

Original Research Article

Effect of carbamazepine in pentylenetetrazole-induced epilepsy in rat model

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Abstract

Purpose: To investigate the effects of carbamazepine on oxidative stress biomarkers and seizure-associated behavioral alterations in pentylenetetrazole (PTZ)-induced epilepsies.

Method: A total of 15 adult male Wistar rats were randomly assigned into three groups ($n = 5$ in each group). Group 1 was the control group and received 0.9 % normal saline, group 2 received PTZ (40 mg/kg intraperitoneal), and group 3 received carbamazepine (CBZ; 50 mg/kg, oral gavage) 1 hour before PTZ administration for 14 days. Seizure latency, frequency, and duration were assessed. Oxidative stress biomarkers, including superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA), were determined in brain homogenates using standard spectrophotometric methods.

Results: Pentylenetetrazole-induced changes in all measured parameters were significant at $p < 0.001$. However, following carbamazepine treatment, the increases in SOD and GSH were significant at $p < 0.05$, whereas the improvements in seizure latency, seizure frequency, seizure duration, and MDA were significant at $p < 0.001$.

Conclusion: The study reveals that modulating oxidative stress may play a potential role in mediating the neuroprotective effects of CBZ, suggesting a promising therapeutic target in epileptogenesis.

Keywords: Carbamazepine, Epileptogenesis, Oxidative stress biomarkers, Pentylenetetrazole-induced kindling, Seizure behavior

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INTRODUCTION

Epilepsy is one of the most prevalent chronic neurological disorders characterized by recurrent spontaneous seizures resulting from abnormal brain hyperexcitability and hypersynchronous activity [1]. It is one of the most widespread neurological diseases in the world, and now ranks as the fourth leading neurological disorder with 50 million patients affected [2]. Existing antiseizure medications remain symptomatic in

nature and do not modify the underlying process of epileptogenesis, despite recent advancements [1]. There is growing evidence that oxidative stress is closely associated with the pathophysiology and progression of epilepsy [2]. Recurrent seizures raise metabolic demand for neurons, causing excessive generation of reactive oxygen species (ROS), mitochondrial impairment, and alteration of cellular redox balance [2]. The resulting oxidative excesses trigger lipid peroxidation, oxidative modification of

proteins, and DNA damage, leading to brain cell injury and increased excitability, which reinforces epileptogenesis [2].

Oxidative biomarkers are frequently used to assess redox imbalance in epileptic models. Malondialdehyde (MDA) is a principal indicator of lipid peroxidation, while superoxide dismutase (SOD) and glutathione (GSH) represent large enzymatic and non-enzymatic defense mechanisms, respectively. Clinical and experimental studies have demonstrated that epileptic conditions are characterized by elevated oxidative stress and diminished antioxidant defenses. This suggests that oxidative damage may play a significant role in the initiation and progression of seizures [3].

Carbamazepine is a first-line antiseizure medication in focal and generalized tonic-clonic seizures. It works primarily by blocking voltage-gated sodium channels, which stabilizes neuronal membranes and limits neuronal repetitive excitation. Besides its antiepileptic activity, more attention has been directed toward the effects of CBZ on oxidative stress pathways, with some studies showing antioxidant properties and other studies showing pro-oxidant properties [1]. Emerging evidence indicates that carbamazepine may affect oxidative stress, but further data on this activity remain controversial. Several studies suggest that long-term treatment with carbamazepine modifies oxidative balance and elevates oxidative damage, but others suggest possible protective or modulatory effects depending on the dose, duration, and experimental conditions [4]. These disparities underscore the importance of comprehensively evaluating redox-mediated effects of carbamazepine in epilepsy models. The pentylenetetrazole (PTZ)-induced model is a well-accepted and standard animal model for epilepsy that allows for the investigation of seizure mechanisms and response to anticonvulsant treatment [1].

Behavioral parameters of seizure latency, frequency and duration are major outcome measures that represent seizure susceptibility, severity, and overall burden. They work in unison with biochemical markers to understand the correlation between molecular changes and functional seizure results [5]. There are many studies investigating either oxidative stress or seizure behavior in isolation, but few have combined both approaches to assess the overall effects of carbamazepine on both behavioral outcomes and oxidative biomarkers [6]. Furthermore, this current study investigated the role of oxidative stress biomarkers (MDA, SOD

and GSH) in addition to measuring seizure-related behavioral alterations (latency, frequency, as well as duration) following carbamazepine administration in a rat model.

EXPERIMENTAL

Animals

A total of 15 adult male Wistar rats (250 – 300 g) were randomly and equally assigned into 3 groups. The animals were maintained under standard laboratory conditions (22 ± 2 °C and 12 h light / dark cycle) with unrestricted access to food and water to acclimatize for one week. Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC; approval no: 4.42), and complied with the internationally accepted guide for the care and use of laboratory animals [7].

Study design

The rats were categorized into three groups ($n = 5$ in each group). Group 1 was the control group and received 0.9 % normal saline (per oral and intraperitoneal), group 2 received PTZ (40 mg/kg intraperitoneal) [8], and group 3 received carbamazepine (CBZ; 50 mg/kg oral gavage) 1 h before PTZ to induce seizures [9]. Treatment was done every 48 hours for 14 days, 1 h before PTZ injection (40 mg/kg, IP).

Induction of epilepsy

Epilepsy was induced using PTZ with a sub-convulsive dose of 40 mg/kg, administered intraperitoneally every 48 h for 14 days. After each PTZ administration, the animals were observed for 30 min, and seizure activity was recorded using video monitoring.

Drug administration

Pentylenetetrazole (PTZ 5g; Santa Cruz, USA) was freshly prepared before each administration by suspending the powder in 0.9 % normal saline solution. Carbamazepine (CBZ; Hof Pharma, India) was prepared as an oral suspension by suspending the powder in distilled water and administered orally with a feeding tube at 50 mg/kg, 1 h before PTZ injection. Treatment was repeated every 48 hours for 14 days.

Sample collection and tissue preparation

On day 17, the animals were euthanized by decapitation, after which the whole brains were rapidly excised and washed with ice-cold normal saline. Brain samples were homogenized in

chilled phosphate-buffered saline (PBS, pH 7.2; HiMedia, India) using a tissue-to-buffer ratio of 1:10 (w/v). Thereafter, the homogenized sample was centrifuged at a relative centrifugal force (RCF) of $1,887 \times g$ (5,000 rpm speed) for 15 min at 4 °C. The obtained supernatants were separated and preserved at -20 °C for further biochemical evaluation.

Evaluation of parameters/indices

Behavioral assessment

Convulsive activity was monitored for 30 min following PTZ injection using a modified Racine scale. Behavioral parameters such as seizure latency, duration and frequency were recorded. Seizure severity was graded according to the scale in Table 1 [8].

Table 1: Grade of seizure severity

Grades	Severity of convulsive episodes
0	No observable response
1	Hyperactivity, restlessness, and vibrissae twitching
2	Head nodding, clonic head movement and myoclonic jerks
3	Unilateral or bilateral limb clonus
4	Clonic convulsions affecting the forelimbs
5	Generalized clonic seizures associated with loss of posture and falling
6	Extension of the hind limbs
7	Fatal outcome

Biochemical analysis

Brain tissue homogenate was obtained to determine levels of MDA, SOD and GSH. Malondialdehyde was determined using thiobarbituric acid reactive substances (TBARS), depending on the generation of MDA-TBA complex measured at 535 nm [10]. Superoxide dismutase (SOD) activity was evaluated using the pyrogallol autoxidation assay [11]. Reduced glutathione (GSH) level was measured following the interaction of sulfhydryl groups with 5,5'-dithiobis-(2-nitrobenzoic acid; DTNB), producing a yellow chromogen measured at 412 nm [12]. All measurements were performed using a UV-visible spectrophotometer, and results were expressed as mmol/L tissue for MDA, U/mL for SOD, and µg/L tissue for GSH.

Statistical analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS, v29.02; SPSS Inc., Chicago, Illinois, USA). Data were presented as mean ± standard deviation (SD), and compared using a one or two-way ANOVA

(Dennett's adjustment for multiple comparison tests). $P < 0.05$ was considered statistically significant.

RESULTS

Behavioral test

Treatment with carbamazepine significantly increases seizure latency and reduces frequency and duration ($p < 0.05$) compared to PTZ alone (Table 2).

Table 2: Effects of carbamazepine on seizure characteristics

Variables	Group 2	Group 3
Latency (sec)	6	81*
Duration (sec)	71	35*
Frequency	11	4*

* $P < 0.05$ vs PTZ alone. Group 2 (PTZ 40 mg/kg), group 3 (carbamazepine 50 mg/kg)

Biochemical profile

Oxidative stress biomarker

Group 2 showed significantly lower SOD and GSH, and higher MDA levels ($p < 0.05$) compared to control. Furthermore, group 3 showed significantly higher SOD, GSH, and lower MDA levels compared to group 2 ($p < 0.05$; Table 3).

Table 3: Effect of carbamazepine on oxidative biomarkers (mean ± SD; n = 5)

Variable	Group 1 (Control)	Group 2	Group 3
SOD (U/mL)	37.4 ± 12	25.06 ± 5.8*	33.44 ± 11**
GSH (µg/L)	24.25 ± 8	17.76 ± 3.8*	22.35 ± 4.05**
MDA (mmol/L)	20.48 ± 5.8	26.86 ± 7.06*	19.94 ± 6.2***

* $P < 0.05$ vs control, ** $P < 0.05$ vs PTZ alone, *** $P < 0.001$ vs PTZ alone. Group 2 (PTZ 40 mg/kg), group 3 (CBZ 50 mg/kg)

DISCUSSION

The present study demonstrated that repeated PTZ administration induced significant behavioral and oxidative alterations, while carbamazepine treatment ameliorated seizure activity and improved oxidative stress biomarkers. Group 2 showed marked generalized tonic-clonic seizures following PTZ administration. These seizures were represented by a more severe average seizure intensity score consistent with stage 5 or 6 in the revised Racine scoring system compared to baseline (group 1). Rats subjected to PTZ

showed significantly shorter latency time compared to the CBZ-treated group, which is in tandem with previous studies [8,13]. It has been established that PTZ-kindling model of seizure is achieved by antagonizing the GABA-A receptors [14]. This impairs inhibitory synapse function, resulting in enhanced neuronal excitability and development of generalized seizures in experimental animals [6,15]. Duration and severity of seizures in group 2 were significantly increased compared to CBZ-treated group which is in agreement with previous reports, demonstrating that PTZ increases both the frequency and duration of seizures [16]. Following treatment with CBZ, there was significant prolongation in convulsion onset, improvement in mean seizure severity and reduction in seizure duration compared to group 2.

A previous study showed that pretreatment with carbamazepine increased latency to seizure initiation and significantly reduced severity of convulsions in the PTZ model [17]. Also, at 50 mg/kg, CBZ demonstrated anticonvulsant effects by elevating seizure threshold and attenuating seizure severity in experimental models [18], consistent with the findings of the present study. It does this by stabilizing the inactivated state of voltage-gated sodium channels, which suppresses high-frequency neuronal firing [19]. Superoxide dismutase (SOD) is a primary component of the endogenous antioxidant network protecting neuronal tissues against ROS generated during epileptic seizures [20]. Findings from this study showed that PTZ administration significantly reduced SOD activity compared to the control group. This reduction may result from excessive ROS generation accompanied by elevated utilization of endogenous antioxidant enzymes during PTZ-induced epileptogenesis [21]. Administration of CPZ significantly increased SOD levels compared to PTZ. This improvement in SOD activity may be secondary to its anticonvulsant action, which likely attenuated seizure-induced ROS generation and oxidative stress [22]. Reduced glutathione (GSH) is one of the major endogenous non-enzymatic antioxidants in the brain, and depletion during epileptogenesis reflects excessive utilization in scavenging ROS generated during recurrent seizures [23].

The present study demonstrated that PTZ significantly reduced brain GSH concentration, compared to control, which is consistent with earlier findings [24]. Following treatment with CBZ, level of GSH was significantly higher compared to group 2, which may be attributed to its anticonvulsant action through modulation of

voltage-gated sodium channels, resulting in reduced neuronal hyperexcitability and seizure-associated oxidative stress [22].

Free radicals are known for initiating lipid peroxidation in cellular membranes. Malondialdehyde (MDA) is one of the major end products generated during the oxidative degradation of polyunsaturated fatty acids, and is widely used as a biomarker of lipid peroxidation and oxidative stress. The PTZ model in rats has been reported to elevate MDA levels [21] and enhance lipid peroxidation with a reduction in antioxidant activity compared to control, consistent with this study. Similar findings have been demonstrated in previous study, supporting the role of oxidative stress in the pathogenesis of epilepsy [25]. The observed increase in MDA of brain homogenate following PTZ administration is consistent with previous studies on PTZ-induced oxidative stress [26], leading to a significant oxidative surge. Furthermore, PTZ-binding has been observed to enhance free radical production by stimulating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to elevated MDA levels [27]. This mechanism may contribute significantly to oxidative stress and subsequent neuronal damage. Treatment with CBZ significantly reduced MDA levels compared to PTZ, which may be attributed primarily to its anticonvulsant action through blockade of voltage-gated sodium channels and suppression of repetitive neuronal firing, thereby reducing seizure-associated oxidative stress and ROS overproduction [28].

Limitations of the study

Despite the observed significant differences, the sample size was relatively small ($n = 5$ per group), which may affect statistical power. Also, biochemical measurements were taken at a single point (day 17), but oxidative stress markers may change over the course of seizure activity. Future studies with larger sample sizes, multiple time-point assessments, and additional biomarkers, such as inflammatory cytokines or histopathological analyses, would better characterize CBZ-mediated neuroprotection and should be pursued.

CONCLUSION

Carbamazepine significantly increased SOD, GSH, and seizure latency, while reducing MDA, seizure frequency, and duration. Therefore, CBZ modulates oxidative stress, suggesting a promising therapeutic target in epileptogenesis.

DECLARATIONS

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None.

Ethical approval

Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC; approval no: 4.42).

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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