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EXPERIMENTAL STUDY / DENEYSEL ÇALIŞMA

The Effect of Varying Doses of Intravenous Paracetamol on the Electrical Activity of the Brain in Penicillin-Induced Status Epilepticus in Rats

Sıçanlarda Penisilin ile Oluşturulan Status Epileptikus Modelinde Farklı Dozlarda Uygulanan İntravenöz Parasetamolün Beyin Elektriksel Aktivitesine Olan Etkisi

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Summary

Objectives: Paracetamol is a widely used analgesic and antipyretic agent. It has been reported that N-arachidonoyl-phenolamine, the active metabolite of paracetamol, reduces epilepsy by activating the endocannabinoid system in some models of experimental epilepsy. Diazepam is a benzodiazepine well known to have anticonvulsant effects. The aim of the present study was to investigate the effects of different doses of paracetamol on penicillin-induced epileptiform activity (PIEA) in rats.

Methods: Rats anesthetized with urethane (1.25 g/kg, intraperitoneal) were placed in a stereotaxic frame. Body temperatures were maintained at 37°C by a heating blanket. An epileptic focus was produced by 500 IU Penicillin G (PGP) injection into the soma-motor cortex using a hole drilled into the cranium. Paracetamol (100, 150 and 300 mg/kg, respectively) and diazepam (5 mg/kg) were administered thirty minutes after PGP injection, and their effects on the epileptiform activity were examined comparatively. Electrocorticographic activity was monitored for two hours.

Results: Intracortical injection of PGP (500 units) induced epileptiform activity in all groups of rats. Diazepam caused a statistical significant decrease in the epileptiform activity in the 40th minute after PGP injection. Paracetamol (100 mg/kg) application did not influence the PIEA ($p>0.05$). However, 150 and 300 mg/kg IV paracetamol had a statistically significant effect on the antiepileptic activity ($p<0.001$).

Conclusion: The results of the present study indicated that 150 and 300 mg/kg doses of paracetamol had an effect on PIEA. Further studies are needed to understand the reasons for this effect.

Key words: Acetaminophen; diazepam; epilepsy; paracetamol; penicillin.

Özet

Amaç: Parasetamol yaygın olarak kullanılan analjezik ve antipiretik bir ajandır Parasetamolün aktif metaboliti olan N-araşidonil-fenolamin bazı deneysel epilepsi modellerinde endokannabinoid sistemi aktive ederek epileptik aktiviteyi azalttığı bildirilmiştir. Diazepam antikonvülzan etkileri iyi bilinen bir benzodiazepindir. Bu çalışmanın amacı, sıçanlarda penisiline bağlı gelişen epilepside, farklı dozlardaki parasetamol ve diazepamın etkilerini karşılaştırmaktır.

Gereç ve Yöntem: Üretan (1.25 g/kg, intraperitoneal) anestezisi altındaki sıçanlar stereotaksiki cihazına yerleştirildi. Vücut ısısı bir ısıtıcı battaniye ile 37°C'de muhafaza edildi. Kraniyuma açılan bir delik içinden somatomotor kortekse 500 IU penisilin G (PGP) enjeksiyonu ile epileptik odak oluşturuldu. Parasetamol 100, 150 ve 300 mg/kg ve diazepam 5 mg/kg dozlarında epileptiform aktivite üzerine karşılaştırmalı etkileri penisilin enjeksiyonunda 30 dakika sonra uygulanarak incelendi. Elektrokortigografi aktivitesi iki saat süreyle izlendi.

Bulgular: İntrakortikal PGP (500 IU) enjeksiyonu tüm gruplarda epileptiform aktivite oluşturdu. Diazepam (5 mg/kg, intravenöz) penisilin enjeksiyonundan sonraki 40. dakikada epileptiform aktivitede istatistiksel olarak anlamlı derecede azalma sağladı. Parasetamolün 100 mg/kg dozunda penisilin ile oluşturulan epileptiform aktiviteye etkisi yoktu ($p>0.05$). Ancak 150 ve 300 mg/kg *i.v* parasetamol antiepileptik aktivite üzerinde istatistiksel olarak anlamlı derecede etkili bulundu ($p<0.001$).

Sonuç: Bu çalışmanın sonuçları parasetamolün 150 ve 300 mg/kg dozlarının penisiline bağlı epileptiform aktivite üzerinde etkisi olduğuna işaret etmektedir. Bu etkinin nedenlerini anlamaya yönelik ileri çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Asetaminofen; diazepam; epilepsi; parasetamol; penisilin.

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Introduction

Epilepsy is a seizure disease characterized by partially or totally synchronized abnormal electrical activity of the cerebral neurons.

It is a limited-time epileptic activity (extreme and/or hypersynchronous) of the neurons in the brain. Epileptic seizures occur upon a disruption in the coordination between inhibition and excitation of the central system (CS). The prevalence of epilepsy is 0.7% of the population, and seizures tend to continue in 20-30% of those receiving medical therapy. Despite having many antiepileptic drugs (AEDs), new molecules and drugs are required to fill the treatment gaps. The incidence of status epilepticus (SE) is between 0.01% and 0.041%. Anticonvulsant drugs fail to end SE in 31-50% of the cases.[1-3]

In resistant cases, continuous IV anesthetics having serious side effects such as respiratory depression, arterial hypotension and immunosupression may be necessary in treatment arrangements.[4-6] For this reason, rigorous attention should be paid while administering anesthesia in generalized convulsive SE.[7] Anticonvulsant drugs known to be effective in SE, such as benzodiazepines, propofol and barbiturates that enhance gamma-aminobutyric acid (GABA)-mediated neuronal inhibition, may be ineffective too.[8-10] Alternative treatment options have become available for cases where anticonvulsant treatments are ineffective.

In a study conducted in 1987 and utilizing generalized and focal models of epilepsy induced by intraperitoneal (IP) and IC PGP, the effects of paracetamol (IP 150-140 mg/kg), along with some other non-steroidal anti inflammatuar drugs (NSAIDs), were examined and it was shown that paracetamol decreased seizure frequency significantly, delayed or blocked spike activity and seizure onset and lessened ECoG voltage output.[11] Similarly, in a study employing an epilepsy model induced by pentylenetetrazole (PTZ), it was shown that paracetamol (300 or 450 mg/kg) was effective in decreasing seizure frequency in rats.[12]

The aim of the present study was examine the effects of different doses of IV paracetamol on epileptic activity in IC PGP-induced ESE animal models. In cases having a high morbidity and mortality such as SE, additional benefit to be gained from paracetamol which is a cheap, reliable, easily applicable drug would be very important.

Materials and Methods

In the present study where the experimental animals were approved by the ethical committee, 35 male Wistar Albino rats aged between 12-16 weeks and weighing 200±50 gr were used. Rats were obtained from our Experimental Animals Laboratory and kept at standard laboratory conditions (12 hours day and night rhythm, room temperature 20-22°C). The rats were kept in plastic cages (7 animals/ cage) and were allowed free access to food and water throughout the experiment. The rats that did not meet the experimental research standards were excluded from the study. At the end of the procedure, the animals were sacrified using an intracardiac air injection. During the procedure, rectal body temperatures were monitored and kept at 37°C using an electrical blanket (Harward Homeothermic Blanket System). After numbering the animals from 1 to 35, the animals were divided into 5 groups (each containing 7 animals) by simple random sampling method.

Groups:
1st Group (Control Group): No medication. IV 1 ml SF administration
2nd Group: (5 mg/kg IV diazepam)
3rd Group: (100 mg/kg IV paracetamol)
4th Group: (150 mg/kg IV paracetamol)
5th Group: (300 mg/kg IV paracetamol)

All the invasive procedures were carried out under anesthesia. The rats not meeting the experimental research standards were excluded. Anesthesia was induced by 1.25 g/kg IV urethane (25% solution) prepared just before the experiment. Distilled water was used as a solvent. After the onset of anesthesia, the heads of the rats were shaved and rats were placed in a stereotaxic frame. On the head skin, 4-5 cm incisions were made along the midline rostra-caudal level of the scalp. After removing the tendons and fascia, small bleeding points were coagulated with an electrocautery probe. A total of 3 holes (1 for recording, 1 as a reference (negative), 1 for IC PGP) having 2 mm diameters each were opened using a surgical drill on the left hemisphere of the scalp with the coordinates given below.

Hole no 1 (for the recording electrode) was opened at the intersection 1 mm to the anterior of bregma and 2 mm lateral to the sagital suture, hole no 2 (for the reference electrode) was opened at the intersection 5 mm posterior to bregma

and 2 mm lateral to the sagittal suture while hole no 3 (for IC PGP) was opened at the intersection 3 mm lateral and 2 mm posterior to bregma. While drilling the holes, necessary care was taken not to harm the brain but drill only the scalp. The activity recorded by the electrodes was transferred online to Biopac System MP150 electrocardiography equipment data recording system. The analog signals obtained from the cortex by Biopac System MP150 were turned into numerical data and sent to a computer using a USB cable. The brain activity was viewed online using AcqKnowledge® (v: 3.02) and recorded on a computer to use during post-experiment analysis. IC injections to the brain were performed 3 mm lateral, 2 mm posterior and 4 mm ventral to the bregma using a Hamilton micro-injector. During injections, necessary care was taken not to damage the vein with the tip of the injector.

ESE was induced by IC PGP injection under anesthesia in all the animals including those in the control group. In order to create an epileptiform activity, PGP (500 unit (IU) dose and 200 IU/µl) was administered in a total volume of 2.5 µl from the third hole (3 mm lateral, 2 mm posterior to the bregma) opened on the left cortex by positioning the Hamilton micro-injector 4-5 mm long ventrally. Intravenous administrations were carried out by inserting 24 G intracatheter into the tail veins of the rats 30 minutes after IC PGP administrations.

First of all, the baseline activities of all the rats used in the experiments were recorded. In order to see whether IC injection caused any change in the brain activity, SF was administered to the somatomotor cortex using the coordinates of the IC PGP injection. When compared to the baseline recording, it was seen that there was no change in the brain ECoG activity records of the SF group. 2-4 minutes after PGP injection, an epileptiform activity characterized by bilateral spikes and spike-wave complexes in ECoG occurred. The frequencies of the spikes became stable in 20-30 minutes and the activity lasted for 3-4 hours. As the epileptiform activity occurring as a result of PGP injection became stable in 20-30 minutes, the mean of the values obtained during the 10-minute period between the 20th and 30th minute of PGP injection were recorded as the 1st minute value, and then the spike frequency averages were recorded for a period of 1 minute at every 10 minutes. Statistical analyses were carried out using the values recorded for a period of 1 minute, and time indicators at the graphics were adjusted accordingly.

Statistical analysis

Using AcqKnowledge® software (v: 3.02) and its macro features, the electrophysiological recordings were segmented into one minute time periods and number of spikes per minute were calculated. The same procedure was repeated for all the recordings obtained from all the animals. After turning all the electrophysiological recordings into numerical data, these data were evaluated statistically using SPSS v12.0. In all the groups, ANOVA and Post Hoc LSD tests were used to analyze the spike frequency values. The values of

Table 1. The mean spike frequencies recorded in each group (mean frequency±SEM)

Groups	Mean±SEM			
	n	Before	Min.10	Min.90
I. group	5	41.60±4.19	40.60±3.50	37.00±8.03
II. group	6	36.50±3.99	22.16±2.80	9.33±5.43
III. group	6	37.83±4.57	35.16±5.19	28.83±9.57
IV. group	7	37.81±1.79	28.77±2.32	10.72±1.65
V. group	7	35.31±1.09	26.22±2.11	10.87±1.12

1st Group (Control Group): No medication
2nd Group: 5 mg/kg IV diazepam
3rd Group: 100 mg/kg IV paracetamol
4th Group: 150 mg/kg IV paracetamol
5th Group: 300 mg/kg IV paracetamol

As the epileptiform activity occurring as a result of PGP injection became stable in 20-30 minutes, the mean of the values obtained during the 10-minute period between the 20th and 30th minute of PGP injection were recorded as the 1st minute value, and then the spike frequency means were recorded for a period of 1 minute at every 10 minutes.

the study group are stated in the graphics and the text as mean±SEM (Standard Error of Mean). Differences where p value was below 0.05 based on the results obtained in the tests were regarded as significant.

Results

Epileptiform activity was induced by IC PGP injection in all the groups. Within 3-5 minutes after PGP injection, an epileptiform activity characterized with bilateral spikes and spike wave complexes started. The epileptiform activity became stable in a mean of 30 minutes and lasted for a mean of 3-4 hours.

As it took 20-30 minutes for the PGP-induced epileptiform activity to become stable, the mean of the values obtained during the 10-minute period between the 20th and 30th minute of PGP injection was recorded as the 1st minute value while assessing the spike frequency, and then the spike frequency means were recorded for a period of 1 minute at every 10 minutes. Statistical analyses were carried out using the values recorded for a period of 1 minute, and time indicators at the graphics were adjusted accordingly. The mean spike frequencies recorded at every 10 minute in each group are shown as the mean frequency±SEM in Table 1.

In PGP+Diazepam group, 5 mg/kg IV diazepam was administered by the tail vein of the rats 30 minutes after IC PGP injection. Starting from the 15th minute of diazepam injection, epileptiform activity was lowered significantly in the diazepam group when compared to the control group ($p<0.05$). The mean spike frequency (spike/

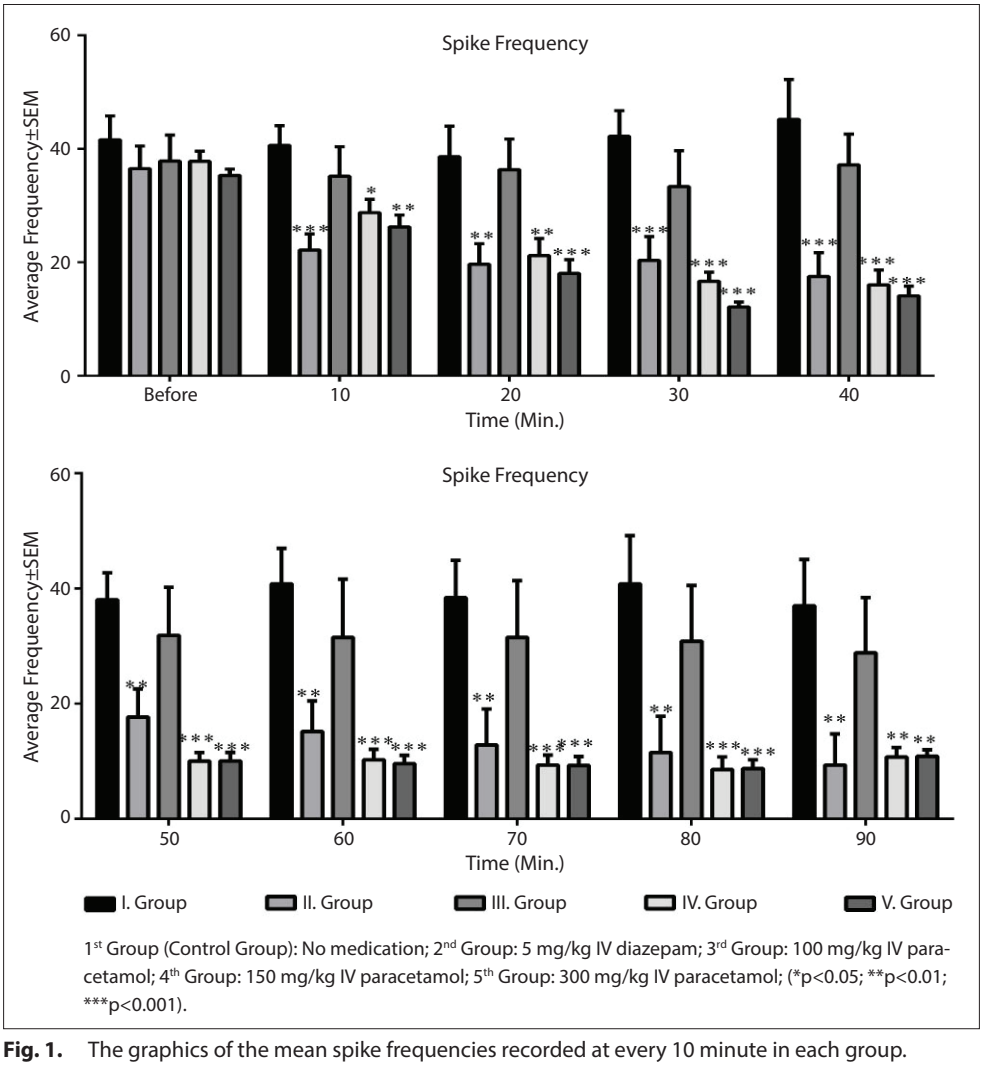

Fig. 1. The graphics of the mean spike frequencies recorded at every 10 minute in each group.

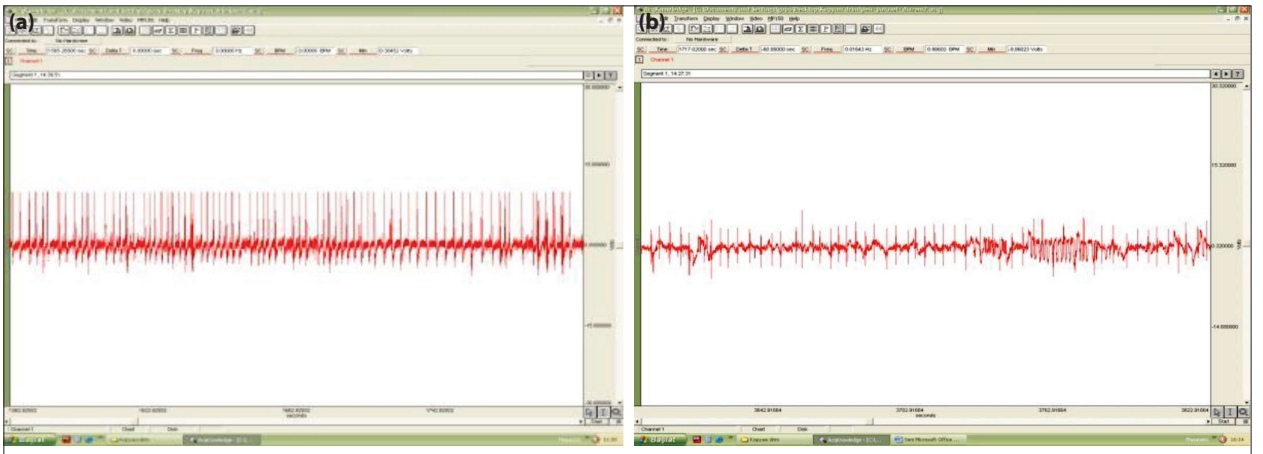

Fig. 2. Before the treatment with 300 mg/kg IV paracetamol **(a)** and after **(b)** samples of the ECoG recordings.

min., mean±SEM) was 40.60±3.5 in the control group and 22.16±2.8 in the diazepam group (Table 1). The statistically significant decline in the epileptic activity continued until the end of the experiment (Figure 1).

In PGP±100 mg/kg IV paracetamol group, 100 mg/kg paracetamol was administered 30 minutes after IC PGP injection. Compared to the control group, there was no statistically significant difference in epileptic activity during the 90 minutes recording time ($p>0.05$). The mean spike frequency (spike/min., mean±SEM) was 40.6±3.5 in the control group and 35.16±5.2 in PGP+100 mg/kg IV paracetamol group (Table 1, Figure 1).

Compared to the control group, 150 and 300 mg/kg IV paracetamol lowered epileptiform activity statistically significantly starting from 10 minutes after paracetamol injection in the paracetamol group ($p<0.05$). While the mean 10 min. spike frequency (spike/min., mean±SEM) was 40.60±3.5 in the control group, it was 28.77±2.32 ($p<0.05$) in the 150 mg/kg IV paracetamol group, and 26.22±2.11 ($p<0.01$) in the 300 mg/kg IV paracetamol group ($p<0.01$). The statistically significant decline in the epileptic activity continued until the end of the experiment (Table 1, Figure 1, Figure 2).

Discussion

In the present study, epileptic spikes and spike-wave complexes were observe 2-4 minutes after injecting 500 IU PGP IC. The epileptic activity became stable in 30 minutes and remained stable for more than 3 hours. During the time from PGP injection to epilepsy onset, no statistically significant difference was observed between the groups ($p>0.05$). Similarly, the groups did not show any difference in terms of the spike frequency recorded during the 30 minutes after PGP injection ($p>0.05$). There are many studies in the literature on PGP-induced epileptiform activity and its features.[13,14] This has been approved through many studies conducted in our country too.[15-18] The features of the epileptiform activity obtained by IC injection of PGP in the present study were consistent with the data present in the literature. ESE model obtained by PGP partially resembles to the seizures in humans.[19]

In acquired epilepsy cases, physiological and/or biochemical changes that form a base for seizure induction in the brain occur. During the process, the anatomical, physiological and biochemical changes in the CS result in a chronic epileptic condition called "epileptogenesis". None of the AEDs known today is effective in preventing the epileptogenesis process.[20]

There are various types of experimental models used in epilepsy studies. Among these models, models induced chemically by convulsant agents (PTZ, bicuculline, picrotoxin, PGP, etc.) or by electrical stimulation in animals genetically predisposed to epilepsy are used frequently.[21] According to Edmonds et al.,[19] the advantages of penicillin model in experimental epilepsy are as follows:

1. PGP causes focal seizures in vertebrate ranging from fish

to man.
2. Seizure inducing is rapid and easily registered. The activity begins in the initial 15 minutes after the application and continues for a number of hours that follow.
3. Photomorphological changes after the local application of penicillin are rarely found.
4. The speed of activity spreading from the focus and intensity of clinical manifestation of the seizure are directly dependent on the dosage of penicillin administered.
5. Penicillin induced seizures are not resistant to anticonvulsants.
6. The indicated seizure completely disappears 24 hours after the application of penicillin.[19]

The PGP activity we obtained in our study was consistent with the criteria listed above. In a study conducted in 1987 and utilizing generalized and focal models of epilepsy induced by IP and IC PGP, the effects of paracetamol (IP 150-140 mg/kg), along with some other NSAIDs, were examined and it was shown that paracetamol decreased seizure frequency, delayed or blocked spike activity and seizure onset and lessened electrocortical voltage output significantly.[11] In a similar study employing an epilepsy model obtained by administration of PTZ, it was shown that paracetamol (300 or 450 mg/kg) was effective in decreasing seizure frequency in rats.[12]

In terms of the spike frequency of the epileptic activity created by PGP, it was seen that there was no statistically significant difference only between the paracetamol 100 mg/kg group and the controls. However, the spike frequency of the diazepam, paracetamol 150 and 300 mg/kg groups decreased to such an extent that there was a significant difference between the groups and the paracetamol 100 mg/kg group. In PGP-induced epilepsy models employed in previous years, administration of paracetamol before the onset of seizure activity was considered as a preventive treatment and IV 150 mg/kg paracetamol administration yielded an antiepileptic activity having a statistically significant level. However, with a more apparent effect in high doses such as 300-450 mg/g, paracetamol has been observed to prolong the onset of seizure activity and decrease the frequency of spike activity for approximately 30 minutes.[22]

Moreover, the effect of paracetamol on the epileptiform activity was compared to that of penicillin in different dose groups. Diazepam, whose anti-epileptiform effect is definitely known, is a drug used in comparative groups in many studies. As it is known that diazepam statistically significantly decrease the epileptiform spike frequency induced by IV PGP, a paracetamol group and a diazepam group was created to compare the effect of paracetamol on the spike frequency.

As a result, 150 and 300 mg/kg dosed of paracetamol have positive effects on PIEA. Further studies are needed to understand the reasons for this effect.

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