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EXPERIMENTAL STUDY / DENEYSEL ÇALIŞMA

Presentation to Affirmative Effect of Doxcycyline in Pentylene Tetrazole Induced Seizures Models

Doksisiklinin Pentilentetrazol İndükte Nöbetler Üzerine Olan Olumlu Etkisinin İncelenmesi

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Summary

Objectives: There were some experimental and clinical evidence that inflammation in the brain is likely to predispose epileptogenesis. Also, it is thought to be that oxidative stress can play a role in epilepsy. Both laboratory and clinical studies have demonstrated the antiinflammatory-antioxidant properties of doxycycline. We aimed to highlight the anticonvulsant action of doxycycline based on clinical, laboratory and EEG findings in animal models.

Methods: 36 rats were randomly divided in two groups. Group A for EEG recordings and Group B for behavioral assesment. 35 mg/kg pentylene tetrazole (PTZ) used for EEG recording and 70 mg/kg PTZ used for behavioral evaulations. For behavioral evaluations we assessed first myoclonic jerk time (FMJ) and Racine convulsion scores (RCS).

Results: The groups were evaluated according to EEG records and severety of seizures. It was found to be doxcycyline is effective both on the time of the first myoclonic jerk' (FMJ) latency and Racine convulsion scale (RCS) scores. Besides, doxcycyline significantly decreased to the percentage of spikes on electroencephalography (EEG) records. Also In current study, it was detected that Malone dialdehit (MDA) levels decreased and superoxide dismutase (SOD) activity increased in doxcycyline-treated groups.

Conclusion: In our study, we evaluated that the anticonvulsant effects of doxycycline showed a dose dependent protective effect against PTZ-induced seizures in rats by its anti-inflammatory and neuroprotective properties.

Key words: Doxcycyline; malonedialdehit; pentylenetetrazole; superoxide dismutase.

Özet

Amaç: Daha önce yapılan çalışmalarda enflamasyonun epilepsi patogeneziyle olan ilişkisi gösterilmiştir, buna göre oksidatif stresin de epilepsi patogeneziyle ilişkili olabileceği düşünülebilir. Literatürde doksisiklinin hem antienflamatuvar hem de antioksidan etkilerini inceleyen çalışmalar bulunmaktadır. Bu çalışmanın amacı doksisiklinin muhtemel antikonvulzan etkilerini elektroensefelografi kayıtları (EEG), laboratuvar bulguları ve davranışsal bulgular eşliğinde incelemektir.

Gereç ve Yöntem: Otuz altı sıçan rastgele iki gruba ayrıldı. Grup A EEG kayıtları, Group B ise davranışsal değişikliklerin incelenmesi için belirlendi. 35 mg/kg pentilentetrazol (PTZ) EEG kayıtları için, 70 mg/kg PTZ davranışsal değişiklikler oluşturmak için kullanıldı. Davranışsal değişikliklerin değerlendirilmesinde ilk miyoklonik jerk zamanı ve Racine konvulziyon skalası kullanıldı, EEG kayıtlarının değerlendirilmesinde ise spike yüzdeleri kullanıldı.

Bulgular: Yapılan istatistiksel analiz sonucunda doksisiklin verilen gruplarda spike yüzdeleri, ilk miyoklonik jerk zamanları ve Racine konvulziyon skoları açısından doksisiklinin olumlu etkileri tespit edildi. Ayrıca çalışmamızda doksisiklin verilen grupta malonedialdehit seviyeleri düşmüşken, süperoksitdismutaz seviyelerinin artmış olduğu görüldü.

Sonuç: Sunulan çalışmada doksisiklinin PTZ indükte konvulziyonlar üzerine olumlu etkileri olduğu görülmüştür, bu etkisini de antienflamatuvar ve nöroprotektif özellikler yoluyla olabileceğini düşünmekteyiz.

Anahtar sözcükler: Doksisiklin; malonedialdehit; pentilentetrazol; superoksit dismutaz.

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Introduction

International League Against Epilepsy (ILAE) defined an epileptic seizure as transient occurance of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition.[1] Epileptogenesis refers to a process in which an initial brain-damaging insult triggers a cascade of molecular and cellular changes that eventually lead to the occurence of spontaneous seizures.[2] Brain inflammation has gained recognition as a crucial contributor to epileptogenesis. Brain inflammation seems to be an intrinsic feature of the diseased hyperexcitable brain tissue where spontaneous and recurrent seizures originate. Following a convulsant challenge or epileptogenic brain injury, cytokines, prostaglandines, and inflammatory molecules together with their receptors are induced in neurons and activated glial cells, as well as in endothelial cells of blood-brain barrier, in the brain region of focal injury. Brain inflammation contributes significantly to determine seizure threshold in susceptible brain regions, thus playing a role in seizure precipitation and their recurrence.[3] Brain inflammation induced by an epileptogenic injury has a rapid onset (<30 min), it can persist for several days thus outlasting the initial precipitating event, and it is inefficiently opposed by endogenous anti-inflammatory mechanisms.[4]

Doxycycline is a long-acting second generation tetracycline-class antibiotic. It can pass to CSF well and exerts its effect in 1-3 hours when it is given orally and in 30 minutes when it is administered intravenously. Its action mechanism is based on the characteristics that tetracyclines inhibit protein synthesis by acting ribosome levels, 16S rRNA.[5] Both some laboratory and clinical studies have demonstrated anti-oxidant properties of doxycycline. Furthermore, it promotes neuronal survival, inhibits microglial activation and reduces reactive gliosis in some animal models.[6,7] According to these information, the aim of our study was to investigate doxycycline's anti-convulsant effects with EEG records, biochemical findings and clinical (behavioral) assesment.

Materials and Methods

Animal and Laboratory

The experimental procedures employed in present study were approved by Ege University Animal Ethics Comittee. All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals, as confirmed by National Institues of Health (U.S.) 36 male (18 of them for EEG recording and 18 of them are for behavioral studies) Sprague–Dawley rats, weighing 200–250 g each were utilized for this study. The rats were kept on a 12 hour –12 hour light–dark cycle (light from 07.00 to 19.00), in quiet rooms, with 22–24 °C ambient temperature. They were fed by standard laboratory food and tap water ad libitum.

Experimental procedures

36 rats were randomly divided in two groups: Group A for EEG recordings and Group B for behavioral assesment. In Group A; Rats were deeply anesthetized. Then, a small hole was opened with a drill stereotaxically. The electrodes (Polyamide-coated stainless steel wires, 0.1 mm diameter and electrical resistance <1Ω/10 mm) were implanted on dura over left frontal cortex (2.0 mm lateral to the midline, 1.5 mm anterior to the bregma) and the reference electrode was implanted over cerebellum (1.5 mm posterior to the lambda, on midline)[8,9] for EEG recording. Then, electrodes were fixed by using dental acrylic (Dental acrylic is a mixture of numerous alloys using for dental restoration). Rats were deeply anesthezied by ketamine (80 mg/kg) and xylazine (4 mg/kg) intraperitoneally (i.p.) After 10 days from the electrode replacement were fixed, 24 rats were divided randomly into 3 groups (n=6): Group A1, A2, A3.

Group A1 was administered saline i.p, Group A2 was administered 100 mg/kg doxycycline (Tetradox, Actavis) i.p and, Group A3 was administered 200 mg/kg doxycycline i.p. The drugs were administered 30 minutes prior to pentylentetrazol (PTZ) (35 mg/kg, i.p.) injection. All groups were administered 35 mg/kg PTZ and EEG was recorded. EEG recordings were taken in awake rats in a special container after 5 minutes from PTZ administeration.

The duration of EEG recording was 60 minutes (Figure 1).[10,11] The signals were amplified 10,000 times and filtered with a range of 1–60 Hz. EEG records were taken by using the Biopac MP 150 amplifier system and spike percentage was evaluated. Two clinical neurophysiologists scored the EEG data for spike percentage. We defined "spike percentage" as the percentage of 1-second bins with at least one spike-wave in them.[12] We affirmed the electrode location histologically following euthanization.

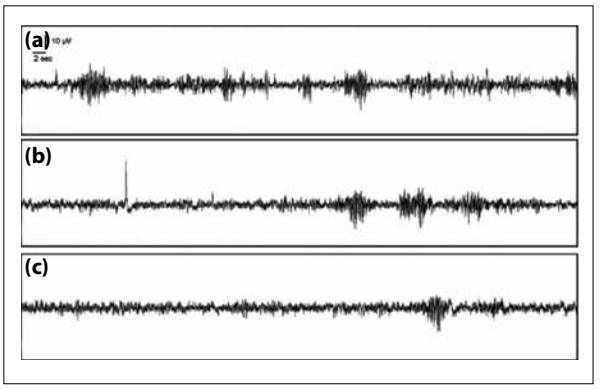


Fig. 1. EEG recording **(a)** PTZ (35 mg/kg) and saline, **(b)** PTZ (35 mg/kg) and 100 mg/kg doxycycline group; **(c)** PTZ (35 mg/kg) and 200 mg/kg doxycycline group.

Then the groups were rearranged with different 18 rats (Group B) and these rats were then divided into 3 groups (n=6): Group B1 was administered saline i.p, Group B2 was administered 100 mg/kg doxycycline (Tetradox, Actavis) i.p and, Group B3 was administered 200 mg/kg doxycycline i.p. The drugs were administered 30 minutes prior to PTZ (70 mg/kg, i.p.) injection. Racine's Seizure Scale (RCS) score and latency times of 'first myoclonic jerk' (FMJ) was used to evaluate the seizures (for only PTZ 70 mg/kg) as follows: 0 = no seizure; 1 = twitching of vibrassae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myclonic jerks; 4 = tonic clonic seizure while the animal remained on its feed; 5 = tonic–clonic seizure with loss of the righting reflex; 6 = lethal seizure. Rats were observed for latency times of FMJ as previously described.[13] The onset times were recorded as seconds. Almost all animals showing tonic generalized extension were died. The observation period for PTZ-induced seizures were limited with 30 minutes duration.[13] After this duration, the animals were euthanized and brain tissues were removed for biochemical analysis.

Measurement of brain lipid peroxidation (MDA)

Lipid peroxidation was determined in brain tissue samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactiıve substances (TBARS).[14] Briefly, trichloroacetic acid and TBARS reagent were added to the tissue samples, then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve using tetraethoxypropane and expressed as nmol/gr protein.

Determination of brain SOD activity

Total SOD activity was determined according to the method of Sun et al. The principle of the method is 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. SOD activity was given as units per milligram protein (U/mg protein).

Statistical analysis

Results were expressed as a mean±standard error of mean (SEM). Data analyses were performed by utilizing SPSS version 15.0 for Windows. The RCS score, FMJ time were evaluated by one-way analysis of variance (ANOVA). Post-hoc Bonferonni test was utilized to identify differences between the experimental groups. The value of $p<0.05$ was accepted as statistically significant.

Results

Evaluation of brain lipid peroxidation (MDA)

In 100 mg/kg and 200 mg/kg doxycycline-treated groups, brain MDA levels (42.4±4.7 nmol/gr, 36.1±7.7 nmol/gr, respectively) significanlty decreased when compared with saline-given group (93.7±4.8 nmol/gr, $p<0.0001$) (Figure 2).

Evaluation of brain SOD activity

In 100 mg/kg and 200 mg/kg doxycycline-treated groups, brain SOD levels (0.087±0.012 U/mg protein, 0. 11±0.018 U/mg protein, respectively) significanlty increased when compared with saline-given group (0.038±0.007 U/mg protein, $p<0.001$).

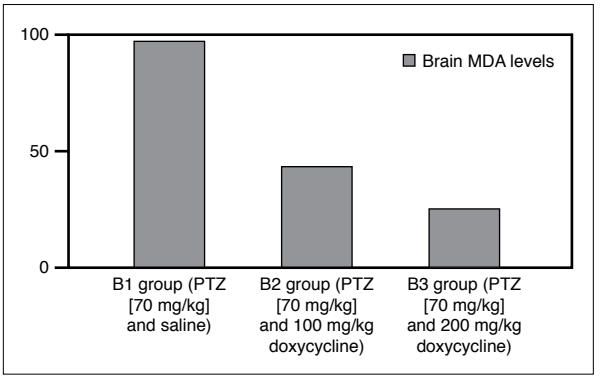


Fig. 2. Brain MDA levels in saline-given and doxcycyline-treated groups.

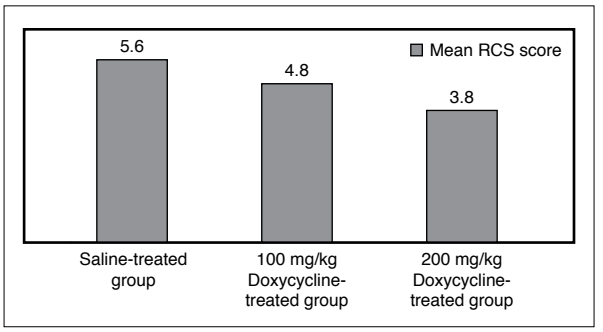


Fig. 3. Mean Racine's Seizure Scale (RCS) scores in saline-given and doxcycyline-treated groups.

Evaluation of the seizures

The Racine seizure scale score and FMJ latency time were measured in saline and doxcycyline-treated groups. RCS score was 5.6±0.2; FMJ latency time is 72.2±5.4 sec in saline-given group. 100 mg/kg and 200 mg/kg doxycycline treatment decreased RCS scores (4.8±0.2 and 3.8±0.2, $p<0.01$, $p<0.000$) and increased FMJ latency time (89.8±4.3, 115.5±5.6, $p<0.05$, $p<0.000$) when compared with saline-given group (Figure 3 and Table 1).

Evaluation of EEG records

Percentage of spikes were calculated as %73.3±4.7 in saline-given group. 100 mg/kg and 200 mg/kg doxycycline treatment significantly decreased spike percentage (%55.5±7.3 and %37.8±8, $p<0.05$, $p<0.01$) when compared with saline-given group (Figure 1).

Discussion

Doxycycline is one of the second generation tetracyclines that are known to prevent neuronal and oligodendroglial cell death in some in vitro models.[15] In previous studies it is presented that doxycycline has neuroprotective effects.[6] Though, the mechanism of epileptogenesis is not understood completely, the seizures have been reported to occur among patients with chronic inflammatory diseases.[16] Proinflammatory cytokines are induced rapidly in rodent brains during epileptic activity.[17] Seizures increase steady-state levels of mitochondrial O_2^-, a central mediator of oxidative stres.[18] The cytotoxic mechanism by which reactive oxygene species (ROS) induce neuronal damage may involve direct oxidative attack on cellular macromolecules and initiation or propagation of free radical chain reaction, ultimately leading to macromolecular damage.[19] Proteins, lipids and DNA are sensitive targets of ROS. As seizure induced inflammation is associated with free radical production and oxidative stress, there is also an increase in lipid peroxidation level.[20] Lipids are the major target of oxidative damage that occurs during seizures. Polyunsaturated fatty acids present in phospholipids of biological membranes are highly susceptible to oxidation by ROS.[21] Oxidation of fatty acids alters the structure of the cell membrane, cause changes in fluidity and permeability. Malondialdehyde is one of the products of lipid peroxidation and is therefore a good indicator of the rate of lipid peroxidation.[22]

The presence of lipid peroxidation following seizures has been demonstrated with MDA levels in our study. In doxycycline-treated group, MDA levels significanlty decreased and SOD levels significantly increased in a dose dependent manner when compared with saline-given group.

Recent studies demonstrated that doxycycline has protective effects on ischemic and degenerative brain diseases.[23,24] Hydrogen peroxide (H_2O_2) is formed whenever O_2^- is generated because of its rapid conversion to H2O2 by superoxide dismutase (SOD) enzyme.[25] H_2O_2 has moreless effect than superoxide group and it is counteracted for cell been converting by such as catalase, peroxidase and glutation. Causes of increases to effect of SOD contribute to render superoxide groups harmless.[26,27] Researchers have observed contradictory results in the levels of SOD, either in acute or chronic models.[28-30] SOD is one of the major anti-oxidants. In current study, SOD levels increased in doxycycline treated when compared with saline-given. This proves doxycycline's anti-oxidant effect on PTZ induced seizure

Table 1. First Myoclonic Jerk' (FMJ) latency time of groups

Groups	FMJ latency time (sec)
PTZ (70 mg/kg) and saline (B1 Group)	72.2±5.4
PTZ (70 mg/kg) and 100 mg/kg doxycycline (B2 Group)	89.8±4.3*
PTZ (70 mg/kg) and 200 mg/kg doxycycline (B3 Group)	115.5±5.6#

Data were expressed as mean±SEM. *$p<0.05$, #$p<0.0001$, **$p<0.01$ (different from saline-treated group).

model of rats. In our study, we also detected both 100 and 200 mg/kg doxycycline's affirmative effects on seizures by EEG recordings, it decreases spike percentage and seizure severity.

In conclusion, following the treatment with doxycycline, latency time for FMJ significantly increased; RCS scores and spike percentage decreased in a dose-dependent manner. This results demonstrate that doxycycline preserves from PTZ-induced seizures and decreases oxidant stress which occurs because of seizures.

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