The Protective Effects of Vitamin B$_{12}$ on Pentylenetetrazole-Induced Seizures in Rats

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Abstract

Objective: Epilepsy is defined as a short-lived paroxysmal disorder of the brain functions observed in seizures by sudden, abnormal and hypersynchronous discharges of a group of neurons in the central nervous system. Vitamin B$_{12}$ derivatives are complex organometallic cofactors used by a limited number of enzymes. B$_{12}$ vitamins are involved in many cellular functions, both glial and neuronal, in the central and peripheral nervous system. The aim of this study was investigate the effects of vitamin B$_{12}$ on pentylenetetrazole-induced seizures in rats.

Methods: In our study, 18 240-280gr male Wistar albino rats were used. Animals were divided into three groups: control (n=6), 50μg/kg vitamin B$_{12}$ (n=6) and 100μg/kg vitamin B$_{12}$ (n=6). Serum physiologic to first group and other two groups were administered for seven days at the indicated doses of vitamin B$_{12}$ intraperitoneally. On the seventh day, pentylenetetrazole (PTZ) was intraperitoneally injected at 70mg/kg 45 minutes after drug administration. The animals were observed for 30 min. Stages were determined according to the Racine seizure scale and the first myoclonic jerk time (FMJ) was recorded in seconds. After the procedure, brain tissues were removed of animals. After routine histological follow-up, serial sections from brain tissues were stained with toluidine blue. The hippocampal CA1, CA2 and dentate gyrus regions were evaluated histopathologically. The data analyses were performed with SPSS Version 21.0 for Windows and were evaluated using a one-way analysis of variance (ANOVA).

Results: The results of epileptic behavior were evaluated according to Racine convulsion scale, the difference between the control and 50μg vitamin B$_{12}$ group was statistically significant (p<0.01). The first myoclonic jerk time was considered, the difference between the control and 50μg vitamin B$_{12}$ was statistically significant (p<0.001). When the groups were evaluated histopathologically, it was statistically significant that 50μg B$_{12}$ treatment reduced neuronal damage in CA1, CA2 and dentate gyrus regions (p<0.05).

Conclusion: This study suggests that vitamin B$_{12}$ therapy may reduce epileptic seizures and post-seizure neuronal damage.

Abbreviations: PTZ: Pentylen Tetrazole; FMJ: First Myoclonic Jerk; AEDs: Antiepileptic Drugs; RCS: Racine’s Convulsion Scale; CA: Cornu Ammonis; DG: Dentate Gyrus; SEM: Standard Error of Mean

Keywords: Epilepsy; Pentylenetetrazole; Vitamin B$_{12}$

Introduction

Epilepsy is the one of most common and critical neurological disorder that affects millions of people worldwide [1]. This neurological disorder is defined the repeated occurrence of bursts of electrical activity (seizures) in specific brain areas such as limbic system and cerebral cortex [2]. Various antiepileptic drugs (AEDs) are widely used both long-term combined therapy and mono therapy in epilepsy. Drug resistant (i.e., pharmacoresistant or medically intractable) epilepsy is defined as failure to achieve seizure control despite adequate trials of antiepileptic drug (AED) therapy [3] and approximately one-third of epileptic patients do not respond efficiently to present AEDs [4]. Many available AEDs may also cause toxicity [5]. Therefore, more influential and safer new therapeutics are necessary.

Vitamin B12 is needed for the development and initial myelination of the central nervous system as well as for the maintenance of its normal function [6]. Promoting neurite growth, neuroregeneration and antinoception properties
Experimental animals

The present study was performed in Cumhuriyet University Animal Laboratory after approval of Local Ethics Committee. Healthy adult male, weighing 230-250 g, Wistar albino (n=18) were used. All animals were fed by a standard laboratory diet and they could drink water whenever they requested. Rats were capable of normal activity in the cages, 22 ± 2°C, humidity (50-70%) and 12 hours of night/day. All animals were kept under observation for a few days before the study to decide if they are healthy or not.

Drug administration

Vitamin B12 and pentylentetrazol were dissolved in physiological saline. The drugs were purchased from Sigma-Aldrich Co., St Louis, MO, USA. Solutions were freshly prepared and they were no statistically significant differences between 100µg/kg vitamin B12 and physiological saline. The seventh day, pentylentetrazol (PTZ) (70 mg/kg, i.p.) was injected 45 min after the last vitamin B12 injection to induce seizures. Racine's Convolusion Scale (RCS) were used to evaluate the seizures stages as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks; 4 = tonic-clonic seizure while the animal remained on its feed; 5 = tonic-clonic seizure with loss of the righting reflex; and 6 = lethal seizure. Rats were observed for both to evaluate Racine's Convolusion Scale (RCS) and to record first myoclonic jerk (FMJ) onset times which coincide inception stage3 [13]. The observation period for PTZ-induced seizures was limited to 30 min in duration [14]. Two hours after, the animals were terminated using the decapitation method and brain tissues were removed.

Materials and Methods

Experimental procedure

Eighteen rats were divided randomly into three groups for behavioral and histological assessments. Group1 was given saline intraperitoneally (i.p.), group2 50µg/kg vitamin B12, i.p. and group3 100µg/kg vitamin B12, i.p. for 7 days. The seventh day, pentylentetrazol (PTZ) (70 mg/kg, i.p.) was injected 45 min after last vitamin B12 injection to induce seizures. Racine's Convolusion Scale (RCS) were used to evaluate the seizures stages as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks; 4 = tonic-clonic seizure while the animal remained on its feed; 5 = tonic-clonic seizure with loss of the righting reflex; and 6 = lethal seizure. Rats were observed for both to evaluate Racine's Convolusion Scale (RCS) and to record first myoclonic jerk (FMJ) onset times which coincide inception stage3 [13]. The observation period for PTZ-induced seizures was limited to 30 min in duration [14]. Two hours after, the animals were terminated using the decapitation method and brain tissues were removed.

Histopathological evaluation

Formalin-fixed brain sections (5µm) were stained with toluidine blue stain to quantify the number of dark neurons. All sections were examined and photographed with Olymopus C-5050 digital camera at Olymopus BX51 microscope. In hippocampal CA1, CA2 (Cornu Ammonis) and DG (Dentate gyrus) regions, dark neurons and survival neurons were counted in six sections per studied animal (n=3 for each group) by an image analysis system (Image- Pro Express 1.4.5, Media Cybernetics, Inc. USA). The numbers of dark neurons were given as percentage (toluidine blue stained neurons*100/survival neuron). The observers blinded to the study groups accomplished all histological assessments.

Statistical analysis

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

Results

Evaluation of groups in terms of RCS and FMJ Onset Times

When the Racine scores were calculated between the groups, there were statistically significant differences between the saline (5.5 ± 0.2) and 50µg/kg vitamin B12 (3.6 ± 0.4)(p <0.01). However there were no statistically significant differences between the saline (5.5 ± 0.2) and the 100µg/kg vitamin B12 (4.5 ± 0.4) group than in the saline (p >0.05). In addition, there were no differences between the 50µg/kg vitamin B12 and 100µg/kg vitamin B12 (p > 0.05) (Figure 1). There were statistically significant differences (p < 0.001) between saline (83.3 ± 9.9 s) and 50µg/kg vitamin B12 (176.6 ± 16.7 s) groups in terms of FMJ onset times. There were no statistically significant differences between 100µg/kg vitamin B12 (109.3 ± 11.2) and saline in terms of FMJ onset times.

In addition, there were differences between 50µg/kg vitamin B$_{12}$ and 100µg/kg vitamin B$_{12}$ statistically (p<0.01) (Table 1).

Table 1: Fist myoclonic jerck (FMJ) onset time as seconds(s). Data were expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FMJ onset time (s)</th>
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<tbody>
<tr>
<td>PTZ (70mg/kg) and saline (group 1)</td>
<td>83.0 ± 9.9</td>
</tr>
<tr>
<td>PTZ (70mg/kg) and 50µg/kg vitamin B$_{12}$ (group 2)</td>
<td>176.6 ± 16.7*#</td>
</tr>
<tr>
<td>PTZ (70mg/kg) and 100µg/kg vitamin B$_{12}$ (group 3)</td>
<td>109.3 ± 11.2</td>
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Evaluation of groups in terms of dark neurons

Dark neurons were identified by the neuronal shrinkage, cytoplasmic eosinophilia, nuclear pyknosis, and surrounding spongiosis in total hippocampal formation (Figure 2A,2E,2I) and CA1 (Figure 2B,2F,2J), CA2 (Figures 2C,2G,2K) and DG (Figures 2D,2H,2L) hippocampal regions’ formation. Administration of Vitamin B12 at the dose of 50µg/kg significantly prevented production of dark neurons due to PTZ induced seizures in CA1, CA2 and DG regions of hippocampus (p< 0.05) (Figures 3A-3C). However there was no significant difference between at the dose of of 100µg/kg vitamin B$_{12}$, and PTZ group in CA1, CA2 and DG regions of hippocampus in point of dark neurons (p>0,05) (Figures 3A-3C). In addition, there was no significant difference between at the dose of 50µg/kg and at the dose of 100 µg/kg vitamin B$_{12}$ in CA1, CA2 and DG regions of hippocampus in point of dark neurons (p>0,05).

Discussion

In the present study, on the one hand subchronic administration of vitamin B$_{12}$ significantly decreased RC and also increased FMJ. Vitamins have been considered important patterns in controlling certain types of seizures or even preventing adverse effects of AEDs [15,16]. There are various studies indicating an association between vitamin B12 deficiency and EEG abnormalities in epilepsy [17,18]. In addition, there are increasing evidences indicate neuroprotective effects of vitamin B$_{12}$ in peripheral and central nervous systems. In the previous studies, researchers evaluated the neuroprotective actions of vitamin B$_{12}$ in rats with sciatic and corneal nerves crush injury models [19,20]. In addition, a study indicated that vitamin B$_{12}$ has anti-apoptotic effect on peripheral neuron injury by increasing Bax protein and reducing Bcl-2 protein [21]. Furthermore, studies have shown that vitamin B$_{12}$ is able to protect cortical neurons and retinal cell cultures against glutamate cytotoxicity [22]. Also vitamin B$_{12}$ show antiepileptic activity in penicillin-induced model via GABAergic receptor system [23]. On the other hand, neuroprotection is very important as a promising therapy for preventing and treating epilepsy [24]. These findings show possibility potential of vitamin B$_{12}$ in the treatment epilepsy. However, the potential use of vitamin B$_{12}$ in the treatment of epilepsy is not enough yet.

On the other hand, these findings show possibility potential of vitamin B$_{12}$ in the treatment epilepsy. However, the potential use of vitamin B$_{12}$ in the treatment of epilepsy is not enough yet. In present study showed that after PTZ-induced seizures
damaged to hippocampals neurons of the rats were prevented by vitamin B\textsubscript{12}. Dark neurons, previously were considered as histological artifacts in neuro surgical biopsies [25] but later, they were seen after brain trauma [26].

Dark neurons have basophilic appearance and morphological changes and might be seen after hypoglycemia, ischemia, stress and epilepsy [27,28]. Epilepsy has also been introduced as an important cause of dark neuron production [29]. The results of present study showed that PTZ-induced seizures were resulted in dark neuron production in the hippocampal regions which were confirmed by various studies [30,31]. Several studies have also confirmed hippocampal damages created by seizures [32,33]. The results of present study were consistent with previous studies in which it was shown that PTZ-induced seizures were followed by production of dark neurons in the brain tissues.

**Conclusion**

The results of the present study showed that vitamin B\textsubscript{12} decreased epileptic seizures as well as preventing neural damage after PTZ-induced seizure in rats. These results support the beneficial effect vitamin B\textsubscript{12} on the nervous system. Further studies are required for determining the protective effect and mechanism of vitamin B\textsubscript{12}.

**Financial Disclosure**

No funding agency had any part in this study.

**Conflict of Interest**

The authors declare no competing interests.

**References**


