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Research Article

# The Effects of Erythropoietin on the Penicillin Induced Epileptiform Activity in Rats [1]

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## Abstract

Erythropoietin (Epo), a cytokine hormone produced in the kidney, promotes the formation of red blood cells in the bone marrow. The penicillin-induced epilepsy model is a commonly used experimental model for epilepsy research. The present study was conducted to elucidate the effect of Epo on penicillin-G (500 IU/2.5 µl dose, intracortically (i.c)) -induced epileptiform activity in anesthetized adult Wistar-Albino rats (n=39). The animals were randomly divided into four groups as three treatment groups (groups 1-3) and a control group (no drug application). Rats in groups 1, 2 and 3 were intraperitoneally administered 2.000, 4.000 and 6.000 IU Epo/kg, respectively. The effects on penicillin G induced epilepsy were compared across groups using electrocorticography. Epo at 2.000 IU/kg did not cause a significant change ($P>0.05$) in epileptiform spike-wave activity (number/min) and/or amplitude (µV) values, whereas the average number of spike-waves per minute and seizure severity decreased significantly in the 4.000 and 6.000 IU/kg Epo groups compared with the control ($P<0.05$). Consequently, the results of the present study show that administration of Epo has a dose-dependent antiepileptic effect in penicillin induced model of epilepsy in rats.

***Keywords:*** *Erythropoietin, Electrocorticography, Epilepsy, Penicillin, Rat*

# Sıçanlarda Penisilin ile Oluşturulan Epileptiform Aktivitesi Üzerine Eritropoietinin Etkileri

## Özet

Eritropoietin (Epo), böbreklerde sentezlenen ve kemik iliğinde eritrosit üretimini sağlayan bir sitokin hormonudur. Deneysel epilepsi araştırmalarında, genel olarak penisilin ile oluşturulan epilepsi modeli kullanılmaktadır. Çalışmamızda, anestezi altındaki yetişkin Wistar-Albino türü sıçanlarda (n=39), Penisilin-G (intrakortikal (i.c) olarak, 500 I.U/2.5 µl dozda) ile oluşturulmuş epileptik aktivite üzerine Epo'nun etkileri araştırıldı. Sıçanlar, üç tedavi grubu (grup 1-3) ve bir kontrol grubu (ilaç uygulanmadı) olarak rastgele dört farklı gruba ayrıldı. Grup 1, 2 ve 3'de bulunan sıçanlara, intraperitonal olarak sırayla 2.000, 4.000 and 6.000 IU Epo/kg'lık dozlarda Epo uygulandı. Gruplar arasında, penisilin G ile oluşturulan epilepsi üzerine Epo'nun etkisi elektrokortikografi kullanılarak karşılaştırıldı. Kontrol grubu ile Epo grubu karşılaştırıldığında, 4.000 ve 6.000 IU/kg Epo uygulaması, dakika başına diken dalgaların ve dikenlerin ortalama sayısı ve nöbet şiddetini anlamlı ($P<0.05$) derecede azaltır iken, 2.000 IU/kg Epo uygulamasında epileptiform diken dalga akitivitesi (sayı/dk) ve/veya genlik (µV) değerlerinde anlamlı bir değişime neden olmadı ($P>0.05$). Sonuç olarak yapılan çalışma, Epo'nun sıçanlarda penisilin ile oluşturulmuş deneysel epilepsi modeli üzerine uygulanmasının, doz bağımlı antiepileptik etkiye neden olduğu ortaya çıkarmıştır.

***Anahtar sözcükler:*** *Eritropoietin, Elektrokortikografi, Epilepsi, Penisilin, Sıçan*

## INTRODUCTION

Epilepsy is a clinical condition characterized by spontaneous recurrent seizures of cerebral origin [1]. As a common chronic neurological disorder, it affects 1-3% of the population, and approximately 10% of the general population has one or more seizures during their lifetime [2]. Experimental investigations of epilepsy in animal models have contributed important information regarding epilepsy pathogenesis [3,4]. Experimental epilepsy is induced by

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penicillin, topically or intracortically (i.c.) administered at the surface of the cortex. The penicillin-induced epilepsy model has been used in numerous studies. Penicillin causes acute focal epileptic activity similar to that which decreases the activity of the GABA inhibitory system in the brain and increases glutamate, which becomes the main excitatory neurotransmitter in the brain [5-9]. Researchers [6-9] continue to study the antiepileptic effects of agents in animal models of experimental epilepsy, but the therapeutic effectiveness of these agents may not be the same in humans [10,11].

Erythropoietin (Epo), a hematopoietic glycoprotein cytokine hormone produced in the kidney, promotes red blood cell formation in bone marrow and is expressed in other tissues, including the nervous system. Epo mediates a number of biological actions in the central nervous system (CNS), where it is also neuroprotective [12-15]. Epo can cross the blood–brain barrier (BBB) via a receptor-mediated mechanism [16]. Uzum et al.[17] have shown that Epo pretreatment confines BBB leakage to the cerebellum and cortical areas and lessens the intensity of tonic-clonic seizures during pentylentetrazol-induced seizures.

In recent years, many studies have investigated the presence and protective effect of Epo and the erythropoietin receptor (EpoR) on neurons, demonstrating both epileptic and antiepileptic effects of Epo in different experimental animal models [17-23]. However, no study has shown the effects of Epo in a penicillin-induced experimental epilepsy model. Here, we report the effect of Epo at various doses on epilepsy after seizure

## MATERIAL and METHODS

### *Experimental Procedures*

A total number of thirty-nine adult male Wistar-Albino rats (200-250 g; 12-14 weeks) were used in this study. These rats were taken by Duzce University Medical and Surgical Research Center, Duzce-Turkey before experiment and they were housed in groups of 4-5 per cage (42x26x15 cm) in a room with controlled temeparature (21±2°C) and relative humudity (60±5%) with lights on from 8:00-20:00. This study was approved by the Duzce Animal Care and Usage University Ethics Committee (Approval Number: 2009-24). Animal handling during all experiments was consistent with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23).

Rats were randomly assigned to the following groups: (1) 500 IU penicillin (2.5 µl, i.c.) control group (n=10); (2) 500 IU penicillin (2.5 µl, i.c.) + 2.000 IU/kg Epo (n=10); (3) 500 IU penicillin (2.5 µl, i.c.) + 4.000 IU/kg Epo (n=9); and (4) 500 IU penicillin (2.5 µl, i.c.) + 6.000 IU/kg Epo (n=10) groups. All rats were anesthetized with 1.25 g/kg intraperitoneal urethane (Sigma Aldrich Co., St. Louis, MO, USA) and placed in a stereotaxic frame (Harvard Apparatus, Holliston, MA, USA). The left cerebral cortex was exposed by craniotomy. Two Ag-AgCl ball electrodes were placed over the left somatomotor cortex (first electrode: 2 mm lateral to sagittal suture, 1 mm anterior to bregma; second electrode: 2 mm lateral to sagittal suture, 5 mm posterior to bregma). The common reference electrode was fixed on the right pinna. Electrocorticography (ECoG) recordings were continuously monitored. The signals from the electrodes were amplified and filtered (0.1-50 Hz bandpass) using bio-amplifiers (BioAmp; AD Instruments, Bella Vista NSW, Australia). Then the ECoG signal was digitized at a sampling rate of 1024 using a four-channel data acquisition system (PowerLab 8/SP; AD Instruments). Baseline activity was recorded for 10 min in each group.

An epileptic focus was produced by intracortical injection of penicillin G (500 IU/2.5 µl) in all animals. Using a Hamilton microsyringe (type 701 N; Hamilton Co., Reno, NV, USA), penicillin was injected into the left sensorimotor cortex (2 mm posterior to bregma, 3 mm lateral to the sagittal suture, and 1 mm beneath the brain surface) at an infusion rate of 0.5 µl/min. Epileptiform activity was observed by ECoG for 5-6 min. Activity reached a constant level within 30 min following the administration of penicillin G and lasted for 3-5 h. After about 30 min when the spike-waves become stable, the rats were given intraperitoneally Epo at a dose of 2.000, 4.000, or 6.000 IU/kg. All recordings were displayed and stored using a computer. Spike frequencies and amplitudes for each animal were automatically calculated and measured using the data-acquisition Chart v.5.1.1 system (PowerLab software; AD Instruments). The frequency and amplitude of epileptic activity were analyzed offline.

### *Statistics*

The frequency and amplitude values acquired from animals in all groups were converted to a scaling percentage in a time-dependent manner. The percentage changes were used for statistical analyses and graphics. All statistical procedures were performed using SPSS statistical software package version 12.0 (SPSS, Inc., Chicago, IL, USA). Data are expressed as means±SD. The data were analyzed by one-way analysis of variance followed by Tukey's post hoc test to correct for multiple comparisons of treatments. Statistical significance was accepted at $P<0.05$.

## RESULTS

The penicillin-induced epileptiform discharges were characterized by bilateral spikes and spike-wave complexes on a background of ECoG activity *(Fig. 1)*. Data comprising mean spike frequency and mean spike amplitude, and latencies to onset of epileptiform activity in all experimental groups during 120 min record following penicillin injection. Epo was administered 30 min after

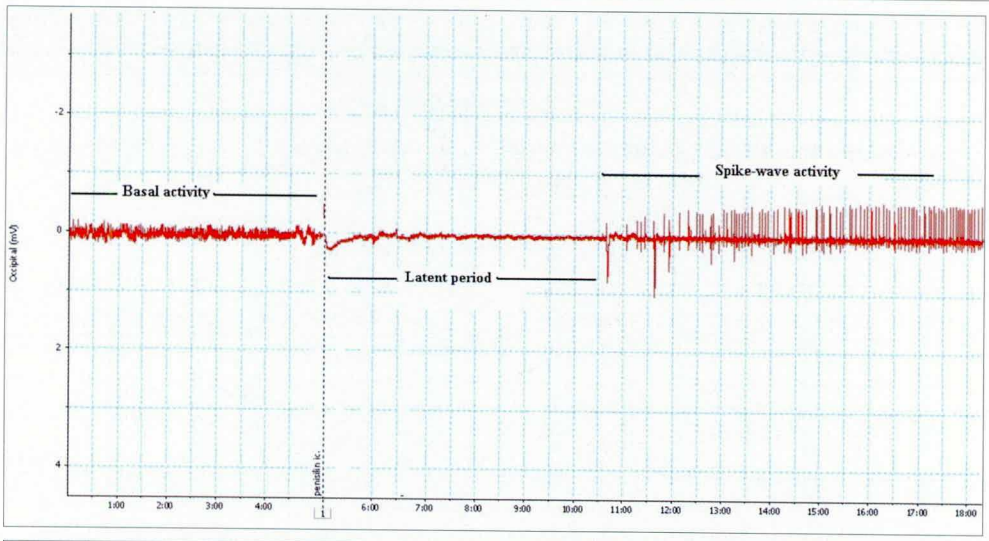


**Fig 1.** Changes in ECoG activity after administration of penicillin G
**Şekil 1.** Penisilin G verilmesinden sonra ECoG aktivitesinde değişiklikler

***Table 1.*** *Number of spike or spike-wave discharges per minute (number/minute) at each dose during each period (mean±SD and P values)*
***Tablo 1.*** *Farklı dozlarda uygulanan EPO nun, her bir periyot aralığındaki dakikadaki diken dalga deşarjı sayıları (sayı/dk) (ortalama±SD ve P değerleri)*

| Treatment Period (min) | Erythropoietin Treatment (dose)[1] | | | | P Value[2] |
|---|---|---|---|---|---|
| | Control group (n:10) | 2.000 IU/kg (n:10) | 4.000 IU/kg (n:9) | 6.000 IU/kg (n:10) | |
| Baseline | 31.78±10.3 | 27.70±11.0 | 34.64±13.2 | 34.80±11.2 | - |
| 1-10 | 32.09±13.2 | 27.34±12.0 | 29.92±9.2 | 32.61±17.5 | 0.229 |
| 11-20 | 33.58±12.9 | 24.38±13.5 | 26.11±8.0[a] | 29.33±15.1 | 0.018[a] |
| 21-30 | 30.44±11.1 | 22.35±12.3 | 23.35±6.4[a] | 24.64±15.1[b] | 0.034[a,b] |
| 31-40 | 30.44±12.0 | 20.81±11.5 | 22.16±5.5[a] | 23.25±13.2[b] | 0.027[a,b] |
| 41-50 | 29.45±12.6 | 16.88±13.5 | 18.95±5.9[a] | 19.73±14.7[b] | 0.034[a,b] |
| 51-60 | 27.72±13.0 | 16.01±13.6 | 15.11±9.0[a] | 15.94±15.3[b] | 0.037[a,b] |
| 61-70 | 26.47±13.5 | 14.93±13.4 | 14.27±9.1 | 17.31±16.9 | 0.099 |
| 71-80 | 24.71±13.2 | 13.31±12.5 | 13.70±9.3 | 18.29±20.0 | 0.224 |
| 81-90 | 22.71±11.4 | 12.07±11.4 | 13.07±9.3 | 17.97±22.0 | 0.310 |
| 91-100 | 20.42±8.8 | 11.99±12.6 | 12.23±9.6 | 13.08±17.1 | 0.196 |
| 101-110 | 19.25±7.4 | 12.21±13.3 | 12.09±10.0 | 11.80±14.2 | 0.191 |
| 111-120 | 16.87±5.2 | 10.86±12.7 | 11.76±10.1 | 8.18±11.2 | 0.133 |

*[1] Values are the mean±SD for rats in each group, [2] Statistical significance; P<0.05; [a,b] P<0.05: Compared with control*

the penicillin injection. The mean spike-wave frequencies of each group are shown in *Table 1*. The mean number of spike-waves per minute wave frequencies in 4.000 IU and 6.000 IU Epo doses between 21-30, 41-50, 51-60 minutes time period were significantly (P<0.05) decreased than control groups. Also, the spike wave frequencies in 4.000 IU Epo dose between 11-20 min time period were significantly (P<0.05) decreased than control groups. However, at 2.000 IU, Epo produced no significant change in the spike-wave frequency of epileptiform activity *(Table 1)*. Additionally, there were no significant differences between all groups of Epo in terms of spike wave amplitude (μV) of penicillin-induced epileptiform activity (P>0.05) *(Table 2)*.

# DISCUSSION

In the present study, we investigated the antiepileptic effects of Epo in penicillin induced epilepsy in rats. This is the first study to demonstrate that Epo has an antiepileptic effect in the penicillin G-induced experimental epilepsy model. Epo at doses of 4.000-6.000 IU/kg inhibited the rate of spikes and spike-waves.

Many researchers [18,19,23] have investigated the effects of Epo in different experimental models of epilepsy. In a kainic acid (KA)-induced seizure model in rats, Kondo et al.[18] used intraventricular infusion of anti-Epo antibody

**Table 2.** *Spike-wave amplitudes (μV) at each dose during each period (mean±SD and P values)*
**Tablo 2.** *Farklı dozlarda uygulanan EPO nun, her bir periyot aralığındaki diken dalga amplitütleri (μV) (ortalama±SD ve P değerleri)*

| Treatment Period (min) | Erythropoietin Treatment (dose)[1] | | | | P Value[2] |
|---|---|---|---|---|---|
| | Control Group (n:10) | 2.000 IU/kg (n:10) | 4.000 IU/kg (n:9) | 6.000 IU/kg (n:10) | |
| Baseline | 0.66±0.2 | 0.96±0.4 | 0.85±0.2 | 0.85±0.4 | - |
| 1-10 | 0.71±0.3 | 0.99±0.4 | 0.89±0.3 | 0.86±0.4 | 0.979 |
| 11-20 | 0.71±0.3 | 0.88±0.3 | 0.87±0.3 | 0.82±0.3 | 0.713 |
| 21-30 | 0.75±0.3 | 0.84±0.2 | 0.88±0.4 | 0.78±0.4 | 0.598 |
| 31-40 | 0.75±0.3 | 0.77±0.3 | 0.85±0.4 | 0.78±0.4 | 0.533 |
| 41-50 | 0.74±0.3 | 0.65±0.4 | 0.80±0.4 | 0.69±0.2 | 0.319 |
| 51-60 | 0.76±0.3 | 0.60±0.4 | 0.61±0.6 | 0.49±0.1 | 0.266 |
| 61-70 | 0.76±0.2 | 0.60±0.5 | 0.55±0.6 | 0.46±0.2 | 0.226 |
| 71-80 | 0.72±0.2 | 0.58±0.4 | 0.50±0.5 | 0.39±0.2 | 0.190 |
| 81-90 | 0.67±0,2 | 0.56±0.4 | 0.45±0.4 | 0.30±0.2 | 0.182 |
| 91-100 | 0.65±0.2 | 0.50±0.5 | 0.43±0.4 | 0.26±0.2 | 0.168 |
| 101-110 | 0.62±0.2 | 0.49±0.5 | 0.41±0.4 | 0,32±0,2 | 0.231 |
| 111-120 | 0.69±0.2 | 0.48±0.5 | 0.41±0.4 | 0,28±0,2 | 0.110 |

[1]*Values are the mean± SD for rats in each group,* [2]*Statistical significance; P<0.05*

to reveal the antiepileptic effect of endogenous Epo and intraventricular infusion of anti-neuropeptide Y antagonist to eliminate the neuroprotective effect of exogenous Epo. Chu et al.[19] studied the effects of Epo (5.000 IU/kg) in a lithium-pilocarpine-induced status epilepticus (SE) model and reported that Epo administration during the latent period following SE prevented BBB leakage, neuronal death, and microglia activation in the dentate hilus, CA1, and CA3; inhibited the generation of ectopic granule cells in the hilus and new glia in CA1; and reduced the risk for developing spontaneous recurrent seizures. Another study [23] also demonstrated that Epo administration reduced seizure activity in the lithium-pilocarpine-induced SE model. Sozmen et al.[22] showed that Epo significantly decreased neuronal cell death in CA1, CA2, CA3, and the dentate gyrus of the hippocampus.

In literature, it has been shown that Epo/erythropoietin receptors (EpoR) has anti-toxic, anti-oxidant, anti-inflammatory and anti-apoptatic effects in different tissues [24,25] *in vivo* and *in vitro* studies. The Epo/ EpoR plays an important role in neurodevelopment and neuroprotection. Also, it is thought that Epo increases the choline acetyltransferase enzyme activity and reduce the epileptic activity with cholinergic effects in neurons [26]. Also, Epo has neurotrophic properties for neuronal stem cell mobilization in damaged regions [27]. In the epilepsy process, cellular events underlying the neuroprotective effects of Epo are dependent on an increase in the total number of (EpoR) and anti-apoptatic (Bcl-2, Bcl-w) molecules, and the total number of pro-apoptatic (Bim, Bid) molecules in hippocampal neurons [28]. Furthermore, Sargin et al.[29] have determined that early intervention with Epo prevents microgliosis caused by neurodegenerative changes. Won et al.[30] demonstrated that Epo protects spinal GABAergic neurons against KA-excitotoxic damage in rat spinal cord cell cultures. They [30] found that post-treatment with Epo for 48 h after KA-induced injury remarkably enhanced the expression of EpoR and glutamate decarboxylase 67, which is an isoform of a GABA-producing enzyme diminished by KA. They suggested that the neuroprotective effect of post-treatment Epo on the GABAergic neurons is mediated by signal transduction involving the EpoR-dependent Janus kinase 2 pathway. We showed an anticonvulsant effect of Epo in a penicillin-induced epilepsy model by antagonizing suppressed GABA inhibition. In addition, Morishita et al.[31] reported that Epo protected cultured neurons from glutamate neurotoxicity mediated by N-methyl-D-aspartate receptors, in a dose and time dependent manner; glutamate-dependent neuronal cell death was reduced by low Epo doses administered 24 h before glutamate exposure, whereas high Epo doses were not effective. In a more recent study, pretreatment with Epo 24 h before the experiment antagonized glutamate-mediated astrocyte water permeability in mice, thereby reducing neurological symptoms [32].

Attempts have been made to explain the mechanism of action of Epo in experimental models of epilepsy. Our observations provide direct evidence that Epo has dose-dependent antiepileptic effects in a penicillin G induced epilepsy model. We revealed that Epo at doses of 4.000-6.000 IU/kg was effective in reducing the frequency, without changing the amplitude, in this model of epilepsy. However, Epo may produce different results in different experimental epilepsy models, in different brain areas, with

different routes of administration, or at different treatment doses. Our findings represent a first attempt to study the antiepileptic role of Epo in penicillin-induced epilepsy in rats. Epo clearly decreased the frequency of penicillin-induced epileptiform activity in a dose-dependent manner, without changing the amplitude of epileptiform activity.

Further studies are needed to clarify the exact mechanisms of Epo at the cellular and molecular levels in various experimental animal models. Epo may be the most promising agent identified thus far for neuroprotection and neuroregeneration in many neurological and psychiatric conditions.

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## Conflict of Interest

The authors declare that there are no conflicts of interest. All authors approved the final manuscript.

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