

The Effects of Pregabalin on Cerebral Cortical Oxidative Stress of Rats on Pentylenetetrazole Induced Epileptic Seizure

Sıçanlarda Pentilentetrazol ile Oluşturulan Epileptik Nöbet Modelinde Pregabalinin Beyin Korteksinde Oksidatif Stres Üzerine Etkileri

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Summary

Objectives: In this experimental model of epileptic seizure induced by pentylenetetrazole (PTZ), we aimed to investigate the effect of pregabalin, (PGB), (Lyrica(R)) in the brain cortex tissues and on superoxide dismutase (SOD) and catalase (CAT) activities as well as nitric oxide (NO) and malondialdehyde (MDA) levels, which are indicators of oxidative stress.

Methods: Forty male Wistar rats were randomly divided into four equal groups. The first group was used as a control group. The second group received a single dose administration of PTZ. Third and fourth groups were given, via gastric gavage, doses of 100 mg/kg body weight/day of PGB, divided into two, for two days. Seizure is obtained by applying intraperitoneally (ip) 50 mg/kg body weight of PTZ to groups II and IV. After one hour of PTZ administration, all rats were sacrificed and brain cortex tissues were taken. SOD and CAT activities and NO and MDA levels were studied in the brain cortex tissues.

Results: MDA levels and SOD activity of the PGB and PGB+PTZ groups were significantly lower than the control group ($p=0.005$, $p=0.001$ and $p=0.005$, $p=0.004$, respectively), and NO level of the PGB and PGB+PTZ groups were significantly higher than the control group ($p=0.001$, $p=0.001$, respectively). CAT levels between the groups were similar.

Conclusion: Our study results indicate that PGB prevents oxidative stress and increases NO levels in the rat brain cortical tissues during epileptic seizure. Increased NO may contribute to PGB's antiepileptic effect.

Key words: Epilepsy; oxidative stress; pregabalin; pentylenetetrazole.

Özet

Amaç: Pentilentetrazol (PTZ) ile epileptik nöbet oluşturulan bu deneysel modelde, beyin korteksinde, oksidatif stresin göstergeleri olan süperoksit dismutaz (SOD), katalaz (CAT) aktiviteleri, nitrikoksit (NO) ve malondialdehit (MDA) düzeyleri üzerine pregabalinin (PGB) etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Wistar cinsi 40 adet erkek sıçan rastgele 4 eşit gruba ayrıldı. İlk grup kontrol grubu olarak kullanıldı ve tek doz PTZ uygulanan grup, ikinci grup olarak kabul edildi. Üçüncü ve dördüncü gruba 100 mg/kg vücut ağırlığı/gün PGB ikiye bölünmüş dozlarda intragastrik yolla iki gün verildi. Grup II ve grup IV'e intraperitoneal (i.p.) (50 mg/kg vücut ağırlığı) PTZ uygulanarak nöbet oluşturuldu. PTZ uygulamasından bir saat sonra tüm sıçanlar sakrifiye edildi ve beyin korteksleri alındı. Beyin korteks dokularında SOD ve CAT aktiviteleri ile NO ve MDA düzeyleri çalışıldı.

Bulgular: PGB ve PGB+PTZ gruplarında MDA düzeyi ve SOD aktivitesi, kontrol grubundan istatistiksel olarak daha düşük saptandı (sırasıyla $p=0.005$, $p=0.001$ ve $p=0.005$, $p=0.004$). PGB ve PGB+PTZ gruplarının NO düzeyi, kontrol grubundan istatistiksel olarak daha yüksekti (sırasıyla $p=0.001$, $p=0.001$). Gruplar arasındaki CAT düzeyleri benzerdi.

Sonuç: Çalışmamızın sonuçları, PGB'nin oksidatif stresi önlediğini ve epileptik nöbet sırasında sıçan beyin korteks dokularında NO düzeylerini arttırdığını göstermiştir. Artmış olan NO düzeyinin PGB'nin antiepileptik etkisine katkı sağladığını düşünmekteyiz.

Anahtar sözcükler: Epilepsi; oksidatif stres; pregabalin; pentilentetrazol.

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Introduction

Epilepsy, which is the third most common chronic brain disorder, is a neurological disorder affecting approximately 8 out of 1000 people. The pathophysiology of epilepsy, however, has remained elusive in many aspects. Consequently, adequate seizure control is still lacking in approximately one third of patients.^[1] The etiology of neuronal death is triggered by a seizure under the influence many factors. These include genetic factors, intracellular electrolyte metabolism disorders leading to increased glutamatergic cell toxicity, mitochondrial dysfunction, oxidative stress, reduced growth factor, and increased cytokine concentration.^[1-4] Experimental and clinical studies have suggested that free radicals may be involved in the pathogenesis of epilepsy. Oxidative stress is known to occur following acute seizure activity but its contributions during epileptogenesis are largely unknown.^[1] Formation of oxidative stress in the central nervous system has been shown in several animal models of epilepsy such as penicillin, kainate, pilocarpine, and pentylenetetrazol models.^[5-8] A number of experimental studies have reported that neuroprotective and antioxidant activity of some antiepileptic drugs inhibit free oxygen radicals.^[9-11] New generation antiepileptic drugs (AEDs) have been developed with improved safety/tolerability profiles.^[12] Pregabalin (PGB), an AED, is specific modulator of the alpha2delta subunit of voltage-dependent calcium channels and inhibits presynaptic excitatory neurotransmitter release via this modulation.^[13]

In this study, we aimed to explore the effects of PGB on superoxide dismutase (SOD) and catalase (CAT) activities and nitric oxide (NO) and malondialdehyde (MDA) levels in the rats' brain cortex which experimentally created epileptic seizures.

Materials and Methods

Animals and treatment

Forty male Wistar albino rats (aged 8-12 weeks), weighing 200 to 250 g, were obtained from Laboratory Animal Production Unit of Suleyman Demirel University and were used in the experiment. All procedures and tests were carried out on the rats in accordance with "Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Suleyman Demirel University, Animal Ethical Committee." Rats were placed in a room controlled at a temperature of 21 ± 2 °C and a humidity of $60\pm 5\%$ in

which a 12 h/12 h light/dark cycle was maintained for 1 week before the start of the experiment. A commercially balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum.

Rats were divided randomly into four experimental groups as following:

Group 1: Control group (n=10); distilled water was administered to the rats five times by gavage way at a dosage of 2.0 mg/kg at 12-hour intervals. One hour after the last dosage, physiological saline (PS) was administered intraperitoneally (ip) at a dosage of 2.0 mg/kg.

Group 2: Pentylenetetrazole (PTZ) group (n=10); distilled water was administered to the rats five times by gavage way at a dosage of 2.0 mg/kg at 12-hour intervals. One hour after the last dosage, PTZ (diluted with PS, 2.0 mg/kg) was given intraperitoneally at a dosage of 50.0 mg/kg, thus an epileptic seizure group was obtained.^[14]

Group 3: Pregabalin (PGB) group (n=10); PGB (Lyrica, Pfizer, diluted with distilled water, 2 mg/kg) was administered to the rats five times by gavage way at a dosage of 50.0 mg/kg at 12-hour intervals. Pregabalin was taken from pharmacy. One hour after the last dosage, PS was administered intraperitoneally at a dosage of 2.0 mg/kg.

Group 4: Pentylenetetrazole-Pregabalin (PTZ-PGB) group (n=10); PGB (Lyrica, Pfizer, diluted with distilled water, 2 mg/kg) was administered to the rats five times by gavage way at a dosage of 50.0 mg/kg at 12-hour intervals. One hour after the last dosage, PTZ (diluted with PS, 2.0 mg/kg) was given intraperitoneally at a dosage of 50.0 mg/kg, thus an epileptic seizure group was obtained.^[15]

Anesthesia and tissue samples

Rats were stopped feeding one night before the procedure, and one hour after the administration of PTZ and PS, ketamin hydrochloride (ketamine HCl, 90 mg/kg) and xylazine (10 mg/kg) were administrated intraperitoneally. Then, all rats were sacrificed and brain cortex tissues were taken. Cortical brain samples were stored in deep freezer at -20 °C.

Biochemical procedure

The frozen tissue samples of the brain cortex were thawed, weighed, and homogenized (Ultra Turrax T25, Germany) (2

ml Tris-HCl buffer), in 50 mM Tris-HCl buffer (pH: 7.5) keeping in ice bath. MDA and NO levels were measured from the homogenates. Then, homogenates were centrifuged in +4 °C cold-centrifuge at 5000 cycle/minutes for 30 minutes, and supernatants were obtained.

SOD activity determination

Total SOD activity was determined according to the method of Sun et al.^[16] The principle of this method is based briefly on the inhibition of nitrobluetetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed units per milligram protein (U/mg protein).

CAT determination

Catalase activity was measured according to the method of Aebi.^[17] The principle of the assay is based on the determination of the rate constant k (dimension: s^{-1} , k) of hydrogen peroxide decomposition. By measuring the absorbance change per minute, the constant rate of the enzyme was determined (k/g protein).

NO determination

Nitric oxide measurement is very difficult in biological specimens, because it is easily oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-) which serve as index parameters of NO production. The method for assay of brain cortex nitrite and nitrate levels was based on the Griess reaction.^[18] Samples were initially deproteinized with Somogyi reagent. Total nitrite ($NO_2^- + NO_3^-$) was measured by spectrophotometry (Shimadzu, UV-Pharmaspec 1700, Japan) at 545 nm after conversion of NO_2^- to NO_3^- by copperized cadmium (Cd) granules. A standard curve was established by a set of serial dilutions (10^{-2} - 10^{-3} mol/L) of sodium nitrite. Results were expressed as micromole per gram wet tissue ($\mu mol/g$ wet tissue).

MDA determination

MDA levels, an indicator of free radical generation which increases because of the lipid peroxidation, were estimated using the Draper and Hadley double heating method.^[19] The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA was calculated by the absorbance coefficient of the

MDA-TBA complex (absorbance coefficient $\epsilon = 1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$) and is expressed as nanomoles per gram wet tissue (nmol/g wet tissue).

The protein content of the brain cortex tissue was determined using the Lowry method.^[20]

Statistical analysis

Comparison of groups was calculated by using Kruskal-Wallis test. For subgroup analysis, Mann-Whitney U-test was used. The data was presented as median (minimum-maximum) and the significance level in the analysis was determined as $p < 0.05$. SPSS 15.0 (Chicago, IL) was used in statistical analysis.

Results

In this study, the measured SOD and CAT activities and MDA and NO levels in the rat brain cortex as shown in Table 1.

MDA levels of the PTZ group were significantly ($p = 0.034$) higher than the control group (Fig. 1a). Whereas, SOD and NO levels demonstrated no difference between the groups ($p = 0.096$ and $p = 0.405$, respectively) (Fig. 1b, c).

MDA level and SOD activity of the PGB group were determined to be significantly lower than the control group ($p = 0.005$ and $p = 0.005$, respectively) (Fig. 1a, b). NO levels of the PGB group were significantly ($p = 0.001$) higher than the control (Fig. 1c).

MDA level and SOD activity of the PGB+PTZ group, were significantly lower than the control group ($p = 0.001$, $p = 0.004$, respectively), and it's NO levels were significantly ($p = 0.001$) higher than the PTZ group (Fig. 1a-c).

NO levels of the PGB+PTZ group were significantly ($p = 0.003$) higher than the PGB group (Fig. 1c). No differences were determined between the groups regarding MDA and SOD activities ($p = 0.059$ and $p = 0.762$, respectively) (Fig. 1a, b). CAT activities between the groups were detected as being similar ($p = 0.880$) (Fig. 1d).

Discussion

This is the first study that explores whether PGB and oxidative stress may be related to the model of epileptic seizure in the brain cortex.

Table 1. SOD and CAT activities, MDA and NO levels in the rat brain cortex

Groups	Group1 Control Median (minimum- maximum) (n=10)	Group 2 PTZ Median (minimum- maximum) (n=10)	Group 3 PGB Median (minimum- maximum) (n=10)	Group 4 PTZ+PGB Median (minimum- maximum) (n=10)	p values between the groups
SOD (U/mg protein)	0.05 (0.02-0.06)	0.05 (0.04-0.05)	0.04 (0.03-0.05)	0.04 (0.03-0.04)	<0.001
					1-2 0.096
					1-3 0.005
					1-4 0.004
					2-3 0.003
2-4 0.001					
3-4 0.762					
CAT (k/g protein)	0.03 (0.01-0.06)	0.04 (0.03-0.09)	0.04 (0.00-0.08)	0.04 (0.01-0.07)	0.880
					1-2 0.034
MDA (nmol/g wet tissue)	57.74 (53.45-63.17)	61.51 (57.03-71.87)	52.81 (45.40-55.88)	47.95 (43.32-54.73)	<0.001
					1-3 0.005
					1-4 0.001
					2-3 0.001
					2-4 0.001
3-4 0.059					
NO (μ mol/g wet tissue)	0.70 (0.58-0.86)	0.80 (0.54-0.99)	0.98 (0.88-1.10)	1.12 (1.03-1.48)	<0.001
					1-2 0.405
					1-3 0.001
					1-4 0.001
					2-3 0.001
2-4 0.001					
3-4 0.003					

MDA, one of the productions of the lipid peroxidation, has been detected at higher levels in the PTZ groups than the control group. This result is in accordance with the literature findings.^[9,11,14,15,21,22]

However, the results concerning antioxidant enzyme activities are somewhat conflicting. Discrepancies can be explained by methodological differences, or elevated enzyme activities may be reduced because of oxidative stress. For example, Ilhan et al.^[15,23] argues that SOD activities do not change in a rat's brain with experimentally-induced seizure with PTZ. Obay et al.^[14] have showed that SOD and CAT activities have decreased in a rat's brain with epileptic seizure models that were induced by PTZ. Freitas et al.^[24] determined that with SOD and CAT activities no changes occurred in the rats' hippocampus when an epileptic seizure had been induced by pilocarpine. In this study, it was shown that SOD and CAT activities were similar between the control group and the PTZ groups.

The relationship between epilepsy and NO is not clearly understood. De Luca et al.,^[25] Kaputlu, and Uzbay^[26] have discussed that if NO production was inhibited, seizures could not occur. In contrast, some authors have suggested that endogenous NO could prevent seizures.^[27] This discrepancy suggests that the relationship between epilepsy and NO is complex.

NO may contribute to the neuronal excitability by affecting other neurotransmitter systems (e.g. dopamine, GABA, and the other excitatory amino acids). NO may exert proconvulsive or neurotoxic effects by increasing glutamate, and may produce anticonvulsive or neuroprotective influences by supplying GABA to the tissues.^[28] Noh et al.^[29] reported that the ketogenic diet was effective in experimental nitric oxide synthase (NOS) to knock out the mouse's hippocampus and as an antiepileptic via the releasing of the endogenous NO levels to increase the expression of NOS. Stimulation of the NMDA receptors causes NO production. NO can regulate NMDA receptors because inhibitory feedback

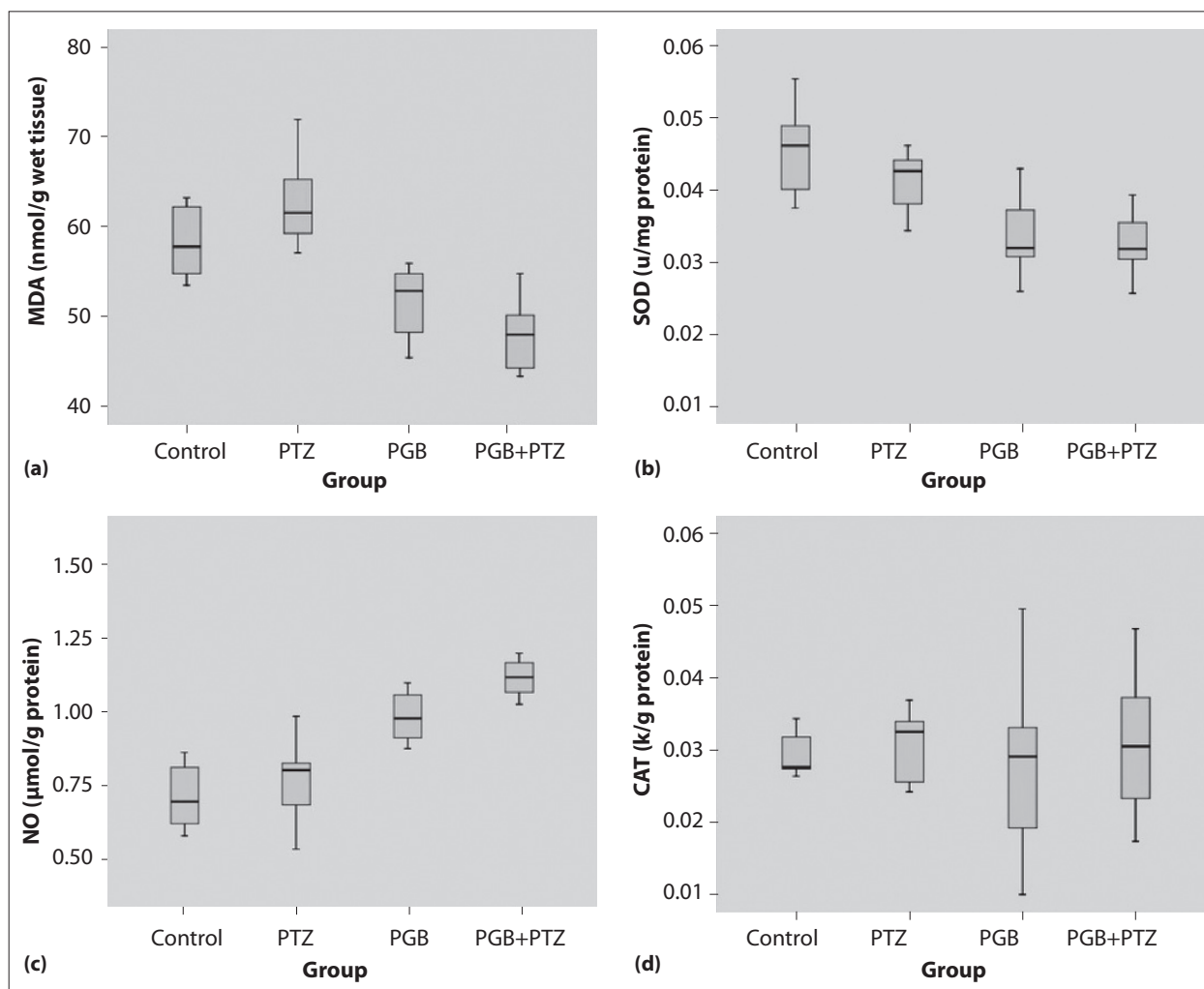


Fig. 1. (a) The values malondialdehyde of cerebral cortex. (b) The superoxide dismutase activity of cerebral cortex. (c) The nitrous oxide levels of cerebral cortex. (d) The catalase activities of cerebral cortex.

affects the sulfhydryl group located on the redox modulator domain. So, NMDA receptor activities decrease and the neurons are saved from overstimulation.^[27,30] Ferraro et al.^[28] showed that decreasing NO levels affected the stimulation of the NMDA receptors and increased the excitatory neurotransmission in the brain cortex and hippocampus.

Some recent reports showed a relation between GABA and NO. High NO levels potentially affect the releasing of the GABA, and increases the postsynaptic GABA-dependent chloride flows in GABA-A receptors. In contrast, low NO concentrations may affect GABAergic transmission as an inhibitory effect.^[31]

A decrease in NO amounts is followed by a corresponding

decrease in the GABA levels via an increase in the GABA transaminase activity.^[32] Naziroglu et al.^[21] showed no differences in the NO levels in the brain cortex of rats when seizures were induced with PTZ. Ilhan et al.^[15,23] in two studies documented an increase in NO levels in the brain cortex of rats that had seizures induced with PTZ. In this study, it was observed that there is no difference in the rat brain cortex NO levels between the PTZ and control groups. However, MDA and SOD activities measured were lower in the PGB and PGB+PTZ groups than in both control and PTZ groups with statistically significance. As well, NO levels were detected as increased in PGB and PGB+PTZ groups compared with the control and PTZ groups. Decreases in MDA levels showed that PGB prevents oxidative stress, but in groups with PGB, oxidative stress does not occur while

NO levels are increasing. The question is: does NO contribute to the PGB's antiepileptic effect?

The decrease in MDA levels showed that it prevents peroxynitrite, a reactive form of NO, which is one of the free radicals. The increased NO levels can affect the anticonvulsive or neuroprotective effect instead of the neurotoxic or proconvulsive effect.

PGB affects the normalization of the hyperexcited neurons, and its effects are more pronounced on hyperexcited neurons than on non-excited neurons.^[13,33] MDA levels of the PGB and PGB+PTZ groups were lower than the control group, and NO levels of the PGB and PGB+PTZ groups were higher than the control group, statistically. The decrease in MDA levels and the increase in NO levels were most marked in the PGB+PTZ group (as shown in Table 1). This result shows that PGB is more effective in the seizure group as compared to the non-excited group regarding the prevention of oxidative stress. The antioxidant effect of PGB is more pronounced on the hyperexcited neurons and concomitant with the increase of the NO levels; this occurrence leads to the question of whether NO may contribute to the PGB's antiepileptic effect. Sharpe et al.^[34] showed that NO prevents the organic elements from the oxidation of Fenton and Haber-Weiss reactions in live brain tissues. The increase of NO levels in groups with PGB may affect freeing of oxygen radicals for scavenging and its contribution as an antioxidant effector. Thus, the substrates of the antioxidant enzymes such as SOD and CAT are decreased and so their activities may be decreased. Therefore, in this study, an increase in the NO levels and a concomitant decrease of the SOD activities detected in groups with PGB may be an important result. Furthermore, it has strengthened the idea that PGB may stimulate NO production and contributes to the neuroprotective effect. We concluded that an increase of the NO levels caused by PGB has contributed to PGB's antiepileptic effect. However, this relationship should be questioned by further studies using the NOS inhibitors.

If we look at AEDs treatment and the relationship with oxidative stress in clinical or experimental studies, it is hard to conclude whether some AEDs may decrease or increase oxidative stress. Some studies suggest that in the old-generation AEDs, oxidative stress may occur; however, new-generation AEDs may not cause oxidative stress, and

may even prevent it. Our results support this conclusion. For example, Pavone et al.^[35] demonstrated that gabapentine, lamotrigine, tiagabine, and levetiracetam were not different from the control group on the MDA levels and production of the free oxygen radicals, whereas, in primary rat astrocytes cultures, carbamazepine, topiramate, and oxcarbazepine had significantly higher MDA levels and the production of the free oxygen radicals than in the control group. Also, tiagabine and levetiracetam had no effect on the increase of NO levels; however, the rest of the AEDs were affected by an increase in the NO levels as dose-dependent. Naziroglu et al.^[21] showed that topiramate had no significant effect on MDA and NO levels in rat brain cortex. Bashkatova et al.^[11] reported that PTZ-induced MDA and NO levels have decreased with phenobarbitale, lamotrigine, phenazepam, meksidol, and α -tocopherol treatment.

Conclusions

This study indicates that PGB may prevent oxidative stress during the epileptic seizure in the rat brain cortex. PGB is one of the new-generation AEDs, and is frequently used in treatment of epilepsy and central or peripheral neuropathic pain. Its relationship with oxidative stress needs to be supported by further clinical or experimental studies.

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