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Effect of the cyclooxygenase-2 inhibitor tenoxicam on pentylenetetrazole-induced epileptic seizures in rats

Siklooksijenaz-2 inhibitörü tenoksikam'ın pentilentetrazol ile oluşturulan epileptik nöbetler üzerine etkisi

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SUMMARY

Objective: Epilepsy is a short-term paroxysmal disturbances of brain functions observed between sudden, abnormal and hypersynchronization discharges and seizures of a group of neurons in the central nervous system. The nonsteroidal anti-inflammatory tenoxicam is chemical agent that selectively inhibits type 2 cyclooxygenase (COX2), which converts arachidonic acid to prostaglandins (PGs). The aim of this study was to investigate the effect of the cyclooxygenase-2 inhibitor tenoxicam on pentylenetetrazole on epileptic seizures. **Method:** Eighteen Wistar Albino male rats (220±20 g) were divided into three groups: control (n=6), 10 mg/kg/day tenoxicam (n=6) and, 20 mg/kg/day tenoxicam (n=6). Tenoxicam was administered intramuscularly for ten days. On the tenth day, pentylenetetrazol (PTZ) was injected intraperitoneally at 70 mg/kg after 45 minutes of drug administration and the animals were observed for 30 min. Stages were determined according to the Racine seizure scale (RC) and the first myoclonic jerk time (FMJ) was recorded in seconds. After completing procedure, whole brain tissues were removed and stained with toluidine blue stain. The number of dark neurons with chromatin aggregation in hypocampal CA1 and dentate gyrus (DG) was determined as percentage. **Results:** Epileptic behavior were evaluated according to the RC, 10 mg/kg of tenoxicam significantly reduced the seizure stage compared to the control ($p<0,05$). In addition, 10 mg/kg tenoxicam significantly increased the FMJ compared to the control ($p<0,05$). According to the histopathological findings, neuronal damage was increased in CA1 region of 20 mg/kg of tenoxicam group compared to control, whereas neuronal damage was reduced significantly in the dentate gyrus of 10 mg/kg and 20 mg/kg of tenoxicam groups ($p<0,05$).
Conclusions: This study shows that dose-dependent administration of tenoxicam might have a potential to reduce epileptic seizures and post-seizure neuron damage.
Keywords: Epilepsy, cyclooxygenase-2 inhibitor, tenoxicam, pentylenetetrazole

ÖZET

Amaç: Epilepsi merkezi sinir sisteminde bir grup nöronun ani, anormal ve hipersenkronize deşarjları ile nöbetler halinde gözlenen beyin fonksiyonlarının kısa süreli paroksimal rahatsızlığı olarak tanımlanır. Nonsteroid anti inflamatuarlardan olan tenoksikam araşidonik asidi prostaglandinlere dönüştüren tip 2 siklooksijenaz (COX2)

enzimini selektif olarak inhibe eden kimyasal bir ajandır. Bu çalışmanın amacı siklooksijenaz-2 inhibitörü tenoksikam'ın pentilentetrazol ile oluşturulan epileptik nöbetlere üzerine etkisini araştırmaktır.
Yöntem: Çalışmamızda 18 tane 220-240 gr Wistar Albino erkek sıçan kullanılmıştır. Hayvanlar kontrol (n=6), 10 mg/kg/gün tenoksikam (n=6) ve 20 mg/kg/gün tenoksikam (n=6) olmak üzere üç gruba ayrıldı. On gün süre ile kontrol grubuna çözücü ve diğer iki gruba belirtilen dozlarda tenoksikam intramusküler olarak uygulandı. Onuncu gün ilaç uygulamalarından 45 dk sonra pentilentetrazol (PTZ) 70 mg/kg intraperitoneal olarak enjekte edildi. Hayvanlar 30 dk boyunca gözlemlendi. Racine nöbet skalasına göre evreleri belirlendi ve ilk miyoklonik jerk zamanı (FMJ) saniye olarak kaydedildi. İşlem bitiminden sonra hayvanların beyin dokuları alındı. Beyin dokuları rutin histolojik takip sonrası toluidin blue boyası ile boyanandı. Hipokampüste CA1 ve dentat girus bölgelerinde nöronal hasarı gösteren 'Dark nöron' sayıları yüzde olarak belirlendi.
Bulgular: Epileptik davranış sonuçları Racine nöbet skalasına (RC) göre değerlendirildiğinde, 10 mg/kg tenoksikam kontrole göre nöbet evresini anlamlı olarak azalttı ($p<0,05$). Ayrıca 10 mg/kg tenoksikam kontrole göre ilk miyoklonik jerk zamanını anlamlı olarak arttırdı ($p<0,05$). Histopatolojik olarak gruplar değerlendirildiğinde, CA1 bölgesinde 20 mg/kg tenoksikam kontrole göre nöronal hasarı arttıdığı, buna karsışık dentat girus bölgelesinde 10 mg/kg ve 20 mg/kg tenoksikam nöronal hasarı anlamlı olarak azalttı ($p<0,05$).
Sonuç: Bu çalışma tenoksikam uygulamasının epileptik nöbetleri ve nöbet sonrası nöron hasarını doz bağımlı olarak azaltabileceğini göstermektedir.
Anahtar sözcükler: Epilepsi, Siklooksijenaz -2 inhibitörü, tenoksikam, pentilentetrazol

INTRODUCTION

Extensive experimental and clinical data show that activation of inflammatory pathways is a crucial factor contributing to the pathogenesis of seizures in diverse forms of epilepsy from different etiologies [1-3]. These data suggest an important role of inflammation on epilepsy development, known as epileptogenesis and the and including the initiation of seizures [1, 2, 4, 5]. In addition, several anti-inflammatory drugs have been reported to exert antiepileptic actions[1, 2, 5]. On the contrary, there are inverse data have been declared about the relation between inflammation and epilepsy. Therefore, more studies are needed to fully understand this relation [5].

Cyclooxygenase (COX) is the rate-limiting enzyme in PG synthesis and is a major target of nonsteroidal anti-inflammatory drugs [4]. Local signaling is necessary to generate of new neuronal or glial cells within the central nervous system[6]. Microenvironment changes, damage and/or inflammation may effect cell differentiation and proliferation[6]. There are two COX isozymes have been identified: COX-1 and COX-2. COX-1is constitutively expressed in almost all tissue types whereas COX-2 is highly regulated by different endogenous and exogenous signals[6] and predominantly expressed isoform in the brain [9]. The specific inhibitor of COX-2 has been shown to protect against epileptogenesis and neuronal damage in experimental rat models [4, 7, 8]. Although little is known about how the COX-2 mechanism regulates neuronal signaling in epileptogenesis, applied COX-2 inhibitors are claimed to have anti-epileptogenic and neuroprotective effects in experimental models[9]. Today, tenoxicam, a nonsteroidal anti-inflammatory drug, is rather frequently prescribed because it is a selective inhibitor of COX-2 [10, 11].

The present study aimed to investigate the effects of tenoxicam on pentylenetetrazole-induced seizures and to demonstrate the neuroprotective effect of tenoxicam on neuronal damage after pentylenetetrazole administration. Our results show that dose-dependent administration of tenoxicam might have a protective effect to reduce epileptic seizures and post-seizure neuron damage.

MATERIALS AND METHODS

Experimental Animals

All experimental protocols were performed in accordance with the guidelines for the local ethics committee on the care and use of animals. All mice were housed and bred 12 h light/12 h darkness in polypropylene cages and in a temperature of 20-22ºC, with free access to both food and water. 18 Wistar albino adult male rats (220±20 g) were used.

Drug administration

Tenoxicam were dissolved in physiological saline. Solutions were freshly prepared on the days of the experiments.

Experimental procedure

Eighteen rats were divided randomly into three groups for behavioral and histological assessments. Group 1 was given saline intramuscularly (i.m.), group 2: 10 mg/kg/day tenoxicam (i.m.), and group 3: 20 mg/kg/day tenoxicam (i.m.) for ten days. The tenth day,

pentylentetrazol (PTZ) (70 mg/kg) was injected intraperitoneally 45 min after last tenoxicam injection to induce seizures. Racine's Convulsion Scale (RCS) were used to evaluate the seizures stages as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks; 4 = tonic-clonic seizure while the animal remained on its feed; 5 = tonic-clonic seizure with loss of the righting reflex; and 6 = lethal seizure. Rats were observed for both to evaluate Racine's Convulsion Scale (RCS) and to record first myoclonic jerck (FMJ) onset times which coincide inception stage3 [12]. The observation period for PTZ-induced seizures was limited to 30 min in duration [13]. Two hours after, the animals were terminated using the decapitation method and brain tissues were removed.

Histopathological evaluation

Formalin-fixed brain sections (4 μm) were stained with toluidine blue stain to quantify the number of dark neurons. All sections were examined and photographed with Olympus BX51 microscope. Dark neurons and survival neurons were counted in six sections per studied animal (n=6 for each group) in the ImageJ (Fiji) program (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997-2014). The number of dark neurons were given as percentage (toluidine blue stained neurons∗100/survival neuron). The observers blinded to the study groups accomplished all histological assessments.

Statistical analysis

The results were expressed as a mean ± standard error of mean (SEM). The data analyses were performed with SPSS Version 21.0 for Windows. The RCS score, FMJ time and dark neurons were evaluated using a one-way analysis of variance (ANOVA). A post hoc Tukey test was utilized to identify the differences between the experimental groups, and a value of $p < 0.05$ was accepted as statistically significant.

RESULTS

Evaluation of groups in terms of RCS and FMJ onset times

When the Racine scores were calculated between the groups, there were statistically significant differences between the saline (5.5 ± 0.2) and 10 mg/kg (4.3 ± 0.4) ($p< 0.05$) and there were no statistically significant differences between 20 mg/kg tenoxicam (5.5 ± 0.2) and saline ($p>0,05$). In addition, differences between the 10 mg/kg tenoxicam and 20 mg/kg tenoxicam were significant statistically ($p< 0.05$) (Fig.1).

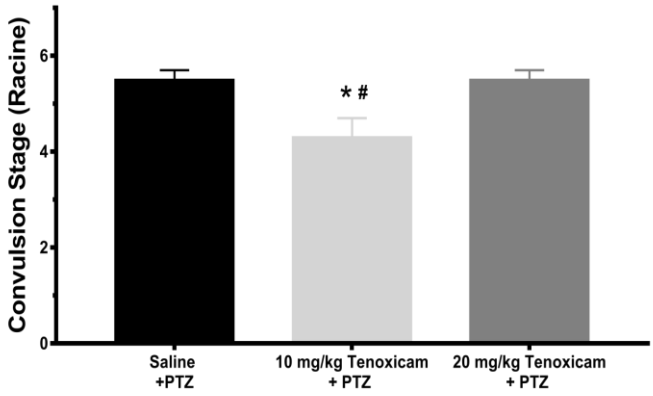

Figure 1 Racine Convulsion Scale, Data are presented as mean ± SEM .*$p < 0.05$, 10 mg/kg tenoxicam + PTZ group compared with saline + PTZ. #$p < 0.05$, 10 mg/kg tenoxicam + PTZ group compared with 20 mg/kg tenoxicam + PTZ group.

There were statistically significant differences (p< 0.01) between saline group (39,3 ± 6.3 s) and 10 mg/kg tenoxicam (87,3 ± 12 s) group in terms of FMJ onset times. On the contrary, there were no statistically significant differences between 20 mg/kg tenoxicam (51,5 ± 2,5) and saline group in terms of FMJ onset times (p>0.05). In addition, there were differences between 10 mg/kg tenoxicam and 20 mg/kg tenoxicam statistically (p<0,05) (Table 1).

Table 1. Fist myoclonic jerk (FMJ) onset time as seconds(s). Data were expressed as mean ± SEM. *p < 0.01comparedto PTZ-saline group.#p <0,05 compared to PTZ and 20 mg/kg tenoxicam group.

Groups	FMJ onset time(s)
PTZ (70 mg/kg) and saline (group 1)	39,3 ± 6.3
PTZ (70 mg/kg) and 10 mg/kg tenoxicam (group 2)	87,3 ± 12*#
PTZ (70 mg/kg) and 20 mg/kg tenoxicam (group 3)	51,5 ± 2,5

Evaluation of groups in terms of dark neurons

There are varied criteria used to identify the dark neurons, contains neuronal shrinkage, nuclear pyknosis, chromatin aggregation, intense (dark) staining of perikaryal, dendritic and axonal cytoplasm, and surrounding spongiosis in hippocampal formation (Fig. 2). Administration of tenoxicam at the dose of 10 mg/kg significantly prevented production of dark neurons due to PTZ induced seizures in DG regions of hippocampus (p< 0.05; Fig. 3A) but did not significantly prevent production of dark neurons in CA1 regions of hippocampus (p> 0,05; Fig. 3B). Also 20 mg/kg tenoxicam significantly decreased dark neurons in DG region of hippocampus (p< 0,05; Fig. 3A) but interestingly increased dark neurons in CA1 region of hippocampus (p< 0,05; Fig. 3B). In addition, there was significant difference between at the dose of 10 mg/kg and at the dose of 20 mg/kg tenoxicam in CA1 and DG regions of hippocampus in point of dark neurons (p< 0,05; Fig. 3. A-B).

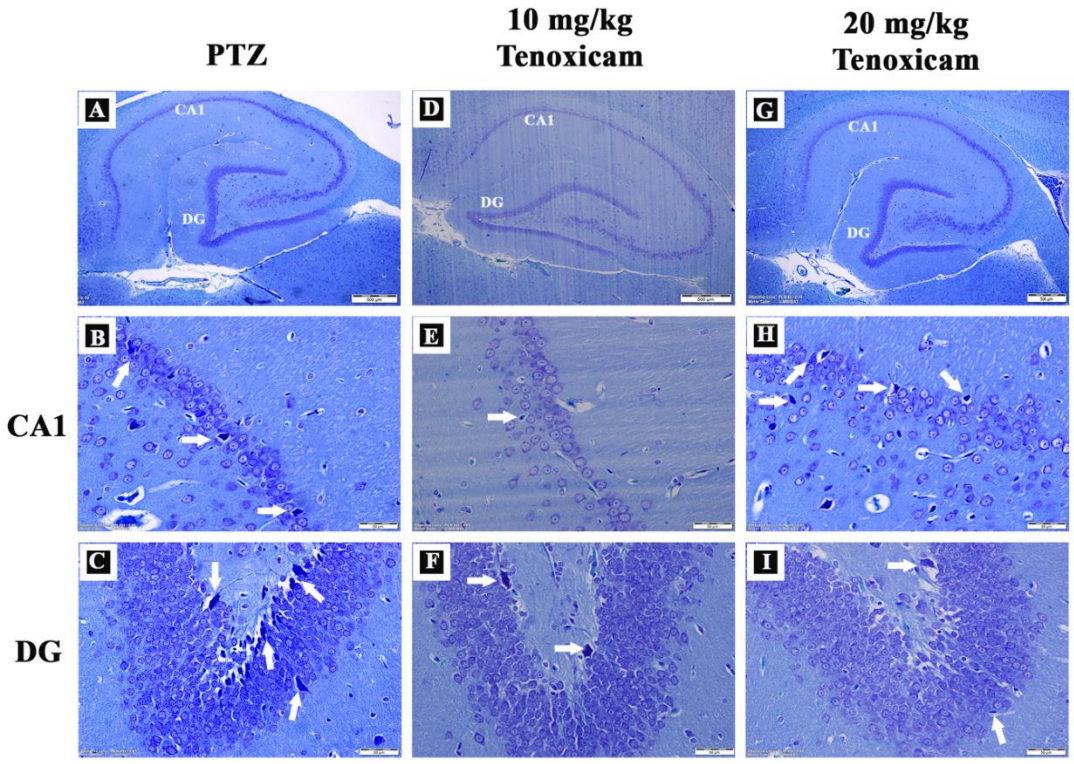

Figure 2 Toluidine blue staining of coronal hippocampus sections are showing the different areas of the hippocampal formation; Cornu Ammonis 1 (CA1) and dentate gyrus (DG) regions. A-C; PTZ group. D-F; 10 mg/kg Tenoxicam group. G-I; 20 mg/kg Tenoxicam group. Dark neurons are presented with arrows among pyramidal cells in CA1 and DG regions.

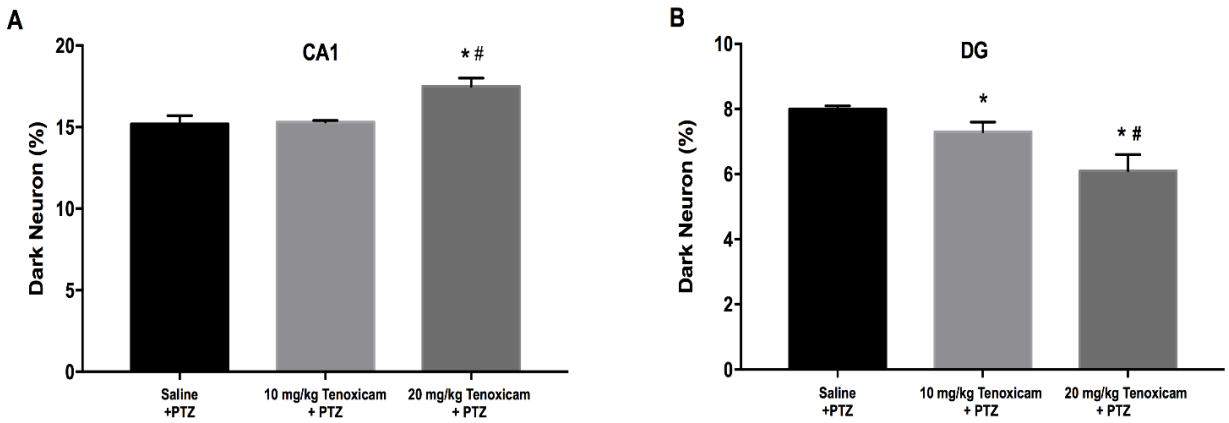

Figure 3 Comparison of dark neuron numbers per area in CA1 (A) and DG (B) areas between groups. Data are presented as mean ± SEM .*P <0.05 in comparison with control group. #P <0.05 20 mg/kg tenoxicam+PTZ compared to 10 mg/kg tenoxicam+PTZ.

DISCUSSION

Various studies in different animal models of epilepsy and epilepsy patients with hippocampal sclerosis have shown that COX-2 expression was induced after seizures [6, 14-17]. Furthermore, the concentrations of prostaglandins (PGs) increased in the cerebrospinal fluid of epilepsy, hippocampal sclerosis and febrile seizures patients [18]. In the present study, chronic administration of low dose tenoxicam significantly decreased RC and also increased FMJ but this effect disappeared at high dose. This suggests that the activation of COX-2 has a central role in the genesis of epilepsy, as well in the pathways targeted by new anti-epileptogenic drugs.

Moreover, COX-2 expression regulates prostaglandin E2 (PGE_2) synthesis, which plays crucial role in synaptic signalling during seizures and was detected high level in the cerebrospinal fluid of patients with epilepsy, hippocampal sclerosis and febrile seizures [6]. Several COX-2 selective inhibitors such as nimesulide, parecoxib, rofecoxib, NS-398, SC-58125 etc., have been tested in experimental epilepsy models[19]. Published data indicate that the effects of COX-2 inhibitors are contradictory in epileptic animal models. Some previous researches showed that the drugs that inhibit COX-2 activity such as nimesulide and indomethacin treatment played an anticonvulsant role and reduced hippocampal cell death in experimental epilepsy models[15, 17, 18]. Using SC-58125, a specific COX-2 inhibitor, inhibited the upregulation of inflammatory cytokines and reduced COX-2 and PGE_2 levels and neuronal apoptosis[20, 21, 22]. On the contrary, using another selective COX-2 inhibitor NS-398 generated proconvulsant effects and increased neuronal mortality and damage in mice [23]. In this study, pretreatment with tenoxicam significantly decreased the stage and duration of pentylentetrazole-induced seizures.

On the other hand, in this present study showed that after PTZ-induced seizures damage to hippocampal neurons of the rats were prevented by tenoxicam as previous studies, which were treatment with different COX-2 inhibitors [6, 20-24]. In addition, the results of this present study showed that PTZ-induced seizures were resulted in dark neuron production in the hippocampal regions, as observed by previous studies [25, 26]. The affected neurons, which are distributed among the healthy neurons and microenvironment, are called dark neurons [27]. Initially, although dark neurons were thought to be histological artifacts in neurosurgical biopsy specimens, it was later noticed that they were formed after brain trauma[28]. Dark neurons have characteristic features such as basophilic appearance and morphological changes and have been shown in hypoglycemia, ischemia, stress, as well as in epilepsy[29]. It is suggested that depolarization, glutamate release or receptor activation are more efficient in the mechanism of dark neuron production[27], in addition, epilepsy is also an important cause of dark neuron production [26, 27-29]. There are many reports that confirmed hippocampal damages caused by seizures [26, 30, 31]. The results of this study were consistent with previous studies showing that dark neurons were produced in brain tissues following PTZ-induced seizures.

The results of the present study showed that low dose of tenoxicam decreased epileptic seizures as well as preventing neural damage after PTZ-induced seizure in rats. These results support the beneficial effect tenoxicam on the nervous system. Further studies are required for determining the mechanism of tenoxicam on this effects.

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