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Effects of epilepsy and antiepileptic drugs on nitric oxide, lipid peroxidation and xanthine oxidase system in children with idiopathic epilepsy

Ebru Arhan [a,*], Ayse Serdaroglu [a], Bahadır Ozturk [b], H. Serdar Ozturk [c], Aysima Ozcelik [a], Nese Kurt [a], Ebru Kutsal [a], Neriman Sevinc [c]

[a] Gazi University School of Medicine, Department of Pediatric Neurology, İncirli-Ankara, Turkey
[b] Selcuk University School of Medicine, Department of Biochemistry, Turkey
[c] Ankara University School of Medicine, Department of Biochemistry, Turkey

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ABSTRACT

The aim of this study is to investigate the effects of epilepsy, valproic acid and oxcarbazepine on nitric oxide levels, lipid peroxidation and xanthine oxidase levels in newly diagnosed epileptic children and healthy controls. A total of 49 patients with newly diagnosed idiopathic epilepsy and 15 healthy children were enrolled in this study. Of these 49 patients, 16 children were treated with valproate and 16 treated with oxcarbazepine. Nitric oxide, malondialdehyde and xanthine oxidase levels prior to antiepileptic drug therapy were measured in the serum. Blood samples were drawn before antiepileptic drug therapy and after 3 and 6 months of the antiepileptic drug treatment. Nitric oxide levels were statistically higher in the newly diagnosed epileptic patients. In oxcarbazepine group, the nitric oxide and malondialdehyde levels were found to be decreased. No statistically significant differences were noted in nitric oxide, malondialdehyde and xanthine oxidase levels in valproic acid treated group. Oxcarbazepine which is a frequently used new antiepileptic drug in childhood epilepsy may modify nitric oxide levels and lipid peroxidation. These results suggest that decreased lipid peroxidation would play a role in the mechanism of antiepileptic effects by oxcarbazepine treatment.

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1. Introduction

Oxidative stress is characterized by an imbalance between oxidative and antioxidative mechanisms in the living organism. This is either due to accumulation of excessive reactive oxygen species or presence of inadequate antioxidants. Oxidative stress might have some essential role in the etiology of multiple disease processes.[1]

Accumulating evidence supports the hypothesis that epilepsy is mediated by oxidative stress, leading to abnormal structural alterations of cellular proteins, membrane lipids, DNA and RNA.[2–4] The reactive oxygen species arising from molecular oxygen originate from different sources such as mitochondria, xanthine oxidase (XO) system. Superoxide anion radical (O_2^-), a major radical in cellular systems, producer enzyme may increase through the proteolytic conversion of xanthine dehydrogenase to XO and produce enormous amount of O_2^-. Moreover, oxygen radicals interfere with many cellular elements, leading to damage of cellular structure and functions, and the end-point could be cellular death. Lipids are known to be the most sensitive biomolecules to free radical generated damage. Malondialdehyde (MDA) is the end product of lipid peroxidation and can have hazardous effects on nearby and remote tissues.

Nitric oxide (NO), a small diffusible gaseous messenger, synthesized from the amino acid L-arginine by the enzyme NO synthase, appears to play a crucial role in a number of physiological and pathophysiological processes in the brain, including the modulation of neuronal plasticity, cerebral blood-flow, cognitive and behavioral functions, as well as, its involvement in neurological disorders such as ischemia and epilepsy.[5,6] NO synthesis seems to be defective when there is endothelial damage. Endothelial damage is accepted as the hallmark in the etiology of various diseases. The role of NO in epilepsy has been examined in a number of in vivo and in vitro studies, however, the obtained results are still contradictory, reporting both pro- and anti-convulsant properties of NO.[7]

Most of the studies which evaluated the lipid peroxidation and levels of NO, lipid peroxidation and antioxidant status were done in children receiving antiepileptic treatment. Clinical studies evaluating oxidant status in children with epilepsy before drug treatment are rare. While one of these studies found increased lipid peroxidation, others found no change in oxidant markers in newly diagnosed epileptic patients.[8–10]

* Corresponding author. Yunus Emre Cad. Yigitler Sok., No. 9/3, 06010 İncirli-Ankara, Turkey. Tel.: +90 312 3232 995.
E-mail address: petekarhan@yahoo.com.tr (E. Arhan).

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A number of recent reports indicate that many antiepileptic drugs used in children have miscellaneous effects on the oxidative status.[11–13] It was suggested that newer antiepileptic drugs may be less likely to exert adverse effects on oxidant–antioxidant status, but very few reports are in favor of this suggestion.[14] Valproate (VPA) was the first antiepileptic drug that has been investigated in several studies.[8–12,15] Oxcarbazepine (OXC), a keto-derivative of carbamazepine, is a new antiepileptic drug whose structure closely resembles that of carbamazepine.[16] Its advantages over carbamazepine include better tolerability,[17,18] a lower risk of allergic cutaneous reactions, a lower propensity for drug–drug interactions, and fewer metabolic effects. These advantages are attributable to the different biotransformation of OXC. In contrast to carbamazepine, OXC has a different metabolic pathway in the liver. Thus, OXC was suggested as a safe antiepileptic drug since it has less adverse effects.

In the present study, we investigated the effect of epilepsy and antiepileptic drugs (an older antiepileptic drug; VPA and a new antiepileptic drug; OXC) on serum MDA, NO and XO levels in children with newly diagnosed idiopathic epilepsy. We hypothesized that a newer antiepileptic drug, OXC, might show favorable effects in this subset of children who have excessive endothelial damage and reactive oxygen species formation.

2. Material and methods

A total of 49 patients (21 girls and 28 boys) aged 5–12 years, with newly diagnosed idiopathic epilepsy and 15 healthy children of similar age and gender served as controls, were enrolled in this study. Of the 49 patients with newly diagnosed idiopathic epilepsy, 14 had tonic seizures, 8 had absence seizures, 15 had secondary generalized seizures, 3 had simple partial seizures, 6 had complex partial seizures and 3 had atomic seizures. Of these 49 patients, 16 children were treated with valproate and 16 treated with OXC and 17 were treated with different antiepileptic drugs. Patients with symptomatic and syndromic epilepsy, mental motor retardation and underlying a chronic disorder were not included in the study. The patients were not taking previous medication trials prior to OXC use. The patients were not taking other medications at the time of study. There were no acute medical conditions such as infection, physical exertion and trauma at the time of blood drawn.

Descriptions of seizure type followed the criteria of the International League Against Epilepsy.[19] The antiepileptic drug dose used was chosen according to generally accepted guidelines.[20] VPA and OXC were administered twice daily. Control of epilepsy was good in all patients enrolled, and after the first month of treatment, no patients exhibited more than two seizures during treatment.

Blood samples were taken to measure NO, MDA and XO levels prior to antiepileptic drug therapy.

After obtaining the written informed consent, blood samples were drawn before antiepileptic drug therapy and after 3 and 6 months of the antiepileptic drug treatment. Complete blood count and liver function tests were also evaluated at the same time. Blood samples were obtained from the patients in the interictal period. Levels of NO, XO and MDA were measured in fasting blood serum samples of the patients and controls. NO level (μmol/mL) was determined according to the method based on the diazotization of sulfanilic acid by NO at acid pH and subsequent coupling to N-(1-naphthyl-ethylenediamine).[21,22] XO activity (IU/mL) was determined by measuring uric acid formation from xanthine substrate at 293 nm.[21] MDA level (nmol/mL) was measured by the thiobarbituric acid reactive substances method.[23,24]

The study was approved by the local ethic committee of Gazi University School of Medicine. Informed consent was obtained from all the parents.

Statistical analysis was performed using the Statistical Package of Social Science (SPSS), Version 13.0 (SPSS Inc., Chicago, IL). Data were expressed as mean ± standard deviation. The Tukey test was used to analyze the variance among groups. Statistically significant differences obtained from Tukey test analysis were further tested by the Student's *t*-test comparisons between groups. Chi-square test was used to compare the proportions. Repeated measures design was used to evaluate the data of each groups measured at different times. *p*-Values of <0.05 were considered statistically significant.

3. Results

Ages of children in all groups ranged between 3 and 12 years. The mean age of the patients and both average age and its distribution according to sexes did not differ between the groups (Table 1).

Comparison of the NO, MDA and XO levels between control group and newly diagnosed epileptic patients are given in Table 2. NO levels were statistically higher in the newly diagnosed epileptic patients (Table 2, $p < 0.005$). MDA and XO levels were not different between control group and epileptic patients.

The NO, MDA and XO levels of both the control group and epileptic patients before and after 3 and 6 months of VPA and OXC treatment are presented in Table 2. Whereas XO and MDA levels in the control and pretreatment groups were found to be similar, serum NO was found to be increased in the epileptic group.

As for the group receiving VPA treatment, no statistically significant differences were noted in NO, MDA and XO levels in either the third or sixth month of the treatment when compared with the pretreatment levels (Fig. 1a–c). When pretreatment levels were compared with the control group, NO levels were significantly higher in the VPA treated group. NO, MDA and XO levels were similar in both control group and pretreatment values of the VPA group (Table 3).

Table 1
Distribution of groups according to sex and gender.

	Control group (*n*: 15)	VPA treated group (*n*: 16)	OXC treated group (*n*: 16)	*p*-Value
Age (years)	8.80 ± 3.12	9.5 ± 2.46	8.43 ± 3.25	0.71
Sex (M/F)	6/9	5/10	9/7	0.65

Table 2
Mean ± SD values for NO, NOS, XOD and MDA levels of newly diagnosed epileptic children and control groups.

Groups	NO (μmol/mL)	XOD (IU/mL)	MDA (nmol/mL)
Control (*n*: 15)	11.83 ± 3.23	113.00 ± 32.18	2.22 ± 0.46
Newly diagnosed Epilepsy group (*n*: 49)	24.94 ± 15.50	126.76 ± 62.48	2.28 ± 0.84
p-Value	0.001	0.453	0.744

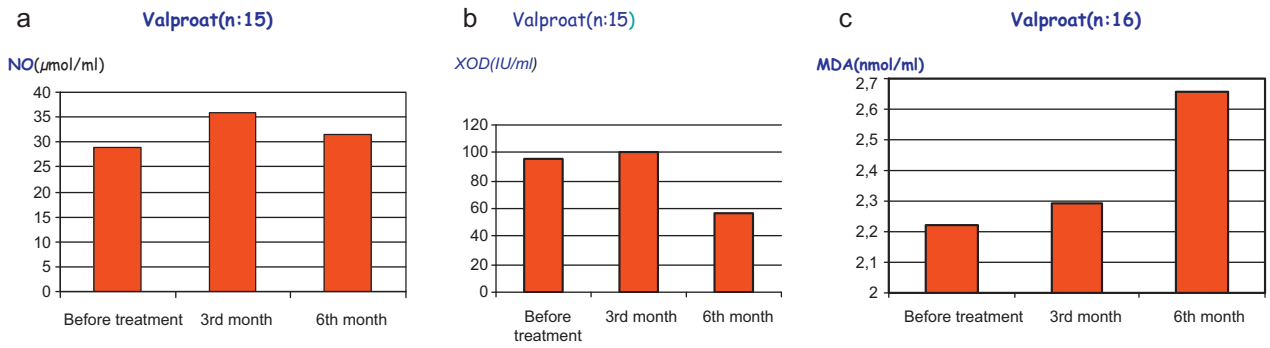


Fig. 1. (a–c) NO, MDA and XOD levels before and after 3 and 6 months of treatment with valproate, respectively.

Table 3
NO, XO and MDA levels in control group and epileptic children before and after 3 and 6 months of treatment.

	Control (*n*: 15)	Valproate group (*n*: 16)			OXC group (*n*: 16)		
		Before treatment (1)	Third month (2)	Sixth month (3)	Before treatment (1)	Third month (2)	Sixth month (3)
NO (μmol/mL)	11.83 ± 3.23	28.93 ± 13.06	35.96 ± 11.98	31.44 ± 8.73	32.83 ± 19.76	19.16 ± 8.04	31.98 ± 11.99
XOD (IU/mL)	113.00 ± 32.18	96.41 ± 35.01	100.10 ± 21.13	57.08 ± 26.98	167.00 ± 73.27	159.15 ± 41.01	161.76 ± 51.3
MDA (nmol/mL)	2.22 ± 0.46	2.22 ± 0.74	2.29 ± 0.91	2.66 ± 0.67	1.91 ± 0.77	1.21 ± 0.22	1.48 ± 0.43
p-Value		NO: 1 vs. 2: 0.464, 1 vs. 3: 0.367, 2 vs. 3: 0.434; XOD: 1 vs. 2: 0.675, 1 vs. 3: 0.197, 2 vs. 3: 0.165; MDA: 1 vs. 2: 0.245, 1 vs. 3: 0.507, 2 vs. 3: 0.476			NO: 1 vs. 2: 0.043, 1 vs. 3: 0.452, 2 vs. 3: 0.016; XOD: 1 vs. 2: 0.342, 1 vs. 3: 0.651, 2 vs. 3: 0.675; MDA: 1 vs. 2: 0.016, 1 vs. 3: 0.107, 2 vs. 3: 0.052		

In the group receiving OXC, the NO levels were found to be decreased in the third month of the treatment compared with those of the pretreatment group. However, this decrease became insignificant in the sixth month of the treatment (Fig. 2a). With regard to the XO levels, no differences were noted in either the third month or sixth month when compared with the pretreatment levels (Fig. 2b). The MDA levels were significantly decreased in the third and sixth month. The third and sixth month levels were similar (Fig. 2c). NO and XO levels were significantly higher in OXC treated group before treatment was initiated in comparison with the control group (Table 3).

When patients with newly diagnosed epilepsy were divided into two groups according to the type of epilepsy (partial or generalized), nitric oxide, XO and MDA levels did not differ between the groups.

The biochemical tests performed on the patients and control group were within normal limits. There was no correlation between the lipid peroxidation status and liver function tests (AST, ALT, GGT).

4. Discussion

Oxidative stress is one of the predisposing factors in childhood epilepsy. In vivo detection of free radicals is difficult because of their short lifetimes and lipid peroxidation markers are used as indicators of oxidative stress in clinical and experimental studies. The brain is more vulnerable to injury by lipid peroxidation products than other tissues and lipid peroxidation is an index of neuronal damage of cell membrane phospholipids.[25] Most of the studies about oxidative stress in epileptic children were done to evaluate the effects of antiepileptic drugs on oxidative status. The number of studies evaluating the role of epilepsy on the oxidative status is scarce. On the other hand, there are only a few studies evaluating the effect of new generation antiepileptic drugs on the oxidative stress in epileptic children.[14]

4.1. Effect of epilepsy on nitric oxide, XO and MDA levels

In the baseline measurement, we evaluated the role of epilepsy on NO, XO and lipid peroxidation.

In our study, the levels of serum NO were found to be significantly high in epileptic children. Although the role of NO in the pathophysiology of epilepsy remains unclear and debatable this observation may suggest that NO induces the neuronal loss and reactive glial proliferation and thus it could be potentially involved in the pathogenesis of epilepsy. Previous experimental studies have reported that inhibition of NO prevents convulsion.[26] Ribeiro et al. showed that experimental methylmalonate-induced

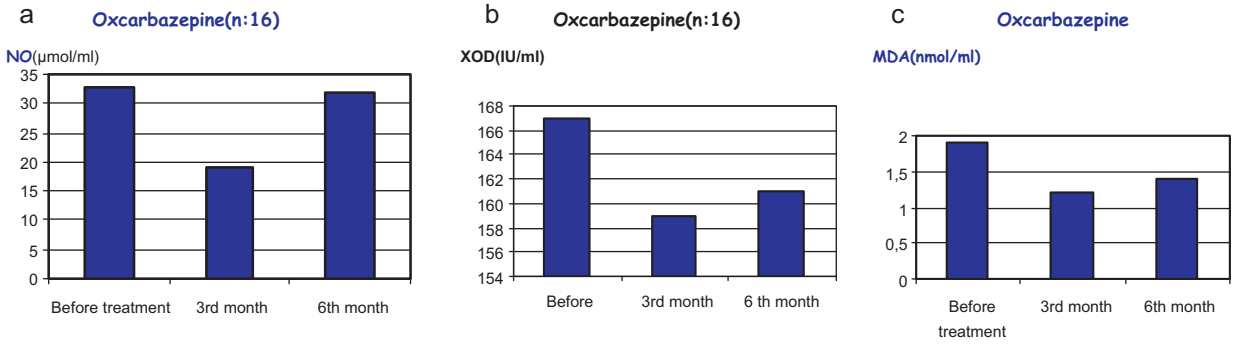


Fig. 2. (a–c) NO, MDA and XOD levels before and after 3 and 6 months of treatment with OXC, respectively.

seizures are alleviated in NO deficient mice.[27] de Vasconcelos et al. reported that NG-nitro-L-arginine increased severity of seizures and L-arginine improved damage of seizures.[28] In addition, in an experimental study, Rundfeldt et al. concluded that two NO inhibitors (NG-nitro-L-arginine and NG-nitro-L-arginine methyl ester) had been as effective as an antiepileptic drug clinically.[29] However, there are also studies claiming the opposite, that is, endogenius NO may play a neuroprotective role.[30] These contradicting findings indicate that the relationship between NO and epilepsy is complex.[31] Consequently, more research will be needed before a final conclusion about the possible contribution of NO to the pathophysiology of epilepsy can be known.

Studies evaluating the effect of epilepsy on lipid peroxidation have conflicting results. Recently, some studies observed an increase in lipid peroxidation in the brain of animals probably caused by recurrent seizures.[32,33] Increased lipid peroxidation of brain tissue has been reported in ferric chloride and kainate-induced epilepsy in rats.[4,34] In contrast with these studies, our results showed no difference in lipid peroxidation between epileptic patients and healthy control subjects. Similarly, Michoulas et al. found no difference in urinary levels of 15-F2T-isoprostane, a marker of oxidative stress in newly diagnosed epileptic patients and healthy controls.[9] Verrotti et al. reported no difference in oxidative status between epileptic patients and control group.[10] In a previous study by Yuksel et al., increased serum lipid peroxidation in epileptic children was found before treatment.[8] Yis et al. found significantly decreased lipid peroxidation in the erythrocytes of newly diagnosed epileptic patients.[35] Our results suggest that epileptic disorder per se does not seem to cause an increase of lipid peroxidation, as also observed in some other studies in which no change in oxidant markers was found in newly diagnosed epileptic patients.[9,13]

In the present study we found no difference in XO levels between control group and epileptic children. This finding made us consider the possibility that the reason for the change in lipid peroxidation is concominant with the alteration in antioxidant enzyme activity. XO is a major potential source of oxygen free radicals. The burst of XO mediated free radical generation in the tissue is triggered by a large increase in substrate formation, which occurs secondary to degradation of adenine nucleotides.

4.2. Effect of antiepileptic drugs on NO, XO and MDA levels

Growing body of evidence observed that antiepileptic drug therapy can cause an increase in oxidant markers.[36–38] VPA was the first antiepileptic drug that has been investigated in several studies.[8–12] Previous studies evaluating the effects of VPA on MDA in epileptic children showed conflicting result. Our study showed that VPA itself does not appear to cause alterations of oxidative status in epileptic patients. Similar to our results, Verrotti et al. reported that VPA therapy does not effect oxidative status in epileptic children who remained non-obese during treatment.[10] Yuksel et al. found increased serum lipid peroxidation in epileptic children receiving VPA for 13 months when compared to control group and the pretreatment levels.[8] A recent study showed that oxidative stress has a potential role on VPA induced hepatotoxicity.[15] Sobaniec et al. found insignificant elevations of MDA concentrations in patients treated with VPA.[11] Michoulas et al. also reported higher urinary levels of 15-F2T-isoprostane, a marker of oxidative stress in epileptic children treated with VPA.[9] In contrast with these studies, lower MDA concentration in patients receiving VPA than in the controls was reported by Cengiz et al.[12] At the same time alterations in NO levels were demonstrated in children receiving VPA therapy. We found no changes in NO levels during VPA therapy. Michaelis et al. reported that VPA inhibited angiogenesis by a mechanism involving a decrease in e-NO synthase expression in human umbilical vein endothelial cells.[39] Researchers reported that antiepileptic efficiency of VPA may work through its ability to release NO.[40,41] On the other hand, it has been reported that NO might mediate the anticonvulsive effects of other antiepileptic agents, but not VPA.[42,43] NO probably does not contribute to the anticonvulsive efficacy of VPA.[44] Peker et al. demonstrated an increase in serum NO levels in epileptic children receiving VPA.[45] Karabiber et al. have also shown that nitrite and nitrate levels were significantly higher in epileptic children who were treated with VPA.[41] However, in their study, the control group consisted of children who had also taken antiepileptic medication but did not get any pharmacotherapy for the last 6 months and were seizure free. Unlike them, we selected our subjects from healthy children. In a recent study it has been shown that children receiving VPA have significantly higher levels of urine 15-F2t-isoprostane than those treated with carbamazepine or clobasam or control subjects receiving NO medications.[9] In our study, malondialdeyde and NO levels of patients receiving VPA did not show significant changes during the treatment were not different from the control group. This finding suggests that lipid peroxidation and NO levels in epileptic patients increase during VPA treatment but this does not reach to pathological levels. To better comment on this finding, it will be better to follow the same patients for a longer time.

The effects of newer generation antiepileptic drugs on oxidative stress have been investigated only in a few studies.[14] In our study, patients treated with OXC had lower levels of MDA levels in the third month of the treatment. After 3 months of OXC administration, serum MDA and NO levels were significantly lower than the baseline measurements. Although our data is generally suggestive of a beneficial effect of OXC in this particular setting, some drawbacks of our study exist. We did not have a placebo group in our study design. Hence, we were not able to verify and strengthen the results of our study. Despite these, our preliminary data is the first in the English published work about the antioxidant effects of OXC in a selected population of epileptic children. It is obvious that double-blind randomized investigations with long term use of antiepileptic drugs are warranted.

5. Conclusion

In conclusion, the present study demonstrates that epilepsy per se does not seem to cause an increase of lipid peroxidation but provides evidence that NO related oxidative damage may be involved in epilepsy. Our results also indicate decreased lipid peroxidation with a newer antiepileptic drug use; OXC, in newly diagnosed epileptic children. We may speculate that the decrease in lipid peroxidation may be responsible for the anticonvulsant effect of OXC. Detailed investigations will be needed to ascertain whether OXC has indeed long-term benefits in this subset of patients. To elucidate this phenomena, more advanced prospective long term clinical studies are required.

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