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Research Article/ Araştırma Makalesi

The Effect of Ceftriaxone on Penicillin-Induced Epileptiform Activity in Rats: An Electrophysiological Study

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Purpose: Epilepsy is a set of chronic neurological disorders characterized by seizures associated with abnormal and uncontrolled neuronal activity of the brain. Glutamate is the main excitatory neurotransmitter in the central nervous system. Excitatory amino acid transporter-2 (EAAT2), one of the major glutamate transporters, is responsible for total glutamate intake. Ceftriaxone is a β-lactam antibiotic that increases EAAT-2 expression and functional activity. This study aims to investigate the effects of ceftriaxone on penicillin-induced epileptiform activity by using electrocorticography (ECoG) in anesthetized rats.

Method: In this study, 35 Wistar male rats were used. The rats were divided into five groups of 7. In group 1, 2.5 µL 500 IU of penicillin intracranially (i.c.) and 1 ml saline solution and intraperitoneally (i.p.) were given, respectively. In group 2, 200 mg/kg, i.p. of ceftriaxone was administered 30 minutes after penicillin. In group 3, 400 mg/kg of ceftriaxone was administered i.p. 30 minutes after penicillin. 500 mg/kg of sodium valproate was administered i.p. following 30 minutes of penicillin in group 4. In group 5, 400 mg/kg, i.p. of ceftriaxone and 500 mg/kg, i.p. of sodium valproate were administered 30 minutes after penicillin. After the surgical procedure the rats were placed in a stereotaxic device and electrocorticogram recordings were captured for 210 minutes.

Results: The acute treatment of ceftriaxone reduced spike-wave frequency and spike-wave amplitude of penicillin-induced epileptiform activity in the rats.

Conclusion: These findings suggest that acute ceftriaxone had an anticonvulsant effect on penicillin-induced focal onset epileptic activity. Ceftriaxone may have an anti-epileptogenic potential.

Keywords: Experimental epilepsy, Ceftriaxone, Electrocorticography

1.INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by seizures associated with abnormal and uncontrolled neuronal activity in the brain.[1] It affects about 1% of the world's population.[2] Although many antiepileptic drugs are used, they can't prevent seizures in 20-30% of patients.[3] Glutamate, the main excitatory neurotransmitter in the central nervous system (CNS), is the most abundant amino acid in the mammalian brain.[4] Glutamate is essential in many processes, such as learning, memory, cognition, and emotion.[5] Regulation of extracellular glutamate levels is necessary to maintain appropriate neuronal activity and function. In epilepsy, there is potential dysregulation of glutamatergic mechanisms and dysfunction of neuronal, glial, and/or neuronal-glial interactions. Glutamate is removed from the synaptic cleft by several high-affinity excitatory amino acid transporters (EAAT1-5). EAAT1 and EAAT2 are expressed in astrocytes and glial cell types.[6] It has been shown that mRNA and EAAT2 protein levels are decreased in the hippocampi of drug-resistant temporal lobe epilepsy patients with hippocampal sclerosis.[7] Homozygous EAAT2-deficient mice are characterized by increased extracellular glutamate concentration in the brain and show fatal spontaneous seizures.[8] Similar changes in EAAT2 expres-

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sion were seen in some different animal models of epilepsy.[9,10] Furthermore, it is noteworthy that ceftriaxone exerts an impact on EAAT2 expression. Ceftriaxone, a third-generation cephalosporin in the group of β-lactam antibiotics, is frequently used in skin and soft tissue infections, meningitis, pneumonia, and hospital-acquired infections.[11] It is of great interest as it modifies the course of various neurodegenerative diseases with multiple mechanisms. Ceftriaxone treatment has been shown to increase EAAT2 expression and cell viability and reduce glutamate-induced apoptotic cell death in primary rat cortical cell cultures.[12] It has been shown that ceftriaxone causes anticonvulsant effects by activating EAAT2, removing glutamate, and decreasing glutamate's concentration in the synaptic cleft.[13] EAAT2 is also known as glutamate transporter 1 (GLT-1). It is a protein that is a glutamate transporter in the CNS. GLT-1 is primarily expressed in astrocytes, which are non-neuronal cells in the brain. GLT-1 eliminates the neurotransmitter glutamate's potentially harmful effects by removing it from the synaptic cleft. The stimulation of GLT-1 expression by ceftriaxone is thought to rely on NF-kappa B (NF-κB, Nuclear Factor kappa B). When NF-kB is activated, it binds to the GLT-1 promoter region and increases transcription of this gene,[14] thereby reducing the concentration of glutamate in the synaptic cleft and decreasing the possible neurotoxic consequences of excessive glutamate.[15] Additionally, the ceftriaxone treatment has been shown in specific trials to reduce neuronal damage and enhance spatial memory and learning.[16] It has also established a beneficial effect in a model of pentylenetetrazole (PTZ)-induced convulsions.[17,18] There are also studies that cannot confirm the anticonvulsant effect of ceftriaxone.[19]

Animal models used in experimental studies of epilepsy shed light on its pathogenesis.[20] Experimental epilepsy induction is performed with penicillin applied topically or intracortically to the surface of the cortex. Penicillin induces acute focal epileptic activity similar to decreasing the activity of the γ-aminobutyric acid (GABA) inhibitory system in the brain and increasing glutamate, which has become the main excitatory neurotransmitter in the brain.[21] Penicillin diminishes the inhibitory effect of GABA, leading to overstimulation of nerve cells.[22] In this scenario, the impact of glutamate becomes more pronounced. Glutamate is the main excitatory neurotransmitter in the central nervous system and usually enhances nerve transmission. Due to the diminished inhibitory effect of GABA caused by penicillin, the effect of glutamate is amplified, paving the way for epileptic activity. Consequently, penicillin triggers epileptic activity by disrupting the balance of neurotransmitter levels in the brain.[23] This type of epileptiform activity model is frequently employed in laboratory studies to comprehend epilepsy mechanisms and investigate the effects of antiepileptic treatments.

The aim of this study is to investigate the effects of ceftriaxone on penicillin-induced epileptiform activity by using electrocorticography (ECoG) in rats.

2. MATERIALS and METHODS

2.1.Animals

In this study, 35 Wistar albino male rats weighing 200-250 g were used. Rats were housed in a 12-hour light-dark cycle (light between 07:00 and 19:00) and quiet rooms at 22-24°C ambient temperature. They were fed ad libitum with standard laboratory chow and tap water.

The animals were randomly divided into five groups (n=7) as follows.

(1) Penicillin (500 IU, 2.5 µl, intracranially (i.c.)),

saline solution 1 ml intraperitoneally (i.p.)

(2) Ceftriaxone (200 mg/kg, i.p.), penicillin (500 IU, 2.5 µl, i.c.)

(3) Ceftriaxone (400 mg/kg, i.p.), penicillin (500 IU, 2.5 µl, i.c.)

(4) Sodium valproate (500 mg/kg i.p.) plus penicillin (500 IU, 2.5 µl, i.c.)

(5) Ceftriaxone (400 mg/kg i.p.), sodium valproate (500 mg/kg i.p.), penicillin (500 IU, 2.5 µl, i.c.).

All experimental procedures were carried out based on the principles set in the European Union Directive (2010/63/EU). The experimental procedures of the study were approved by the Ethics Committee of the Tokat Gaziosmanpasa University, Tokat (2020-HADYEK-25).

2.2.Chemicals

1.25 mg/kg urethane (Sigma-Aldrich, USA) dissolved in distilled water 30 minutes before administering penicillin (25% solution) i.p. Penicillin G Potassium (Pen-G 1.000.000 IU vial, I.E., Ulagay, Turkey) is dissolved in distilled water to a concentration of 500 IU. to produce epileptiform activity. It will be in a volume of 2.5 µL administered i.c. 500 mg/kg sodium valproate (Depakin 400 mg/4 mL ampoule, Sanofi, France) and ceftriaxone 200 mg/kg and 400 mg/kg (Novosef, 1 g vial, Zentiva, Czechia) were dissolved with distilled water at the appropriate concentration and injected i.p.[24] Additionally, they were administered 30 minutes after the injection of penicillin.

2.3.Surgical Procedure

After anesthesia with urethane, the rats were placed in the stereotaxic device and fixed (Harvard Stereotaxic Instrument). Then an incision was made approximately 3 cm in the rostrocaudal plane. After the soft tissue over the left somatomotor cortex was removed, the skull bone was removed by thinning with a touring engine. For electrophysiological recordings, two Ag/AgCl ball electrodes were utilized, and one Ag/AgCl clamp electrode was used for grounding purposes. The positive electrode was placed 1 mm anterior to the bregma, 2 mm lateral to the sagittal suture, and the negative electrode was placed 5 mm posterior to the bregma and 2 mm lateral to the sagittal suture. A ground electrode was applied to the right ear. The rats' body temperature was kept at 37 °C throughout the experiment with a homeothermic blanket attached to a rectal probe (Harvard Instrument, USA). The preoperative and postoperative periods involved continuous monitoring of electrocorticographic (ECoG) activity, with the researcher actively observing and intervening to address any potential complications. Detailed observations were conducted by the researcher to identify possible complications during surgical procedures. In the event of any adverse effects attributed to the surgical intervention, immediate intervention by the researcher was implemented to ensure the improvement of records' quality and reliability after the surgical procedure.

The activity was recorded with electrodes placed on the MP 150-CE (Biopac Systems, USA) interface, upgraded to the MP 150 EEG-100C (Biopac Systems, USA), and transferred to the data recording system. The analog signals received from the cortex were converted into a digital value with the MP 150. Then it was transferred to the computer with the help of a USB cable. Brain activity viewed with AcqKnowledge 3.9.1 (Biopac Systems, USA) software. After the registration period ended, the frequency and amplitude of the epileptiform activity recordings were analyzed.

2.4.Induction of Epileptiform Activity

While the rats were in the stereotaxic unit, 2.5 µL of penicillin dissolved in distilled water was administered to the left somatomotor cortex, 3 mm lateral, 2 mm posterior, and 2 mm ventral from bregma[24] using a Hamilton microinjector (710 SNR, infusion rate 0.5 µL/min). The epileptiform activity caused by the internal administration of penicillin started to be recorded 1-2 minutes after the injection. First-group rats were given penicillin and saline solution. After 30 minutes of penicillin, the second and third groups were given 200 and 400 mg/kg of ceftriaxone, respectively, i.p. The fourth group was given sodium valproate after 30 minutes of penicillin. 30 minutes after penicillin administration, ceftriaxone 400 mg/kg, i.p. was given from the right, and sodium valproate 500 mg/kg, i.p. was given on the left to the 5th group.

2.5.Electrophysiological Processes

The experimental procedures were performed in the Gaziosmanpaşa University Faculty of Medicine Physiology Laboratory. Animals were brought to the laboratory one day before the experiment to facilitate their adaptation to the new environment and reduce stress. The recording of epileptiform activity induced by the administration of penicillin started after 2 minutes. ECoG activity was recorded for 210 min. Epileptiform activity stabilized after the 20th and 30th minutes of penicillin injection. The average spike and amplitude values between the 20th and 30th minutes of penicillin injection were accepted as the 1st-minute value. After 30 minutes, the averages of the spike frequency and amplitudes of the 1-minute slices were taken at 10-minute intervals. The 180-minute recording obtained 30 minutes after penicillin injection was divided into 10-minute periods. The number of spikes and the average number of spikes per minute were calculated by counting the peak-to-peak amplitudes.

2.6.Statistical Analysis

Statistical analysis was performed using 1-minute values taken at 10-minute intervals. These results were analyzed using the SPSS (Statistical Package for Social Sciences) 26.0 program for Windows. Since there were more than two independent groups, the Kruskall-Wallis test was used to compare continuous quantitative data between groups. Then the Man Whitney-U test was additionally performed to determine the differences. To identify the difference between repeated measurements within groups, the Wilcoxon test was applied. The obtained results were evaluated at a 95% confidence interval and a significance level of 5%. Data for all experimental groups used in the study were expressed as mean ± standard error of the mean (SEM). A p-value below 0.05 was considered significant.

3.RESULTS

3.1.Spike Frequency

In the acute penicillin epilepsy model, the mean spike frequency was 97.50 ± 1.78 spikes/min after penicillin microinjection. The mean spike frequency of the ceftriaxone (200 mg/kg and 400 mg/kg) and sodium valproate plus ceftriaxone 400 mg/kg groups after penicillin injection significantly reduced over 180 minutes (Table 1). The mean spike frequency of epileptiform activity in penicillin plus 200 and 400 mg/kg ceftriaxone groups were 65.21 ± 1.83 and 65.77 ± 2.23 spike/min, respectively. In addition, the mean spike frequency of the ceftriaxone (200 and 400 mg/kg) groups was statistically significantly lower than the group in which sodium valproate was administered after penicillin microinjection ($p<0.001$; 65.21 ± 1.83 spike/min vs. 93.71±2,18 spike/min and 65.77 ± 2.23 spike/min vs. 93.71±2,18 spike/min, respectively). The mean spike frequency of sodium valproate plus ceftriaxone 400 mg/kg group the following penicillin microinjection was significantly

decreased compared to the penicillin plus sodium valproate and penicillin group ($p<0.001$; 65.38 ± 2.52 spike/min vs. 93.71±2,18 spike/min and 65.38±2.52 spike/min vs. 97.50 ± 1.78 spike/min, respectively). The groups receiving ceftriaxone at doses of 200 mg/kg and 400 mg/kg exhibit a statistically significantly lower mean spike frequency compared to the group where sodium valproate is administered following penicillin microinjection. This suggests that ceftriaxone is more effective when compared to sodium valproate. The sodium valproate plus ceftriaxone (400 mg/kg) group demonstrates a statistically significantly lower mean spike frequency when compared to the group receiving sodium valproate after penicillin microinjection, as well as the group receiving only penicillin. This indicates that this combination is more effective than sodium valproate alone or penicillin alone.

The mean spike frequency values and the percentage change in spike frequency according to the groups for 180 minutes were presented in (Table 1, Figure 1) The first spike frequency value of the sodium valproate group after penicillin microinjection was higher than the first spike frequency value of the penicillin group. The 10th-minute spike frequency value of the penicillin-administered ceftriaxone 200 mg/kg group was lower than the 10th-minute spike value of the penicillin group. After the 10th minute, the spike frequency value of the penicillin-administered ceftriaxone 200 mg/kg group started to decrease significantly ($p<0.05$). The spike frequency value of sodium valproate plus ceftriaxone 400 mg/kg administered following the microinjection of penicillin decreased compared to the penicillin group ($p<0.05$). After penicillin microinjection, the 130th-minute spike frequency value of the penicillin plus sodium valproate plus ceftriaxone 400 mg/kg treatment group was lower than the same-minute value of the penicillin-applied sodium valproate group ($p<0.05$). At the same time, the spike frequency value of the penicillin plus sodium valproate plus ceftriaxone 400 mg/kg and alone penicillin plus ceftriaxone 400 mg/kg groups decreased the following 140th-minute compared to both the penicillin and sodium valproate groups following penicillin microinjection. The spike frequency value of the ceftriaxone 200 mg/kg group decreased significantly following 170th-minute after penicillin microinjection ($p<0.05$) (Figure 1). It has been observed that both ceftriaxone groups (200 mg/kg and 400 mg/kg) lead to a significant decrease in the mean spike frequency over 180 minutes compared to the groups treated with penicillin. Particularly, in the ceftriaxone 200 mg/kg group, a noticeable decline has been observed from the 10th minute onwards. The sodium valproate plus ceftriaxone 400 mg/kg group induces a significant reduction in the mean spike frequency over 180 minutes compared to the penicillin group. This combination has proven to be more effective than other treatment groups.

Figure 1.

*Graph of changes in spike Frequency over time in all groups. A statistically significant decrease was found in the ceftriaxone + sodium valproate group at 70 minutes and after (P = .043 < .05) (Multiple comparison tests were used. *P < .05, all groups were compared with the first-minute value).*

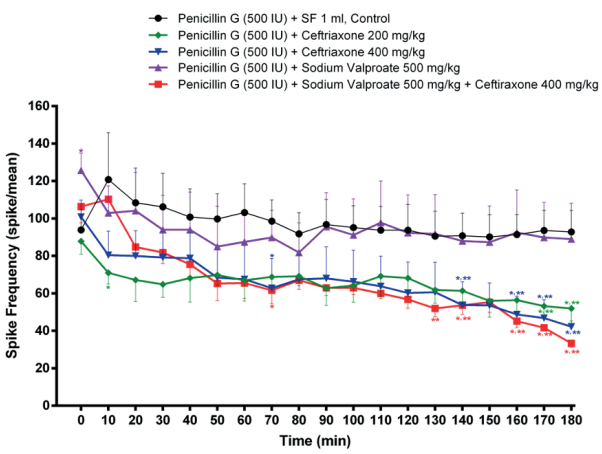


Table 1.
Percentage changes in spike frequency values according to the groups for 180 minutes (spike/min)

Groups	Penicillin	Ceftriaxone 200 mg	Ceftriaxone 400 mg	Sodium valproate 500 mg	Ceftriaxone + Sodium valproate
1st	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
30st	110.78±8.26	73.80±5.37*	76.81±8.77*	72.94±10.3*	74.25±9.82*
60th	108.68±5.87	77.40±11.9*	64.53±13.2*	68.30±9.56*	55.74±9.67*
90th	102.62±5.04	72.04±8.94	66.21±12.9*	74.6±10.59	59.73±12.6*
120th	99.02±5.15	78.44±10.13	58.64±13.4*	71.58±11.70	55.68±12.9*
150th	96.35±5.56	66.62±11.67	55.69±11.3*	67.52±10.8*	49.12±10.5*
180th	99.05±4.39	60.80±8.25*	53.49±11.12*	68.86±10.35*	46.34±9.82*

The mean difference is significant at the 0.05 level.
Values expressed as mean±SD.
* $p<0.05$ different compared to penicillin group,
** $p<0.05$ different compared to penicillin plus sodium valproate group.

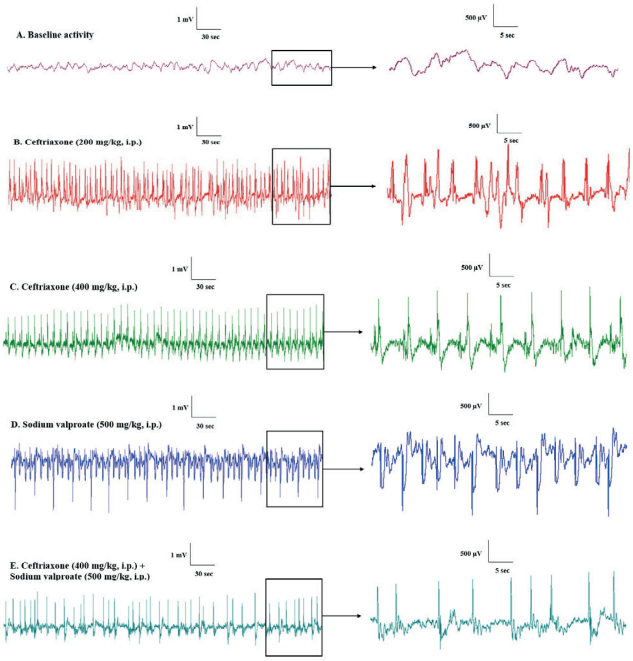
Although the spike frequency values of the penicillin group at all minutes were not significantly different from the first spike frequency value ($p>0.05$) in the penicillin plus ceftriaxone 200 mg/kg and ceftriaxone 400 mg/kg group, those significantly decreased compared to the first spike every 10 minutes for 180 minutes ($p<0.05$). In the sodium valproate applied group after penicillin microinjection, the frequencies of the all-minute spike decreased compared to the baseline, but the frequency of spikes significantly decreased after 60th minutes. Following the microinjection of penicillin, the spike frequency of the group that received ceftriaxone 400 mg/kg plus sodium valproate significantly decreased after 20th minutes compared to the baseline value.

Table 1 shows the comparison of the mean percentage spike change values of the groups. In the groups of sodium valproate, ceftriaxone (200 mg/kg and 400 mg/kg), and sodium valproate plus ceftriaxone 400 mg/kg administered following the microinjection of penicillin, the percentage change in all spikes during 180 minutes was significantly found to be lower than in the penicillin group ($p<0.05$). However, there was no significant percentage change in spike frequency during 180 minutes in the penicillin group (99.05±4.39 spike/min), the 200 and 400 mg/kg ceftriaxone groups after penicillin microinjection displayed a significant decrease in percentage spike frequency of approximately 40% and 47%, respectively in comparison with their baseline (60.80±8.25 spike/min, 53.49±11.12 spike/min, respectively). Furthermore, the percentage spike frequency change in the penicillin-applied sodium valproate groups group is almost 32% compared to baseline (68.86±10.35 spike/min). After penicillin microinjection, the percentage spike frequency of group sodium valproate plus ceftriaxone 400 mg/kg declined by about 54% in comparison with the baseline (46.34±9.82 spike/min). In conclusion, all treatment groups (sodium valproate, ceftriaxone 200 mg/kg, ceftriaxone 400 mg/kg, and sodium valproate plus ceftriaxone 400 mg/kg) exhibit a significant decrease in the rate of spike frequency compared to the penicillin group over the course of 180 minutes. The reduction in spike frequency is particularly pronounced in the ceftriaxone groups.

All group samples from the 60th and 70th minute of ECoG were obtained from epileptiform activity induced by penicillin as shown in Figure 2.

Figure 2.

A) Baseline activity, B) Ceftriaxone 200 mg, C) Ceftriaxone 400 mg, D) Sodium valproate, E) Ceftriaxone + sodium valproate samples from the 60th and 70th minute of ECoG obtained from epileptiform activity induced by penicillin.



3.2.Spike Amplitude

The mean levels of spike amplitude of groups are shown in Figure 3. The mean spike amplitude value of the penicillin group was significantly higher compared to the other groups ($p<0.001$; 0,111 ± 0,01 µV). Following penicillin microinjection, the mean spike amplitudes of ceftriaxone (200 mg/kg and 400 mg/kg), sodium valproate, and sodium valproate plus ceftriaxone 400 mg/kg groups were significantly decreased compared to the penicillin group ($p<0.001$; 0.063 ± 0.01 µV, 0.057 ± 0.01 µV, 0.060 ± 0.01 µV, 0.047± 0.01 µV, respectively). Moreover, the penicillin-applied sodium valproate plus 400 mg/kg ceftriaxone group mean spike amplitudes were lower than those of the penicillin plus ceftriaxone (200 mg/kg and 400 mg/kg) and sodium valproate groups ($p<0.05$; 0,047±0,01 µV). The mean spike amplitude values and spike amplitude percent change for each group throughout 180 minutes are displayed in Table 2, Figure 3. After penicillin microinjection, the mean amplitude values of the ceftriaxone (200 mg/kg and 400 mg/kg), sodium valproate, and sodium valproate plus ceftriaxone 400 mg/kg groups for 180 minutes for 10 minutes each were significantly lower than the mean amplitude values of the penicillin group ($p<0.05$). Furthermore, the spike amplitude mean of the penicillin plus sodium valproate plus 400 mg/kg ceftriaxone group was lower than the spike amplitudes of the penicillin-administered 200 mg/kg ceftriaxone and sodium valproate groups over 180 minutes ($p<0.05$). In summary, penicillin administration resulted in increased spike amplitude, and the subsequent administration of ceftriaxone, sodium valproate, and their combination led to significant decreases in spike amplitude compared to the penicillin group.

Figure 3.

*Graph of changes in spike amplitude frequency over time in all groups. A statistically significant decrease was found in the ceftriaxone + sodium valproate group at 10 minutes and after (P = .043 < .05) (Multiple comparison tests were used. *P < .05, all groups were compared with the first-minute value).*

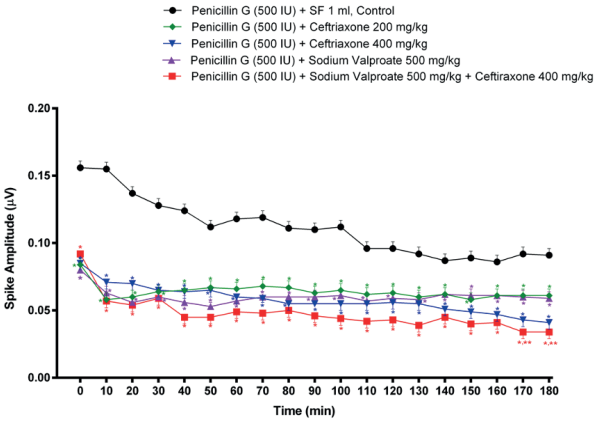


Table 2.
Percentage changes in amplitüde frequency values according to the groups for 180 minutes (spike/min)

Groups	Penicillin	Ceftriaxone 200 mg	Ceftriaxone 400 mg	Sodium valproate 500 mg	Ceftriaxone + Sodium valproate
1st	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
30st	80,16±11,28	73,80±5,37	76,33±3,90	76,91±8,38	72,87±9,30
60th	74,08±13,53	77,40±11,92	66,95±7,41	81,48±13,33	68,24±11,86
90th	68,57±7,84	72,04±8,94	67,84±6,06	79,86±10,22	57,31±7,72
120th	61,54±7,47	78,44±10,13	67,01±9,21	79,56±11,77	54,16±10,91
150th	55,77±7,74	66,62±11,67	58,37±6,14	83,32±8,81*	53,98±9,95#
180th	57,66±9,05	60,80±8,25	52,92±10,13	79,39±10,20	44,62±9,37#

The mean difference is significant at the 0.05 level.
Values expressed as mean±SD.
* p<0.05 different compared to penicillin group,
** p<0.05 different compared to penicillin plus sodium valproate group.

Throughout the 180-minute duration, the spike amplitude values at the 80th minute in the penicillin group exhibited a significant decrease compared to its first spike amplitude values (p<0.05). In the penicillin plus 200 mg/kg ceftriaxone group, the 140th-minute amplitude value decreased significantly compared to the first amplitude value (p<0.05). The decrease in all spike amplitude values after the 10th minute was statistically significant compared to its spike initial amplitude value of the group penicillin administered 400 mg/kg of ceftriaxone (p<0.05). Significant decreases were observed in the penicillin plus sodium valproate group after the 20th minute compared to its initial spike amplitude value (p<0.05). The spike amplitude values of the group treated with penicillin plus sodium valproate plus 400 mg/kg ceftriaxone showed a significant decrease in all minute values during the 180 minutes compared to its initial spike amplitude value (p>0.05). These results indicate differences among treatment groups and highlight which combinations are more effective at specific time points.

The mean percent change in amplitude values of the groups over 180 minutes was evaluated based on time (Table 2). The penicillin group had a 43% change in mean amplitude value at the end of 180 minutes compared to its baseline value (57,66±9,05 μV). In addition, the 200 and 400 mg/kg ceftriaxone groups after penicillin microinjection displayed a decrease in percentage spike amplitude change of approximately 40% and 48%, respectively, in comparison with their baseline (60.70±7.25 μV, 52,92±10,13 μV, respectively). The percentage amplitude change in the penicillin-applied sodium valproate group is approximately 31% compared to its baseline value (79,39±10,20 μV). After penicillin microinjection, the percentage spike amplitude of group sodium valproate plus ceftriaxone 400 mg/kg declined by about 56% in comparison with the baseline (44,62±9,37 μV). These findings suggest that ceftriaxone diminishes the effects of penicillin. Additionally, the group treated with sodium valproate plus 400 mg/kg ceftriaxone appears to be more effective in reducing seizure activity.

4.DISCUSSION

In this study, the impact of ceftriaxone on spike frequency and amplitude alterations in the pathogenesis of epilepsy was examined utilizing the

penicillin-induced epilepsy model. While several studies in the literature have assessed the anticonvulsant effects of ceftriaxone[17,18,25], this research employs a distinct epilepsy model and methodology to comprehensively evaluate its anticonvulsant properties. The investigation demonstrated the anticonvulsant effect of 200 mg/kg and 400 mg/kg ceftriaxone on seizures induced by penicillin in rats. Rats pre-administered ceftriaxone exhibited a dose-dependent reduction in spike frequency and amplitude in electrocorticogram (EcoG) recordings. This positive effect became more pronounced when higher doses (400 mg/kg) of ceftriaxone were administered to the rats.

Epilepsy is a common chronic neurological disease in which motor coordination, sensory perception, and cognitive functions are altered due to the stimulation of neurons.[1] Although the physiopathological basis of epilepsy is not fully understood, it is thought that increased glutamate activity and decreased γ-aminobutyric acid (GABA) inhibitory activity (an imbalance between excitation and inhibition) may be associated with seizures and epileptogenesis.[26] Sodium valproate is an anticonvulsant medication used to treat various types of epileptic seizures, including generalized seizures, absence seizures, and focal seizures. It is thought to potentiate GABA activity by inhibiting the enzymes that catabolize GABA or by blocking the reuptake of GABA into glia and nerve endings. GABA is responsible for reducing the excitability of neurons and preventing excessive neuronal firing, which can lead to seizures.[28] It also modulates voltage-gated sodium channels, which are involved in the generation and spread of electrical impulses in neurons. Blocking these channels can help regulate the abnormal electrical activity that occurs during seizures.[29] In our study, in accordance with the literature, the administration of sodium valproate has been observed to exhibit a reducing effect on penicillin-mediated seizure activity.

Experimental epilepsy models are used to elucidate the pathogenesis of epileptic seizures and to develop new antiepileptic drugs. A simple and quick way to induce epileptic activity is the administration of chemical convulsants such as penicillin.[30] The penicillin model is preferred as an experimental epilepsy model to understand the pathogenesis of epileptic seizures and to develop new antiepileptic drugs. This is useful in triggering and observing epileptic seizures quickly in a laboratory setting. Application of penicillin to the cortex of experimental animals has resulted in recorded ECoG activity resembling acute focal epileptic seizures. These waves resemble interictal spikes observed in humans and this activity persists for approximately 2-4 hours.[31,32] Therefore, it is anticipated that the ECoG activity recorded over 210 minutes in the acut penicillin experimental studies provides ample time for spike analysis. In the penicillin focal epilepsy model, the impacts of different anticonvulsants like barbiturates, diphenylhydantoin, phenobarbital and diazepam, benzodiazepines, levetiracetam, carbamazepine, and ion channel blockers were investigated.[33] Penicillin increases glutamate release by inhibiting the GABAA receptor, resulting in rhythmic epileptiform discharge.[34] This mechanism results in rhythmic epileptiform discharge. The model can be utilized to understand the effects of drugs targeting GABA receptors and to develop drugs that modulate these receptors. In conclusion, the penicillin model is frequently chosen in epilepsy research due to its ability to rapidly induce epileptic seizures in laboratory conditions and to evaluate the effects of various antiepileptic drugs. Penicillins and cephalosporins' ability to induce convulsions has been linked to their blockage of GABA receptors. Among the cephalosporins, ceftriaxone

has demonstrated some antiepileptic indications of epileptogenic activity.[35]

Ceftriaxone, one of the most effective beta-lactam antibiotics, has been studied in vitro models of ischemia and motor neuron degeneration. Beta-lactam antibiotics have been reported to have neuroprotective properties.[36] Ceftriaxone improves neurogenesis and enhances motor function in rats.[37,38] It was shown that ceftriaxone is neuroprotective in several neurological diseases such as Parkinson's disease, Huntington's disease, ALS,[39] and accelerated aging.[40] Rothstein et al. reported that more than five days of ceftriaxone treatment was sufficient to increase EAAT2 (GLT-1; slc1a2) expression.[41] Ceftriaxone also activates the transcription factor nuclear factor-κB (NF-κB). NF-κB then binds to the glutamate transporter-1 (GLT-1) promoter, enhancing the transcription of the GLT-1 gene.[42] The GLT-1 downregulation and glutamate accumulation in the brain has been associated with many neurological diseases, including amyotrophic lateral sclerosis,[43] Alzheimer's disease,[44] several forms of epilepsy,[8] and ischemia/stroke and traumatic brain injury.[45] GLT-1 removes more than 95% of synaptic glutamate in the forebrain of animals.[46] The ceftriaxone treatment after traumatic brain injury has been shown to restore the expression of GLT-1 and reduce post-traumatic seizures in rats.[47] On the other hand, it enhanced GLT-1 expression and its biochemical and functional activity in the brains of rats and mice both in vitro and in vivo.[41]

The results indicate that ceftriaxone antibiotics are neuroprotective and antioxidant, possibly via upregulating GLT-1, which reduces glutamate chemical transmitter and Ca^{+2} overload, the primary processes causing increased reactive oxygen species (ROS) formation in the hippocampus during epileptic seizures.[48] Altas et al. showed that its treatment caused a considerable increase in glutathione peroxidase and superoxide dismutase (SOD) activity while decreasing malondialdehyde (MDA) in ischemia-exposed rat brains.[49] Furthermore, the concentration-dependent increase in ex vivo production of the neuroprotective protein GLT-1 by ceftriaxone indicates its potential to reduce glutamate excitotoxicity by activating metabotropic glutamate receptor (mGluR) receptors.[50-53] The antioxidant properties of ceftriaxone and its ability to reduce glutamate toxicity may unveil its antiepileptic characteristics. This indicates the anticonvulsive efficacy of ceftriaxone in reducing glutamate excitotoxicity during the acute period.

In a limited number of studies, ceftriaxone's antiepileptic activity was documented. Recent research suggests that ceftriaxone may have an anti-epileptic impact because it increases glutamate reuptake by GLT-1.[54] Different effects of it are shown on neurotransmitters involved in epileptogenesis. The primary outcome is a suppression of the GABA signaling pathway, suppressing postsynaptic GABA ion channels and thus reducing GABA-mediated inhibitory transmission. The convulsant effects of beta-lactam antibiotics such as ceftriaxone are shown to be caused by this.[55] In one study, 100 or 200 mg/kg, ceftriaxone treatment for 27 days reduced burning scores, restored motor and cognitive functions, and increased antioxidative activities in a PTZ-induced rat epilepsy model.[13] Uyanikgil et al. demonstrated that ceftriaxone has protective effects on PTZ-induced convulsions.[18] This impact can be explained through improved GLT1 expression and activation.[17,41] Additionally, Hussein et al. reported that ceftriaxone had an antiepileptic effect in the PTZ rat model by increasing oxidative stress markers such as SOD and MDA.[56] Similarly, our study revealed antiepileptic effects associated with both low and high doses of

ceftriaxone.

This study demostrated that ceftriaxone decreased the spike-wave number and spike amplitude in the experimental model of epilepsy induced by penicillin. The effects of ceftriaxone administered acutely at different doses (200 mg/kg and 400 mg/kg) in the penicillin model of epilepsy were investigated. The alone and combined administration of ceftriaxone and sodium valproate significantly affected penicillin-induced seizures. A combination of high-dose ceftriaxone and sodium valproate significantly attenuated spike frequency and amplitude latency in seizures. The effects of ceftriaxone on the experimental model of epilepsy induced by penicillin have not been demonstrated in previous studies, and this study has the potential to provide an original contribution in this context. These findings indicate that ceftriaxone may have potential antiepileptic efficacy, representing a preliminary study in this regard.

5.CONCLUSION

Epilepsy stands out as one of the most prevalent and significant neurological disorders worldwide. The goal of antiepileptic drug therapy is to achieve a seizure-free state with minimal side effects. Our study has demonstrated a protective effect of ceftriaxone on penicillin-induced seizures. This study is the first to investigate the effect of ceftriaxone on penicillin-induced epileptic seizures. However, we believe that more precise and valuable results will be obtained through molecular analyses of the anticonvulsant effects of ceftriaxone. Further research is needed to explore the impact of GLT-1 levels, oxidative stress markers, and other measurement indicators on seizures. The lack of investigation into the potential side effects of ceftriaxone in the study constitutes a limitation of this research. In clinical trials, the safety, efficacy, and potential side effects of ceftriaxone in epilepsy patients should be assessed. Further elucidation of possible adverse effects and their consequences can be provided through more comprehensive biochemical, cellular, and histopathological investigations. Possible questions related to the use of ceftriaxone may include dosage, treatment duration, side effects, and interactions with other antiepileptic drugs. Therefore, further research is necessary to validate these findings for clinical application and establish specific treatment protocols.

Conflicts of Interest

The authors declare there is no conflict of interest.

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Authors' Contributions

ZA: Conceptualization, Project administration, Resources, Software, Visualization, Data curation, Formal Analysis, Resources, Writing.

SO: Conceptualization, Formal Analysis, Data curation, Resources, Software, Validation, Writing – original draft.

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