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Thiamine alleviates cognitive impairment and epileptogenesis by relieving brain inflammation in PTZ-induced kindling rat model

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ABSTRACT

Objective: Epileptogenesis, the process by which the brain becomes epileptic, is related to neuroinflammation, hyperexcitability cognitive deficits. Evidence suggests that improving brain inflammation can inhibit the epileptogenesis process and help the emergence of new drugs for the treatment of epilepsy. Therefore, the PTZ kindling model of epilepsy was utilized to assess the neuroprotective role of thiamine in epileptogenesis.

Methods: Male rats were exposed to PTZ-induced kindling and pretreated with low thiamine (25 mg/kg) or high thiamine (50 mg/kg). Cyclooxygenase (COX-1 and COX-2), interleukin 1-beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and nuclear factor-κB (NF-κB) concentrations in the brain were analyzed using biochemical assays. Cognitive function was evaluated using the passive avoidance test.

Results: Thiamine ameliorated epileptogenesis and enhanced the rats' performance in the passive avoidance test. Also, thiamine significantly decreased the level of neuroinflammatory mediators in the brain induced by PTZ.

Conclusion: These results provide evidence that thiamine alleviates PTZ-induced neuroinflammation and cognitive impairments.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by spontaneous recurrent seizures resulting from hyperexcitability and hypersynchrony of brain neurons [1]. It is one of the most common diseases of the central nervous system that affects approximately 2% of the world population [2]. Antiepileptic drugs (AEDs) are the principal treatment for the disease. However, nearly up to 40% of newly diagnosed epilepsy patients fail to respond to these drugs [3]. Moreover, AEDs cannot modulate the underlying pathophysiology, only have seizure suppression. Epileptogenesis, defined as the normal brain becoming epileptic, involves a process that begins after insult and gradually changes neuronal excitability [4]. This period between the emergence of spontaneous seizures from unprovoked seizures can represent a good opportunity window to change or prevent the progress of the disease. However, there is still no effective treatment that prevents or modifies epileptogenesis. Neuroinflammation has recently been recognized as one of the main etiological factors contributing to epileptogenesis [5]. Mounting evidence from animal and human studies indicates that proinflammatory cytokines and other mediators in the brain play an etiological role in epileptogenesis and the accompanying comorbidity, such as cognitive decline [6,7]. The excessive release of

these inflammatory mediators can significantly increase the calcium permeability in the glutamatergic neurons causing abnormal neuronal hyperexcitability [8]. Therefore, the goal of reducing the levels of neuroinflammatory molecules in the brain points to a potential antiepileptogenic strategy.

Thiamine (vitamin B1) is a water-soluble vitamin that serves as a cofactor for several enzymes involved in neurotransmitters' energy metabolism and biosynthesis [9]. Albeit many organs utilize thiamine, the brain is particularly vulnerable to thiamine deficiency related to an impairment of oxidative metabolism, excitotoxicity, brain damage, and inflammation [10]. Thiamine deficiency is also associated with neurological disorders such as Wernicke-Korsakoff syndrome and neurodegenerative diseases characterized by behavioral, cognitive and neuropathological defects such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [11,12]. Remarkably, cases of Wernicke encephalopathy have been reported, with epileptic seizures improving after thiamine treatment [13,14]. It has also been suggested that severe thiamine deficiency may cause epilepsy in infants [15]. Moreover, Mesdaghinia et al. showed that chronic treatment with thiamine increased the PTZ-induced tonic and clonic seizure threshold and suggested that

thiamine supplementation in classical AEDs may reduce the required drug doses [16]. Despite the encouraging results regarding the anticonvulsant potential of thiamine, its effect on the epileptogenesis and the inflammatory response involved in this process remains unclear. To this end, we aimed to investigate the effect of thiamine pretreatment on epileptogenesis, cognitive function, and brain inflammatory response in the rat PTZ-kindling model.

2. Materials and methods

2.1. Animals

The study was performed with 24 adult male Wistar albino rats (230–250 g), supplied from Animal Research Laboratory of our university. All rats were housed in a 12-hr light/12-hr dark cycle in a humidity (35–60%) and temperature-controlled room (23 ± 2 °C). All procedures were performed in accordance with the guidelines of the Local Ethics Committee for the welfare of experimental animals. (65,202,830–050.04.04–480).

2.2. Experimental design and drug treatments

The experimental design of the study illustratively is shown in Figure 1. Thiamine and pentylenetetrazol (PTZ) were purchased from Sigma-Aldrich (USA) and dissolved in 0.9% saline. All treatments were administered through an intraperitoneal (i.p.). The pretreated rats with thiamine have received different concentrations (25 and 50 mg/kg) before the PTZ injection in the kindling process [17]. Animals were randomized into four groups (n = 6) subjected to the following treatment: (i) The control animals were treated with a placebo (physiological saline 1 mL/kg), (ii) PTZ animals were treated with physiological saline (1 mL/kg) 30 min before each PTZ (35 mg/kg) every other day, (iii) TIA-25 animals received thiamine (25 mg/kg) 30 min before each PTZ injection, and (iv) TIA-50 animals received thiamine (50 mg/kg) 30 min before each PTZ injection. To induce kindling

in rats, PTZ (35 mg/kg, i.p.) was administered as a repeated subconvulsant dose on each alternate day, as described previously [18]. Following each PTZ injection, the seizure behavior was observed for 30 minutes. The severity of seizure response was scored according to a modified Racine's scale as follows [19]: Stage 0: No response, Stage 1: Ear and facial twitching, Stage 2: Head nodding, Stage 3: Myoclonic jerks, Stage 4: Tonic-clonic seizures without loss of postural control, Stage 5: Tonic-clonic seizures with loss of postural control, Stage 6: Tonic-clonic seizures with wild running and jumping, and Stage 7: Lethal seizure. The rats were described as 'kindled' when they have obtained a seizure score of 4 for three consecutive days.

2.3. Passive avoidance test

This test was performed as described previously [20]. The passive avoidance apparatus consisted of a light and dark chamber distinguished by an automatically retractable door. The darkroom floor was made from stainless steel grilles. For habitation, rats were slowly placed on the grilled floor, 5 min to adapt to each rat. During the conditioning phase, after 24 hours, the rats were placed in the lightroom, and when the hind legs of both rats entered the darkroom, the sliding door was automatically closed. Then, the ground grids supplied an electric charge at the foot (0.25 mA, 2 s). At the end of 10 seconds, the rats returned to their cages. After 24 hours, the delay time required for the rat to enter the darkroom was recorded during the retention test in which the electric shock was removed. The cutoff time for the training and retention session was set at 300 seconds.

2.4. Biochemical analysis

The content of COX-1, COX-2, IL-1β, TNF-α, NF-κB was determined in both hippocampus and cerebral cortex on the 30th day of the end of the experiment. The brain tissues were homogenized in an ice-cold phosphate buffer solution (pH: 7.4) through a hand
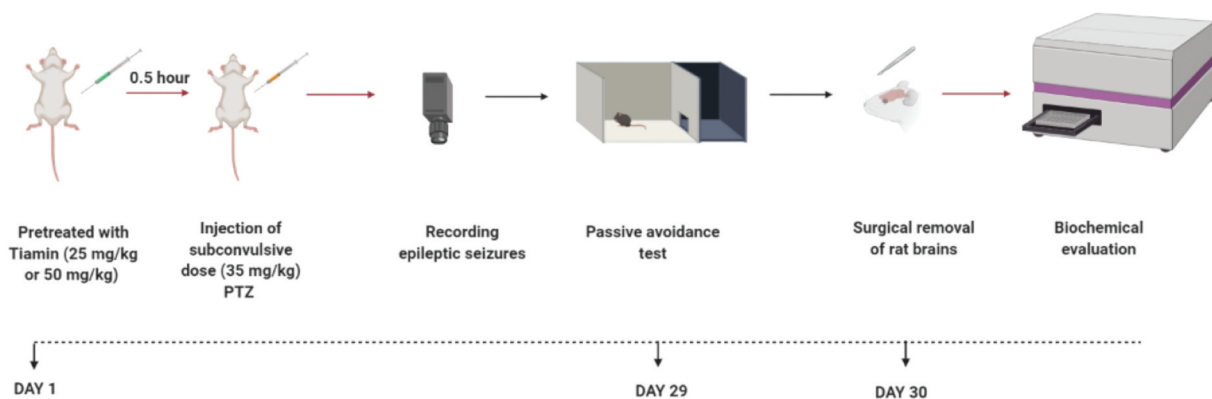

Figure 1. Schematic presentation of the experimental protocol of the study (created with BioRender app, www.biorender.com).

homogenizer. These homogenates were centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant of the centrifuged homogenate was removed, and the protein content was measured using the Bradford protein assay [21]. The brain cytokine levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the instructions given by the manufacturer (YL Biont, Chine). Briefly, after the standard solution and tissue samples were added to the plate, they were incubated at 37 °C for 60 minutes. After the washing step, the staining solutions were added, and the tissue samples were incubated at 37 °C for 15 minutes. Next, after applying the stop solution, the wells were immediately read using an ELISA reader (Thermo Fisher Scientific, Altrincham, UK) at 450 nm.

2.5. Statistical analysis

The data retrieved from each experiment were averaged and expressed as means ± SEM. The Kolmogorov–Smirnov test was performed to determine whether the data were normally distributed. Two-way ANOVA, followed by Bonferroni posttest, was applied for analysis drug X time interaction on the seizure stage. One-way ANOVA with post hoc Tukey test was used in other experiments. Values of $P < 0.05$ were considered statistically significant. Statistical analysis was performed by IBM SPSS Statistical Software Version 22.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Thiamine suppresses PTZ-induced epileptogenesis in rats

First, we asked whether thiamine pretreatment affects epileptogenesis. Our behavioral analyses showed that the PTZ-induced epileptogenesis was effectively suppressed by thiamine pretreatment (Figure 2, $P < 0.05$). It was also found that pretreatment with a 50 mg/kg dose of thiamine was much more effective at reducing the development of PTZ-induced epilepsy in rats ($P < 0.05$).

3.2. Thiamine attenuates PTZ-induced learning deficits in rats

Using a passive avoidance test, we tested thiamine pretreatment against memory deficits caused by PTZ administration. The delay in entering the darkroom on the conditioning day (learning session) was not different between the groups (Figure 3, $P > 0.05$). However, the delay in entering the dark chamber in the retention trial was significantly lower in rats in the PTZ group compared to the control animals ($P < 0.05$). Also, the attenuation in learning deficits
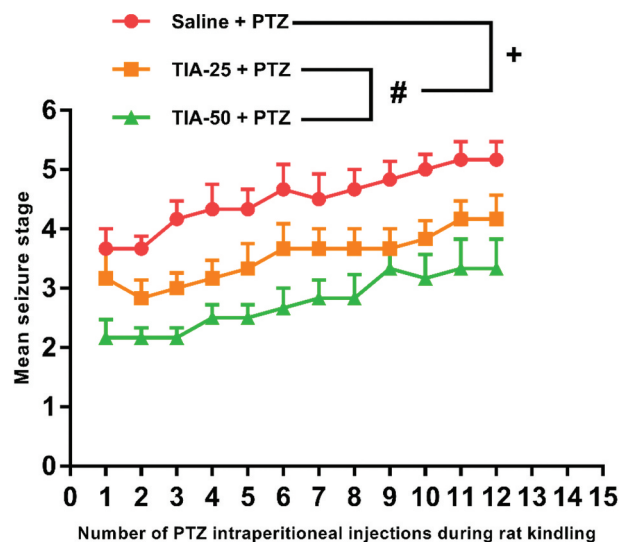

Figure 2. Effect of the pretreatment of thiamine on seizure stage during epileptogenesis process. Values are presented as mean ± SEM. (n = 6 rat in each group). [+]$p < 0.05$ vs PTZ group; [#]$p < 0.05$ vs TIA-25 group.

of the passive avoidance test was significant in animals in the TIA-25 and TIA-50 groups compared to the PTZ group ($P < 0.05$).

3.3. Thiamine inhibits inflammatory mediators in the brain of rats

We then determined the effect of pretreatment with thiamine during the epileptogenic period on the levels of neuroinflammatory mediators in the brain of rats. Statistical analysis showed that PTZ-induced kindling led to an increase in the level of inflammatory mediators in the cortex and hippocampus of rats. Still, these increases were largely ameliorated by pretreatment of thiamine. (Figures 4(a–d) and 5(a–e), $P < 0.05$).

As shown in Figure 4(a,b), compared to the control group, increased levels of cortical and hippocampal COX-1 levels were observed in rats treated with PTZ ($P < 0.05$). Thiamine pretreatment limited these pathological increases in COX-1 levels both in the cortex of rats in the TIA-25 group and the hippocampus of rats in the TIA-50 group. Similarly, cortical and hippocampal COX-2 levels were elevated in PTZ-treated rats (Figure 4(c–d), $P < 0.05$) when compared to the control group. However, COX-2 levels were considerably lower in the cortex and hippocampus of animals receiving thiamine pretreatment (specifically TIA-50) compared to the PTZ group ($P < 0.05$).

As shown in Figure 5(a-b), cortical and hippocampal TNF-α increased in PTZ-treated rats compared to the control group ($P < 0.05$), whereas pretreatment with thiamine, as with COX-1, suppressed the level of the brain TNF-α depending on its concentration of applied. The results presented in Figure 5(c–f) show that pretreatment of thiamine decreased PTZ-induced
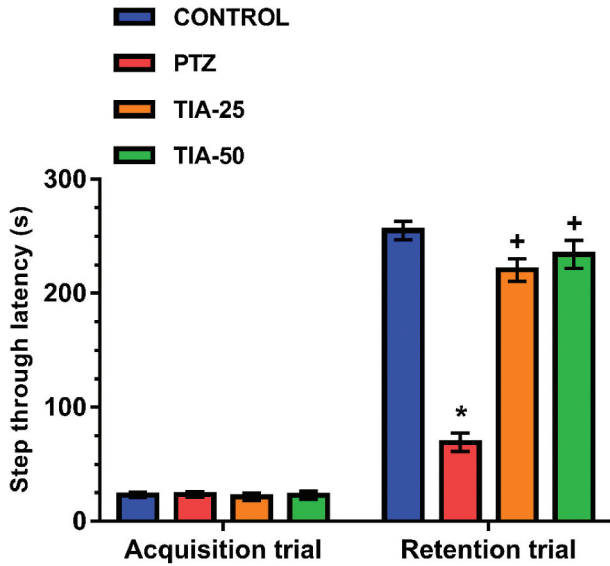
Figure 3. Effect of the pretreatment of thiamine in PTZ-kindled rats on memory performance during the passive avoidance test. Values are presented as mean ± SEM. (n = 6 rat in each group). *p < 0.05 vs Control group; +p < 0.05 vs PTZ group.

4. Discussion

The presence of brain inflammation in epileptogenesis is well documented, and thus targeting neuroinflammation has emerged as a promising approach to the treatment of epilepsy. The current study provides evidence that thiamine pretreatment during epileptogenesis ameliorates neuroinflammation and attenuates behavioral deficits in the PTZ-induced kindling rat model. Thiamine reduced the PTZ-induced increase of inflammatory molecules including COX-1, COX-2, TNF-α, IL-1β, and NF-κB levels in rats' hippocampus and cerebral cortex.

Despite the growing interest in addressing epileptogenesis to completely prevent or modify the disease, there is still no anti-epileptogenic drug. Neuroinflammation is one of the critical findings of epilepsy and epileptogenesis, which can lead to impaired blood brain barrier, abnormal neural connectivity, hyperexcitable neuronal network, and excitotoxicity [22,23]. Such pathological events are enough for the normal brain to turn into the epileptic one. Studies on epilepsy using both in vivo and in vitro experiments have reported that numerous cytokines, enzymes, and other inflammatory mediators are involved in epileptogenesis [24]. Among them, cyclooxygenases (COX-1 and COX-2) are rate-limiting enzymes that catalyze the biosynthesis of prostaglandins and thromboxanes.
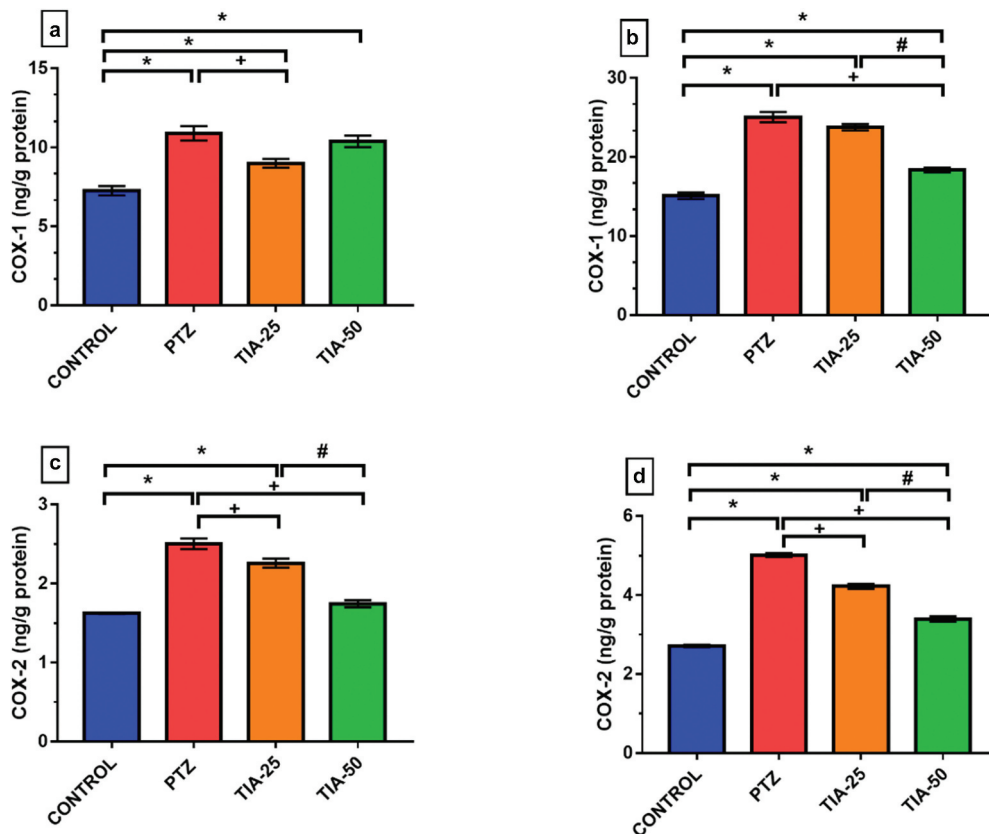
cortical and hippocampal IL-1β and NF-κB levels. Notably, pretreatment with thiamine at 50 mg/kg dose significantly reduced the level of these mediators in the cortex and hippocampus (P < 0.05).

Figure 4. Effect of the thiamine on COX-1 levels in cortex (a) and hippocampus (b); COX-2 levels in cortex (c) and hippocampus (d). Values are presented as mean ± SEM. (n = 6 rat in each group). *p < 0.05 vs Control group; +p < 0.05 vs PTZ group; #p < 0.05 vs TIA-25 group.
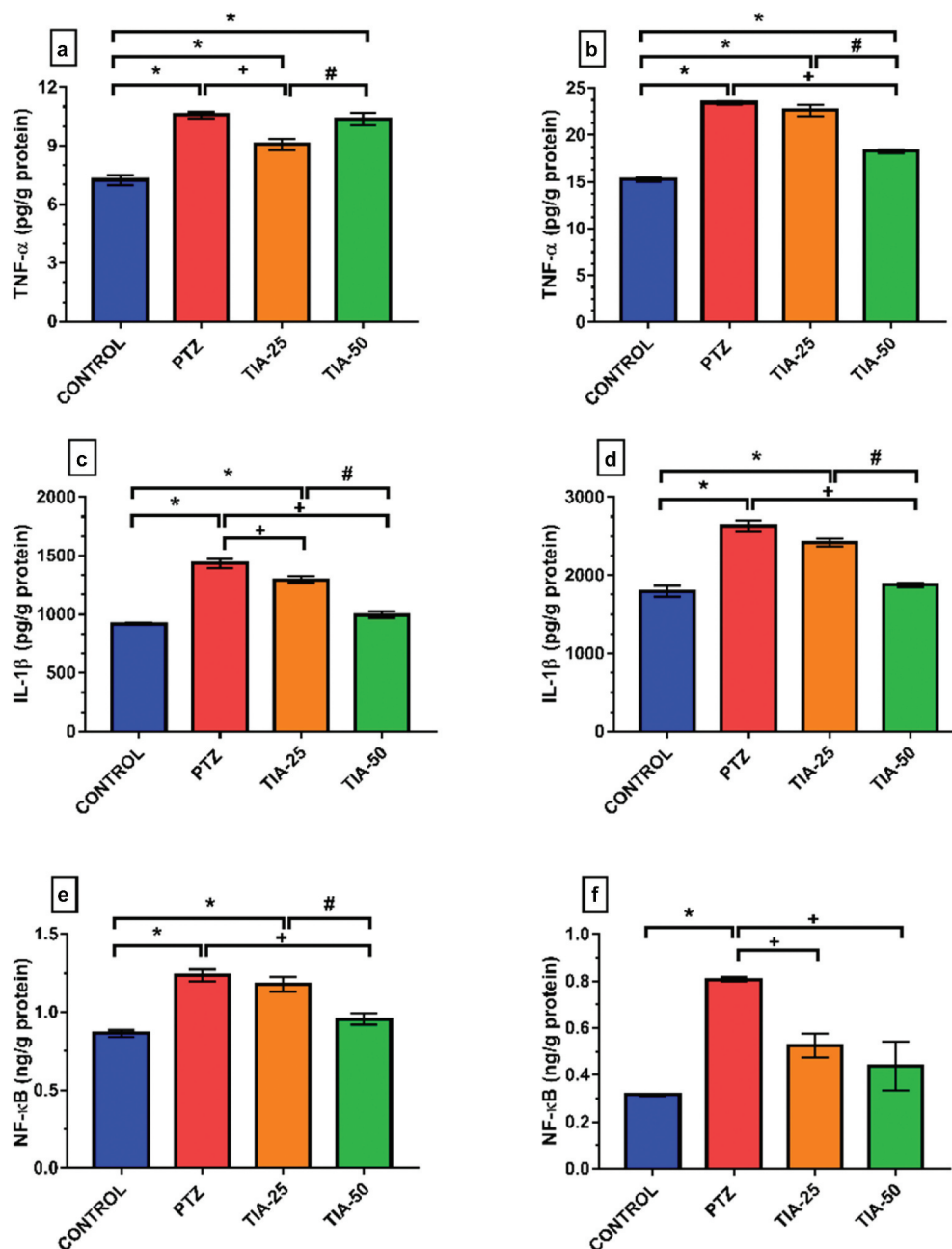
Figure 5. Effect of the thiamine on TNF-α levels in cortex (a) and hippocampus (b); IL-1β levels in cortex (c) and hippocampus (d); NF-κB levels in cortex (e) and hippocampus (f). Values are presented as mean ± SEM. (n = 6 rat in each group). *p < 0.05 vs Control group; +p < 0.05 vs PTZ group; #p < 0.05 vs TIA-25 group.

Its upregulation during seizures has been reported in both in-vitro and in-vivo models of epilepsy [25–27]. Consistent with these findings, we found that PTZ injections increased COX-1 and COX-2 in both the cortex and hippocampus. In addition, thiamine pretreatment decreased the levels of the two COX enzymes in the rat brain. In line with our results, Bozic et al. reported that pretreatment with benfotiamine, a synthetic derivative of thiamine, suppressed COX-2 at both the gene and protein levels in lipopolysaccharide-stimulated murine BV-2 microglia [28]. It has been shown that targeting the COX system with selective and non-selective drugs as an approach to interfering with epileptic seizures may be beneficial [29,30]. Together,

these observations suggest that COX enzymes may be a good target for developing neuroinflammation-targeted therapeutics in the management of epilepsy.

NF-κB is a transcription factor that regulates genes encoding many proinflammatory cytokines such as pro-interleukin-1β (pro-IL-1β), TNF-α, and interleukin-6 (IL-6) [31]. The NF-κB signaling pathway is involved in many pathological processes associated with neurodegeneration in the CNS, including seizures and epilepsy [32]. Consistent with this, we found that PTZ-induced epileptogenesis caused an increase in brain levels of TNF-α and IL-β as well as NF-κB. Thiamine pretreatment attenuated neuroinflammation as evidenced by the reduction in NF-κB,

IL-1β, and TNF-α levels in rat cortical and hippocampal tissues. These results are in line with previous in-vitro studies reporting that thiamine derivatives decreased inflammation by suppressing activation of NF-κB signaling and consequently alleviate the TNF-α transcription [28,33].

Previous studies have reported that specific proinflammatory cytokines such as IL-1β and TNF-α contribute significantly to brain hyperexcitability by altering the balance of GABA and Glutamate [34–37]. Remarkably, thiamine deficiency led to increased concentrations of these cytokines in brain regions, including the cortex and hippocampus [38,39]. This finding suggests that there may be an interaction between thiamine deficiency and seizure formation in an epileptic brain. Supporting this possibility, Fattal-Valevski et al. have reported that severe deficiency of infants' thiamine may cause epilepsy [40]. Moreover, it has been suggested that increased seizure sensitivity caused by lead poisoning in rats may be mediated by changes in thiamine levels [41].

It suggests that inflammation and seizures contribute to neuropsychiatric comorbidities of epilepsy [42]. Moreover, cognitive impairments such as spatial memory deficit frequently manifest in patients with epilepsy [43]. Consistent with our results, it has been shown in previous studies that PTZ-induced epileptogenesis causes learning and memory impairments [44,45]. Chemical kindling induced by PTZ leads to structural pathological changes, oxidative stress, and neuroinflammation in the hippocampus, which is the essential structure of the brain in learning and memory [46,47]. Our results showed that thiamine pretreatment alleviated learning deficits caused by PTZ-kindling. A recent experimental study showed that oral benfotiamine supplementation increased thiamine diphosphate concentrations in the hippocampus and entorhinal cortex, and it improved the STZ-related cognitive deficit in rats [48]. Evidence indicates that thiamine is a neuroinflammation modulator known for removing reactive oxidative species [12,49]. Also, previous studies reported that benfotiamine can exert antiinflammatory effects [50,51]. Considering the constantly increasing oxidative stress and inflammation in the epileptic hippocampus, it is plausible that the neuroprotective effect of thiamine is likely a result of its potent anti-inflammatory effect.

In conclusion, the current study results show that controlling the extent of neuroinflammation with thiamine treatment can abate both epileptogenesis progression and memory deficit. Considering the high cost of acquiring synthetic drugs and the side effects of drug administration, we suggest that such vitamins and vitamin products can be used as an adjunct to antiepileptic drug therapies in managing epileptogenesis and epilepsy. Further studies are needed to address whether thiamine treatment affects other components involved in epileptogenesis, such as multiple molecular changes, decreased neurogenesis, and abnormal synaptic reorganization in the brain.

Authors' contributions

Sebahattin Karabulut contributed to the experimental design, statistical analysis, manuscript writing, and editing. Ahmet Kemal Filiz contributed to the experimental design and experimental procedures. Recep Akkaya contributed to the experimental design, biochemical analysis, and manuscript editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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