Research Article / Araştırma Makalesi

The Protective Effect of Vitamin B12 on Epileptic Seizure Activity in Rats Induced by Penicillin

Penisilin ile İndüklenen Sıçanlardaki Epileptik Nöbet Aktivitesinde B12 Vitamininin Koruyucu Etkisi

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Abstract

Background: Epilepsy, characterized by sudden, abnormal, and uncontrolled neuronal activity in the central nervous system, is a chronic neurological disorder. Vitamin B12 derivatives act as complex organometallic helpers for a select group of enzymes. This vitamin affects many cellular functions in the central and peripheral nervous system. This study aimed to explore how Vitamin B12 impacts penicillin-induced epileptic seizure activity in anesthetized rats through electrocorticography (ECoG).
Materials and Methods: For this study, 35 male Wistar rats were utilized and divided into five groups, each consisting of 7 rats. The groups were as follows: Control +Penicillin; rats received 2.5 µL of 500 IU penicillin intracranially (i.c.) along with 1 ml saline solution intraperitoneally (i.p.), 1 mg/kg Vit B12 +Penicillin; rats were administered 1 mg/kg of Vitamin B12 intraperitoneally (i.p.) along with penicillin, 2 mg/kg Vit B12 +Penicillin; rats were given 2 mg/kg of Vitamin B12 i.p. along with Penicillin, Sodium Valproate (VPA) +Penicillin; rats received 500 mg/kg of sodium valproate i.p. along with penicillin, and 2 mg/kg Vit B12 + VPA +Penicillin; rats were administered 2 mg/kg of Vitamin B12 i.p. and 500 mg/kg of sodium valproate i.p. following 30 minutes after penicillin. After the administration, ECoG recordings were taken from rats placed in the stereotaxic device (for 180 minutes).
Results: The immediate application of the Vit B12 (1 mg/kg and 2 mg/kg), VPA, and VPA plus Vit B12 2 mg/kg resulted in a notable reduction in the spike-wave frequency of penicillin-induced epileptic seizure activit in the rats ($p<0.05$). Moreover, following penicillin microinjection, the mean spike amplitudes of Vit B12 (1 mg/kg and 2 mg/kg), VPA, and VPA plus Vit B12 2 mg/kg groups were lower than those of the penicillin group ($p<0.05$).
Conclusions: These results imply that administering Vitamin B12 acutely demonstrates anticonvulsant effects on penicillin-induced focal onset epileptic activity. The study further proposes that vitamin B12 therapy may possess anti-epileptogenic potential.

Key Words: Epilepsy, Vitamin B12, Penicillin

Öz

Amaç: Merkezi sinir sisteminde ani, anormal ve kontrolsüz nöronal aktivite ile karakterize edilen epilepsi, kronik nörolojik bir hastalıktır. B12 vitamini türevleri, belirli bir grup enzim için karmaşık organometalik yardımcılar olarak görev yapar. Bu vitamin merkezi ve periferik sinir sistemindeki birçok hücresel fonksiyonu etkiler. Bu çalışma, B12 Vitamininin anestezi altındaki sıçanlarda penisilin kaynaklı epileptik benzeri aktiviteyi elektrokortikografi (ECoG) yoluyla nasıl etkilediğini araştırmayı amaçladı.
Materyal ve Metod: Bu çalışma için 35 adet erkek Wistar sıçanı kullanıldı ve her biri 7 sıçandan oluşan beş gruba ayrıldı. Gruplar şu şekildeydi: Kontrol +Penisilin; sıçanlara intrakraniyal (i.c.) olarak 2.5 µL 500 IU penisilin ve intraperitoneal (i.p.) olarak 1 ml salin solüsyonu verildi, 1 mg/kg Vit B12 +Penisilin; sıçanlara intraperitoneal (i.p.) olarak 1 mg/kg Vitamin B12 ve penisilin verildi, 2 mg/kg Vit B12 +Penisilin; sıçanlara intraperitoneal (i.p.) olarak 2 mg/kg Vitamin B12 ve penisilin verildi, Sodyum Valproat (VPA) +Penisilin; sıçanlara intraperitoneal (i.p.) olarak 500 mg/kg sodyum valproat ve penisilin verildi, ve 2 mg/kg Vit B12 + VPA +Penisilin; sıçanlara 2 mg/kg Vitamin B12 i.p. ve 500 mg/kg sodyum valproat i.p. ve penisilin verildi, ilaçlar penisilinden 30 dakika sonra uygulandı. Uygulama sonrasında stereotaksik cihaza yerleştirilen sıçanlardan (180 dakika süreyle) ECoG kayıtları alındı.
Bulgular: Vit B12 (1 mg/kg ve 2 mg/kg), VPA ve VPA artı Vit B12 2 mg/kg'ın uygulanması, sıçanlarda penisilin kaynaklı epileptiform aktivitenin spike dalga frekansında dikkate değer bir azalma meydana getirdi ($p <0,05$). Ayrıca penisilin mikroenjeksiyonunu takiben Vit B12 (1 mg/kg ve 2 mg/kg), VPA ve VPA artı Vit B12 2 mg/kg gruplarının ortalama spike amplitüdleri penisilin grubuna göre daha düşüktü ($p<0,05$).
Sonuç: Bu sonuçlar, B12 Vitamininin akut olarak uygulamasının penisilin kaynaklı fokal başlangıçlı epileptik aktivite üzerinde antikonvülsan etkiler gösterdiğine işaret etmektedir. Çalışma ayrıca B12 vitamini tedavisinin antiepileptojenik potansiyele sahip olabileceğini öne sürüyor.

Anahtar Kelimeler: Epilepsi, B12 Vitamini, Penisilin

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Introduction

Epilepsy is characterized by persistent and uncontrolled recurring seizure activity in the brain, defining it as a chronic neurological condition (1). Epilepsy impacts approximately 1% of the global population (2). Even though many antiepileptic drugs are used, they cannot prevent seizures in 20-30% of patients (3). Roughly 33% of individuals newly diagnosed with epilepsy might experience a form of the condition that proves unresponsive to standard antiepileptic medications, posing significant challenges in treatment (4). Epileptic seizures originate from abnormal synchronized electrical activity among neurons. Seizures arise from an imbalance in the synaptic transmission within brain tissue, where there's a disruption in the equilibrium between excitatory and inhibitory signals (5). Findings from studies conducted on experimental epilepsy models suggest that cellular damage occurring through apoptosis, neuroinflammation, oxidative stress and autophagy causes epilepsy. Nevertheless, the precise cause of epilepsy remains incompletely understood (6). Moreover, numerous epilepsy patients experience cognitive and psychiatric issues, including cognitive deterioration often accompanied by anxiety and depression (7). Epileptogenesis refers to a gradual sequence of molecular and cellular processes that lead to the onset of epilepsy in an otherwise healthy brain. Developing therapies and medications aimed at halting or reducing the progression of epilepsy could greatly enhance the quality of life for those impacted by the condition.

Vitamin B12, also known as cobalamin due to its cobalt-containing molecules, exhibits diverse properties including antinociception and neuroprotection (8). Vitamin B12 plays an important role in the first myelination of the central nervous system and the cellular metabolism of biomolecules. Additionally, it contributes to immune mechanisms within the body (9). Research indicates that vitamin B12 has the potential to promote neurite outgrowth and provide protection to cortical neurons and retinal cell cultures from glutamate excitotoxicity (10). Furthermore, studies have revealed that injection of vitamin B12 diminishes of epileptic seizures in the penicillin-induced in rats (11). Lee et al. demonstrated that this vitamin can prevent epileptogenesis by reducing neuroinflammation and oxidative damage (12). Sodium valproate (VPA) is a commonly prescribed antiepileptic medication utilized by numerous individuals to manage seizures in cases of idiopathic generalized epilepsy. Previous research has suggested that valproic acid (VPA) exhibits superior efficacy in controlling seizures associated with idiopathic generalized epilepsy (IGE) compared to newer antiepileptic drugs (AEDs) such as lamotrigine or topiramate (13). Additionally, there is evidence suggesting that the therapeutic mechanism of valproic acid (VPA) in epilepsy treatment is rooted in its interaction with neurotransmitters such as gamma-aminobutyric acid (GABA), receptor sites, and ion channels (14).

Despite the growing evidence supporting the neuroprotective properties of vitamin B12, its impact on antiepileptic effects has not been conclusively proven. This study was conducted to investigate the effect of vitamin B12 on penicillin-induced seizures. Acknowledging the importance of exploring innovative strategies in epilepsy management, our study was designed to examine the potential impact of systemic administration of vitamin B12, either independently or in conjunction with VPA, on epileptogenesis. Our objective is to assess the effects of vitamin B12 on penicillin-induced epileptic seizure activity in rats, utilizing electrocorticography (ECoG) as the primary investigative method.

Materials and Methods

Animals

This study utilized 35 adult male Wistar albino rats, aged between 4 and 5 months, with weights ranging from 200 to 250 grams. The rats were sourced from the Tokat Gaziosmanpaşa University Animal Laboratory in Tokat Gaziosmanpaşa, Turkey. They were housed under standard conditions, including a 12:12-hour light-dark cycle (lights on from 07:00 to 19:00), maintained at a controlled temperature of 23 ± 2°C, and a humidity level of 35–60%. The rats had access to standard laboratory chow and tap water ad libitum. Experiments were conducted between 9:00 a.m. and 4:00 p.m., adhering to the guidelines established by the Tokat Gaziosmanpaşa Universty Ethics Committee to ensure the ethical treatment of rats (Registry Number: 2020 HADYEK-31).

Chemicals

Penicillin and vitamin B12 were purchased from Sigma-Aldrich Co., St Louis, MO, USA. Sodium valproate was purchased from Sanofi, France. Vitamin B12 and Penicillin were administration after dissolving in physiological saline. All chemical were delivered via intraperitoneal, with applied occurring 30 minutes following penicillin injection.

Experimental procedure

The animals were randomly divided into five groups, each containing seven animals, as outlined below:

Control +Penicillin: Penicillin (500 IU, 2.5 µl, i.c.) plus saline solution 1 ml intraperitoneally (i.p.) (15).

1 mg/kg Vit B12 +Penicillin: Vitamin B12 (1 mg/kg, i.p.) plus penicillin (500 IU, 2.5 µl, i.c.) (16).

2 mg/kg Vit B12 +Penicillin: Vitamin B12 (2 mg/kg, i.p.) plus penicillin (500 IU, 2.5 µl, i.c.) (17).

VPA +Penicillin: Sodium valproate (500 mg/kg i.p.) plus penicillin (500 IU, 2.5 µl, i.c.) (15).

2 mg/kg Vit B12 + VPA +Penicillin: Vitamin B12 (2 mg/kg, i.p.) plus sodium valproate (500 mg/kg i.p.) plus penicillin (500 IU, 2.5 µl, i.c.).

In our study, there was no negative control group in which penicillin was not administered and saline alone was administered. Because only the basic basal activity can be recorded in the recordings obtained from these rats and no spike activity will occur, frequency or amplitude analysis cannot be performed in the negative control group.

Surgical procedure

Following anesthesia with 1.25 g/kg urethane (Sigma-Aldrich, USA), the rats were positioned in the stereotaxic apparatus and securely immobilized (Harvard Stereotaxic Instrument). After this, an approximately 3 cm long incision (along the rostrocaudal plane) was made. Then, the soft tissue covering the left somatomotor cortex was removed, and the skull bone was delicately thinned using a rotary tool. Electrophysiological recordings were conducted using two Ag/AgCl ball electrodes, while one Ag/AgCl clamp electrode served for grounding. A positive electrode was placed bregma (1 mm anterior) and sagittal suture (2 mm lateral). The negative electrode was positioned bregma (5 mm posterior) and sagittal suture (2 mm lateral). A ground electrode was affixed to the right ear. The body temperature of the rats was maintained at 37°C throughout the experiment using a homeothermic blanket connected to a rectal probe (Harvard Instrument, USA).

The activity was recorded using electrodes connected to the MP 150-CE interface (Biopac Systems, USA), which was later upgraded to the MP 150 EEG-100C (Biopac Systems, USA), and then transferred to the data recorder. The analogue blips recorded from the brain cortex were digitized via a MP 150 system. Later signals transferred the laptop computer. Activities in the brain was visualized using (AcqKnowledge 3.9.1 software, Biopac Systems; USA). Following the registration period, the epileptic seizure activity recordings were evaluated (18).

Induction of epileptic seizure activity

First-group rats were given penicillin (2.5 µL, i.c.) and saline solution (1 ml, i.p.). After 30 minutes of penicillin (2.5 µL, i.c.), the second and third groups were given 1 and 2 mg/kg of vitamin B12, respectively, i.p. The fourth group was given sodium valproate (500 mg/kg, i.p.) after 30 minutes of penicillin (500 IU, 2.5 µL, i.c.). 30 minutes after penicillin (500 IU, 2.5 µL, i.c.) administration, vitamin B12 2 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 (15).

Electrophysiological processes

The experimental protocols were executed within the Physiology Laboratory of Tokat Gaziosmanpaşa University's Faculty of Medicine. Recording of epileptic seizure activity, initiated following the administration of penicillin, commenced after a two-minute interval. ECoG activity was monitored for a duration of 180 minutes. The epileptic seizure activity reached a stable state after the 20th and 30th minutes following the injection of penicillin. The average spike and amplitude values recorded between the 20th and 30th minutes following the penicillin injection were regarded as the baseline values for the 1st minute. After the initial 30 minutes, the averages of spike amplitudes and frequency for 1-min intervals were computed at 10-min intervals. The 180-minute recording was segmented into 10-minute intervals. The spike count and the average spike count per minute were determined by tallying the peak-to-peak amplitudes.

Statistical analysis

Statistical analysis was performed using the 1-minute values collected at 10-minute intervals. SPSS (Statistical Package for the Social Sciences) 26.0 software for Windows was employed for this analysis. The differences between groups were examined using the Kruskal-Wallis test. Groups with statistically significant differences and homogeneous subgroups were analyzed using the multiple comparison method. A p-value less than 0.05 was considered statistically significant. The data were presented as mean ± SEM.

Results

The PowerLab system (PowerLab/8 SP ADInstruments, Australia) was used to record ECoG throughout the experiment, following the placement of electrodes. At the onset, a 10-minute baseline activity was recorded, followed by a 120-minute ECoG recording session. The data were digitized using PowerLab Chart v.7.0 software. ECoG recordings from each animal were segmented into 10-minute intervals. The data obtained were analyzed in terms of the frequency of the spike–wave and spike-wave amplitude.

Spike frequency

Following the penicillin microinjection in the acute penicillin epilepsy model, the average spike frequency recorded was 96.83 ± 6.92 spikes per minute. The mean spike frequency of the Vit B12 (1 mg/kg and 2 mg/kg) and sodium valproate plus Vit B12 2 mg/kg groups after penicillin injection significantly reduced over 180 minutes ($p<0.05$) (Figure 1A). The average spike frequency of epileptic seizure activity in penicillin plus 1 with 2 mg/kg Vit B12 groups were 72.62 ± 5.73 and 72.21 ± 6.36 spike/min, respectively. In addition, the average spike frequency of epileptic seizure activity in penicillin plus VPA and penicillin plus 2 mg/kg Vit B12 +VPA groups were 61.45 ± 4.75 and 63.86 ± 5.58 spike/min, respectively. The mean spike frequency of epileptic seizure activity in penicillin plus 2 mg/kg Vit B12 +VPA were higher than those in the penicillin plus VPA group, but this increase was not significant ($p> 0.05$) (Figure 1A).

The mean spike frequency values and the percentage change in spike frequency according to the groups for 180 minutes were illustrated in Figure 2 (A-B). The first spike frequency value of the VPA and 2 mg/kg Vit B12 groups after penicillin microinjection was higher than the first spike frequency value of the control +penicillin group. The 10th-minute frequency value of the all groups was lower than the 10th-min spike frequency mean value of the penicillin group. After the 10th min, the spike frequency value of the penicillin-administered VPA group started to decrease significantly ($p<0.05$). Additively, the spike frequency mean value of sodium valproate plus Vit B12 2 mg/kg administered following the microinjection of penicillin decreased compared to the penicillin group ($p<0.05$) (Figure 2A). After penicillin microinjection, the 110th-minute spike frequency value of the penicillin plus VPA plus Vit B12 2 mg/kg treatment group was lower than the same-minute value of the penicillin-applied

VPA group (p>0.05). At the same time, the spike frequency value of the all groups decreased the following 140th-minute compared to the penicillin group following penicillin microinjection. The spike frequency mean value of the Vit B12 1 and 2 mg/kg groups decreased significantly following 150th-minute after penicillin microinjection (p<0.05).

The spike frequency mean values of the penicillin group at all minutes were not significantly different from the first spike frequency value in the penicillin plus Vit B12 1 and 2 mg/kg groups. But, those significantly decreased compared to the first spike every 10 minutes for 180 minutes. In the VPA applied group after penicillin microinjection, the frequencies of the all-minute spike decreased compared to the baseline. Moreover the frequency of spikes significantly decreased after 60th minutes.

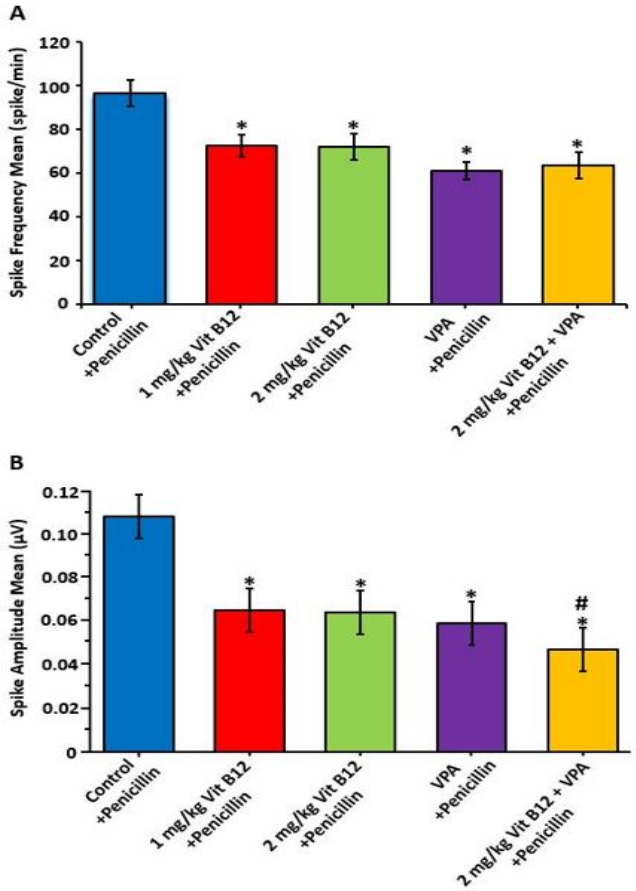

Figure 1. The spike-wave frequency (spike/min) and amplitude (µV) mean values of groups of EcoG activity. **A:** The spike frequency (spike/min) average values. **B:** The spike amplitude (µV) mean values. The vertical bars represent the mean ± SEM. *p<0.05 compared to the Control +Penicillin group. #*p*< 0.05 compared to the VPA +Penicillin group.

A comparison of the mean percentage spike change values of the groups is shown in Figure 2B. In the groups of VPA, Vit B12 (1 mg/kg and 2 mg/kg), and VPA plus Vit B12 2 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 (99.64±5.58 spike/min), the 1 and 2 mg/kg Vit B12 groups after penicillin microinjection displayed a significant decrease in percentage spike frequency of approximately 40% and 38%, respectively in comparison with their baseline (60.14±7.34 spike/min, 62.65±9.83 spike/min, respectively). Furthermore, the percentage spike frequency change in the penicillin-applied VPA groups group is almost 46% compared to baseline (54.48±12.60 spike/min). After penicillin microinjection, the percentage spike frequency of group VPA plus Vit B12 2 mg/kg declined by about 42% in comparison with the baseline (58.34±11.23 spike/min).

Spike amplitude

The mean spike amplitude levels of the groups are shown (Figure 1B). The mean spike amplitude value for the penicillin group was significantly elevated in comparison to the other groups (p<0.05). Following penicillin microinjection, the mean spike amplitudes of Vit B12 (1 mg/kg and 2 mg/kg), VPA, and VPA plus Vit B12 2 mg/kg groups were significantly decreased compared to the penicillin group (p<0.001; 0.065 ± 0.01 µV, 0.064 ± 0.01 µV, 0.059 ± 0.01 µV, 0.047± 0.01 µV, respectively). Moreover, the penicillin-applied VPA plus 2mg/kg Vit B12 group mean spike amplitudes were lower than those of the penicillin plus VPA group (p<0.05; 0.047±0,01 µV) (Figure 1B).

The mean spike amplitude values and the percentage change in spike frequency according to the groups for 180 minutes were illustrated in Figure 3 (A-B). After penicillin microinjection, the mean amplitude values of the Vit B12 (1 mg/kg and 2 mg/kg), VPA, and VPA plus Vit B12 2 mg/kg groups for 180 minutes for 10 minutes each were lower than the mean amplitude value of the penicillin group (p<0.05). Furthermore, the spike amplitude mean of the penicillin plus VPA plus 2 mg/kg Vit B12 group was lower than the spike amplitudes of the penicillin-administered VPA group over 180 minutes (p<0.05) (Figure 3A). During the entire 180-minute duration, the spike amplitude values in the penicillin group showed a notable decrease in comparison to its initial spike amplitude values (p<0.05). In the all groups the 10th-minute amplitude value decreased significantly compared to the first amplitude value (p<0.05). The decrease in the penicillin-applied VPA plus 2mg/kg Vit B12 group spike amplitude values after the 150th minute was statistically significant compared to its spike initial amplitude value of the group penicillin administered VPA (p<0.05) (Figure 3A).

The percent change in amplitude values of the groups over a period of 180 minutes was assessed according to time (Figure 3B). The penicillin group had a 52% change in mean amplitude value at the end of 180 minutes compared to its baseline value (48.47±8.52 µV). In addition, the 1 and 2 mg/kg Vit B12 groups after penicillin microinjection displayed a decrease in percentage spike amplitude change of approximately 46% and 47%, respectively, in comparison with their baseline 54.02±8.12 µV, 53.93±9.26 µV. The percentage

amplitude change in the penicillin-applied VPA group is approximately 31% compared to its baseline value (42.86±10.15 µV). After penicillin microinjection, the percentage spike amplitude of group VPA plus Vit B12 2 mg/kg declined by about 72% in comparison with the baseline (28.26±10.28 µV**)**.

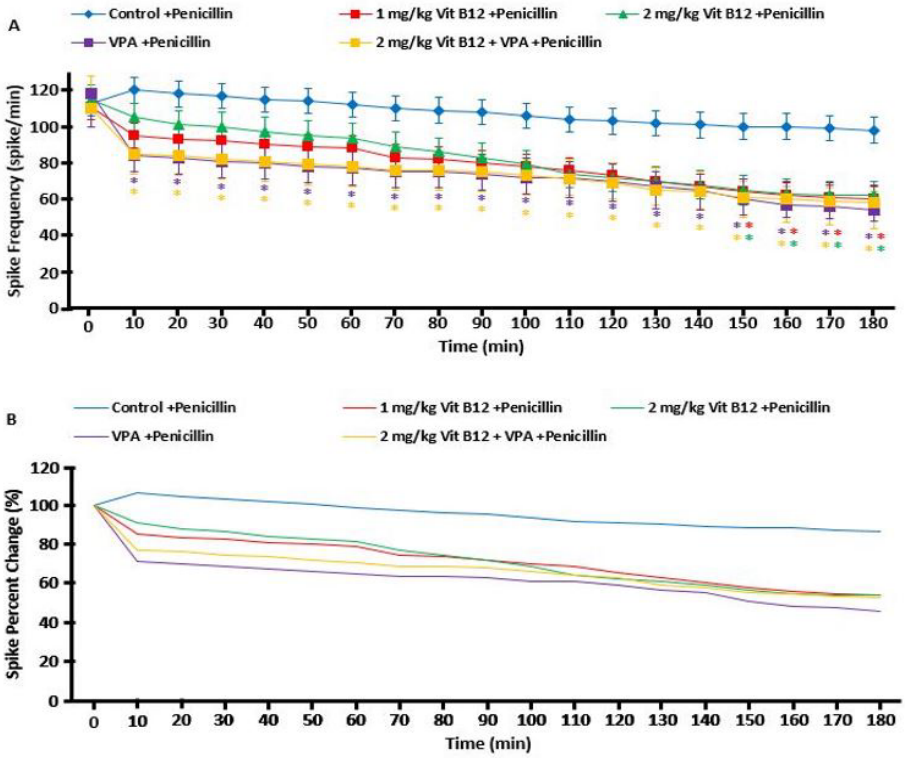

Figure 2. The spike frequency (spike/min) mean values and spike-wave frequency percentage change the graph of groups. **A:** The values of spike-wave frequency (spike/min) of groups obtained from EcoG recording after penicillin injection. *p<0.05 compared to the control +penicillin group. **B:** The spike percent change graph of groups.

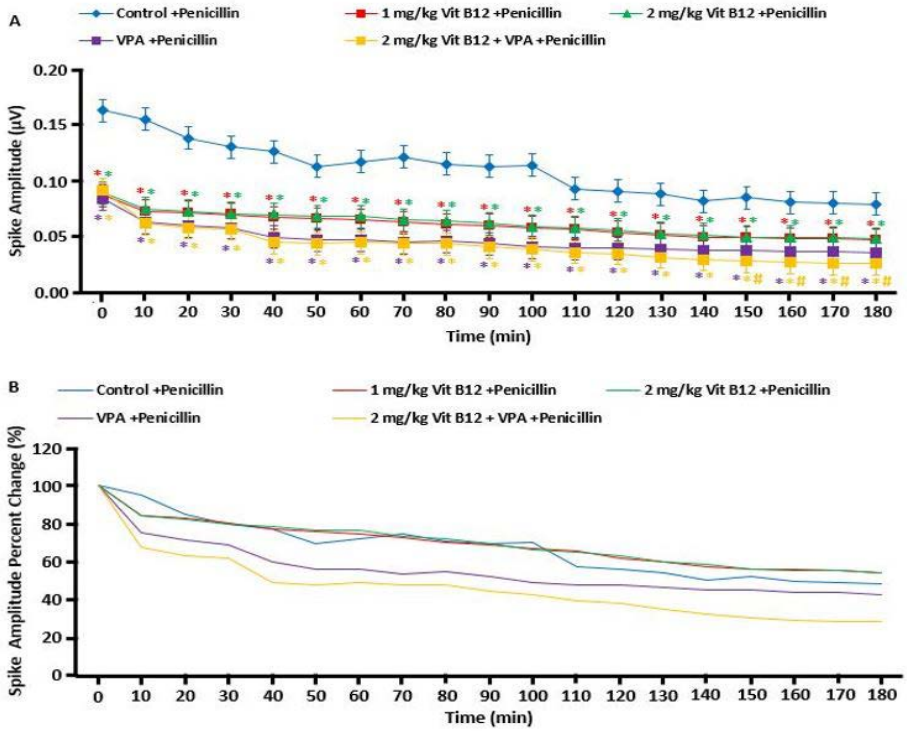

Figure 3. The spike amplitude (µV) mean values and spike amplitude percentage change the graph of groups. **A:** The values of spike amplitude (µV) of groups obtained from EcoG recording after penicillin injection. **B:** The spike amplitude percentage change graph of groups. *p<0.05 compared to the Control +Penicillin group. #*p*< 0.05 compared to the VPA +Penicillin group.

Discussion

Based on recent relevant articles, in the present study represents the primary report on the curative effectiveness of vitamin B12, either lonesome or in combination VPA, on epileptic seizure activity in an experimental epilepsy model ınduced by penicillin. The principal findings of the existing investigation are such as: (1) the mean values of spike frequency plus amplitude significantly decreased in the groups administration of B12 vitamin application; (2) In addition, the mean spike amplitude value in the vitamin B12 plus VPA administered group decreased significantly compared to the VPA group. In general, these findins indicate that vitamin B12 alone or combined with VPA demonstrated significant neuroprotection against penicillin-induced epileptogenesis.

There is a growing belief that certain vitamins may aid in the management of specific seizure types and mitigate the adverse effects associated with antiepileptic drugs (19, 20). Several studies have demonstrated a correlation between vitamin B12 deficiency and abnormalities in electroencephalogram (EEG) readings among individuals with epilepsy (21, 22). Evidence for the neuroprotective effects of B12 vitamin in both the central and peripheral nervous systems is increasingly accumulating. This vitamin demonstrates several impacts on the neural system, encompassing neurotrophic act. Previous researchs have indicated that vitamin B12 fosters nerve cells accretion and facilitates neuron regenerateness in certain animal models (23, 24). In our present working, the reduction of induced by penicillin epileptic seizure activity following B12 precuring can be ascribed to its neuropreservative characteristics. Aligning with our results, previous literature has documented similar findings, indicating that injection of vitamin B12 diminishes the epileptic seizure activity induced by penicillin. Furthermore, the study have demonstrated that vitamin B12 exhibits antiepileptic effects through the GABAA receptor system in the induced by penicillin experimental rat model (11). Our results imply that the GABA-A-benzodiazepine receptor complex system contributes to the antiepileptic seizure activity of vitamin B12.

Studies have assessed the neuroprotective effects of vitamin B12 in rats using models of sciatic and corneal nerve crush injuries (25, 26). In a previous study, researchers showed that vitamin B12 exhibits an anti-apoptosis influence on environmental neuronal damage by upregulating protein expression and downregulating protein expression (27). Additionally, the previous study revealed that vitamin B12 can shield in the brain cortical neurons (28). Moreover, neuroprotection holds significant importance as a hopefuling therapeutic approach for both preventative and remedia in the epilepsy (29). Our findings suggest the potential efficacy of vitamin B12 in epilepsy curative.

Our findings highlight the potential of vitamin B12 in epilepsy treatment. However, the current utilization of vitamin B12 in this context remains inadequate. Our study demonstrated that vitamin B12 prevented penicillin-induced seizures in rats. Furthermore, the mean spike-wave amplitude (μV) value decreased in the vitamin B12 plus VPA administered group compared to the VPA group. These results align with previous studies indicating that vitamin B12 can mitigate penicillin-induced seizures.

Conclusion

In summary, the findings of the current study demonstrate that vitamin B12 reduced epileptic seizures following penicillin-induced seizures in rats. These results show that vitamin B12 contributes to the nervous system. Additional research is necessary to ascertain the protector impact and mechanism of action of vitamin B12.

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Ethical Approval: *This study was carried out in accordance with the rules of research and publication ethics. The study was appro-ved by the Tokat Gaziosmanpaşa University Faculty of Medicine Animal Experiments Local Ethics Committee, numbered 2020 HADYEK-31.*

Author Contributions:
Concept: S.O., Z.C., Z.A.
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Design : S.O., Z.C., Z.A.
Data acquisition: S.O., Z.C., Z.A.
Analysis and interpretation: S.O., Z.C., Z.A.
Writing manuscript: S.O., Z.C., Z.A.
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References

1. Li HH, Lin PJ, Wang WH, Tseng LH, Tung H, Liu WY, et al. Treatment effects of the combination of ceftriaxone and valproic acid on neuronal and behavioural functions in a rat model of epilepsy. Experimental physiology. 2021;106(8):1814-28.
2. Serikawa T, Mashimo T, Kuramoro T, Voigt B, Ohno Y, Sasa M. Advances on genetic rat models of epilepsy. Experimental animals. 2015;64(1):1-7.
3. Moshé SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: new advances. Lancet. 2015;385(9971):884-98.
4. Kwan P, Brodie MJ. Early identification of refractory epilepsy. The New England journal of medicine. 2000;342(5):314-9.
5. Aronica E, Muhlebner A. Neuropathology of epilepsy. Handbook of clinical neurology. 2017;145:193-216.
6. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. Nature reviews Neurology. 2011;7(1):31-40.
7. Lenck-Santini PP, Scott RC. Mechanisms Responsible for Cognitive Impairment in Epilepsy. Cold Spring Harbor perspectives in medicine. 2015;5(10).
8. Reynolds E. Vitamin B12, folic acid, and the nervous system. The Lancet Neurology. 2006;5(11):949-60.
9. Stabler SP. Vitamin B12 deficiency. The New England journal of medicine. 2013;368(21):2041-2.

10. Okada K, Tanaka H, Temporin K, Okamoto M, Kuroda Y, Moritomo H, et al. Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. Experimental neurology. 2010;222(2):191-203.
11. Erfanparast A, Tamaddonfard E. Effects of intracortical microinjection of vitamin B12 on penicillin-induced epileptiform activity in rats. Acta neurobiologiae experimentalis. 2015;75(2):200-7.
12. Lee YJ, Wang MY, Lin MC, Lin PT. Associations between Vitamin B-12 Status and Oxidative Stress and Inflammation in Diabetic Vegetarians and Omnivores. Nutrients. 2016;8(3):118.
13. Mole TB, Appleton R, Marson A. Withholding the choice of sodium valproate to young women with generalised epilepsy: Are we causing more harm than good? Seizure. 2015;24:127-30.
14. Anyanwu E, Harding GF. The involvement of taurine in the action mechanism of sodium valproate (VPA) in the treatment of epilepsy. Acta physiologica, pharmacologica et therapeutica latinoamericana : organo de la Asociacion Latinoamericana de Ciencias Fisiologicas y [de] la Asociacion Latinoamericana de Farmacologia. 1993;43(1-2):20-7.
15. Acungil ZK, Nacar T. Effect of Resveratrol on a Penicillin-Induced Epilepsy Model in Rats. Arch Epilepsy. 2022;28(2):78-84.
16. Albay C, Adanir O, Kahraman Akkalp A, Dogan VB, Gulec MA, Beytemur O. Cyanocobalamin and cholecalciferol synergistically improve functional and histopathological nerve healing in experimental rat model. Ideggyogyaszati szemle. 2020;73(3-4):113-20.
17. Arikan M, Togral G, Hasturk AE, Horasanli B, Helvacioglu F, Dagdeviren A, et al. Histomorphometric and Ultrastructural Evaluation of Long-Term Alpha Lipoic Acid and Vitamin B12 Use After Experimental Sciatic Nerve Injury in Rats. Turkish neurosurgery. 2016;26(6):944-52.
18. Uyanikgil Y, Ozkeskek K, Cavusoglu T, Solmaz V, Tumer MK, Erbas O. Positive effects of ceftriaxone on pentylenetetrazol-induced convulsion model in rats. The International journal of neuroscience. 2016;126(1):70-5.
19. Ayyildiz M, Yildirim M, Agar E. The effects of vitamin E on penicillin-induced epileptiform activity in rats. Experimental brain research. 2006;174(1):109-13.
20. Sawicka-Glazer E, Czuczwar SJ. Vitamin C: A new auxiliary treatment of epilepsy? Pharmacol Rep. 2014;66(4):529-33.
21. Biancheri R, Cerone R, Rossi A, Schiaffino MC, Caruso U, Minniti G, et al. Early-onset cobalamin C/D deficiency: epilepsy and electroencephalographic features. Epilepsia. 2002;43(6):616-22.
22. Incecik F, Herguner MO, Altunbasak S, Leblebisatan G. Neurologic findings of nutritional vitamin B12 deficiency in children. The Turkish journal of pediatrics. 2010;52(1):17-21.
23. Sun H, Yang T, Li Q, Zhu Z, Wang L, Bai G, et al. Dexamethasone and vitamin B(12) synergistically promote peripheral nerve regeneration in rats by upregulating the expression of brain-derived neurotrophic factor. Archives of medical science : AMS. 2012;8(5):924-30.
24. Tamaddonfard E, Farshid AA, Samadi F, Eghdami K. Effect of vitamin B12 on functional recovery and histopathologic changes of tibial nerve-crushed rats. Drug research. 2014;64(9):470-5.
25. Gan L, Qian M, Shi K, Chen G, Gu Y, Du W, et al. Restorative effect and mechanism of mecobalamin on sciatic nerve crush injury in mice. Neural regeneration research. 2014;9(22):1979-84.
26. Romano MR, Biagioni F, Carrizzo A, Lorusso M, Spadaro A, Micelli Ferrari T, et al. Effects of vitamin B12 on the corneal nerve regeneration in rats. Experimental eye research. 2014;120:109-17.
27. Wang D, Zhang P, Li Z, Liu Y. [Effects of mecobalamin on Bax and Bcl-2 in neurons after peripheral nerve injury]. Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2015;33(11):841-3.
28. Akaike A, Tamura Y, Sato Y, Yokota T. Protective effects of a vitamin B12 analog, methylcobalamin, against glutamate cytotoxicity in cultured cortical neurons. European journal of pharmacology. 1993;241(1):1-6.
29. Acharya MM, Hattiangady B, Shetty AK. Progress in neuroprotective strategies for preventing epilepsy. Progress in neurobiology. 2008;84(4):363-404.