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Cerebral protective effects of gabapentin in a rat model of head injury

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ABSTRACT

Objective: Gabapentin reduces neuronal excitability, oxidative stress, release and/or synthesis of inflammatory mediators. The purpose of our study is to explore the emerging biochemical and pathological effects of gabapentin in a rat model of head injury.

Materials and Methods: Thirty-two male Sprague-Dawley rats were used. The rats were randomly assigned into four distinct groups, each consisting of eight subjects: a sham group, a control group, a dexamethasone-treated group, and a gabapentin-treated group. In brain samples, assessment of malondialdehyde (MDA) levels, glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzyme activities were evaluated.

Results: Pathological examinations indicated that the cortical injury resulting from trauma was reduced in the group treated with gabapentin. Traumatic brain injury (TBI) led to a substantial increase in MDA levels within the tissue. Conversely, when examining the tissue SOD and GPx activities, a significant reduction was observed. The assessment of SOD and GPx activities illustrated that both the dexamethasone and gabapentin groups exhibited significantly elevated levels in contrast to the control group.

Conclusion: These findings strongly suggest that gabapentin is effective in a head injury model.

Keywords: Gabapentin, Rat model, Head injury, Antioxidant

1. INTRODUCTION

Gabapentin, a structural analog of gamma-aminobutyric acid, has shown benefit in the treatment of epilepsy, reflex sympathetic dystrophy, and many psychological disorders. It inhibits the secretion of monoamine neurotransmitters (dopamine, adrenaline, and serotonin) [1]. Gabapentin also regulates the N-methyl-D-aspartate (NMDA) receptor and calcium channel. Thus, gabapentin reduces neuronal excitability, oxidative stress, release and/or synthesis of inflammatory mediators by regulating calcium currents [2].

Antioxidant enzymes play a significant role in mitigating oxidative stress by inhibiting the peak levels of reactive oxygen species (ROS) and free radicals, which can severely compromise the integrity of cell membranes through the process of lipid peroxidation. One such enzyme, superoxide dismutase (SOD), has the capability to convert harmful superoxide radicals into hydrogen peroxide (H_2O_2), thereby averting potential cellular damage. Additionally, catalase (CAT) further contributes to cellular protection by transforming lipid peroxidation products into benign end-products: water and oxygen [3].

In the context of neuropathic pain, research utilizing a rat model has demonstrated that gabapentin can effectively decrease the levels of pro-inflammatory mediators, including, interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF) and interleukin-6 (IL-6), while simultaneously elevating the levels of the anti-inflammatory cytokine interleukin-10 (IL-10) [4]. Moreover, recent studies have highlighted gabapentin's ability to reverse the inflammatory processes in established acute mouse models of inflammation [5]. These findings lay the groundwork for our investigation, which aims to explore the emerging biochemical and pathological effects of gabapentin in a rat model of head injury.

2. MATERIALS and METHODS

The ethical considerations surrounding the experimental protocol of our research were meticulously reviewed and subsequently sanctioned by the Ethics Committee at Ankara Training and Research Hospital, as evidenced by Decision Date: 01/11/2024 and Decision No: 794. The animal subjects employed in this investigation were sourced from the Experimental Animal Breeding Laboratory affiliated with Ankara Training and Research Hospital. Furthermore, all experimental procedures were conducted within the confines of the Experimental Animal

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Research Laboratory at the same institution. The biochemical analyses associated with this study were carried out by the proficient staff at the Medical Biochemistry Unit of Beştepe State Hospital, Ankara.

Animals

In the course of our research, we utilized 32 male Sprague-Dawley rats, each weighing between 250 and 300 grams and aged 12 weeks. The rats were randomly assigned into four distinct groups, each consisting of eight subjects: a sham group, a control group, a dexamethasone-treated group, and a gabapentin-treated group. Anesthesia was administered via intraperitoneal injection of ketamine hydrochloride (Ketalar, Pfizer, Istanbul, Turkey) at a dosage of 70 mg/kg, accompanied by xylazine hydrochloride at 7 mg/kg (Rompun, Bayer, Istanbul, Turkey). The heads of the rats were securely positioned in the injury apparatus, ensuring that their jaws were firmly placed against the bottom surface. A closed head injury was created using the method described by Marmarou et al. [6]. For all treatment groups, the cranial impact was applied to the vertex along the midline, just behind the coronal suture. Rats were kept at room temperature with 12 hours light/12 hours dark cycle in the laboratory environment throughout the experiment. All rats had free access to food and water. At 24 hours into the study, animals were decapitated for sampling for biochemical examinations.

Groups and surgical procedures

All surgical interventions were conducted by a single surgeon.

Group I (n=8): Following the administration of anesthesia, an incision was made on the scalp and subsequently closed.

Group II (n=8): In addition to the surgical steps outlined for Group I, participants experienced closed head trauma.

Group III (n=8): Beyond the surgical procedures performed in Group II, participants received a single intraperitoneal dose of dexamethasone (Dekort®, DEVA Holding A.Ş., Istanbul, Turkey) (1 mg/kg) after the trauma.

Group IV (n=8): In addition to the surgical operations described in Group II, participants were administered a specific dose of gabapentin orally (50 mg/kg) (Neurontin®, Pfizer İlaçları Ltd. Şti., Istanbul, Turkey) [7].

Following euthanasia, the cranial bones of the animals, along with the scalp, were retrieved as a single entity. Then, each brain was divided into two hemispheres along the midline, and one hemisphere was allocated for pathological examination, while the other hemisphere was designated for biochemical analysis.

Evaluation of pathological sections

The samples were preserved in a 10% formalin solution, thereafter undergoing standard processing protocols with the support of the LEICA ASP 300S apparatus (Leica Microsystems, Wetzlar, Germany). Subsequently, paraffin blocks were formed, and cross-sections measuring 4 microns were obtained using the LEICA RM 2255 microtome (Leica Microsystems, Wetzlar, Germany). These sections were then stained utilizing hematoxylin and eosin. The final assessments were performed with an OLYMPUS BX51 microscope (Olympus Corporation, Tokyo, Japan).

Later, sections were evaluated in terms of perivascular edema and interstitial edema. All these parameters were calculated in terms of the percentage of the damaged surface, and the classification used was as follows: 0 (no edema), 1 (slight) 5%, 2 (minimal) 6–20%, 3 (moderate) 21–50%, 4 (severe) 51–75%, 5 (very severe) 76–100% [8].

Assessment of MDA levels, GPx, and SOD enzyme activities in brain samples

The brain samples underwent processing through an Ultra Turrax tissue homogenizer (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Each resulting sample was then combined with 1 milliliter of distilled water. The homogenates obtained from this procedure were utilized to analyze the levels of malondialdehyde (MDA) as well as the activities of glutathione peroxidase (GPx) and SOD. It is noteworthy that all experimental procedures were conducted at a temperature of 4°C to ensure sample integrity.

The protein content in the brain samples was quantified employing the Lowry technique, as outlined by Lowry et al. [9]. Subsequently, the concentration of MDA, a biomarker indicative of lipid peroxidation within the brain, was assessed utilizing the Northwest Life Science Specialties's NWKMDA01 assay . The results of this analysis were expressed as micromoles of MDA per gram of protein, providing a precise measurement of lipid oxidation levels [9].

In addition to MDA measurement, the activity of GPx was evaluated using a colorimetric assay kit (Northwest Life Science Specialties, Vancouver, WA, USA)., following the methodology established by Paglia and Valentine [10]. The concentration of GPx was reported in milliunits per gram of protein, facilitating an understanding of the enzymatic activity involved in oxidative stress responses [10].

To further assess antioxidant capacity, the activity of SOD was measured using a dedicated assay kit (Superoxide Dismutase Assay Kit, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The results of the SOD activity evaluation were expressed in units per gram of protein, providing insight into the enzyme's role in mitigating oxidative damage within the brain.

Through these methodological approaches, the levels of MDA and the activities of GPx and SOD were systematically evaluated, contributing to a comprehensive understanding of oxidative stress and its implications in the context of brain health.

Statistical Analysis

Each group had a sample size of 8 so we reported median values along with the interquartile range (25th and 75th percentiles) and used non-parametric tests, as suggested in previous research [11]. For overall group comparisons, we applied the Kruskal-Wallis test. If significant differences were detected, we performed pairwise subgroup comparisons using the Mann-Whitney U test. The significance level (Type I error) was set at 5%, and to account for multiple comparisons, we adjusted p-values using

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the Bonferroni correction. All analyses were conducted in IBM SPSS version 23 (IBM Corp., Armonk, NY, USA).

3. RESULTS

Pathological examination

Pathological data are summarized in Table I. Perivascular and interstitial edema were found to be significantly different between all groups ($p<0.05$). No edema was seen in sham group (Figure 1a). In addition, a statistically significant difference was found between the control and gabapentine groups ($p<0.05$). However, no difference was found between the dexamethasone and gabapentine groups ($p>0.05$). Very severe edema was observed in the control group (Fig. 1 b). Upon analyzing the sections pertaining to the study groups, it was observed that the group receiving gabapentin and dexamethasone exhibited a decrease in perivascular edema, particularly when compared to the control group (Figure 1c). This finding highlights the comparative efficacy of gabapentin in mitigating perivascular edema in the studied subjects (Figure 1d).

Table I. *Mean injury grading scores in samples (Values are presented as the median scores (interquartile range)*

Score	Sham	Control	Dexamethasone	Gabapentin	
Perivascular edema	0 (0-0)	4 (3.75-4.25)	2 (1.75-2.25)	1.5 (0-2)	† ‡
Interstitial edema	0 (0-0)	4 (3.75-5)	2 (1-2.25)	1 (0.75-1.25)	† ‡

† Significant difference between all groups ($p < 0.05$), ‡ No Significant difference between dexamethasone and gabapentine groups ($p >0.05$).

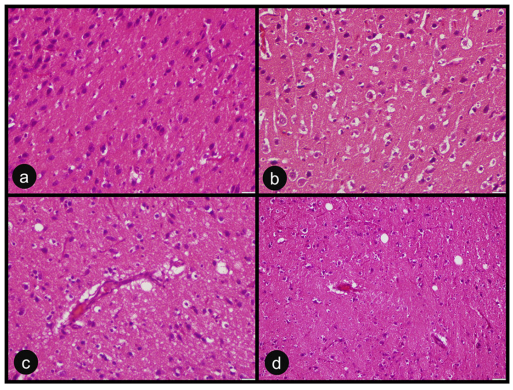


Figure 1. *Presents sections stained with hematoxylin and eosin, illustrating distinct pathological variations across different experimental groups. (a) The Sham group reveals intact brain tissue, indicating a baseline of normality. (b) In contrast, the control group exhibits significant perivascular and interstitial swelling, signifying extensive pathological changes. (c) The Dexamethasone group displays moderate levels of perivascular and interstitial edema, suggesting a partial therapeutic effect. Lastly, (d) the Gabapentin group indicates only mild perivascular and interstitial edema, reflecting a comparatively favorable response to treatment.*

MDA, GPx, and SOD enzyme activity levels by groups

Table II displays the median levels of MDA and the activities of SOD and GPx across each group. Notably, traumatic brain injury (TBI) led to a substantial increase in MDA levels within the tissue. Conversely, when examining the tissue SOD and GPx activities, a significant reduction was observed. Statistical analysis revealed a meaningful difference when comparing MDA, SOD, and GPx levels across all groups ($p<0.05$). To ascertain the origins of this difference, comparisons between the sham and control group were separately conducted, which also displayed a statistically significant difference ($p<0.05$).

The comparative analysis of experimental cohorts revealed no significant statistical disparity between the dexamethasone and gabapentin groups, as indicated by a p-value exceeding 0.05. Moreover, the assessment of SOD and GPx activities illustrated that both the dexamethasone and gabapentin groups exhibited significantly elevated levels in contrast to the control group. Furthermore, both dexamethasone and gabapentin groups demonstrated a statistically significant decrease in mean MDA levels when compared to the control group.

Table II. *SOD, GPx activities and MDA levels in brain tissue of rats (Values are presented as the median scores (interquartile range)*

	Sham	Control	Dexamethasone	Gabapentin
MDA	52.29 (46.45-56.86)	131.2 (129.8-139.11)	84.56 (76.19-90.42)	87 (81.23-95.08)
SOD	16225.57 (13627.38-18505.32)	4002.55 (2781.3-5180.33)	9108.42 (6104.86-12133-63)	11753.84 (9807.23-15060.63)
GPx	20229.62 (18309.35-22094.94)	6071.26 (5113.17-7258.22)	11301.87 (9812.08-13084.08)	11500.55 (9264.19-16296.07)

MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase

4. DISCUSSION

Traumatic brain injury is a major source of morbidity and mortality, placing a serious burden on global health systems. While the primary injury after TBI is immediate due to the impact, secondary injury can develop over days or years as a result of complex pathophysiological processes [12]. Secondary injury after TBI is enhanced by various biochemical processes, the most important of which is inflammation. The neuroinflammatory cascade affects brain structure and function much more than the initial trauma, leading to different TBI outcomes. Although, the purpose of the inflammatory response is to promote tissue repair, when uncontrolled it can cause additional damage [13].

After TBI, the integrity of the central nervous system (CNS) may be compromised, leading to neuronal death, which may affect treatment efficacy. In the event of disruption of the CNS, the entry of migrating immune cells into the CNS for tissue repair may promote secondary injury by increasing neuroinflammation [14]. In addition, the resulting neuronal excitotoxicity and energy metabolism disturbances exacerbate

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neuroinflammation. Stretching of neurons during injury causes excitatory states by activating calcium channels. In this process, decreased cerebral perfusion or ischemic conditions can trigger secondary brain injury [15]. Excess glutamate increases neuronal damage by overstimulating NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Calcium entry activates apoptotic pathways and inflammatory genes. Impairment of glutamate clearance by TNF and IL-1β perpetuates excitotoxicity by affecting astrocyte function [16,17]. Ionic imbalances and increased energy demands are observed after injury; increased interstitial lactate and adenosine, together with decreased glucose levels, signal metabolic crisis. Mouse models have shown both early and late energy metabolism changes, including increased glucose consumption and decreased adenosine triphosphate (ATP) availability. Oxidative stress is usually caused by ischemia. It results from an imbalance between free radicals and antioxidants, which in turn leads to anaerobic metabolism and cellular acidosis. This activates calcium channels, lowers cytoplasmic pH and increases ROS production, which contributes to lipid peroxidation. Microglia regulate oxidative stress by activating enzymes such as nitrogen oxides (NOx) and inducible nitric oxide synthase (iNOS) and produce harmful compounds such as peroxynitrite. ROS impair mitochondrial ATP synthesis, leading to secondary injury. Mitochondrial dysfunction increases oxidative stress and inhibits neuronal repair. Research on antioxidant therapies for TBI is ongoing, as oxidative stress can occur even in mild cases. When the pathological sections of our study were analyzed, we observed that TBI caused very severe perivascular and interstitial edema. Gabapentin treatment showed a significant decrease in these pathological features of trauma. Also, although there was no statistical difference between gabapentin and dexamethasone treatments, the mean values of the gabapentin group were better.

Oxidative damage caused by free radicals has an important place in the etiology of post-traumatic brain damage. Free radicals can damage all kinds of biomolecules such as DNA, lipids and proteins. In our study, the decrease in SOD and GPx levels in the control group indicated that free radicals were produced after trauma. MDA is an important indicator of lipid peroxidation, which indicates excessive damage to the cell membrane, and was detected at high levels in the control group. Gabapentin and dexamethasone treatment significantly decreased MDA levels. The beneficial effects of gabapentin were reported by Emmez et al in the spinal cord rat injury model, and the changes in MDA levels in the trauma experimental groups are consistent with the data of our study [18]. SOD, a critical antioxidant found within various tissues, serves as a vital marker of oxidative stress. This enzyme plays an essential role in neutralizing superoxide free radicals, which are byproducts generated during the energy metabolism processes occurring in mitochondria and peroxisomes. By catalyzing the conversion of superoxide radicals into less harmful molecules, SOD helps to mitigate cellular damage and maintain redox balance, highlighting the importance of this enzyme in protecting biological systems from oxidative stress and its associated deleterious effects. SOD converts highly toxic superoxide radicals to H_2O_2, which is subsequently converted to water and molecular oxygen by tissue CAT and GPx. Together, SOD, CAT and GPx form a family of enzymes collectively involved in the cellular defense mechanism against free radicals [19-21]. It has been previously reported that the activity of SOD and glutathione (GSH) decreased in various pathological conditions, and in our study, a similar trend was observed in activity and concentration after trauma in the control group [22,23]. Gabapentin and dexamethasone treatments significantly increased SOD and GPx activities. Kale et al., examined the beneficial effects of gabapentin in a spinal cord ischemia reperfusion model and reported increased SOD and GPx activities in the gabapentin-treated groups, which is consistent with our study [23]. In addition, the pathological result of our study was supported by the biochemical analysis results. To explain more effects of gabapentin on TBI, further studies are needed.

Conclusion

These findings strongly suggest that gabapentin is effective in a head injury model, as is dexamethasone treatment. In addition, the pathological result of our study was supported by the biochemical analysis results. The shortcoming of our study is that the results were not supported by immunostaining and electron microscopy. Further studies with larger numbers and different dose ranges will provide us with deeper information on the mechanism of action of gabapentin.

Compliance with Ethical Standards

Ethical approval: The experimental protocol of this research were reviewed and subsequently sanctioned by the Ethics Committee of Ankara Training and Research Hospital, as evidenced by Decision Date: 01/11/2024 and Decision No: 794.

Conflict of interest: The authors declare that there are no conflicts of interest.

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Author contributions: OS:Concept, design, supervision and resource, F K K: Materials, data collection and/or processing, analysis and/or interpretation and literature review, O S: Writing and critical review. Both authors approved the final version of the manuscript.

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