ORIGINAL RESEARCH

Edaravone Ameliorates Valproate-Induced Gingival Toxicity by Reducing Oxidative-Stress, Inflammation and Tissue Damage

Şehkar OKTAY, Burçin ALEV, Leyla KOÇ ÖZTÜRK, Sevim TUNALI, Sezin DEMİREL, Ebru EMEKLİ ALTURFAN, Tuğba TUNALI-AKBAY, Serap AKYÜZ, Refiye YANARDAĞ, Ayşen YARAT

ABSTRACT

Valproic acid (2-n-propylpentanoic acid, VPA), the most widely used antiepileptic drug, has potential adverse effects and it can disrupt the oxidant and antioxidant balance. Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one, EDA) is a potent free radical scavenger. In this study, the effect of EDA on gingiva in VPA induced toxicity was investigated. Female Sprague Dawley rats were randomly divided into four groups: control group, EDA (30 mg/kg/day) given group, VPA (0.5 g/kg/day) given group, and VPA+EDA (in same dose and time) given group. EDA and VPA were given intraperitoneally for seven days. Total protein, lipid peroxidation (LPO), sialic acid (SA) and reduced glutathione (GSH) levels and catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), myeloperoxidase (MPO), alkaline phosphatase (ALP), acid phosphatase (ACP), sodium potassium ATPase (Na^+/K^+-ATPase) and tissue factor (TF) activities were determined in gingiva homogenates. The VPA-induced increases were statistically significant for MPO ($p<0.01$), ACP ($p<0.01$), Na^+/K^+-ATPase ($p<0.05$) and TF ($p<0.01$) activities, but not for LPO level and ALP activities. EDA treatment markedly blunted all such elevated anomalies. Conclusively, VPA induced oxidative and inflammatory gingival tissue damage, reactions that were appreciably reversed by concurrent administration of EDA.

Keywords: Gingiva, valproic acid, edaravone, oxidative stress, inflammation

Şehkar Oktay[1], Burçin Alev[1], Leyla Koç Öztürk[1], Sevim Tunalı[2],
Sezin Demirel[3], Ebru Emekli Alturfan[1], Tuğba Tunalı-Akbay[1], Serap Akyüz[3],
Refiye Yanardağ[2], Ayşen Yarat[1]
Department of Basic Medical Sciences[1], Department of Pedodontics[3], Faculty of Dentistry, MarmaraUniversity, 34854, Maltepe, Istanbul, Turkey.
Department of Chemistry[2], Faculty of Engineering, Istanbul University, 34320, Avcilar, Istanbul, Turkey.

Correspondence to:
Şehkar Oktay
E-mail: nsehkar@yahoo.com
ayarat@marmara.edu.tr

INTRODUCTION

Valproic acid (2-n-propylpentanoic acid, VPA) is the most widely used antiepileptic drug in the treatment of certain types of seizures (1). Although VPA has good efficacy and it is often well tolerated, it has several side effects on the neurological, gastrointestinal, hematological and reproductive systems. The most common symptoms of VPA toxicity include anorexia, nausea, vomiting and also hyperammonemic encephalopathy, pancreatitis, elevated liver enzymes, leukopenia, thrombocytopenia, coagulation disorders and development of coma (2). The toxicity of VPA has been investigated in various studies, but the knowledge about the incidence and occurrence of these effects is still unclear (3-7). Besides the damage caused to other tissues, taking VPA can cause primarily gingival overgrowth and other dental problems (8).

Epilepsy is a chronic neurologic disorder and affects 1-3 % of the population. Most epilepsy patients receive long-term

anticonvulsant therapy and may require medications for other transient conditions on body. Both epilepsy and its medical management may affect oral health. Therefore, it is important to consider the adverse effects of anticonvulsant usage on tissues (9). Evidence suggests that the incidence of dental disease is increased in epileptic patients, thus they have tendency of dental disease and increased oral health needs. The positive correlation between severity of seizure and periodontal disease was shown in a study with refractory epilepsy patients (10).

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one, EDA) is a potent free radical scavenger, and has been widely used to treat acute ischemic stroke. In experimental studies EDA has been suggested to show protective effects on inflammation and tissue damage by eliminating the deleterious effects of oxidants in various tissues (11-13). It acts as an antioxidant by quenching hydroxyl radicals and by preventing lipid peroxidation caused by hydroxyl radicals. In this study, the effect of EDA in VPA induced toxicity was investigated on gingiva by some biochemical parameters.

MATERIAL and METHODS

All experimental protocols were approved by the Marmara University Animal Care and Use Committee (134.2013. mar). VPA (Merck, Germany) and EDA (Fluka, Switzerland) were dissolved in saline (0.9% NaCl) seperately. Thirty seven female Sprague Dawley rats were randomly divided into four groups as follows; Control group (n=8), EDA (30 mg/kg/day) given group (n=8), VPA (0.5g/kg/day) given group (n=10), VPA + EDA (in the same dose) given group (n=11). VPA was given to the animals one hour later than the EDA administration everyday. EDA and VPA were administered to the experimental group intraperitoneally for 7 days. On the 8th day of the experiment, animals were fasted overnight and then sacrificed under anesthesia. Gingiva samples were homogenized in saline (0.9% NaCl) in a bowel filled ice cubes for 15 second at high speed by using by Ika- Werkrw-14 homogenizer to obtain 10% (w/v) homogenates. Oxidant and antioxidant parameters; lipid peroxidation (LPO) and reduced glutathione (GSH) levels, superoxide dismutase (SOD), glutathione-S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and myeloperoxidase (MPO) activities, inflammation, bone resorption and thrombosis parameters; sialic acid (SA) levels, Na-K ATPase, alkalen phosphatase (ALP)-acide phosphatase (ACP), tissue factor (TF) activities were determined in gingiva homogenates.

Determination of total protein

The method of Lowry (14) was used to determined total protein levels of gingiva samples. Briefly, alkali proteins are reacted with copper ions and then reduced by Folin reactive. The absorbance of the product was evaluated at 500 nm spectrophotometrically and calculated to express the results of the parameters per protein.

Determination of LPO

Malondialdehyte (MDA), results from LPO, was determined as thiobarbituric acid reactive substances by the method of Yagi (15). The extinction coefficient of 1.56×10^5 M^{-1}cm^{-1} was used and LPO was expressed in terms of MDA equivalents as nmol MDA/mg protein.

Determination of GSH

The method of Beutler (16) was used to determine GSH levels of gingiva samples. Metaphosphoric acid was used for protein precipitation and 5,5′-Dithiobis-2-nitrobenzoic was used for color development. For calculation, the extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} was used and results were expressed as mg GSH/g protein.

Determination of SOD activity

The method based on the ability of SOD to increase the effect of riboflavin-sensitized photo-oxidation of o-dianisidine was used to determine SOD activities in gingiva samples. The absorbance of the product was measured in 460 nm spectrophotometrically. The net absorbance was calculated by measuring absorbances at 0 and 8th minutes of illumunation. The results were expressed as U/mg protein (17).

Determination of GST activity

GST is an antioxidant enzyme that catalyzes the conjugation of GSH. The absorbance of the mixture is measured in 340 nm spectrophotometrically and GST activities were calculated by using the extinction coefficient of 9.6 mM^{-1} cm^{-1}. Results were expressed as U/g protein (18).

Determination of CAT activity

The method of Aebi (19) was used to determine CAT activities of gingiva samples. The method is based on converting hydrogen peroxide (H_2O_2) to water by the effect of CAT. The absorbance was measured in 240 nm spectrophotometrically and results were expressed as U/mg protein.

Determination of GPx activity

The method of Paglia and Valentine (20) was used to determine GPx activities of gingiva samples. Glutathione reductase reduced glutathione disulfide to GSH. During the

oxidation of NADPH to NADP, the decreasing absrbance was measured in 366 nm spectrophotometrically. For calculation, the extinction coefficient of 6.22 $mM^{-1}cm^{-1}$ was used and results were expressed as U/g protein.

Determination of SA

The method of Warren (21) was used to determine SA levels of gingiva samples. The hydrolysate was prepared by incubation of the homogenates with 0.1N H_2SO_4 at 80ºC for 1 hour. In concentrated phosphoric acid, SA is oxidized with sodium periodate and the product of periodate oxidation is coupled with thiobarbituric acid. The formed chromophore is extracted into cyclohexanone and the absorbances of gingiva samples were evaluated in 549 nm spectrophotometrically. The results were expressed as mg SA/g protein.

Determination of MPO activity

The method of Wei and Frenke (22) was used to determine MPO activities of gingiva samples. The method contains a solution of homogenate, phenol, H_2O_2 and 4-Aminoantipyrine. The change in absorbance of reaction mixture per minute at 460 nm was recorded. The results were expressed as U/g protein.

Determination of ALP and ACP activity

The method of Walter (23) was used to determine ALP and ACP activities of gingiva samples. Depending on the pH of the medium, ACP and ALP hydrolyze p-nitrophenyl phosphate to p-nitrophenol. The absorbances were measured in 405 nm spectrophotometrically and ALP and ACP activities were expressed as U/g protein.

Determination of Na⁺/K⁺-ATPase activity

The method of Ridderstap (24) was used to determine Na^+/K^+-ATPase activities of gingiva samples. The reaction solution contained 5 mmol/L KCl, 150 mmol /L NaCl, 2.5 mmol/L $MgCl_2$ and 0.1mmol/L EDTA in 600 ml of 20 mmol/L imidazole buffer. 0,5 ml of ghost suspension was added and the mixture was placed for incubation in 37⁰ for 10 min. Adding 0.1 ml of 2.5 mmol/L disodium ATP was started the reaction. After 1 h, the tubes were cooled and 1ml of 15% trichloroacetic acid was added to stop the reaction. Na^+K^+ ATPase activities were expressed as mmol Pi/ mg protein per hour.

Determination of TF activity

The method of Quick (25) was used to determine TF activities

of gingiva samples. Pooled plasma collected from healthy subjects was used. The reagents were brought to 37ºC which was the reaction temperature. 0.1 ml tissue homogenate with 0.1 ml of 0.02 M $CaCl_2$ was mixed and by adding of 0.1 ml of plasma the clotting reaction being started. Since the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity.

STATISTICS

All statistical analyses were performed using SPSS 15.0 (Statistical Package for the Social Sciences) Software program. Results were expressed as mean ± standart error of the mean (SEM). Groups of data were compared using ANOVA followed by Tukey's multiple comparison tests. p <0.05 values were regarded as significant.

RESULTS

Oxidant and antioxidant parameters

The increase in LPO levels of the VPA group was not statistically significant (Fig.1A). There was a tendency towards decreased antioxidant status in VPA group as evidenced by unsignificant decreases in GSH levels, SOD, CAT, GPx activities compared to controls (Fig.1B,1C,1E,1F). GST activities decreased significantly (p<0.01) in VPA group compared with the control group (Fig.1D). EDA was also increased GSH levels, SOD, GST, CAT, GPx activities and decreased LPO levels unsignificantly (Fig. 1A, 1B,1C,1D,1E, 1F) .

Inflammation, bone resorption and thrombosis parameters

The VPA-induced increases were statistically significant for MPO (p<0.01) (Fig.2A), ACP (p<0.01) (Fig.2B), Na^+/K^+-ATPase (p<0.05) (Fig.2C) and TF (p<0.01) (Fig.2D) activities, but not for SA levels (Fig.2E) and ALP activities (Fig.2F) compared to control group. EDA treatment markedly blunted all such elevated anomalies. TF activity was expressed as seconds. Shortened clot formation time shows increased TF activity. EDA treatment caused to significant decreases in MPO (p<0.01) (Fig.2A), ACP (p<0.01) (Fig.2B), Na^+/K^+-ATPase (p<0.05) (Fig.2C) and TF activities (p<0.01) (Fig.2D) in VPA group. EDA was also decreased SA and ALP levels unsignificantly (Fig.2E and Fig.2F).
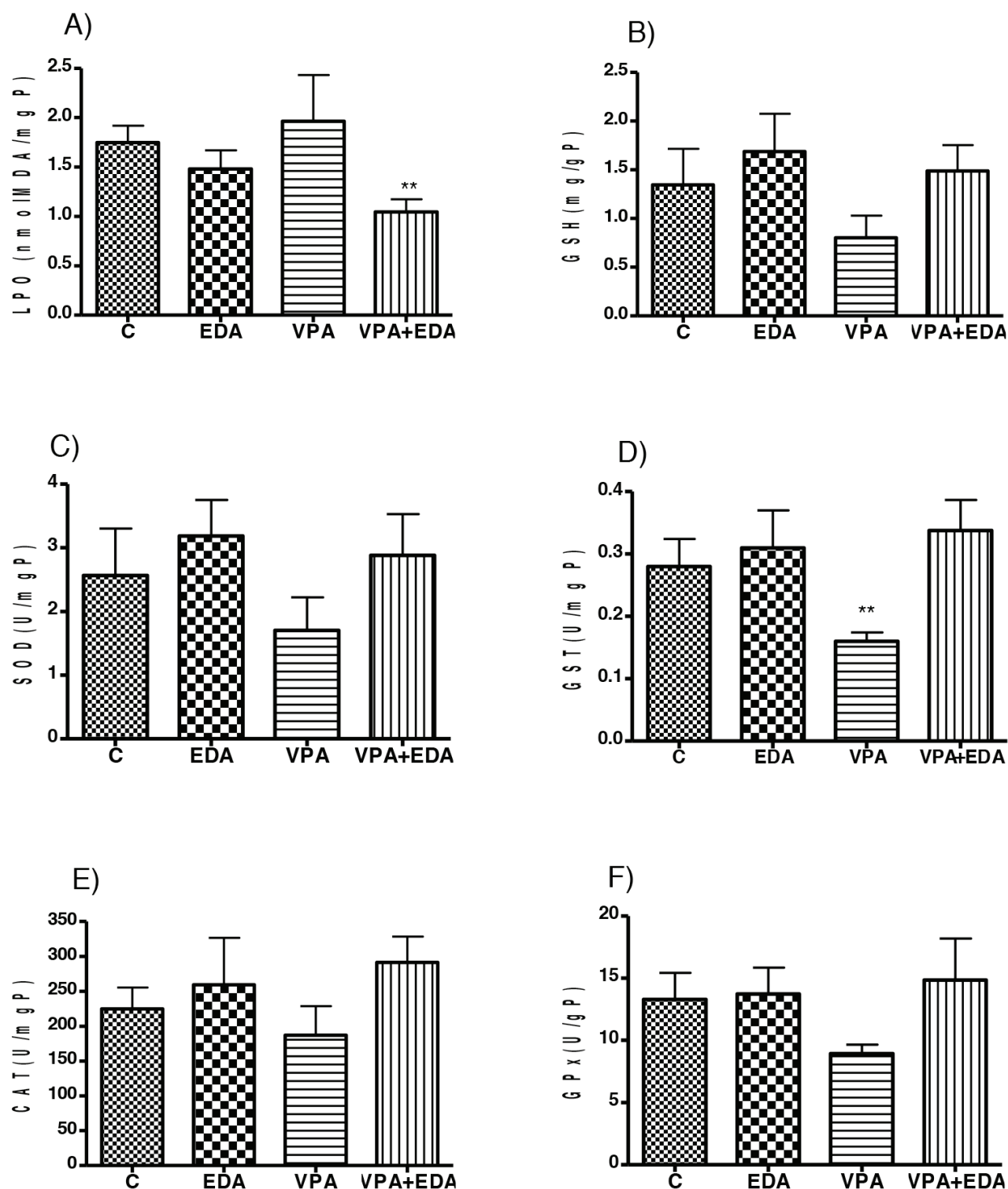
Figure 1. Oxidant and antioxidant parameters in four groups.
Values are given as mean±standard error, C: Control group; EDA: Edaravone given group; VPA: Valproic acid given group; VPA+EDA: Valproic acid and edaravone given group; P:protein; LPO: lipid peroxidation; GSH: glutathione; SOD: superoxide dismutase; GST: glutathione-S-transferase; CAT: catalase; GPx: glutathione peroxidase.
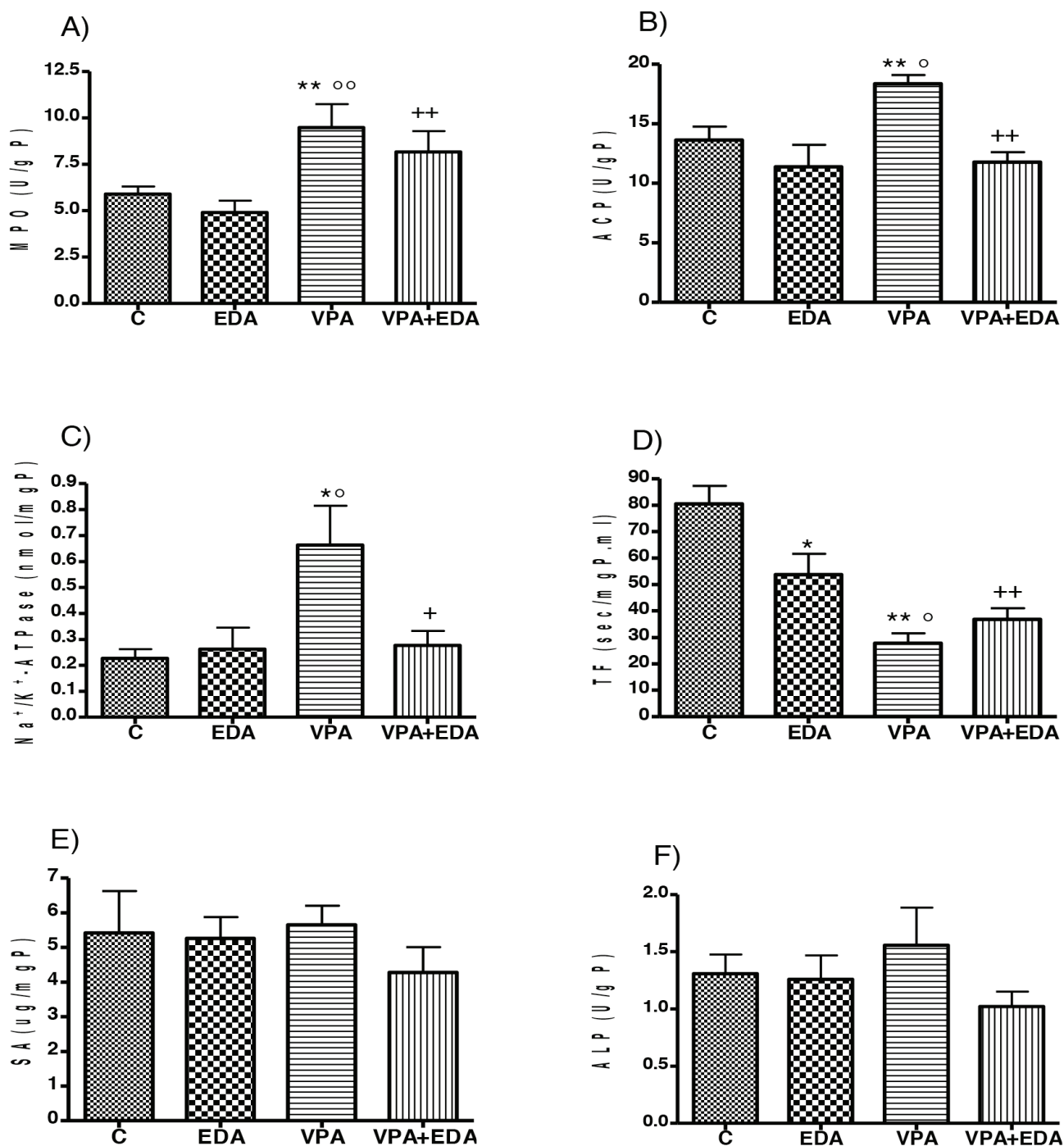**p<0.01 significantly different from group C

Figure 2. Inflammation, bone resorption and thrombosis parameters in four groups.

Values are given as mean±standard error, C: Control group; EDA: Edaravone given group; VPA: Valproic acid given group; VPA+EDA: Valproic acid and edaravone given group; P:protein; MPO: myeloperoxidase; ACP: acid phosphatase; Na^+/K^+ATPase: sodium-potasium ATPase; TF: tissue factor (the lengthening of the clotting time is a manifestation of decreased TF activity); SA: sialic acid and ALP: alkalen phosphatase.

*$p<0.05$, **$p<0.01$ significantly different from group C; °$p<0.05$, °°$p<0.01$ significantly different from group EDA; +$p<0.05$, ++$p<0.01$ significantly different from group VPA

DISCUSSION

VPA is still the broadest spectrum used in antiepileptic drugs for all types of seizures in children and adults, although serious complications may occur in some patients due to toxic side effects of VPA. Gingival enlargement is highly seen in patients with gingivitis and periodontal diseases and is a common adverse effect of drugs such as calcium channel blockers, anti-convulsants, and immunosuppresants. Sodium valproate induced gingival enlargement in a patient with pre-existing chronic periodontitis has been reported (26). Twenty two months old child suffering from gingival enlargement following intake of sodium valproate has been reported (27). The assosiacion between chronic carbamazepine, valproic acid and phenytoin medication on the periodontal condition in epileptic children and adolescents was studied and it was reported that the gingival and sulcus bleeding indices were higher in epileptics than in controls, gingival enlargement was also found in 30% of the epileptic patients (28). In the study of Gurbuz and Tan, epileptic children have been shown to possess mental and motor deficits and also these patients were at risk for oral health due to side effects of anticonsulvant treatment. The dental conditions of epilepsy patients were significantly worse than non-epileptic age-matched groups. Furthermore, poorly controlled epilepsy patients had worse oral health compared to patients who had better controlled epilepsy (29).

Disrupted balance between oxidants and antioxidants cause oxidative stress, which results in the activation of antioxidant system to neutralize the toxicity in the cell. But excessive production of reactive oxygen species (ROS) leads to depletion of antioxidant enzymes, such as SOD, GST, CAT and GPx. These enzymes are part of protection system of the organism against harmful effects of ROS by detoxify peroxides. Antiepileptic drugs increase ROS by several mechanism and cause some damages in certain tissues (2,30,31). In our previous study, significantly decreased GST activities and also, although the results were unsignificant, decreased SOD, CAT and GPx activities were found in the intestinal tissues of VPA group compared to controls (12). The results of this study were consisted with the previous studies related with the VPA toxicity (32-35). GSH is an effective nonenzymatic antioxidant factor in defence system. Although the result was unsignificant, decreased GSH level was found in VPA treated group compared to controls. Reduced capacity of defence system may be a result of decreased levels of antioxidants in gingival tissue. Elevation in LPO levels was frequently seen in VPA treatment (7,30,35,36), which is an evidence of disruption of oxidant-antioxidant balance and

reduced antioxidant defence by peroxides. In the present study, although unsignificantly, increased LPO levels were found in VPA group compared to controls. Edaravone was an effective agent in eliminating the deleterious effects of VPA by increasing antioxidants. Unsignificantly decreased LPO levels and increased GSH levels and SOD, GST, CAT, GPx activities were found in edaravone given VPA groups compared to VPA group. Unsignificant LPO and GSH results may be related with the dose of VPA for the induction of damage in gingival tissue.

SA is a member of neuraminic acid derivatives and plays a crucial role in metabolic events. Increased levels of SA is known as an inflammation marker due to increased levels of sialylated acute-phase glycoprotein (37). MPO is an enzyme found in polymorphonuclear leucocytes and also used as a marker in inflammation. Anticonvulsant drugs may directly influence gingival connective tissues by stimulating an increase in number of gingival fibroblasts and production of connective tissue matrix (38). The inflammation causing the changes in the gingival tissues influence the interaction between drug and fibroblastic activity. Epithelial width, odema, infiltrates of lymphocytes and plasma cells were reported (38). In the present study, increased SA level in VPA group may be a protection way of gingival tissue and significantly increased MPO activity is an evidence of damaged gingival tissue by VPA caused inflammation. Oxidative damage, in turn, leads to the generation of oxidized molecules, which are bioactive and induce inflammation. There is a relationship between oxidative damage and inflammation in several stages during the pathogenesis of many diseases. The antioxidant effect of EDA and following decreasing of inflammation may cause the decrease in SA levels and MPO activities in VPA group.

Epilepsy patients are at risk of fracture due to enzyme-inducing antiepileptic drugs. These drugs alter the metabolism and cause osteopenia and osteomalacia by leading clearance of vitamin D (29). Anticonvulsant drugs cause changes in bone metabolism and calcium levels and these drugs may lead to a decrease in bone mass (39). In previous studies increased ALP activities were reported in serum and different tissues (40,41). Consistent with these results, unsignificantly increased ALP activity was found in VPA group compared to controls. ALP, which is an important enzyme for calcification and altered activities can be useful to detect inflammation. Edaravone administration reduced ALP activity in VPA group in this study. As VPA has been shown to alter bone and calcium metabolism, the patients using VPA may have special dental needs during treatment.

ACP is an enzyme which is widely distributed in tissues. In many diseases, especially prostate cancer, diseases of bone, diseases of blood increased levels of ACP is found to be elevated (42). In the present study, significantly increased ACP activity was found in VPA group compared to controls. Applying of edaravone was effective in decreasing gingival ACP activity in the VPA group.

Na^+/K^+-ATPase is a membrane bound enzyme and has been identified in various human tissues (43). The Na^+/K^+-ATPase, also known as the Na^+/K^+ pump, converts the free energy of ATP into transmembrane ion gradients. Also, it is a signal transducer that modulates cell energy-transducing ion pump on the plasma membrane protein. It does not only maintains the membrane potential of excitable neuron and different cells but also is involved in reabsorption of Na^+ in the kidney (44) and in salivary glands (45). The interaction between the inducing drug and the fibroblastic activity is influenced by the inflammatory changes within the gingival tissues. Increasing gingival fibroblasts DNA synthesis may involve stimulation of Na^+/K^+-ATPase activity including phosphorylation of Na^+ channels. This may be the reason of the significant increase of Na^+/K^+-ATPase activity found in VPA group compared to controls in the present study. Na^+/K^+-ATPase activity was decreased in edaravone applied VPA group compared to VPA. EDA reversed this increase.

TF is a cell membrane component. It is a cellular initiator of coagulation and many tissues have TF activity (46-50). VPA cause hematological problems and coagulation defects are commonly seen in VPA treatment. VPA administration significantly increased TF activity compared to controls and decrased TF activity in gingival tissue was found in edaravone given VPA group compared to VPA group. Edaravone may be useful in reducing trombosis and coagulation problems.

CONCLUSION

To our knowledge, there are few reports published on the effects of antiepileptic drugs on oral tissues. Dentists should carry out dental treatments to epiletic patients under antiepileptic drug treatment. Increased oxidant and inflammation marker levels and decreased antioxidant enzyme activities suggest the damage in gingiva caused by VPA treatment. EDA was effective in these parameters and it may be useful to prevent side effects of VPA in gingival tissues. EDA can be added to the new pharmaceutical formulations and may be used to alleviate stomatitis, gingivitis and buccal inflammations in dental clinics. However, further investigations in details are necessary.

Edaravon, valproik asit nedeniyle oluşan dişeti toksisitesini, oksidatif stresi, inflamasyonu ve doku hasarını azaltarak düzeltmektedir

ÖZ

Valproik asit (2-n-propilpentanoik asit, VPA) yaygın olarak kullanılan antiepileptik ilaçtır, çeşitli insan ve hayvan modellerinde oksidatif etkisi, oksidan ve antioksidan dengeyi bozduğunu bildiren bazı araştırmalar vardır. VPA gastrointestinal, nörolojik, hematolojik ve üreme sistemleri üzerinde potansiyel olumsuz etkilere sahiptir. Edaravone (3-metil-1-fenil-2-pirazolin-5-on, EDA) güçlü bir serbest radikal temizleyicidir. Bu çalışmada, VPA ile oluşturulan toksisite edaravon'un dişeti üzerine etkisi araştırıldı. Sprague Dawley dişi sıçanlar rastgele dört gruba ayrıldı: Kontrol grubu, EDA (30 mg / kg / gün) verilen grup, VPA (0.5 g / kg / gün) verilen grup, VPA + EDA (aynı dozda ve zaman) verilen grup. EDA ve VPA yedi gün boyunca intraperitoneal olarak verildi. Deneyin 8. gününde, tüm hayvanlar gece boyunca aç bırakıldı ve daha sonra anestezi altında sakrifiye edildi. Dişeti örnekleri alınarak homojenize edildi. Dişeti homojenatlarında total protein, lipid peroksidasyon (LPO), siyalik asit (SA) ve indirgenmiş glutatyon (GSH) düzeyleri ve katalaz (CAT), glutatyon-S-transferaz (GST), glutatyon peroksidaz (GPx), süperoksit dismutaz (SOD), miyeloperoksidaz (MPO), alkali fosfataz (ALP), asit fosfataz (ACP), sodyum, potasyum ATPase (Na^+ / K^+ -ATPaz) ve doku faktörü (TF) aktiviteleri tayin edildi. VPA nın oluşturduğu artışlar MPO (p<0.01), ACP (p<0.01), Na^+/K^+-ATPaz (p<0.05) ve TF (p<0.01) aktiviteleri için istatistiksel olarak anlamlı olup, LPO seviyesi ve ALP aktivitesi için değildi. EDA verilmesi bu anormal artışları anlamlı olarak düzeltmiştir. Sonuç olarak VPA'nın oluşturduğu oksidatif ve inflamatuar dişeti hasarı EDA verilmesi ile geri döndürülmüştür.

Anahtar kelimeler: Dişeti, valproik asit, edaravon, oksidatif stres, inflamasyon

REFERENCES

1. Schachter SC. Currently available antiepileptic drugs. Neurotherapeutics 2007; 4: 4-11.

2. Gina L. Jones GL, Popli GS, Silvia MT. Lacosamide-induced valproic acid toxicity. Pediatr Neurol 2013; 48: 308-10.

3. Cengiz M, Yuksel A, Seven M. The effects of carbamazepine and valproic acid on the erythrocyte glutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children. Pharmacol Res 2000; 41: 423-5.

4. National Institutes of Health. Valproic Acid: MedlinePlus Drug Information. Last Accessed Nov 29 2012 http://www.nlm.nih.gov/medlineplus/druginfo/meds/ a682412.html; 2012.

5. Verrotti A, Scardapane A, Franzoni E, Manco R, Chiarelli F. Increased oxidative stress in epileptic children treated with valproic acid. Epilepsy Res 2008; 78: 171-7.

6. Schulpis KH, Lazaropoulou C, Regoutas S, Karikas GA, Margeli A, Tsakiris S, Papaaotiriou I. Valproic acid monotherapy induces DNA oxidative damage. Toxicology 2006; 217: 228-32.

7. Yuksel A, Cengiz M, Seven M, Ulutin T. Changes in the antioxidant system in epileptic children receiving antiepileptic drugs: two-year prospective studies. J Child Neurol 2001; 16: 603-6.

8. Anderson HH, Rapley JW, Williams DR. Gingival overgrowth with valproic acid: a case report. ASDC J Dent Child 1997; 64: 294-7.

9. Pellock JM, Brodie MJ. Felbamate: 1997 update. Epilepsia 1997; 38: 1261-4.

10. Andre LF Costa, Clarissa Lin Yasuda, Wendel Shibasaki, Ana Carla Raphaelli Nahás-Scocate, Claudio Fróes de Freitas, Paulo Eduardo Guedes Carvalho, Fernando Cendes. The association between periodontal disease and seizure severity in refractory epilepsy patients. Seizure 2014; 23: 227-30.

11. Kikuchi K, Tancharoen S, Takeshige N, Yoshitomi M, Morioka M, Murai Y, Tanaka E. The efficacy of edaravone (Radicut), a free radical scavenger, for cardiovascular disease. Int J Mol Sci 2013; 14: 13909-30.

12. Oktay S, Alev B, Tunali T, Emekli-Alturfan E, Tunali-Akbay T, Koc-Ozturk L, Yanardag R, Yarat A. Edaravone ameliorates the adverse effects of valproic acid toxicity in small intestine. Hum Exp Toxicol 2015; 34: 654-61.

13. Emekli-Alturfan E, Alev B, Tunali S, Oktay S, Tunali-Akbay T, Koc-Ozturk L, Yanardag R, Yarat A. Effects of edaravone on cardiac damage in valproic acid induced toxicity. Ann Clin Lab Sci 2015; 45: 166-72.

14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.

15. Yagi K. Assay for blood plasma or serum. Methods Enzymol 1984; 105: 328-37.

16. Beutler E. Glutathione in red cell metabolism. In: A Manual of Biochemical Methods. Editor: Beutler E. Grune and Strattion, New York. 1975, pp 112-114.

17. Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte SOD activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharm 1986; 82: 512-20.

18. Habig WH, Jacoby WB. Assays for differentation of glutathione-S-transferases. Method Enzymol 1981; 77: 398-405.

19. Aebi H. Catalase in vitro. In: Methods of enzymatic analysis. Editor: Bergmeye HU. Verlag Chemie, 1974, pp:121-61974.

20. Paglia DE, Valentine WN. Studies on the quantitative and quantitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-68.

21. Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem 1959; 234: 1971-75.

22. Wei H, Frenkel K. In vivo formation of oxidized DNA bases in tumor promoter- treated mouse skin. Cancer Res 1991; 51: 4443-9.

23. Walter K, Schült C. Acid and alkaline phosphatase in serum (two point method). In Methods of Enzymatic Analysis. Editor: Bergmeyer HU Academic Press, Inc. New York and London. 1974, pp 856-886.

24. Ridderstap AS, Bonting SL. Na^+-K^+-activated ATPase and exocrine pancreatic secretion in vitro. Am J Physiol 1969; 217: 1721-7.

25. Ingram GIC, Hills M. Reference method for the one stage prothrombin time test on human blood. Thromb Haemostasis 1976; 36: 237-8.

26. Joshipura V. Sodium valproate induced gingival enlargement with pre-existing chronic periodontitis. J Indian Soc Periodontol 2012; 16: 278-81.

27. Dhalkari CD, Ganatra PV. Sodium valproate induced gingival enlargement in 22 months old child. J Indian Soc Periodontol 2014; 18: 644-7.

28. Galas-Zgorzalewicz B, Borysewicz-Lewicka M, Zgorzalewicz M, Borowicz-Andrzejewska E. The effect of chronic carbamazepine, valproic acid and phenytoin medication on the periodontal condition of epileptic children and adolescents. Funct Neurol 1996; 11: 187-93.

29. Gurbuz T, Tan H. Oral health status in epileptic children. Pediatr Int 2010; l52: 279-83.

30. Hamed SA, Abdellah MM, El-Melegy N. Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients. J Pharmacol Sci 2004; 96: 465-73.

31. Liu CS, Wu HM, Kao SH, Wei YH. Serum trace elements, glutathione, copper/zinc superoxide dismutase, and lipid peroxidation in epileptic patients with phenytoin or carbamazepine monotherapy. Clin Neuropharmacol 1998; 21: 62-4.

32. Pippenger CE, Meng X, Von Lente F, Rothner AD. Valproate therapy depresses GSH-Px and superoxide dismutase enzyme activity. A possible mechanism for VPA induced idiosyncratic drug toxicity. Clin Chem 1989; 35: 1173.

33. Cotariu DS, Evans JL, Marcus ZO. Early changes in hepatic redox homeostasis following treatment with a single dose of valproic acid. Biochem Pharmacol 1990; 40: 589-93.

34. Chaudhary S, Parvez S. An in vitro approach to assess the neurotoxicity of valproic acid-induced oxidative stress in cerebellum and cerebral cortex of young rats. Neuroscience 2012; 225: 258-68.

35. Raza M, Al-Bekairi AM, Ageel AM, Qureshi S. Biochemical basis of sodium valproate hepatotoxicity and renal tubular disorders: Time dependence of peroxidative injury. Pharmacol Res 1997; 35: 153-7.

36. Tong V, Teng XW, Chang TKH, Abbott FS. Valproic acid I: Time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. Toxicol Sci 2005; 86: 427-35.

37. Schauer R, Kelm S, Reuter G, Roggentin P, Shaw L. Biochemistry and role of sialic acid. In: Biology of sialic acids. Editor: Rsebberg A. Plenum Publishing Corp. New York, 1995.

38. Van der Wall EE, Tuinzing DB, Hes J. Gingival hyperplasia induced by nifedipine, an arterial vasodilating drug. Oral Surg Oral Med Oral Pathol 1985; 60: 38-40.

39. Oner N, Kaya M, Karasalihoglu S, Karaca H, Celtik C,

Tutunculer F. Bone mineral metabolism changes in epileptic children receiving valproic acid. J Paediatr Child H 2004; 40: 470-3.

40. Voudris K, Moustaki M, Zeis PM, Dimou S, Vagiakou E, Tsagris B, Skardoutsou A. Alkaline phosphatase and its isoenzyme activity for the evaluation of bone metabolism in children receiving anticonvulsant monotherapy. Seizure 2000; 11: 377-80.

41. Giray T, Vitrinel A, Comert S, Deniz NC, Erdag GC, Kesler E, Sadıkoglu S, Akın Y. The evaluation of the effects of antiepileptic drugs on bone metabolism. Turk Pediatri Ars 2005; 40: 221-6.

42. Muniyan S, Chaturvedi NK, Dwyer JG, LaGrange CA, Chaney WG, Lin MF. Human prostatic acid phosphatase: structure, function and regulation. Int J Mol Sci 2013; 14: 10438-64.

43. Schwinger RHG, Bundgaard H, Muller-Ehmsen J, Kjeldsen K. The Na, K-ATPase in the failing human heart. Cardiovasc Res 2003; 57: 913-20.

44. Jorgensen PL. Mechanism of Na^+ K^+ pump, protein structure and conformations of the pure Na^+K^+-ATPase. BBA-Rev Biomembranes 1982; 694: 27-68.

45. Kurihara K, Hosoi K, Kodama A, Uhea T. A new electrophoretic variant of alpha subunit of Na^+/K^+-ATPase from the submandibular gland of rats. BBA-Rev Biomembranes 1990; 1039: 234-40.

46. Yarat A, Tunali T, Pişiriciler R, Akyüz S, İpbuker A, Emekli N. Salivary thromboplastic activity in diabetics and healthy controls. Clin Oral Investig 2004; 1: 36-9.

47. Emekli - Alturfan E, Kasici E, Yarat A: Effects of oleic acid on tissue factor activity, blood lipids, antioxidant and oxidant parameters of streptozotocin induced diabetic rats fed a high-cholesterol diet. Med Chem Res 2010; 19: 1011-24.

48. Tunali Akbay T, Guvercin M, Gonul O, Yarat A, Akyuz S, Pisiriciler R, Göker K: Salivary thromboplastic activity may ındicate wound healing ın tooth extraction. Balk J Stom 2010; 14: 141-4.

49. Aktop S, Emekli-Alturfan E, Ozer C, Gonul O, Garip H, Yarat A, Goker K. Effects of ankaferd blood stopper and celox on the tissue factor activities of warfarin-treated rats. Clin Appl Thromb Hemost 2014; 20: 16-21.

50. Mackman N.Tissue-specific hemostasis in mice. Arterioscl Throm Vas 2005; 25: 2273-81.