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2-(3-Acetyloxy-2-Naphthyl)-4-Acetyl-5-Phenyl-1,3,4-Oxadiazoline Suppresses Pentylenetetrazol-Induced Convulsive and Oxidative Activity on Mice

Farelerde Pentilentetrazol ile Oluşturulan Konvülsif ve Oksidatif Aktivitenin 2-(3-Asetiloksi-2-Naftil)-4-Asetil-5-Fenil-1,3,4-Oksadiazol ile Supresyonu

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Abstract

The aim of the study was to investigate the antioxidant effect of 2-(3-acetyloxy-2-naphthyl)-4-acetyl-5-phenyl-1,3,4-oxadiazoline (OXA) in mice brain and liver. In this study we also compared the antioxidant and anticonvulsive effets of OXA with that of valproat (VPA), an antiepileptic drug. OXA (100 $mg.kg^{-1}$) and valproat (150 $mg.kg^{-1}$) were administered i.p. to mice 4 hours and 1 hour respectively prior pentylenetetrazol (PTZ) injection. Animals were sacrificed after tonic and clonic convulsion and brain and liver tissues were immediately removed to analyse for lipid peroxidation (LPO) and glutathione (GSH) levels. Both OXA and VPA significantly decreased LPO levels in brain and liver which were elevated after PTZ administration. PTZ administration caused depletion of GSH in both brain and liver, however OXA and VPA treatment reversed the GSH levels to control. The results indicated that OXA and VPA protected brain and liver tissue against oxidative damage seen at the time of seizures.

Key words: Oxadiazolines, anticonvulsant activity, lipid peroxidation, glutathione.

Introduction

Free radical or oxidative injury is increasingly recognised as an important factor in the pathophisiology of epilepsy (Romero *et.al.*,1991; Torre *et.al.*, 1996). Although antiepileptic drugs are known to affect antioxidant system (Mahle and Dasgupta, 1997; Seçkin *et.al.*, 1999), because of the often limited number of patients studied and the variable duration of antiepileptic treatment, the effects of these drugs with regard to antioxidant activity are far from unequivocal (Cotariu *et.al.*, 1994; Erminio *et.al.*, 1994; Niketic and Ristic, 1995).

Considerable interest has been focused on hydrazones and 1,3,4-oxadiazoles, which have been shown to possess antiinflammatory (Raman *et.al.*, 1989; Kalsi *et.al.*, 1990), antimicrobial (Hassan *et.al.*, 1983; Mir *et.al.*, 1991), antidepressant (Aboul Wafa and El-Metwalli, 1992), and anticonvulsive (Agrawal *et.al.*, 1972; Parmar *et.al.*, 1974; Chaudhary *et.al.*, 1978) activities.

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We also sythesized a new group of 1,3,4-oxadiazolines with anticonvulsive activity in PTZ-induced seizures in mice and showed that 2-(3-acetyloxy-2-naphthyl)-4-acetyl-5-phenyl-1,3,4-oxadiazoline (OXA) was the most potent one (Doğan *et.al.*, 1998).
This study was designed to investigate whether OXA has antioxidant activity in correlation with anticonvulsive activity and to compare these effects with valproat as a standart drug.

Materials and Methods

The experiment was performed on 40 Balb-c mice of either sex weighing 20-25g. They were allowed water and food ad libitum and kept in a room at 22-24 °C with a 12-hour light cycle.
Pentylenetetrazol was puchased from Sigma, sodium valproat was kindly donated by Dogu Ilaç-Incorporation, Turkey.
OXA was synthesized in two steps. Briefly, equimolar amounts of 3-hydroxy-2-naphthoic acid hydrazide (Sigma) and benzaldehyde (Carlo Erba) were refluxed in ethanol for 2 hours and recrystallized from ethanol. Then, the crystals were dissolved in acetic anhydride (Merck) and refluxed at 160 °C for 45 min. This product was poured into cold water, washed with distilled water and recrystallized from ethanol (Doğan *et.al.*, 1998).
The animal protocol was reviewed and approved by the Animal Care and Research Committee of the University of Marmara. Animals were divided into six groups, ten of each. They were injected with ip saline as control (C group), 55 $mg.kg^{-1}$ pentylenetetrazol (PTZ group), 100 $mg.kg^{-1}$ OXA 4 hours prior to saline injection (OXA group), 150 $mg.kg^{-1}$ sodium valproate 20 minutes prior to saline injection (VPA group), 100 $mg.kg^{-1}$ OXA 4 hours prior to PTZ injection (OXA + PTZ group), and 150 $mg.kg^{-1}$ sodium valproate 20 minutes prior to PTZ injection (VPA + PTZ group).
Mice were observed for 60 minutes after the PTZ injection to induce seizures. Seizure latency was defined as the time elapsed from the injection of PTZ to the first two myoclonic jerks of the forelimbs. This has been concluded to be the first sign of the beginning of a seizure activity. Animals devoid of clonic generalized convulsions were considered protected and results were represented as % protection (Parmar *et.al.*, 1974).
Animals were sacrificed after tonic-clonic convulsions. Brain and liver tissues were rapidly removed and homogenized with 150 mM KCl. The MDA content of homogenates was determined by the spectrophotometric method in which the extract was reacted with thiobarbituric acid (Beuge and Aust, 1978). Glutathione was also determined by the spectrophotometric method using Ellman's reagent (Beutler, 1975).
Statistical analysis: All data were expressed as means ± SEM. Student's t-test was used for comparison of groups. Values of $p<0.05$ were regarded as significant.

Results and Discussion

The anticonvulsant activities in terms of protecting general convulsions and delaying the onset of the first myoclonic twitches were close in both OXA + PTZ and VPA + PTZ groups. VPA prolonged PTZ-induced seizure latency to 7.2 min., while OXA enhanced it to 5.1 min. The protection against PTZ-induced seizure were 60% in OXA and 80% in VPA group (Table).
The brain MDA levels of PTZ group were almost twice the control group. Brain GSH levels were found to be significantly decreased in PTZ group. Although OXA and VPA alone increased MDA levels in brain they did not alter the GSH levels. Both drugs significantly decreased the elevated brain MDA levels caused by PTZ. Furthermore they reversed the decreased GSH levels revealed by PTZ (Figs 1, 2).

Both OXA and VPA did not alter the liver MDA and GSH when administred alone. PTZ administration significantly elevated the liver MDA and decreased the GSH levels when compared with control group. Co-administration of OXA and VPA with PTZ reversed these effects (Figs 3, 4).

Free radicals are involved in etiology and pathogenesis of many CNS diseases, such as epilepsy, neuritis, Alzheimer's disease, aging and atherosclerosis of the brain (Cadet, 1988; Delanty and Dichter, 1998). Free radicals are highly reactive chemical species that can react with organic macromolecules leading to cell and tissue damage and consequent functional disruption (Cadet, 1988; Rokyta *et.al.*, 1996). Brain is a logical target of free radical damage, considering the large lipid content of myelin sheats and the high rate of oxidative metabolism (Hiramatsu and Mori, 1981; Romero *et.al.*, 1991). Raevskii *et al.* (1998) observed that the free radical content increased 4-5 fold in the brain cortex at the peak of seizures and anticonvulsant drugs reduced the seizure manifestations and partially prevented the free radical generation.

Glutathione (GSH) has been found in high concentrations in brain and is generally accepted to have an important function in chemical detoxification (Hiramatsu and Mori, 1981; Singh and Pathak, 1990). Berl *et. al* (1959) have reported focal seizures in cat lesions associated with a significant decrease in GSH and epileptogenic lesions showing a lower concentration of GSH than nonepileptogenic lesions on freezing of lateral cortex .

We have recently reported a new group of oxadiazolines with anticonvulsant activity (Doğan *et.al.*, 1998). The most potent of this group was OXA which contains nonsubstituted phenyl moiety. In the same study we showeu that OXA prevented PTZ-induced convulsive as well as oxidative damage in mice brain and liver.The exact anticonvulsive mechanism of OXA is unknown but it prevents PTZ-induced seizures and decrease the oxidative damage induced by PTZ.

In animal models, seizure activity leads to tissue injury through lipid peroxidation (Ueda *et.al.*, 1997; Kabuto *et.al.*, 1998). In a rat model of seizure activity, increased lipid peroxidation was concomitant with the development of epileptiform activity (Tupeev *et.al.*, 1985; Wojciech and Wojciech, 1993). In a parallel with literature we observed increased MDA, an end product of lipid peroxidation and depressed GSH levels in mice brain and liver with PTZ induced seizure activity. Purpura *et. al.* (1960) reported that in cat brains, seizures caused decreases in GSH levels which is the most important endogenous free radical scavenging compound in vivo that can prevent membrane lipid peroxidation.The same association between decreased central nervous system GSH concentration and seizure activity in animals has been demonstrated in a genetic model of human generalized epilepsy (Abbott *et.al.*, 1990).

In this study we compared the antioxidant activity of OXA and VPA against PTZ induced both seizures and oxidative damage in rat brain and liver. We chose VPA as a refence drug because of its potency in inhibiting generalized seizures with a lower ED_{50} value (Ferrendelli *et.al.*, 1989). OXA was effective as 75% of VPA in preventing generalized seizures.

In conclusion, PTZ caused significant increases in MDA and decreases in GSH both in brain and liver tissues whereas pretreatment with OXA and VPA reversed them. This may be a result of the depressed epileptic activity which causes decrease in glutathione and an increase in lipid peroxidation.

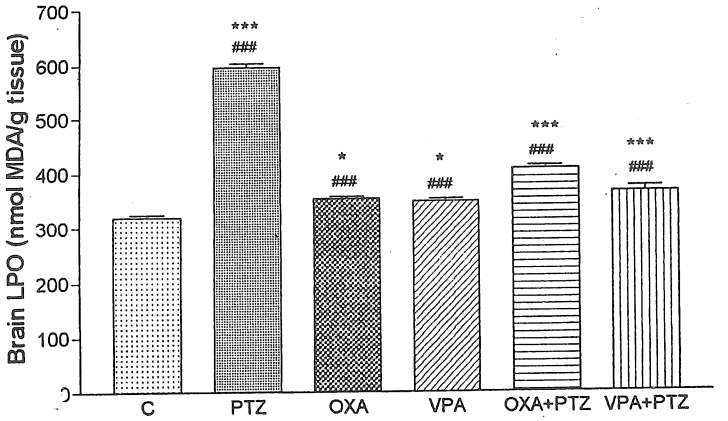

Fig. 1. Brain lipid peroxidation levels.
***: p<0.001, *:p<0.05, compared with control group,
###: p<0.001, compared with PTZ group (Student's t-test).

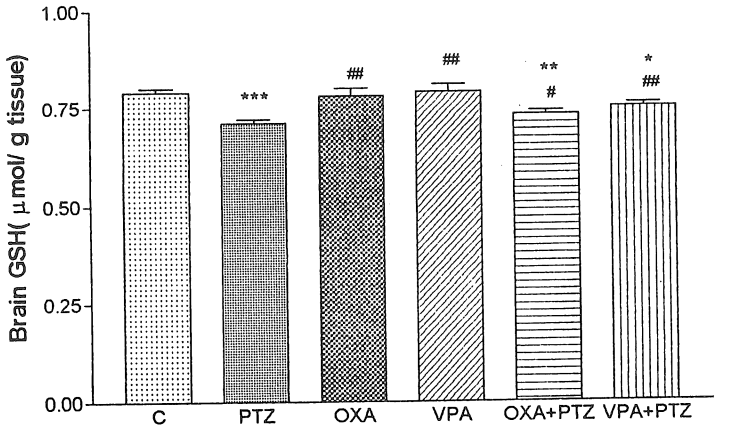

Fig. 2. Brain glutathione levels..
***: p<0.001, **: p<0.01, *:p<0.05, compared with control group,
##: p<0.01, compared with PTZ group (Student's t-test).

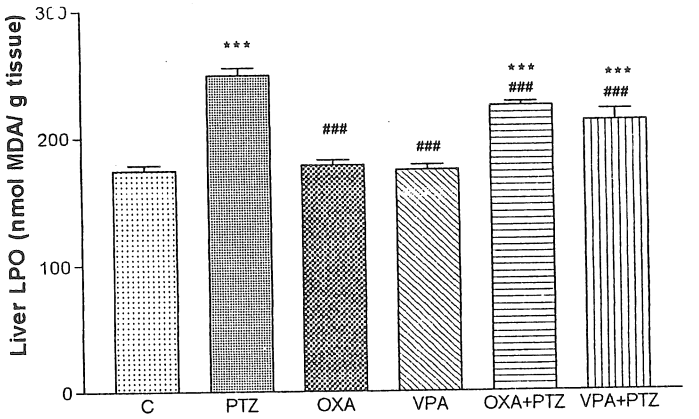

Fig. 3. Liver lipid peroxidation levels.
***: p<0.001, compared with control group,
###: p<0.001, compared with PTZ group (Student's t-test).

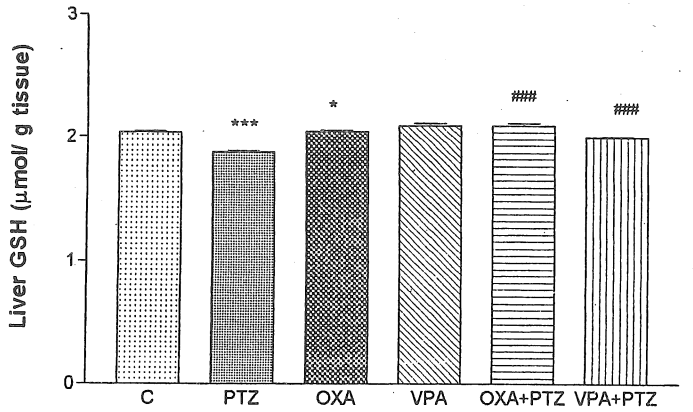

Fig. 4. Brain glutathione levels..
***: p<0.001, **: p<0.01, *:p<0.05, compared with control group,
##: p<0.01, compared with PTZ group (Student's t-test).

Table. Effect of OXA and VPA on seizure latency and protection for PTZ-induced generalized convulsions (%)

	Latency	% Protection
PTZ(n=10)	2.4 ± 0.2	-
OXA + PTZ (n=10)	5.1 ± 0.7 ***	60
VPA + PTZ (n=10)	7.2 ± 1.3 ***	80

***: p< 0.001, compared with PTZ group (Student's t-test)

Özet

Çalışmanın amacı 2-(3-asetiloksi-2-naftil)-4-asetil-5-fenil-1,3,4-oksadiazol (OXA)' ün fare beyin ve karaciğerinde antioksidan etkilerinin incelenmesidir. Çalışmamızda ayrıca OXA'nın antioksidan ve antikonvülsif etkileri antiepileptik bir ilaç olan valproat (VALP) ile karşılaştırıldı. OXA (100 mg.kg^{-1}) ve VALP (150 mg.kg^{-1}), pentilentetrazol (PTZ) injeksiyonundan sırasıyla 4 saat ve 1 saat önce farelere intraperitonal uygulandı. Tonik ve klonik konvülsiyonlardan sonra sakrifiye edilen hayvanların derhal beyin ve karaciğer dokuları çıkarılarak lipid peroksidasyonu (LPO) ve glutatyon (GSH) düzeyleri incelendi. Gerek OXA ve gerekse VALP, PTZ injeksiyonundan sonra beyin ve karaciğerde artan lipid peroksidasyonunu anlamlı olarak düşürdü. PTZ uygulaması beyin ve karaciğerde glutatyon tüketimine neden olurken OXA ve VALP glutatyonu kontrol düzeylerine getirdi. Sonuçlarımız OXA ve VALP'ın beyin ve karaciğer dokusunu nöbet sırasında gelişen oksidatif hasara karşı koruduğunu göstermiştir.

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