

ANTIEPILEPTIC DRUGS AFFECT PROTEIN, LIPID AND DNA OXIDATIVE DAMAGE AND ANTIOXIDANT DEFENSE IN PATIENTS WITH EPILEPSYUTICAJ ANTIEPILEPTIČNIH LEKOVA NA OKSIDATIVNO OŠTEĆENJE
PROTEINA, LIPIDA I DNK I ANTIOKSIDATIVNU ZAŠTITU KOD BOLESNIKA SA EPILEPSIJOM

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Summary

Background: To get more insight into the effects of the most widely used antiepileptic drugs (AEDs) on the prooxidant/antioxidant balance in epilepsy, a comparative analysis of the byproducts of oxidative damage and antioxidant defense mechanisms was performed in patients with epilepsy treated with lamotrigine, carbamazepine and valproic acid.

Methods: Byproducts of oxidative damage to proteins (reactive carbonyl derivatives, RCD and protein thiol groups, P-SH), lipids (urinary isoprostanes, 8-epi-PGF_{2α}) and DNA (urinary 8-hydroxy-2'-deoxyguanosine, 8-OHdG), as well as the activities of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured in 60 patients with newly diagnosed seizure (at illness onset and after 6 months of treatment with lamotrigine, carbamazepine or valproic acid) and in 20 healthy controls.

Results: In patients with epilepsy, RCD, urinary 8-epi-PGF_{2α} and 8-OHdG, together with SOD and GPX activities were significantly increased, while P-SH were only slightly decreased. After 6 months of treatment with AEDs, a decrease was observed in RCD, urinary 8-epi-PGF_{2α} and 8-

Kratak sadržaj

Uvod: U pokušaju da se razjasne mehanizmi kojima najčešće korišćeni antiepileptički lekovi (AEL) utiču na ravnotežu između pro- i antioksidanasa u epilepsiji, u ovom radu je izvedena uporedna analiza pokazatelja oksidativnog oštećenja i antioksidantnih mehanizama zaštite kod bolesnika sa epilepsijom na terapiji lamotriginom, karbamazepinom ili valproičnom kiselinom.

Metode: Pokazatelji oksidativnog oštećenja proteina (sadržaj karbonilnih grupa, RCD i koncentracija proteinskih tiol grupa plazme, P-SH), lipida (urinarni izoprostani, 8-epi-PGF_{2α}) i DNK (urinarni deoksiguanozin, 8-OHdG), kao i aktivnosti antioksidantnih enzima, superoksid dizmutaze (SOD) i glutathion peroksidaze (GPX) određivani su kod 60 bolesnika sa novodijagnostikovanom epilepsijom (nakon prvog napada i posle 6 meseci, na terapiji lamotriginom, karbamazepinom ili valproičnom kiselinom) i kod 20 zdravih osoba.

Rezultati: Kod bolesnika sa epilepsijom, koncentracija RCD, 8-epi-PGF_{2α} i 8-OHdG, kao i aktivnosti SOD i GPX su značajno povećane, dok je koncentracija P-SH umereno smanjena. Nakon šestomesečne terapije AEL, uočeno je

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List of abbreviations: AEDs – antiepileptic drugs, 8-epi-PGF_{2α} – urinary isoprostanes, GPX – glutathione peroxidase, ILAE – International League Against Epilepsy, 8-OHdG – 8-hydroxy-2'-deoxyguanosine, P-SH – protein thiol groups, RCD – reactive carbonyl derivatives, ROS – reactive oxygen species, SOD – superoxide dismutase.

OHdG to values slightly higher or similar to the control, while P-SH remained unchanged. A decrease was also observed in SOD and GPX activities, although they remained significantly increased compared to controls.

Conclusions: The results of this study have shown that treatments with lamotrigine, carbamazepine and valproic acid affect the prooxidant/antioxidant balance in patients with epilepsy.

Keywords: antiepileptic drugs, antioxidant activity, epilepsy, oxidative damage, oxidative stress

Introduction

The important role of oxidative stress and reactive oxygen species (ROS) in seizure disorders has emerged in the last decade (1–4). Still, the question of whether oxidative stress is a cause or a consequence of seizure remains unanswered. Thus, it has been reported that increased generation of free radicals or reduced activity of antioxidative defense mechanisms can cause some forms of epilepsy and, in addition, increases the risk of seizure recurrence (2, 5–7). On the other hand, there are several experimental and clinical studies showing that seizure results in free radical production and oxidative damage to proteins, lipids and DNA (4, 8, 9).

The prooxidant/antioxidant balance in epilepsy is not only modulated by seizures per se, but also by antiepileptic drugs (AEDs) (10–13). Namely, AEDs are metabolized to generate reactive metabolites with the capability of covalent binding to macromolecules, such as proteins or other vital biomolecules, and hence eliciting systemic toxicity (2, 14). Furthermore, it has been shown that long-term use of AEDs, such as carbamazepine, phenobarbital or valproic acid, increases free radical formation and causes oxidative damage within neuronal cells (6, 15, 16). However, it remains unclear whether the prooxidant challenge of AEDs is associated with significant adverse effects on the antioxidant defense system, since major antioxidant enzyme activities, superoxide dismutase (SOD) and glutathione peroxidase (GPX), have been reported to be increased, unchanged or decreased (1, 2, 7, 14).

According to AEDs ranking from 2007, carbamazepine, lamotrigine and valproic acid are among the most commonly prescribed antiepileptic drugs in epilepsy (13, 17). These antiepileptic drugs are considered the first-line drugs, meaning the most effective AED for certain types of seizure (18–20). As other conventional anticonvulsants, they reduce neuronal excitability through effects on ion channels and synaptic function (21). Studies on the effect of AEDs on prooxidative/antioxidant balance in patients with epilepsy have, so far, mainly focused on valproic acid and carbamazepine treatments and their effect on the lipid peroxidation process and antioxidant defense mechanisms (1, 11, 22, 23). Interestingly, the extent

of protein oxidative damage has not been evaluated, while DNA oxidative damage has only been examined in children receiving valproic acid therapy and recently, in one study, on adult patients receiving valproic acid, carbamazepine and levetiracetam (16, 24). Furthermore, data on lamotrigine effects on oxidative stress in patients with epilepsy are scarce.

Zaključak: Terapija lamotriginom, karbamazepinom i valproičnom kiselinom utiče na ravnotežu između prooksidanasa i antioksidanasa kod bolesnika sa epilepsijom.

Ključne reči: antiepileptični lekovi, antioksidativna aktivnost, epilepsija, oksidativno oštećenje, oksidativni stres

In order to get more insight into the effects of the most widely used AEDs on prooxidant/antioxidant balance in epilepsy, in this study we performed a comparative analysis of the byproducts of oxidative damage and antioxidant defense mechanisms in adult patients with epilepsy treated with lamotrigine, carbamazepine and valproic acid.

Materials and Methods

Selection of study participants

We enrolled 60 patients with newly diagnosed epilepsy. The initial diagnostic procedures included physical examination, electroencephalography and neuroimaging (CT scan and MRI), performed in all 60 patients, within first 24 hours after seizure. The subjects were 29 (48%) women and 31 (52%) men, aged between 20 and 46 years (33.02 ± 13.46). Diagnosis of epilepsy was confirmed and limited to focal or generalized tonic-clonic seizures, according to the ILAE classification (25). Out of the total number of patients, focal seizure (motor or complex) occurred in 27 patients (45%), while 33 patients (55%) had primary generalized tonic-clonic seizures. None of the patients had series of seizures or status epilepticus. Exclusion criteria for the study group were abnormal neurological examination, abnormal cerebral CT scan and MRI, psychiatric or progressive neurologic disorders, thyroid diseases and other endocrinopathies, liver, heart or kidney diseases, or diseases that could influence the level of oxidative stress, such as diabetes mellitus, arterial hypertension and malignancies. In addition, smokers and alcohol or narcotic abusers were excluded.

Based on the type of seizure and AED prescribed according to ILAE Treatment Guidelines (26), patients were divided into 3 groups: 20 patients receiving lamotrigine (200 mg/day), 20 patients receiving

carbamazepine (800 mg/day in 18 pts and 1200 mg/day in 2 pts) and 20 patients receiving valproic acid (1000 mg/day). Plasma and urine for biochemical analyses were obtained from all 60 patients after the first seizure and after a seizure-free 6-month period on appropriate AED. The control group consisted of 20 non-epileptic, otherwise healthy subjects, matched for sex and age. None of the patients or controls received any antiepileptic drugs or antioxidant supplementation and there were no dietary differences between these two groups. All subjects gave written informed consent to participate in the study. The study was approved by the ethics committee of the University of Belgrade School of Medicine and the research was carried out in compliance with the Declaration of Helsinki.

Plasma and urine sampling and analytical procedure

Peripheral venous blood (5 mL) for analysis was collected in tubes over trace element-free heparin immediately after admission to the Emergency Unit and a after 6-month period on appropriate AED. Plasma was separated at 3000 rpm at 4 °C during 15 min. The supernatant was collected, aliquoted and stored at -80 °C for enzyme measurement. Determination of carbonyl and protein-thiol groups was performed immediately after blood collection and plasma separation. Presence of hemolysis was followed by measurement of plasma hemoglobin, and all patients with hemoglobin concentration >50 mg/L were excluded from the study. Routine biochemical profiles were measured using an auto-analyser.

For 8-epi-prostaglandin $F_{2\alpha}$ and 8-hydroxy-2'-deoxyguanosine measurement, urine was collected from all patients and controls. Samples of 20 mL were centrifuged, aliquoted and stored at -80°C. Butylated hydroxytoluene was added to prevent oxidation during processing.

Laboratory methods

Determination of protein reactive carbonyl derivatives in plasma. Plasma protein reactive carbonyl derivatives (RCD) were determined using the method of Levine et al. (27) and expressed as $\mu\text{mol/g}$ proteins. Protein concentration was determined by the method of Lowry et al. (28).

Determination of plasma thiol groups. The amount of plasma thiol (P-SH) groups was determined according to the method of Jocelyn (29) and expressed as μmol per gram of proteins ($\mu\text{mol/g}$ prot).

Measurement of urinary 8-epi-prostaglandin $F_{2\alpha}$. Urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-Epi-PGF $_{2\alpha}$) was determined by enzyme immunoassay (Bioxytech

Urinary 8-Epi-prostaglandin $F_{2\alpha}$ kit; Oxis Research, Portland, OH, USA). The results were standardized against urinary creatinine concentrations and expressed in ng/mg creatinine.

Measurement of urinary 8-hydroxy-2'-deoxyguanosine. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was determined by enzyme immunoassay (Bioxytech 8-OHdG-EIA kit; Oxis Research, Portland, OH, USA). The results were standardized against urinary creatinine concentrations and expressed in ng/mg creatinine.

Enzyme assays. Cu, Zn SOD activity in the plasma was measured by the method of Misra and Fridovich (30). SOD activity was determined using the calibrating curve generated by the use of standard solutions of purified SOD.

GPX activity was determined by the coupled assay procedure of Gunzler et al. (31). One unit of enzyme activity is reported as μmol NADPH oxidized per minute, assuming $6.22 \times 10^3/\text{L/mol/cm}$ to be the molar absorbance of NADPH at 340 nm.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 10.0; SPSS Inc, Chicago, IL, USA) and Microcal Origin software (version 5.0). For each variable, values were expressed as median and confidence interval. The differences between examined parameters, at the onset of epilepsy and after 6 months of treatment with lamotrigine, carbamazepine or valproic acid, were calculated using the Wilcoxon's test for paired data. A p value <0.05 was considered statistically significant.

Results

General characteristics of the study population

The study was performed in 60 newly diagnosed patients with epilepsy, on appropriate AEDs treatment. Out of the total number of patients, focal seizure was observed in 27 (45%), while 33 patients (55%) had generalized tonic-clonic seizures. Patients were divided into 3 groups of 20, depending on the AED they received: lamotrigine, carbamazepine or valproic acid.

Effect of AED on byproducts of oxidative protein damage in plasma

The data on the influence of lamotrigine, carbamazepine and valproic acid on protein oxidative modifications in patients with epilepsy are presented in Table I. RCD levels were significantly increased in patients at the onset of epilepsy ($p = 0.006$, $p =$

Table I Byproducts of oxidative damage in controls and patients with epilepsy on different antiepileptic drugs.

Controls (n=20)		Lamotrigine (n=20)		Carbamazepine (n=20)		Valproic acid (n=20)	
		First seizure	After 6 months	First seizure	After 6 months	First seizure	After 6 months
RCD ($\mu\text{mol/g}$ protein)	0.505*	0.702	0.642	0.827	0.631	0.711	0.478
%	100	139**	92***	164	76	141	67
<i>p</i>		0.006 a	NS b	0.001	0.023	0.001	0.005
P-SH/P (mmol/g proteins)	7.07	6.12	5.71	6.16	6.42	5.91	6.19
%	100	87	93	87	104	84	105
<i>p</i>		NS	NS	NS	NS	NS	NS
8-Epi-PGF _{2α} (ng/mg creatinine)	0.60	1.81	0.53	1.32	0.53	1.27	0.49
%	100	302	29	220	40	212	39
<i>p</i>		0.001	0.001	0.001	0.001	0.001	0.001
8-OHdG (ng/mg creatinine)	6.71	20.7	7.68	14.7	5.31	19.5	4.54
%	100	309	37	219	36	291	23
<i>p</i>		0.001	0.001	0.001	0.001	0.001	0.001

* data are expressed as median

** percentage of change when compared to controls

*** percentage of change when compared to illness onset

^a statistical significance when compared to controls

^b statistical significance when compared to illness onset

RCD – protein reactive carbonyl derivatives

P-SH/P – protein thiol groups per protein concentration

8-Epi-PGF_{2 α} – 8-epi-prostaglandin F_{2 α}

8-OHdG – 8-hydroxy-2'-deoxyguanosine

NS – no statistical significance

0.001 and $p = 0.001$) in comparison to healthy controls. In patients receiving lamotrigine, RCD levels only slightly decreased after 6 months of treatment ($p > 0.05$), while in patients on carbamazepine or valproic acid monotherapy there was a significant decrease ($p = 0.023$ and $p = 0.005$, respectively) (Table I, Figure 1A). Only in case of valproic acid RCD levels reached control values (Figure 1A).

Plasma protein thiol groups were only slightly decreased at the onset of epilepsy (Table I). As shown in Figure 1B, independently of the type of antiepileptic drug, P-SH content remained unchanged 6 months after treatment ($p > 0.05$) (Figure 1B).

Effect of AED on byproducts of lipid oxidative damage

The extent of lipid oxidative damage was determined by measurement of urinary 8-epi-PGF_{2 α} , a group of bioactive prostaglandin F₂-like compounds that are generated by oxidatively catalysed reaction of arachidonic acid. Data on the effect of lamotrigine, carbamazepine and valproic acid on urinary 8-epi-

PGF_{2 α} excretion in patients with epilepsy and healthy controls are presented in Table I. As shown, urinary 8-epi-PGF_{2 α} excretion was significantly, more than 2-fold, increased in patients with epilepsy at the onset of illness ($p = 0.001$). Independently of AED used (lamotrigine, carbamazepine or valproic acid), after 6 months of treatment, a significant decrease ($p = 0.001$) was observed in urinary isoprostanes excretion, to values similar to controls (Table I, Figure 1C).

Effect of AED on byproducts of oxidative damage to DNA

The data on the effect of antiepileptic drugs on urinary excretion of 8-OHdG, a product of oxidatively modified DNA base guanine and reliable marker of oxidative damage to DNA, are presented in Table I. As shown, urinary excretion of 8-OHdG was significantly increased ($p = 0.001$) in patients with epilepsy at illness onset when compared to controls. After 6 months of treatment with either lamotrigine, carbamazepine or valproic acid, 8-OHdG excretion significantly decreased ($p = 0.001$) to values slightly different from those in the control group (Table I, Figure 1D).

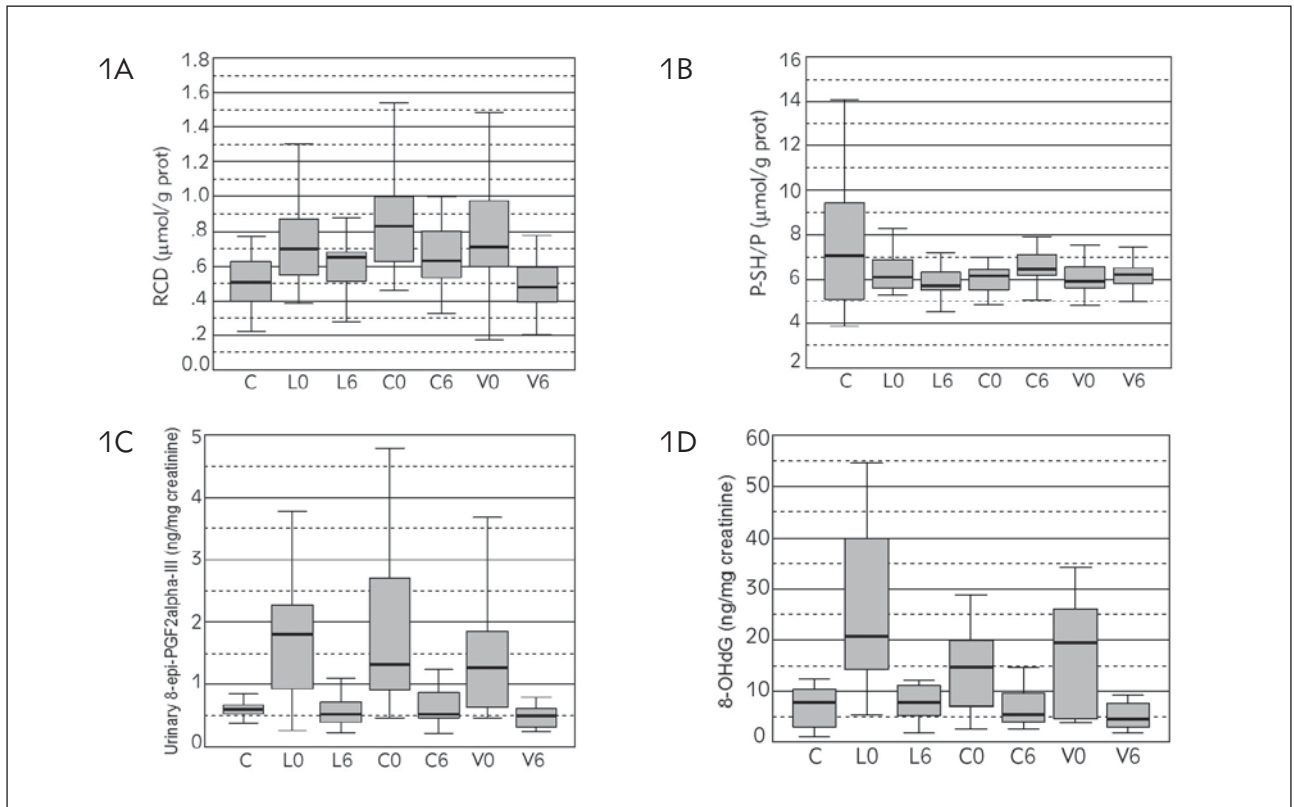


Figure 1 Protein, lipid and DNA oxidative damage in patients with epilepsy on different AEDs. A. Protein reactive carbonyl derivatives (RCD) in patients with epilepsy on different AEDs; B. Protein thiol groups (P-SH/P) in patients with epilepsy on different antiepileptic drugs; C. Urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF $_{2\alpha}$) in patients with epilepsy on different AEDs; D. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) in patients with epilepsy on different AEDs.

L0 – at illness onset in patients who will receive lamotrigine; L6 – after 6 months of lamotrigine monotherapy; C0 – at illness onset in patients who will receive carbamazepine; C6 – after 6 months of carbamazepine monotherapy; V0 – at illness onset in patients who will receive valproic acid; V6 – after 6 months of valproic acid monotherapy.

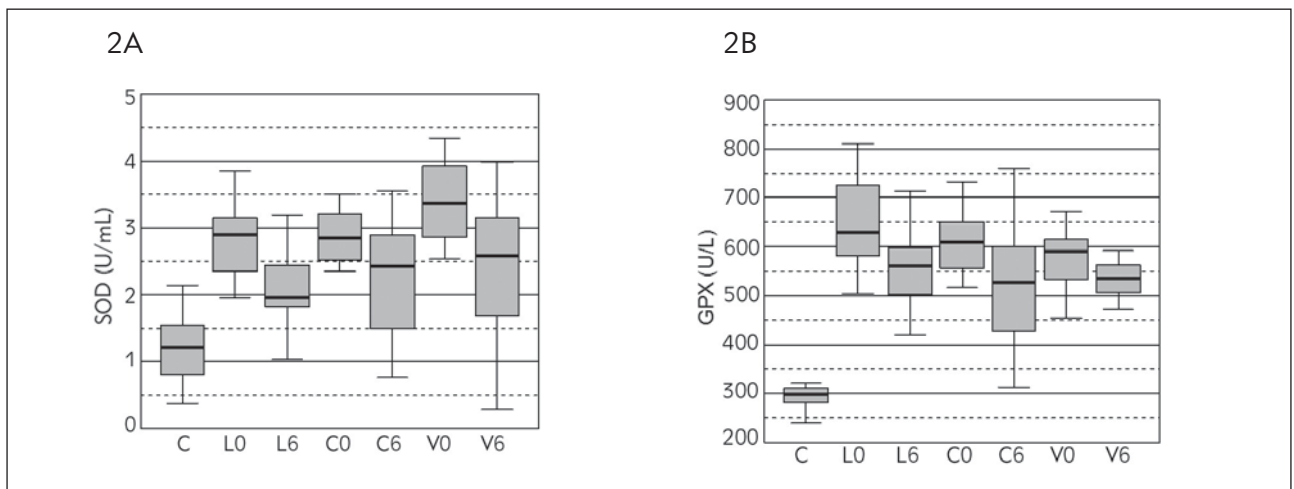


Figure 2 Superoxide dismutase and glutathione peroxidase activity in patients with epilepsy on different AEDs. A. Superoxide dismutase (SOD) activity in patients with epilepsy on different AEDs; B. Glutathione peroxidase (GPX) activity in patients with epilepsy on different AEDs.

L0 – at illness onset in patients who will receive lamotrigine; L6 – after 6 months of lamotrigine monotherapy; C0 – at illness onset in patients who will receive carbamazepine; C6 – after 6 months of carbamazepine monotherapy; V0 – at illness onset in patients who will receive valproic acid; V6 – after 6 months of valproic acid monotherapy.

Table II Antioxidant enzyme activities in the plasma of controls and patients with epilepsy on different antiepileptic drugs.

Controls (n=20)		Lamotrigine (n=20)		Carbamazepine (n=20)		Valproic acid (n=20)	
		First seizure	After 6 months	First seizure	After 6 months	First seizure	After 6 months
SOD (U×10 ³ /L)	1.21*	2.89	1.96	2.84	2.42	3.37	2.58
%	100	239**	68	235	85	279	77
p		0.001 ^a	0.001 ^b	0.001	0.044	0.001	0.026
GPX (U/L)	298	629	561	608	526	590	536
%	100	211	89	204	87	198	90
p		0.001	0.045	0.001	0.039	0.001	0.048

* data are expressed as median

** percentage of change when compared to controls

*** percentage of change when compared to illness onset

^a statistical significance in comparison to controls

^b statistical significance when compared to illness onset

SOD – superoxide dismutase

GPX – glutathione peroxidase

Effect of AED on plasma antioxidant enzyme activities

Table II summarizes the data on major antioxidant enzyme activities in controls and patients with epilepsy at illness onset and after 6 months of treatment with lamotrigine, carbamazepine and valproic acid.

Superoxide dismutase activity was significantly increased in patients with epilepsy at illness onset in comparison to controls ($p = 0.001$). In all three groups of patients, on lamotrigine, carbamazepine or valproic acid monotherapy, a significant decrease in SOD activity ($p = 0.001$, $p = 0.044$ and $p = 0.026$, respectively) was observed (Table II). Still, SOD activity remained increased compared to controls, independently of AED applied ($p=0.001$) (Figure 2A).

Glutathione peroxidase activity was also significantly increased in patients at the onset of epilepsy when compared to controls ($p = 0.001$). Glutathione peroxidase activity was decreased in patients receiving either lamotrigine, carbamazepine or valproic acid ($p=0.045$, $p=0.039$ and $p=0.048$, respectively) (Table II). Although GPX activity decreased from illness onset, similarly to SOD, after 6 months of treatment with AEDs (lamotrigine, carbamazepine or valproic acid) the activity of GPX was still nearly 2-fold higher than in the control group ($p=0.001$) (Figure 2B).

Discussion

Our study has shown that treatments with carbamazepine and valproic acid positively affect the prooxidant/antioxidant balance in patients with epilepsy, while lamotrigine showed a weaker effect in decreasing the oxidative damage of plasma proteins. Still, decreased plasma RCD content and slightly increased levels of protein thiol groups, together with a significant decrease in 8-epi-PGF_{2α} and 8-OHdG urinary excretion, indicate a decrease in protein, lipid

and DNA oxidative modifications in patients receiving all three AEDs. Activities of SOD and GPX in plasma of these patients remain significantly higher than in healthy controls after a seizure free 6-month treatment with antiepileptic drugs.

In recent years, important advances have been made in the diagnosis and treatment of seizure disorders. It has been shown that, apart from other known mechanisms in epileptogenesis, oxidative stress and generation of reactive oxygen species (ROS) are implicated in seizure disorders (3, 4). Namely, it has been shown that seizure results in increased protein, lipid and DNA oxidative modifications, followed by increased activity of major antioxidant enzymes (4). Furthermore, Devi et al. (12) have indicated the ability of antioxidants to reduce the seizure manifestations and the accompanying biochemical changes (i.e., markers of oxidative stress), further supporting the role of free radicals in seizures and highlighting a possible role of antioxidants as an adjunct to antiepileptic drugs for better seizure control. Recently, a possible association between high levels of oxidative stress and valproic acid has been suggested, possibly as a consequence of its biotransformation or deficiency of antioxidant defense systems (7). Hamed et al. (2) suggested that carbamazepine is a better anticonvulsant for the control of free radical related seizures when compared to valproic acid. Interestingly, there are not enough data on the effect of antiepileptic drugs on oxidative modifications of proteins, lipids and DNA, as well as antioxidant enzyme activities.

Although oxidative damage to proteins is one of the major mechanisms underlying neuronal cell damage (32) and the consequences may be impaired enzymatic activity and modified membrane and cellular function depending on the nature of the vulnerable protein component and the attacking radical species (33), there are no data on the effect of AEDs on protein oxidative damage.

The presence of free radical-initiated reactions of side chains of amino-acid residues is indicated by the DNPH-reactive carbonyl group of proteins (34). In accordance with previous studies, significant increase in the DNPH activity of plasma proteins has been shown in patients with epilepsy at illness onset (4). Six months of AEDs treatment resulted in a reduction in oxidative protein damage, although various antiepileptic drugs exhibited different effects on RCD content. Namely, while treatment with carbamazepine or valproic acid induced a sound decrease in RCD content, lamotrigine failed to show such a strong effect. The concentration of protein sulphhydryl groups in plasma, another marker of protein oxidative damage, is reduced in patients with epilepsy, but only slightly modified by lamotrigine, carbamazepine or valproic acid treatments. Still, the pattern of changes after AED therapy resembles those in RCD group levels, with carbamazepine and valproic acid showing a more pronounced antioxidant effect in comparison to lamotrigine. Present results on decreased carbonyl content and thiol oxidation appear to prove the first demonstration that decreased oxidative damage of plasma proteins is present in patients with epilepsy receiving carbamazepine and valproic acid.

Apart from protein oxidative damage, lipid peroxidation is one of the most biologically important free radicals reaction. If unopposed with an effective local antioxidative defense system, peroxidative injury to plasma phospholipids may lead to severe cell damage. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage (12). The increased susceptibility of the brain to oxidative damage highlights the importance of understanding the role of oxidative stress in the pathophysiology of seizures, as well as the effect of AEDs on prooxidant/antioxidant balance. 8-epi-prostaglandin $F_{2\alpha}$ (8-Epi-PGF $_{2\alpha}$), a reliable marker of free radical-induced lipid peroxidation, has so far been studied in animal models of epilepsy and in only one study in adult patients with epilepsy, which showed a significant increase in 8-Epi-PGF $_{2\alpha}$ urinary excretion after a first unprovoked seizure (4, 9). Our data on decreased levels of urinary 8-Epi-PGF $_{2\alpha}$ in patients with epilepsy after a 6-month seizure-free treatment with lamotrigine, carbamazepine or valproic acid suggest that oxidative lipid damage, which results from seizure activity and may play an important role in seizure-induced death of vulnerable neurons, is diminished by these AEDs. In contrast to the differential effects of various AEDs on protein oxidation, this strong effect was consistent for all three AEDs tested.

DNA is also a major target of constant oxidative damage from endogenous oxidants. Although numerous defense systems protect cellular macromolecules against oxidation, there is a high rate of damage to DNA. Currently, 8-hydroxydeoxyguanosine, a

product of oxidatively modified DNA base guanine, is being used as a sensitive marker for oxidative DNA damage (35). Namely, 8-OHdG is produced and accumulates at the area of injury, it is secreted into the bloodstream and is finally excreted in the urine (16). So far, it has been shown that urinary excretion of 8-OHdG is increased in adult patients with epilepsy, after a first unprovoked seizure (4). DNA oxidative damage has also been examined in children receiving valproic acid therapy (16). Namely, in their study on the effect of valproic acid monotherapy on oxidative DNA damage, Schulpis et al. (16) have suggested that increased 8-OHdG levels reflect an increased rate of oxidative damage and repair of DNA of both liver and neuronal cells. They even suggested supplementation with an antioxidant agent that may be beneficial, not only for the liver function, but also for the brain cells, passing the blood-brain barrier. Recently, Varoglu et al. (24) have shown that serum 8-hydroxyguanine levels were higher in patients taking valproic acid, carbamazepine and levetiracetam therapy during the first two months in comparison to controls. Our data on decreased levels of 8-OHdG in patients with epilepsy treated with lamotrigine, carbamazepine or valproic acid, after a 6-month seizure-free period, indicate the effect of these AEDs on decreased levels of oxidative DNA damage in brain tissue. The most pronounced effect on decrease in DNA oxidation was observed for valproic acid.

Majority of studies on the association between antiepileptic drugs and oxidative stress have focused on the question of the involvement of antioxidant enzymes. Extracellular SOD represents a major defense system against superoxide, being also a target for oxidative damage (36). Previous studies on SOD activity have mainly been performed in children with epilepsy on different AEDs, but they reported no significant difference in SOD activity between children with epilepsy and controls (1, 10, 37, 38). Recently, Yis et al. (39) have found a positive correlation between SOD activity and duration of valproic acid treatment in children with epilepsy. Results of this study have shown that, after an initial increase after seizure, there is a significant decrease in SOD activity only in patients receiving lamotrigine, while carbamazepine and valproic acid exhibited a less strong effect. Furthermore, after 6 months of treatment with AEDs, the activity of this antioxidant enzyme remains significantly higher than in the control group.

The data on another major antioxidant enzyme, GPX, seem to be conflicting too (1, 3, 40). Extracellular GPX is presumed to work as part of a traditional glutathione cycle (41). The tripeptide glutathione is involved in the disposal of peroxides by brain cells and in the protection against reactive oxygen species, while its content in brain cells depends strongly on the availability of precursors for glutathione (42). *In vitro* extracellular GPX reduces organic hydroperoxides, phospholipid hydroperoxides and hydrogen peroxide

(43). Studies in mice that overexpress extracellular GPX suggest a protective extracellular antioxidant activity for extracellular GPX (44). Although the results of this study have shown a decrease in plasma GPX activity, the effect of all three AEDs is rather weak. Therefore, plasma GPX activity in patients with epilepsy receiving lamotrigine, carbamazepine or valproic acid remains much higher than in controls. These data are in agreement with the findings of Cengiz et al. (1) who suggested that GPX upregulation, in children with epilepsy receiving carbamazepine and valproic acid, might be a consequence of induced GPX synthesis in the liver as a compensatory mechanism for decreased glutathione levels in the same group of patients.

It is important to note that high plasma antioxidant activities observed in patients with epilepsy at various time points in this study (immediately after a seizure and after AED treatment) might be caused by different molecular mechanisms. Namely, the initial increase of SOD or GPX activity after a seizure might be an adaptive phenomenon due to increased systemic oxidative stress or a consequence of leakage of these enzymes from damaged tissues, particularly from muscle cells. On the other hand, the persistent increase in antioxidant enzyme activities 6 months after AED therapy most probably reflects upregulated antioxidant enzyme synthesis. This might be a secondary phenomenon due to enhanced CYP-450 free radical production, at least for carbamazepine since it undergoes CYP-450 metabolism as a substrate (20). Although valproic acid undergoes the same metabolism as carbamazepine, it acts as a CYP-450 inhibitor, contrary to carbamazepine. However, recent results of Dong et al. (45) indicated that valproic acid exerts an antioxidant effect by regulating the expression of eukaryotic translation initiation factor 4A isoform 1. Since lamotrigine undergoes metabolism via glucuronidation, with minimal CYP-450 involvement, the

mechanism by which it induces antioxidant enzymes is still ambiguous.

It remains elusive whether a causative relationship exists between the relatively high antioxidant enzyme activities and the lowering of byproducts of oxidative stress after 6 months of AED treatment. Although the lack of seizure, which per se induces oxidative damage, could explain the lowering of byproducts of oxidative damage, it is tempting to speculate that upregulated antioxidant enzyme activities after AED treatment are at least partially responsible for the normalization of byproducts of oxidative stress. Such an assumption is limited by the lack of an appropriate control group which, in this case, would hypothetically be patients with epilepsy without any therapy during a six-month follow-up or non-epileptic patients on 6-month AED therapy (eg. carbamazepine in trigeminal neuralgia treatment). Further studies in animal models of epilepsy could help elucidate the mechanisms involved in the possible regulation of prooxidant/antioxidant balance by AED.

In conclusion, data on the overall decrease in protein, lipid and DNA oxidative damage to values similar to controls, in a drug-dependant manner, indicate a positive effect of AED on the prooxidant/antioxidant balance in adult patients with epilepsy. Namely, AED mediated seizure control decreases seizure-induced oxidative damage. Besides, upregulated antioxidant enzyme activities after AED treatment are at least partially responsible for the normalization of byproducts of oxidative stress.

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Conflict of Interest Statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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