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Original Research

Vitamin D3 Treatment Reduces Epileptic Neuronal Damage by Inhibiting Apoptosis and Increasing Vitamin D Receptor Expression in an *In Vivo* Epileptic Model

Yin-yue Nie[1,†], Lu-yue Huang[1,†], Lu-chuan Wang[2,†], Pei Zeng[1], Chao Gong[1], Lin Song[3], Jin Guo[1,*], Shaobo Zhou[4,*]

[1]Heilongjiang Provincial Key Laboratory for Children's Neurorehabilitation, College of Rehabilitation Medicine, Third Affiliated Hospital of Jiamusi University, 154002 Jiamusi, Heilongjiang, China
[2]Department of Pathophysiology, College of Basic Medicine, Jiamusi University, 154002 Jiamusi, Heilongjiang, China
[3]Department of Rehabilitation Medicine, The Fourth Affiliated Hospital of Harbin Medical University, 150000 Harbin, Heilongjiang, China
[4]School of Science, Faculty of Engineering and Science, University of Greenwich, ME44TB Chatham, UK
*Correspondence: guojin8002@163.com (Jin Guo); s.zhou@greenwich.ac.uk (Shaobo Zhou)
[†]These authors contributed equally.
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Abstract

Background: Vitamin D (VitD) deficiency is prevalent in more than half of patients treated with antiepileptic drugs. The number of seizures decreases by more than 40% after vitamin D3 supplementation. This study aimed to investigate the antiepileptic effects of vitamin D3 by using an *in vivo* epileptic model. **Method**: Sprague–Dawley rats received pentylenetetrazole (i.p.) treatment to induce epilepsy and were then treated with sodium valproate, VitD, or a combination of VitD and paricalcitol. **Results**: Vitamin D3 treatment improved epileptic behavior, as evidenced by increased latency time and a significant reduction in epileptic scores on the seventh day after pentylenetetrazole challenge. Improvements in cell morphology and reduced neuronal damage were observed as well as decreased apoptosis rates caused by epilepsy. Although no significant changes in the calcium-sensing receptor (CaSR) were observed in any group, the level of VitD receptor (VDR) significantly increased in groups treated with vitamin D3 alone, and with paricalcitol and sodium valproate. **Conclusions**: The study demonstrated the effect of vitamin D3 on reducing neuronal damage caused by epilepsy. The neuroprotective effects of vitamin D3 treatment may be attributed to the inhibition of cell apoptosis and the increase in the expression of VitD receptors induced by epilepsy.

Keywords: epilepsy; vitamin D3; apoptosis; vitamin D receptor; rats

1. Introduction

Epilepsy is the fourth most prevalent neurological disorder and affects approximately 50 million people worldwide [1]. Epilepsy is caused by abnormal firing of neurons in the brain due to various causes, and its clinical manifestations include persistent repetitive seizures, transience and rigidity. Almost one-third of epileptic patients do not respond well to antiepileptic drugs, and their side effects are associated with cognitive impairment, psychiatric problems and recurrent epileptic adverse reactions [2,3]. Vitamin D (VitD) deficiency exists in more than 37%–54% of patients treated with antiepileptic drugs [4–6] and in 86.8% of epileptic patients with 14 years of history of epilepsy. However, analysis of retrospective data from Saudi patients indicated a 40% reduction in seizures after VitD supplementation [7]. To date, clinical trials have not been conducted to assess the efficacy of VitD in the treatment of epilepsy [8].

Optimal VitD status is essential to maintain calcium homeostasis and bone health by regulating cell differentiation, cell growth, immunomodulation and hormonal control. Theactive form of VitD, namely, 1,25-dihydroxycholecalciferol (calcitriol, 1,25-(OH)2-VitD3), activates the VitD receptor (VDR) to alter the transcription rate of target genes for biological responses. VDRs are found in nearly all cells in the human body. In the nervous system, VDRs exist in the cerebellum, thalamus, hypothalamus, basal ganglia and hippocampus [9–11] and regulate epileptic activities by releasing brain-inhibitory neurotransmitters [12]. VitD3 showed anticonvulsant efficacy (37.5 and 75 µg/kg) by increasing the level of the hippocampal seizure threshold in the seizure model of rats or mice induced by pentylenetetrazol (PTZ) [13–15]. In comparison, 10 µg/kg paricalcitol (a VDR agonist) exerted a protective effect on PTZ-induced convulsion [16]. VitD demonstrated cellular proliferation, differentiation regulation, Ca^{2+} homeostasis, etc. [12,14,17]. These processes may be mediated by calcium-sensing receptors on the membrane (CaSR) [18,19]. CaSR regulates endogenous excitability, synaptic transmission and neuronal activity, which are involved in the pathogenesis of neurological diseases such as epilepsy [20]. Furthermore, the lev-

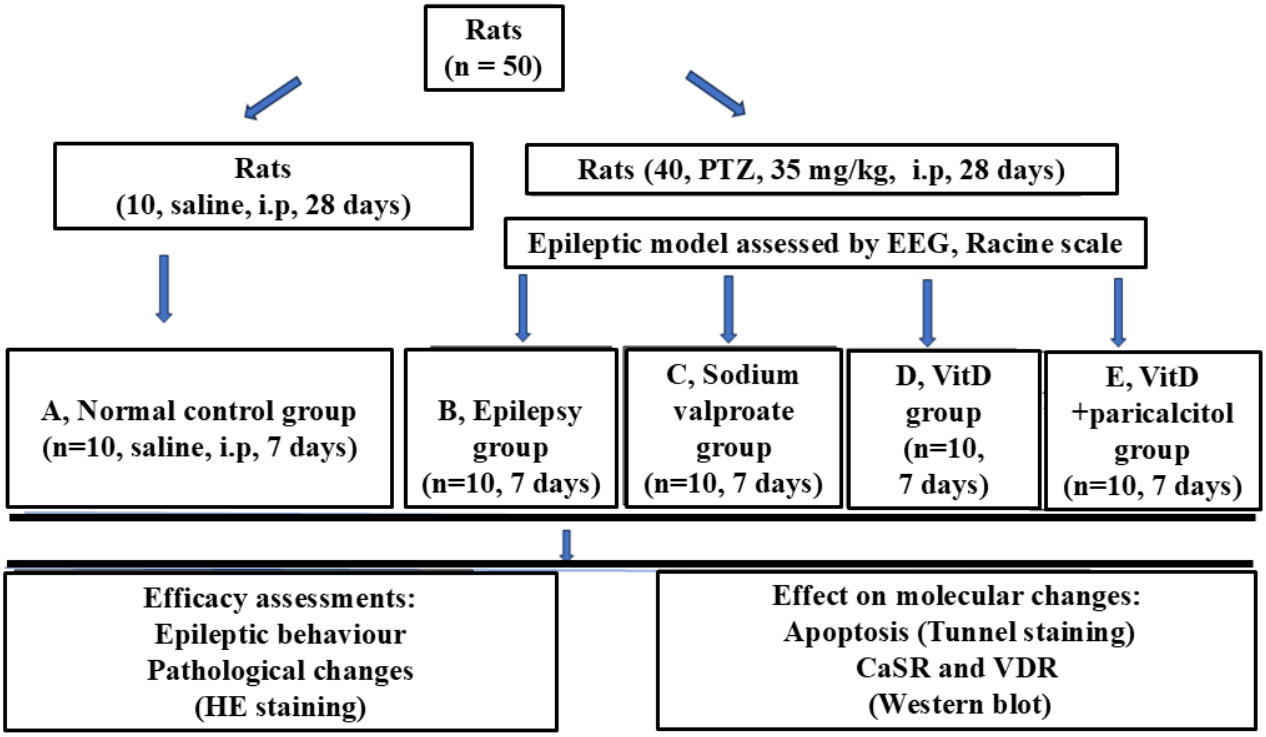

Fig. 1. Experimental scheme. PTZ, pentylenetetrazol; EEG, Electroencephalography; VitD, Vitamin D; CaSR, calcium-sensing receptor; VDR, VitD receptor; HE, Haematoxylin–eosin staining.

els of polyamine (a CaSR activator) are high in the cortex of kainic acid-induced epilepsy [21]. VitD deficiency in epilepsy patients is associated with long-term use of antiepileptic drugs and seizures [5–7]. The use of Vit D supplements has shown a synergetic effect of anticonvulsants in a study using ketogenic diet therapy [22]. VitD can improve intestinal calcium absorption to increase Ca^{2+} concentration in the brain and reduce neuronal excitability.

VitD supplement benefits in treating epilepsy [7,22], especially in patients with VitD deficiency. However, no study has examined its efficacy as an individual agent in the treatment of epilepsy. This pilot study aimed to investigate the antiepileptic effect of VitD in an *in vivo* epileptic model.

2. Materials and Methods

The study was carried out based on the schematic figure illustrated below (Fig. 1).

2.1 Animals

Based on our previous protocol, the study used 50 male Sprague–Dawley rats weighing 200–250 g [23]. All rats were kept in smooth bottom plastic cages at (22 ± 2) °C with a 12-h light/dark cycle and 60% humidity. They were fed standard laboratory food and given tap water ad libitum. Animal experiments were carried out using the Guideline for the Care and Use of Laboratory Animals' published by the Chinese National Institutes of Health. The Ethics Committee of Third Affiliated Hospital in Jiamusi University approved the study. Rats for Western blot evaluation were killed by cervical dislocation. Rats for the Tunnel assay were anesthetised with inhaled isoflurane (26675-46-7, MilliporeSigma, Burlington, MA, USA) (5% for induction and 2% for maintenance) according to our previous study [24] and American Veterinary Medical Association guidelines for animal sacrifice. All efforts were made to alleviate suffering.

2.2 Drug Preparations

VitD 3 powder (cholecalciferol, CAS: 67-97-0, Solarbio, Beijing, China) was dissolved in 10% Dimethylsulfoxide (DMSO) and then diluted in 90% corn oil. Paricalcitol (an agonist of the VitD receptor) was purchased from MedChemExpress (CAS: HY-50919, Monmouth Junction, NJ, USA) was dissolved similar to VitD did. PTZ was purchased from Sigma–Aldrich (CAS: 54-95-5, St. Louis, MO, USA) and dissolved in saline to reach a final concen-

tration of 10 g/L. Sodium valproate was purchased from Sigma–Aldrich (CAS: 1069-66-5) and dissolved in saline to a final concentration of 100 mg/mL.

2.3 Experimental Design

A rat epileptic model was prepared based on our previous reports [25] and other works [15,24]. Rats were randomly assigned to five groups (n = 10 per group). In the control group (A), rats received daily saline treatment for 28 continuous days, followed by DMSO (i.p. 10% DMSO solution in corn oil, and the given dosage was 75 µg/kg body weight) daily for further 7 days [15]. The remaining rats were given PTZ (i.p. 35 mg/kg) [25] daily for 28 days to induce epilepsy and were further divided into four groups: epilepsy group (B), which received only DMSO daily for 7 days; epilepsy + sodium valproate group (C); epilepsy + VitD group (D); and epilepsy + VitD + paricalcitol group (E). The following treatment was given daily in the following seven days. The epilepsy group (B) was given DMSO only; the epilepsy + sodium valproate group (C) received sodium valproate (200 mg/kg) [26] orally for 7 days as a positive treatment group [16]; the epilepsy + VitD group (D) was given VitD 3 (10 µg/kg) treatment through intraperitoneal (i.p.) injection daily for 7 days, and the dosage was based on previous study [15]; and the epilepsy + VitD + paricalcitol group (E) was treated with VitD 3 (10 µg/kg) and paricalcitol (VitD receptor agonist, 10 µg/kg) daily for 7 days, and the dosage was based on a previous study [16]. At the end of the treatment, each rat was administered with one additional PTZ treatment. The severity of rat flare-up behavior was independently assessed by two experienced individuals.

2.4 Behavior of PTZ-Induced Kindling Rats

The rats were placed in cages and observed for 60 minutes after PTZ injection. The frequency and duration of seizures were also assessed. According to Racine's proposed criteria [27], seizure activities were evaluated using a method described in our previous study [24]. The intensity of seizure was assessed as follows: stage 0, no convulsions; stage 1: rhythmic corners of the mouth, facial twitching; stage 2: myoclonic jerking or head nodding; stage 3: head twitching, forelimb clonus with standing; stage 4: limbs twitching, clonic–tonic seizure; and stage 5: generalised tonic–clonic seizures and absence of reflexes. Seizure levels for each rat were recorded daily. Electroencephalography (EEG) data were evaluated using the method described in our previous study [25]. Isoflurane was administered to the rats at a concentration of 5% for anaesthesia induction and maintained at 1%–2%. Three 1.2 mm-diameter stainless-steel screw electrodes were separately positioned in the bilateral temporal lobes and the right frontal lobe of the rats, with the latter as the reference electrode. EEG recordings were conducted for up to 90 minutes by utilizing EEG recorder (Z2N-F-20-C, NCC Electrophysiology, Shanghai, China, https://www.shnccmedical.com/). If rats maintained an epileptic behavior (at stage 2 or above) for 5 consecutive days, then they were considered a successful kindling model and used for subsequent analysis [24,25].

2.5 Haematoxylin–Eosin Staining

Rats were decapitated at the end of the experiment. The brains were removed as previously described [25]. Paraffin sections were taken from the brain tissues of rats in each group and dewaxed with conventional xylene in water. The sections were used for haematoxylin–eosin staining and washed with 1% hydrochloric acid alcohol differentiated, washed with water, re-stained with 1% eosin alcohol, and subjected to conventional gradient alcohol dehydration; the sections were made transparent and sealed. Pathological changes in the CA1 cortical and hippocampal regions were observed under a light microscope (ECLIPSE Ni, Nikon, Melville, NY, USA).

2.6 Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Staining Detection

Paraffin sections of rat brain tissues in each group were used to detect nerve cell apoptosis according to the instructions of the TUNEL kit (CAS: MK1015, Boster Biotechnology, Wuhan, China) [25]. Under an optical microscope, the nucleus of positive cells emerges, and their shapes are round, crescent or irregular. Six observed fields were randomly selected to record the number of positive cells and the total cells in each view. Apoptotic rate was calculated for each field by using the following equation: apoptosis rate (%) = positive cell number/total cell number $\times$ 100.

2.7 Western Blot Analysis

The hippocampal tissues of the rats were extracted and placed in a glass homogenate tube [24]. The sample was added with protein lysis buffer for low-temperature grinding and cracked on ice for 30–40 min. The proteins were then centrifuged at 4 °C and 12,000 rpm for 20 min. The supernatant was added to the protein loading buffer, boiled in boiling water for 5 minutes, cooled and stored in a refrigerator at –20 °C. Protein was extracted for Western blot analysis [26]. Protein lysis buffer was added to extract proteins. About 20 µg of proteins were loaded onto 15% polyacrylamide gel electrophoresis, and the separated proteins were transferred onto a Polyvinylidene Fluoride (PVDF) membrane with 100 V for 2 h. The PVDF membrane was placed in a blocking buffer at 37 °C for 1 h. The membrane was incubated with anti-calcium-sensing recepter (CaSR) (CAS: 19125-1-AP, 1:1000; Proteintech Group; Wuhan, China) and anti-VDR (CAS: K110777P, 1:1000; Solarbio; Beijing, China) at 4 °C overnight. After repeated washing, the membrane was incubated with an alkaline phosphatase-labelled anti-rabbit IgG antibody (CAS: A0352, 1:5000, Beyotime, Shanghai, China) for 1 h. After the membrane was washed,

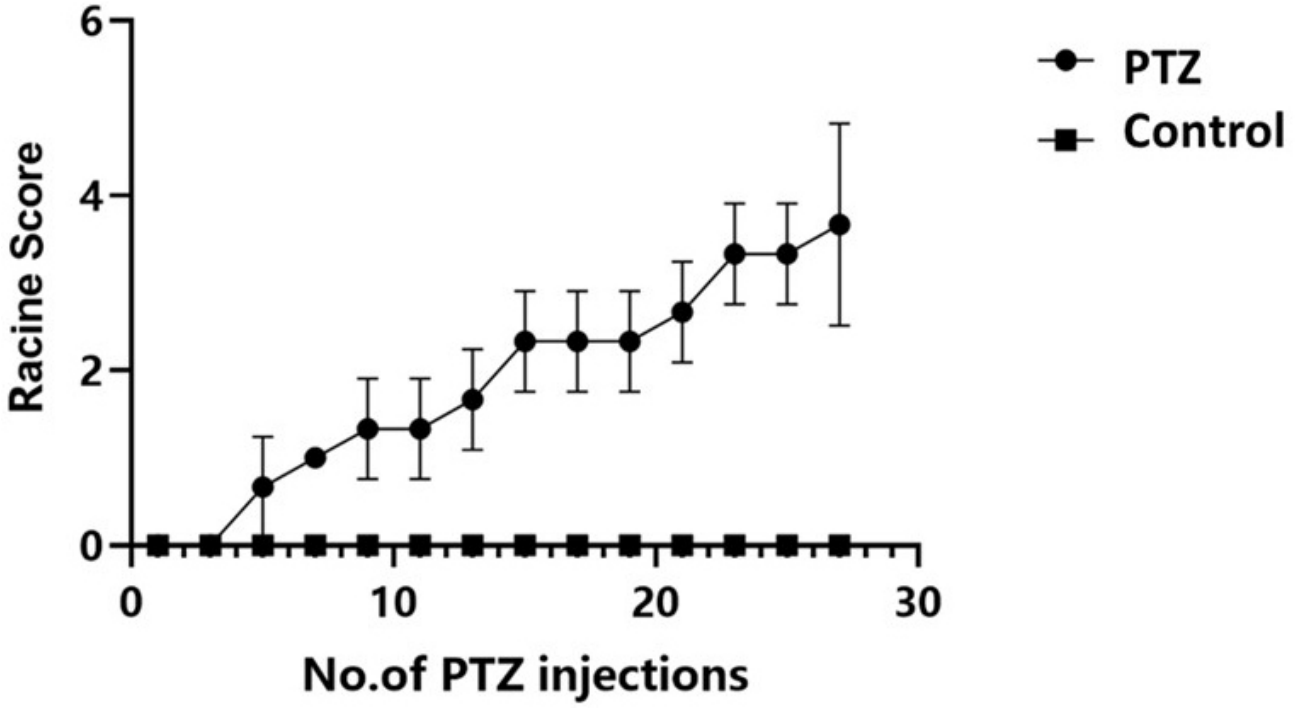

Fig. 2. The Racine score during the number of PTZ injections (mean ± SE, n = 5). SE, Standard Error.

the developer was added, the colour in the darkroom was observed and the optical density of each protein band was analysed. The original figures of Western blot can be found in the **Supplementary Materials**.

2.8 Data Analysis

All data are reported as means ± standard error (mean ± SE) and analysed with SPSS version 23.0 software (IBM SPSS Statistics, New York, NY, USA). *T*-test was used to evaluate differences between the two groups. Differences in Gaussian distribution data among multiple groups were compared by one-way ANOVA. For non-normally distributed data in the analysis of farer-up grade, apoptosis, and expression of CaSR and VDR, the differences between the two groups were evaluated using Mann–Whitney U test. Kruskal–Wallis test was used for multiple groups. A p value of <0.05 indicated statistical significance.

3. Results

3.1 VitD Treatment Improved Epileptic Behaviour

Rats injected with PTZ experienced seizure symptoms such as rhythmic chewing, nodding, tail swinging and forelimb clonus with standing, rolling, jumping, neighing and uncontrolled postures. Seizure behaviour was assessed using Racine scoring method [27]. Rats with epileptic seizures at or above stage 2 were suitable as models of epilepsy. The Racine score during PTZ injections is shown in Fig. 2. The Racine score reflected epileptic behavior, which increased significantly with increasing times of PTZ injection.

These rats were also monitored for changes in EEG waveforms (Fig. 3) before being treated with sodium valproate, a drug used to treat epilepsy, VitD alone, or in combination with paricalcitol for another seven days. The EEG of the control group showed regular waves of nerve electrical activity, and the primary rhythm wave did not change. However, the EEG of the epilepsy group showed spikes of high amplitude or irregular waveforms of the slow spinous complex waves.

At the end of the treatments, rats received one more injection of PTZ to induce recurrence of epilepsy. The latency of the seizure (minutes difference between the completion of a seizure and the start of the subsequent seizure) was analysed to assess the efficacy of the treatments (Fig. 4a). Sodium valproate treatment had the highest efficacy in reducing epileptic symptoms by improving the latency period (3 times), followed by VitD plus paricalcitol treatments (2.5 times) and VitD alone treatments (2 times). They also significantly reduced the epileptic Racine Score on day seven after PTZ challenges (Fig. 4b).

3.2 VitD Treatment Ameliorated Nerve Cell Damage Caused by PTZ

The morphology of nerve cells in the rat cortex and hippocampus are shown in Fig. 5a,b, respectively. Cortical nerve cells were intact in the control group, with a clear outline, regular shape and size. Compared with the control group, the cortical nerve cells in the epilepsy group were of abnormal shape, with a fuzzy outline, obvious nucleus pycnosis, karyolysis and missing cell structures. Compared with the epilepsy group, pathophysiological damage (e.g.,

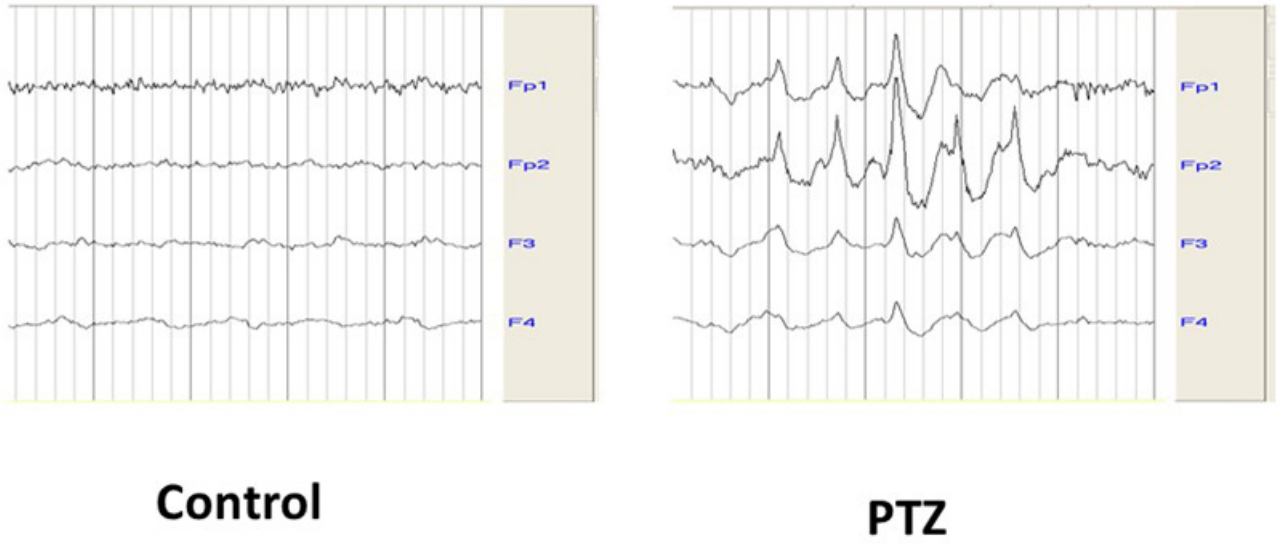

Fig. 3. Changes in EEG waveform and amplitude of rats in control and PTZ group.

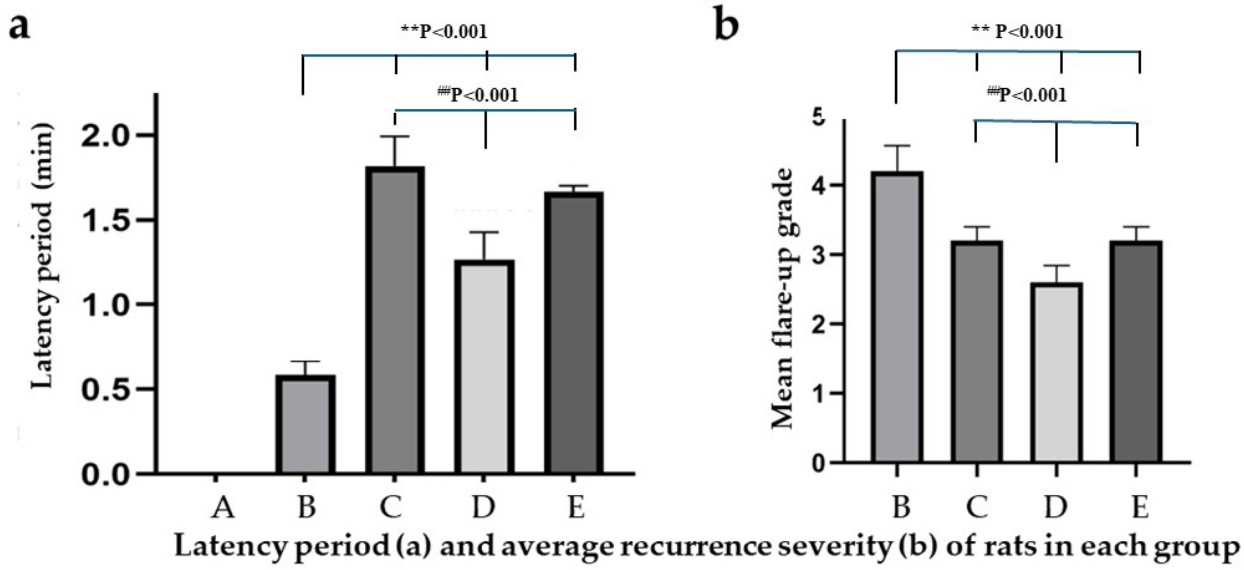

Fig. 4. Seizure latency (a) and average severity of recurrence (also known as flare-up grade after challenge with PTZ); (b), for each group at the end of the experiment. Note: A, Control group; B, Epilepsy group; C, Epilepsy + sodium valproate group. D, epilepsy + VitD group; E, epilepsy + VitD + paricalcitol group; $^{**}p < 0.001$ group B *vs* group C, D, or E, respectively; $^{\#\#}p < 0.001$ group D *vs* group C or E, respectively, n = 5.

cortical nucleus pycnosis and karyolysis) may be mitigated in epilepsy treated with the sodium valproate group, the VitD group, and the VitD plus paricalcitol group, respectively.

Qualitative inspection of the control group tissue indicated intact nerve cells in the hippocampus without apparent damage and abnormal morphology. In the epilepsy group the cell spacing appeared visibly looser, and evidence for cell damage was detected. According to hematoxylin-eosin (HE) staining of brain tissue from two rats of each group, the epilepsy animals treated with sodium valproate, with VitD alone or with paricalcitol groups exhibited less damage and morphological changes compared to the epilepsy group (not quantified).

3.3 VitD Treatment Reduced PTZ-Induced Apoptosis

Images of apoptosis of rat nerve cells after treatments are shown in Fig. 6A–E. Apoptotic cells are stained brown in the TUNEL assay. The apoptosis rate (Fig. 6F) was calculated by the number of five randomly observed optical fields. Apoptosis rate of groups treated with sodium valproate, VitD and VitD plus paricalcitol was significantly lower than that of the epilepsy group ($p < 0.01$).

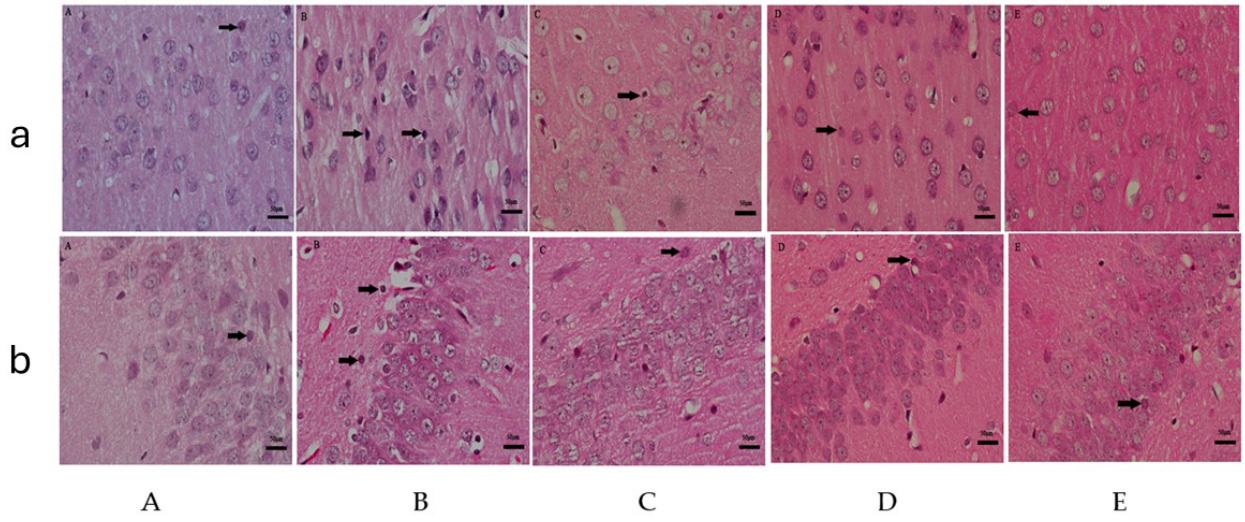

Fig. 5. Morphological images of HE staining in the cerebral cortex (a, top panel) and hippocampus (b, bottom panel) of rats under a light microscope (40 × 10). Note: A, Control group; B, Epilepsy group; C, Epilepsy + sodium valproate group. D, epilepsy + VitD group; E, epilepsy + VitD + paricalcitol group; arrows show the indicated changes, and the bar represents 50 µm. HE, hematoxylin-eosin. Scale bar: 50 µm.

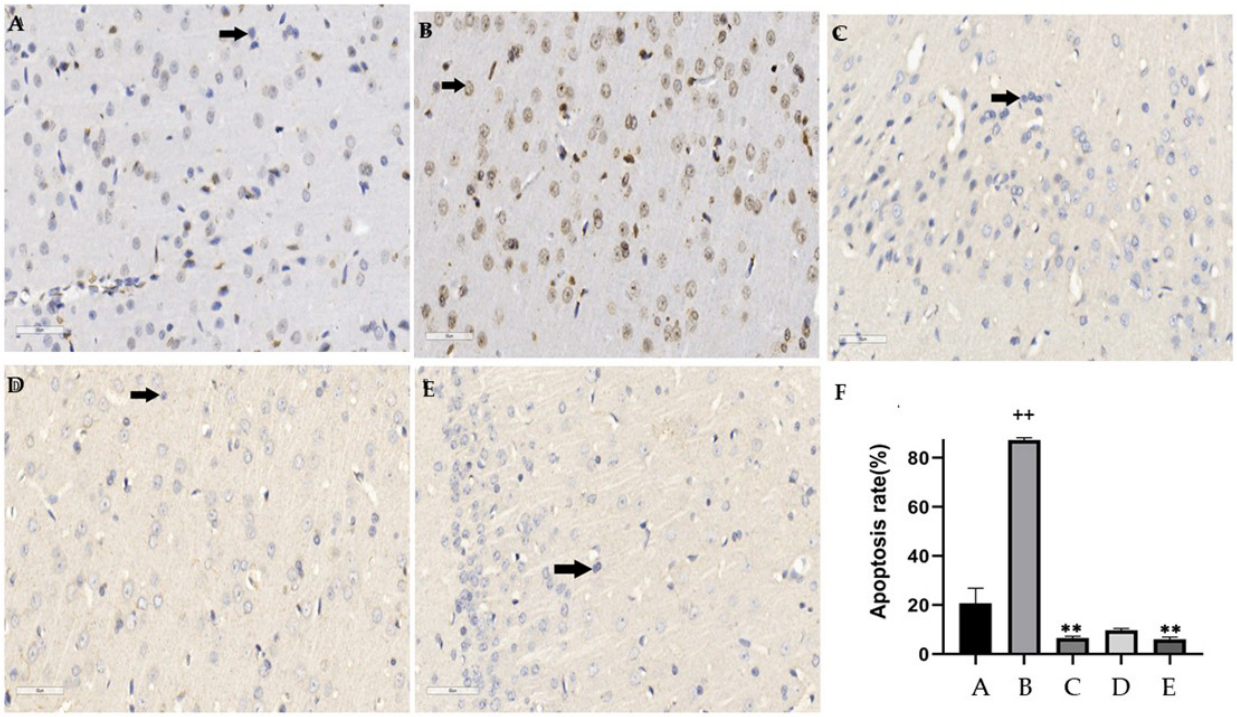

Fig. 6. Images of apoptosis of nerve cells assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (A–E) and comparison of the apoptosis rate (F) calculated by the five observed fields in the brain tissue of each group. Note: A, Normal group; B, Epilepsy group; C, Epilepsy + sodium valproate group. D, epilepsy + VitD group; E, epilepsy + VitD + paricalcitol group. Differences between groups (F) were evaluated using the rank sum test (for nonparametric data). The results represent the mean ± SE for five experiments. Arrow shows the apoptosis cell. $^{++}p < 0.01$, *vs.* other groups; $^{**}p < 0.01$, *vs.* D group; n = 6; scale bar: 50 µm.

3.4 The Expression of the CaSR and VDR Protein

The results (Fig. 7) show no significant changes in CaSR among all groups. However, there were substantial increases in VDR after VitD alone or with paricalcitol and in sodium valproate treatments.

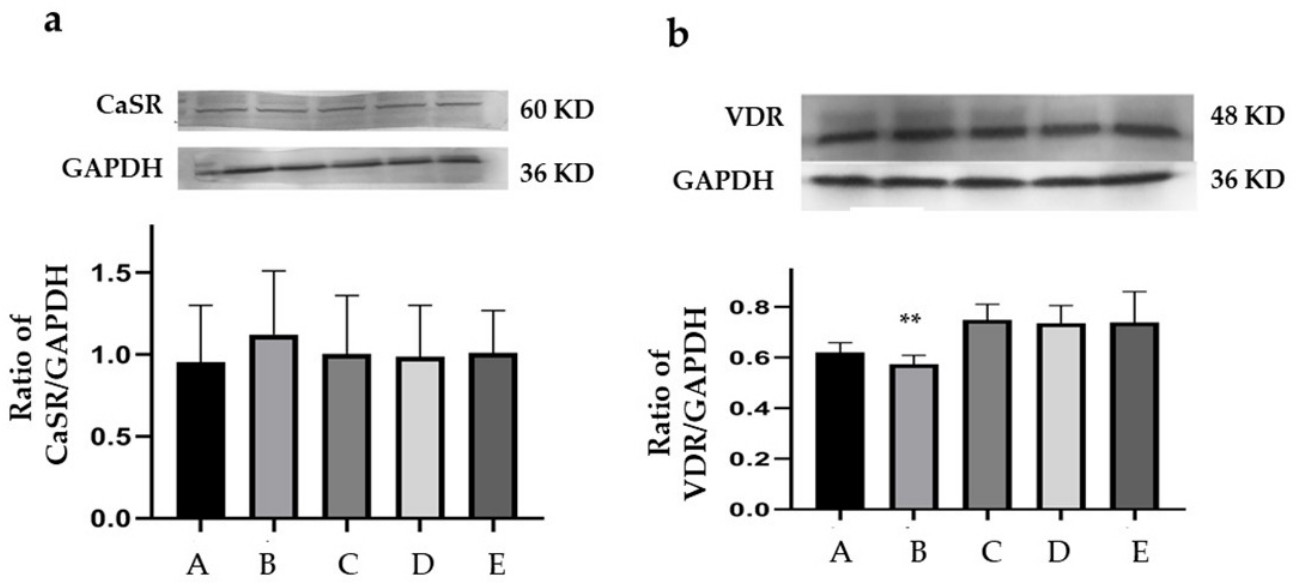

Fig. 7. Western blot analysis of CaSR (a), VDR (b) protein expression in hippocampi from rats with different treatments. Note: A, Control group; B, Epilepsy group; C, Epilepsy + sodium valproate group. D, epilepsy + VitD group; E, epilepsy + VitD + paricalcitol group. Top panel, Western blot protein bands of the expression of CaSR, VDR in each group. Bottom panel bar graphs show the effect of VitD on CaSR, VDR, and expression. The results represent the mean ± SE. VDR: $^{**}p < 0.01$, *vs.* C, D and E groups, respectively; n = 5. VitD, Vitamin D; CaSR, calcium-sensing receptor; VDR, Vitamin D receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase.

4. Discussion

Long-term use of antiepilepsy drugs (AEDs) can lead to adverse side effects and drug resistance among 30% to 40% of patients with epilepsy [2]. VitD supplementation improved efficacy of long-term AED treatment [13,15,16]. This finding may be due to compensation for the VitD lost during epileptic treatment. The advantages of using VitD include less toxicity, few side effects and easy availability at a relatively low price compared to pharmaceuticals.

This study used a subconvulsive dose (35 mg/kg) of PTZ to ignite an animal model of chronic epilepsy [25]. PTZ was injected intraperitoneally once a day for 28 consecutive days. The Racine score (Fig. 2) increased progressively in line with the sequential delivery of PTZ injections. According to the standard of the Racine scoring method, rats in the experiment all had several consecutive epileptic seizures of stages 2–4. The EEG in the PTZ treatment group showed spikes of high amplitude or irregular waveform in the slow spinous complex wave (Fig. 3). The morphological changes in the cortex and the hippocampal regions exhibited signs of severe damage, indicating the success of developing an *in vivo* rat model for epilepsy (Fig. 5). Based on the above observation, it can be confirmed that the epilepsy model has been successfully prepared.

After VitD treatment, seizure latency increased (Fig. 4a), while the severity of recurrence of VitD was also shown of rats was (Fig. 4b). Furthermore, the efficacy of the antiepileptic effect in improving the histological structure of the neuron in the cortex and the hippocampal regions (Fig. 5a,b); moreover, a significant reduction in the rate of apoptosis was found in the hippocampal region (Fig. 6A–F). The correlation between VitD deficiency and epilepsy has been continuously reported in recent years. VitD can also affect neuronal excitability at the threshold level by fine-tuning Ca^{2+} and Cl^{-} currents across the neuronal cell membrane, thereby changing the conductance of L-type calcium channels and chloride channels [8]. Excess calcium ion levels will increase nerve excitability, leading to neuron depolarisation and increased risk of epilepsy. VitD could reduce the flow of calcium ions into cells and involve in the synthesis of calcium-binding protein to protect nerve cells [13]. An appropriate amount of VitD can maintain the homeostasis of intracellular and extracellular calcium ions, thus reducing abnormal discharge from neurons and protecting nerve cells [28]. In 1984, Siegel *et al.* [13] first reported the anticonvulsant properties of 1,25-(OH)2-VitD3 and found that the administration of active VitD3 could increase seizure threshold in the rat hippocampus. Furthermore, an animal study by Kalueff *et al.* [14] showed an anticonvulsant effect of 1,25-(OH)2-VitD3 in chemically induced seizures in mice.

The effect of VitD exhibited a downward trend of CaSR expression of CaSR ($p > 0.05$, Fig. 7a) but a significant stimulation of VDR expression ($p < 0.05$, Fig. 7b). The protective effect of VitD was strengthened, indicating that VitD can reduce the expression of CaSR; the downregulation of CaSR expression might contribute to the neuroprotective effect of VitD. CaSR is widely expressed throughout the brain and affects synaptic transmission and neurotransmitter releases [9,10]. In cultured hippocampal neu-

rons, blocking CaSR inhibits dendritic growth, while activating CaSR activates spontaneous releases of neurotransmitters. CaSR in the parathyroid glands senses the systemic Ca^{2+} level and regulates the secretion of parathyroid hormones (PTH) to maintain calcium homeostasis. Although CaSR mutations do not result in an altered systemic PTH or serum Ca^{2+} level associated with seizures, the impact of CaSR activities on epilepsy progresses [20]. To investigate the role of VDR in epilepsy treatment, paricalcitol, a selective VitD receptor agonist, was introduced. Paricalcitol increases serum VitD levels in a controlled and subtle way. In this experiment, VitD alone or in combination with paricalcitol for 7 consecutive days, nerve cell damage and necrosis in the hippocampal tissues of epileptic rats were apparently reduced, which is consistent with previous studies [13,29]. The expression of the VDR protein in the brain tissue from these two groups was significantly higher than that in the epilepsy group and the sodium valproate group (Fig. 7b). This suggested that VitD could alleviate nerve cell damage in the hippocampal tissues of epileptic rats by regulating the expression level of VDR.

In vivo CaSR and VDR regulate calcium homeostasis; for example, CaSR governs the release of PTH in response to changes in extracellular calcium. By contrast, VDR mediates the effects of calcitriol, the active metabolite of VitD [30]. In reverse, the impact of VitD on the reaction of CaSR and VDR, such as CaSR, helps to maintain the homeostasis of calcium ions in the extracellular space (10–3 M) and the cytosol (10–7 to 10–8 M) and controls many processes, such as cell secretion, apoptosis, chemotaxis, cell proliferation, cytoskeletal rearrangement, ion channel activity, gene expression control and cell differentiation [31,32]. Therefore, CaSR balance *in vivo* would be critical for many pathophysiological processes in multiple organs, including the parathyroid gland, kidney, heart, bone, brain and skin. For example, in previous research, we found a high expression of CaSR, which was associated with a high apoptosis rate in injured hippocampal neurons and cardiomyocytes [32–34].

CaSR is a G-protein-coupled receptor that plays a crucial role in calcium homeostasis by sensing free calcium levels in the blood and regulating parathyroid hormone secretion accordingly. CaSR binds to various G proteins in a tissue-specific manner and activates multiple signalling pathways to regulate different intracellular activities [35]. Calcium is a universal signalling vector for biological information and one of the most specific and selective messengers. It participates in multiple signalling cascades critical for cell survival, differentiation and death. In addition, calcium controls several signalling pathways within cells, including those that regulate cell growth and death [19,28]. VitD regulates calcium homeostasis (e.g., 1,25-(OH)2-VitD3 may act on the regulation of the transcriptional activity of the CaSR and VDR genes [36].

According to Fig. 4, VitD improved the flare-up grade in comparison with sodium valproate and VitD + paricalcitol but could not improve the apoptosis rate (Fig. 6F), even though all the treatments with sodium valproate, VitD alone or treated with paricalcitol reduced apoptosis significantly than the model group with epilepsy without any given treatment. These are possible reasons regarding the higher apoptosis rate in the VitD treatment group than in the VitD plus paricalcitol and sodium valproate groups. Despite VitD's ability to reduce flare-up severity, the higher apoptosis rate in the VitD treatment group highlights the complexity of its effects on cellular processes. VitD has dual role as a neuroprotectant and a modulator of apoptosis so it can reduce seizure severity while promoting apoptosis under certain conditions. Adding paricalcitol or sodium valproate modulates these effects, leading to a balanced outcome regarding cell survival. Further investigation into the specific pathways involved could provide additional insights.

VitD alone or treated with paricalcitol reduced apoptosis *in vivo* (Fig. 6F). The epilepsy-induced apoptotic pathway can have several harmful effects. Recurrent transient seizures can cause progressive loss of hippocampal neurons and loss of spatial memory [37]. VitD has a neuroprotective effect on kainic acid-induced hippocampal apoptosis in rats. The main pathological manifestations of epilepsy are neuronal cell death and glial cell loss [38]. During epileptic seizures, mitochondria in the brain are prone to destruction. In epilepsy-induced brain injury, mitochondrial permeability occurs, and pro-apoptotic proteins are released from the mitochondria, resulting in downstream Caspase-3 activation and cell apoptosis [23]. Previous studies on the pathogenesis of epilepsy were based mainly on cell necrosis and apoptosis, which explained the death of neurons during seizures. In our TUNEL staining, VitD, sodium valproate and paricalcitol groups had significant reductions in neural cell apoptosis in epileptic rats. Hence, VitD inhibited the apoptosis of the *in vivo* epileptic model, but the mechanism needs further exploration. For example, research should explore the role of the phosphoinositol-3 kinase/serine-threonine protein kinase (PI3K/Akt) pathway because its activation is critical for the survival of neurons by inhibiting cell apoptosis [39].

VitD deficiency exists in more than half of patients treated with antiepileptic drugs [4–6] and with a 40% reduction in seizures after VitD supplementation in humans [7,40] as well as in an animal study [13]. This commonly VitD deficiency among individuals with epilepsy could be due to medication, lifestyle and dietary habits as well as the broader implications for health and treatment strategies [4–6,41,42]. Many antiepileptic drugs, such as phenytoin, phenobarbital and carbamazepine, are enzyme inducers and increase the metabolism, thereby affecting the absorption of VitD in the liver and leading to lower levels in the bloodstream. Individuals with epilepsy may have lifestyle restrictions that limit their time outdoors and lead to a sedentary

lifestyle due to concerns about seizure triggers or medication side effects, such as photosensitivity. This can result in less sunlight exposure, which is necessary for the skin to synthesize VitD. Some individuals with epilepsy may have dietary restrictions or poor dietary habits that result in inadequate intake of VitD-rich foods, such as fatty fish, fortified dairy products, and eggs. Chronic seizures and the associated stress on the body can lead to changes in bone metabolism, increasing the demand for VitD and calcium for bone health. People with epilepsy may have other health conditions that affect VitD levels, such as gastrointestinal disorders that impair nutrient absorption or kidney issues that affect VitD metabolism. The efficacy of VitD in epilepsy treatment has not been warranted [8]. VitD deficiency was present in 54% of enzyme-induced patients and 37% of non-enzyme-induced AED [4]. This could be because AEDs destroy VitD stores in the body. VitD nutritional status should be monitored for managing epilepsy and reducing the risk of VitD deficiency, such as osteoporosis and osteomalacia. Until now, evidence remains limited on the efficacy of VitD in epilepsy treatment, but the prevalence of VitD deficiency remains high among patients with epilepsy. This provides good evidence for using VitD in the treatment of epilepsy, although further research is required to verify our results.

This study firstly established an epileptic animal model and then assessed the epileptic behaviour of the animals when they received vitamin D3 treatments and contrasted them with its agonist, paricalcitol, and the positive medication valproate. The results showed that vitamin D3 treatments (1) improved epileptic behavior, (2) reduced the apoptosis rate, and (3) decreased neuron damage caused by epilepsy.

5. Conclusions

This study showed that VitD improved epileptic behavior and delayed the occurrence of epilepsy *in vivo*. It decreased neuron apoptosis and improved the survival rate of epileptic neurons *in vivo*. VitD increased the expression of VDR. Further research is needed on the role of CaSR and the PI3K/Akt pathway and the optimal intervention dose in efficient epileptic treatment.

Availability of Data and Materials

Data are available: Zhou, Shaobo (2023), 'Investigation into the neuroprotective effect of vitamin D *in vivo* epileptic model', Mendeley Data, V1, https://data.mendeley.com/datasets/7fmkxkv3zn/1.

Author Contributions

JG and SZ designed and supervised the research study. YN, LH, and PZ performed the research. LW, LS and CG provided help and advice on the animal model set up and study. YN, LH, CG, and LS analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal study protocol was approved by the Ethics Committee of the Third Affiliated Hospital in Jiamusi University, on 29/10/2019 for studies involving animals.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/JIN25483.

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