatocyte system it can be induced to enhance toxicity or inhibited to decrease toxicity (24).

MATERIALS and METHODS

In this study, 24 adult female Wistar-Albino rats weighing 200 to 250 gr., obtained from Experimental Research Institute of Dicle University (DÜSAM) were used. The female rats coupled with male rats and on the other morning examined for cervical plug. The female rats with positive cervical plug were accepted having gestation. The female rats randomly assigned to four groups. All groups were given standart laboratory chow and tap water. The first group was the control group fed standart laboratory chow and tap water. The second group injected VPA. VPA (Valproic acid; Na valproate Sigma P 4543) was given 300 mg/kg after diluation with saline solution on 8, 9 and 10^{th} days of gestation via subcutan injection in loose connective tissue of the right leg. The third group was VPA and Vit. E group. Vit E was given 250mg/kg by diluating olive oil via nasogastric intubation, whereas VPA was given 300 mg/kg. before one our administration of Vit E (Vitamin E, α-Tocopherol, Sigma T-3251) on 8, 9 and 10^{th} days of gestation. The fourth group was VPA and FA group. VPA was given 300 mg/kg also on 8.9. and 10.days of gestation and FA (Folic Acid, Pteryoglutamic acid vit M, Sigma F 8798) was given 400 microgram ordinarily in drinking water per day during pregnancy. On the 20^{th} day of gestation female rats were sacrified. The liver was removed from each goup and fixed neutral buffered formalin solution for 24 h., some of these samples were fixed in bouin solution. The paraffine sections of the biopcies were cut approximately 5μm in thickness. These sections were histochemically stained with Hematoxylen-Eosine (H&E) and Periodic Acide Shiff (PAS). Microphotographs were being taked after the microscopic examination of stained sections.

RESULTS

In the slides of liver sections of the control group stained with Hematoxylene-Eosine, the appearanc of the liver was histologically normal (Figure 1a).

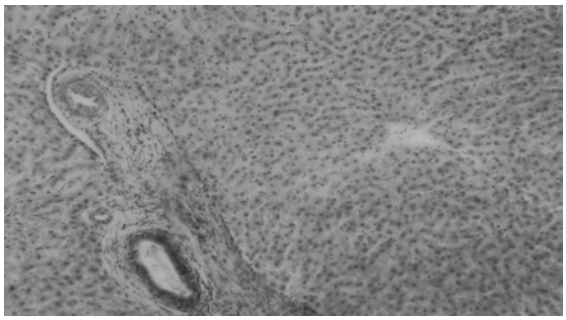

Figure 1 a. (Control group) normal liver appearance.

In the slides of liver sections of valproic acid group stained with Hematoxylene-Eosine; perivenullar dilatation, swelling of Kuppfer cells, microvesicular steatosis and degeneration in acidophilic structures of hepatocytes were observed. In another liver section of the same group, degeneration and inflammation at the periportal zone, mononuclear cell infiltration, necrotic areas and picnosis, affected liver homogenity, reactive and degenerative alterations in hepatocytes were also observed (Figure 1b).

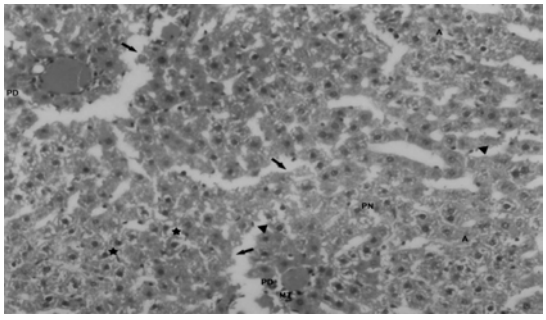

Figure 1 b. (VPA group) Perivenullar Dilatation (→), Swelling in Kupffer cells (►) Microvesicular Steatosis (*),Acidophilic degeneration in some areas of hepatocytes (**A),**Periportal Degeneration (**PD**), Mononuclear cell infiltration (**Mİ),** Picnosis (**PN**)**.** (H.E.Originalmagnification X 82).

In the sections stained with PAS, heterogenity in the distribution of glycogen, dens mononuclear cell infiltrations at periportal zone, particularly arround the V. hepatica interlobularis, were observed. In addition, swelling in Kuppfer cells, perivenullar dilatation, degeneration in hepatocytes, decreasing in the clarity of classic liver-lobule construction, multivesicular steatosis at some area were examined (Figure2).

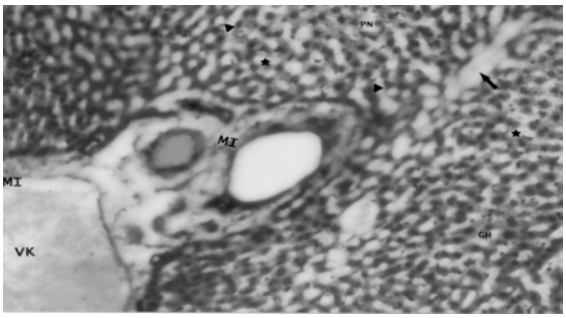

Figure 2. (VPA group) Heterogenity in distrubution of glycogen (**GH),** Mononuclear cell infiltration around V. Hepatica İnterlobularis (**Mİ**), Swelling in Kupffer cells(►),Perivenullar Dilatation (→),Piknosis (**PN**), Microvesicular Steatosis (*),Congestion in V. Porta İnterlobularis (**VK**). (PAS, Original magnification X82).

Degeneration in hepatocytes associated with progress on to pycnosis and necrosis, degeneration and congestion in V.hepatica interlobularis at periportal zone, increasing in connective tissue, infiltration and oedema in periportal zone were observed (Figure 3).

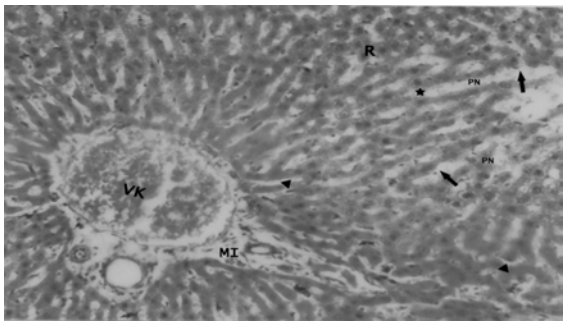

Figure 3. (VPA+FA group) Congestion in interlobular veins at perportal area(**VK**), Periportal Mononuclear cell infiltration (**Mİ),** Very few degeneration in the structure of Remark cell lines (**R**), Picnosis in some areas of hepatocytes (**PN**), Swelling in Kupffer cells (►), Microvesicular Steatosis (*),Perivenullar Dilatation (→) (H.E. Original magnification X82)

In the liver sections stained with Hematoxylene-Eosine, congestion in arteries and veins at periportal zones, inflammation at moderate grade, mononuclear cell infiltration, mild degeneration in the construction of Remark cell lines, pycnosis at some areas of hepatocytes, swelling of Kuppfer cells, congestion in V.centralis, increasing in vascularization, dilatation in perivenullar sinuzoides, severe decrease in microvesicular steatosis were examined (Figure 3).

In the liver sections stained with PAS, heterogenity at the distribution of glycogen in hepatocyte cytoplasm, mononuclear cell infiltration in periportal zone and swelling of Kuppfer cells were seen.

Mild congestion in V. centralis, moderate degeneration in hepatocytes,necrotic areas in some places,mild degeneration in the construction of Remark cell lines, monocellular infiltration, mild increase in connective tissue were observed (Fig 4).

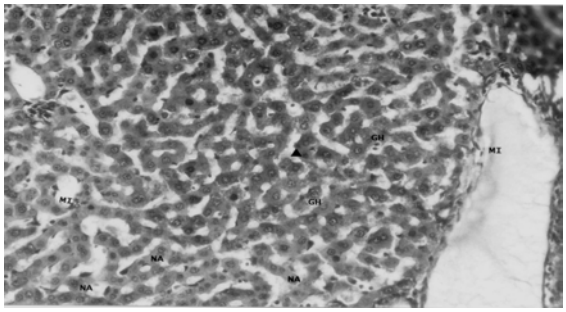

Figure 4. (VPA+FA group) Heterogenity in distrubition of glycogen(**GH**), Mononuclear cell infiltration in periportal area (**Mİ**), Swelling in Kupffer cells (►), Mononuclear cell infiltration in some areas of liver parenchyme near V.centralis (**Mİ**), Necrotic areas in some areas of hepatocytes (**NA**).(PAS, Original magnification X82).

In the liver sections stained with Hematoxylene-Eosine,mild congestion in V.centralis, we observed mild degeneration in the construction of Remark cell lines and dilatation at some perivenullar sinuzoides, mild swelling in Kuppfer cells,normal-like appearance of hepatocytes, no microvesicular steasosis congestion in arteries and veins in periportal zone, mild infiltration of mononuclear cells.(Fig 5,6)

In the liver sections stained with PAS, nearly normal-like appearance of V.centralis, very mild microvesicular steatosis, mild dilatation in perivenullar sinuzoides,normal-like appearance of the structure of hepatocytes and mild heterogenity in their distribution of glycogen, swelling and hyperplasia in Kuppfer cells, mild degree of mononuclear cell infiltration in periportal zone were seen.

Mild degeneration in hepatocytes and dilatation in some sinuzoides, mild congestion in V.centralis, normal-like appearance of the structure of Remark cell lines, and periportal zone, and mild increase in connective tissue were observed (Fig.5, 6).

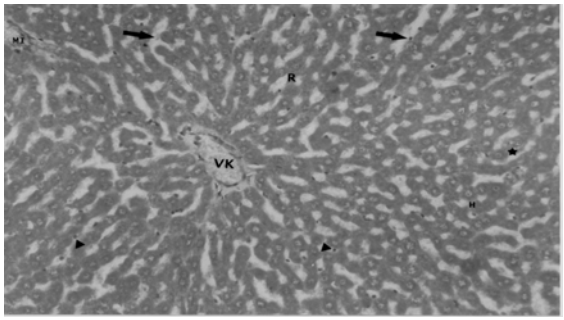

Figure 5. (VPA+Vit E group) İncreasing rate of congestion in V.Centralis (**VK**), Few degeneration in the structure of Remark cell lines **(R),** Dilatation in some areas of perivenullar sinuzoides (→), A few swelling in Kupffer cells (►),The appearance of hepatocytes nearly normal (**H**), Microvesicular Steatosis is almost non present **(***),The grade of mononuclear cell infiltration is little in periportal area (**Mİ**) .(H.E. Original magnification X82).

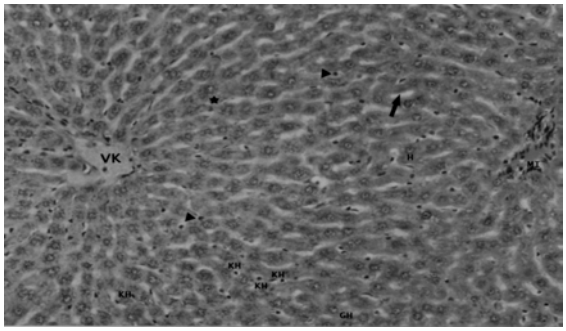

Figure 6. (VPA+Vit E group) Moderate congestion in V.Centralis (**VK**), Microvesicular Steatosis is very few (***),** Little dilatation in perivenullar sinuzoides (→), Little heterogenity in the glycogen structure of hepatocytes (**GH**), Little mononuclear cell infiltration in periportal area (**Mİ**), Kuppfer cell hipertophy +hiperplasia (**KH**). (PAS, Original magnification X82).

DISCUSSION

The mechanism by which VPA causes hepatic damage is uncertain; hepatotoxicity is suspected to result from the formation of toxic VPA metabolites (25,26,27,28) or altered antioxidant enzyme activities (29).

Published reports on valproate-induced hepatotoxicity and renal tubular disorder provide sufficient information on the pathological changes induced in these cells. However, none of them has described these changes as a function of time of exposure (30). But liver biopsy on some patients taking valproate therapy indicated hepatic necrosis, hepatitis, centerlobular necrosis, cirrhosis, hepatomegaly with fatty infiltration, cholestasis and inflamation, as well as end-stage liver disease, with multinodular changes and fibrosis (31). However, the information on these cases is scantly and variable.

As we worked only with liver biopcies in our study, we determined that the findings we viewed that the histopathological changes of VPA on liver were similar at large rate with the studies of Jeavons P.M.et al (1982) almost Quereshi and his friends hadn't determined before.

Pathomorphological changes in mouse liver and kidney during prolonged valproate administration (30).

Most studies on hepatic disorder report microvesicular steatosis in periportal regions of the liver lobule after a single dose of valproate (32,33). Our study was paralel with the results of Kochen, Siemens and Graf (28,29).

Folic acid is converted, in the precence of ascorbic acid, in the liver and plasma to its metabolic active form (tetrahydrofolic acid) by dihydrofolate reductase. A large proportion of the biotransformation is hepatic. Folate deficiency can be linked to scurvy (34,35).

The action of folic acid on the regenerative response in the liver has not yet been elucidated (36), however it is not known whether folic acid can inhibit cell proliferation.

In the study of M. Komatsu and friends, they couldn't explained whatever if folic acid has regenerative changes on liver and whether folic acid inhibits the cell proliferation. Although we viewed that there has been proliferation and regeneration almost little on the cells of hepatocytes and Kuppfer cells of the liver according to the effect of folic acid. Accompanying with these results we determined the proliferative and regenerative effects of folic acid on the liver which M.Komatsu and friends hadn't explained before (36).

The present concessus, therefore, is that diet low in methionin and devoid of choline and folate are sufficient to independently induce tumor formation in rats, primarly in the liver (37).

As we also observed the presence of pycnotic and necrotic areas which VPA had made because of the absence of folic acid in the VPA group, even there was almost no necrotic areas and there was a decrease in pycnosis in VPA+FA group. As it had a supporting quality in the study which Lombardi and friends had made via the level of light microscopy.

Chronic treatment with VPA has been shown to decrease the levels of antioxidants such as vitamin E and glutathion peroxidase further suggesting the role of oxidative stres . Moreover, recent studies have shown protective effect of vitamin C and vitamin E against VPA induced hepatotoxicity (7).

We didn't explored the results even if VPA decreased the realing of antioxidants which Juriva-Romet M.et al (1996) mentioned hence we examined the effects of folic acid and Vitamin E on liver hepatotoxicity instead of Vitamin E and glutathion peroxidase which had been mentioned. Almost together it was clarified that Vitamin E and C decreased hepatotoxicity that Juriva-Romet M. et al (1996) mentioned in their literatur. Under these source of results we also viewed by using folic acid and vitamin E of which are antioxidants decreased the hepatotoxicity on liver tissue. We determined that our study was paralel with the results of Juriva-Romet M.et al (1996) which they had recorded that the antioxidants decrease the liver hepatotoxicity.

The present study examines the effect of such known free-radical scarengers, vitamin E

on sodium valproate toxicity in the rat hepatocyte model. These scavengers would be expected to reduce any free radical metabolities and thus prevent lipid peroxidation and toxicity.The decrease in toxicity could simply be a consequence of Vit E stabilizing the hepatocyte membrans, allowing less lactate dehydrogenase (LDH) to be released into the surrounding media and thus decreasing the LDH index (3).

We couldn't obtained the amount of LDH biochemically in our study. But we determined that the finding vesicular steatosis was more at the level of light microscopy in VPA group. We saw that there was almost no microvesicular steatosis in VPA+Vit E group. According to these results, it supports the idea mentioned in Buchi K.N. et al (1984) that the realising inhibition of LDH is due to Vit E and decreases the microvesicular steatosis at the rank of the light microscopy (3).

When we compared the VPA + FA and VPA+E groups,we viewed that there was decrease of microvesicular steatosis in liver parenchyme in Vit E group, the swelling of Kuppfer cells was less and almost normal appearence, a decrease of mononuclear cell infiltration in artery, venule and connective tissue at periportal area as similar. As a result of these findings it was beleived that vitamin E has more protective effect when comparing with folic acid of preventing the liver hepatotoxicity according to folic acid.

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