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RESEARCH ARTICLE

DOES THE PREGABALIN SHOW NEUROPROTECTION IN A HEAD INJURY RAT MODEL?

Ömer Şahin[1], Fatma Karaca Kara[2]

[1] Bestepe State Hospital, Department of Neurosurgery, Ankara, Turkey
[2] Bestepe State Hospital, Department of Medical Biochemistry, Ankara, Turkey

ORCID: O.S. 0000-0001-9689-0068; F.K.K. 0000-0002-4379-3085.

Corresponding author: Ömer Şahin, **E-mail:** dromersahin060@gmail.com
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ABSTRACT

Purpose: Traumatic brain injury (TBI) encompasses both primary and secondary injury mechanisms that contribute to its overall pathogenesis. Pregabalin is used in the treatment of neuropathic pain. Treatments targeting excitotoxicity may be useful in the posttraumatic period. Purpose of this study is to examine the possible neuroprotective effects of pregabalin administered in a rat model of head trauma.
Materials and Methods: 32 male Wistar rats were used. The rats were randomly assigned into four distinct groups, each consisting of eight subjects: a sham group, a trauma group, and groups receiving treatment with methylprednisolone (MPSS) and pregabalin. In Brain Samples, assessment of malondialdehyde (MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzyme activities were evaluated
Results: Pathological analysis showed that pregabalin treatment lessened the trauma-induced damage to the brain tissue. There was a notable statistical discrepancy in MDA levels when comparing the trauma group with the others (P=0.000). Both the Pregabalin and MPSS groups exhibited elevated GPx and SOD levels relative to the trauma group, with the differences being statistically significant (respectively GPx and SOD; P=0.002, P=0.001).
Conclusion: Pregabalin and MPSS protected the brain against traumatic injury by attenuating lipid peroxidation and inflammatory process

Keywords: Pregabalin, Rat model, Head injury, Antioxidant

INTRODUCTION

Traumatic brain injuries cause the highest morbidity and mortality rates among all traumatic injuries worldwide. In the United States of America, there are 1.4 million cases of head trauma and 50000 deaths per year (1,2). TBI encompasses both primary and secondary injury mechanisms that contribute to its overall pathogenesis. While primary injuries occur instantaneously due to mechanical forces and are beyond our control—leading to disruptions in the functionality of blood vessels, neurons, and their axonal connections—secondary injuries present an opportunity for intervention (3). The secondary injury phase is characterized by a cascade of deleterious processes, including oxidative stress, excitotoxicity, mitochondrial dysfunction, inflammatory responses, damage to the blood-brain barrier and subsequent brain oedema (4,5). Among these secondary mechanisms, excitotoxicity plays a crucial role; it refers to the pathological consequences arising from excessive or prolonged stimulation of neurons by excitatory neurotransmitters such as glutamate, ultimately leading to neuronal cell death (6).

Pregabalin is used in the treatment of neuropathic pain associated with various medical conditions, including diabetic neuropathy, neuralgia, and complex regional pain syndrome, as noted by Tassone DM (7). Although its chemical composition resembles that of gamma-aminobutyric acid (GABA), pregabalin does not function like GABA nor does it interact with GABA receptors. Research indicates that pregabalin binds to the α2-δ subunit of voltage-gated calcium channels. This strong binding action results in a reduction of Ca2+ influx at presynaptic nerve terminals, subsequently diminishing the release of several neurotransmitters, including glutamate and noradrenaline, as reported by Joshi I and Shneker BF (8,9).

Treatments targeting excitotoxicity may be useful in the posttraumatic period. The aim of this research is to examine the possible neuroprotective effects of pregabalin administered in a rat model of head trauma. For this purpose, the effects of pregabalin were evaluated by biochemical and pathological analysis.

MATERIALS AND METHODS

Experimental groups

The research was conducted at the Experimental Animal Laboratory of Ankara Training and Research Hospital, under the auspices of the Experimental Animal Ethics Committee of the same institution (Date: 01.11.2024, Approval No: 0086). A total of thirty-two male Wistar rats, weighing between 240 and 305 grams, were included in the study. Animals were divided into 4 groups, each of eight animals, with all assignments carried out blindly to ensure objectivity.

The sham group underwent only a scalp incision. In the second group, designated as the trauma group, the rats experienced closed head trauma. The third group, referred to as MPSS, received an immediate single dose of MPSS sodium succinate (Prednol, Mustafa Nevzat, Istanbul, Turkey) at 30 mg/kg via intraperitoneal injection right after the trauma incident. Finally, the fourth group, labeled as the pregabalin group, was administered pregabalin at 30 mg/kg via gavage (10,11,12).

Type of surgery

Surgery was performed under general anaesthesia. The anaesthetist anaesthetised the rats with a mixture of IM ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and xylazine (Rompun; Bayer, Istanbul, Turkey) (50 mg/kg ;10 mg/kg). The procedures were performed in prone position. Animals were subjected to closed head trauma using the method described by Marmarou et al: A blunt object weighing 500 g was freely dropped from a height of 1 m through a copper tube onto a metal disk above the animal's skull (13). After induction of head trauma, the metal disk was removed. Twenty-four hours after the study began, the animals were humanely sacrificed via using 100 mg/kg of ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) intraperitoneal injection.

The cranial bones and overlying scalp were carefully extracted as a single unit. After midline separation, one brain hemisphere was assigned for pathological evaluation, while the contralateral hemisphere was reserved for biochemical assays.

Evaluation of Pathological Sections

The samples were fixed in 10% formalin and processed using standard protocols with a LEICA ASP 300S. After embedding in paraffin, 4-µm sections were cut on a LEICA RM 2255 microtome, stained with hematoxylin and eosin (H&E), and examined under an OLYMPUS BX51 microscope.

Measurement of MDA, GPx, and SOD enzyme activities

Tissue samples underwent homogenization with 1 ml of distilled water using an Ultra Turrax tissue homogenizer. The resulting homogenates were utilized to assess levels of MDA, SOD, and GPx activity. All procedures were conducted at a temperature of 4°C. The protein content in the brain samples was quantified employing the Lowry method as outlined by Lowry et al (14). The extent of lipid peroxidation in the brain was evaluated by measuring MDA concentration. MDA levels were quantified using the NWLSS NWKMDA01 assay provided by Northwest Life Science Specialties, with results expressed as micromoles per gram of protein. GPx activity in the brain was determined through a colorimetric assay kit based on the protocol developed by Paglia and Valentine (15). GPx concentrations were reported as milliunits per gram of protein. Furthermore, SOD activity was analyzed with a specialized superoxide dismutase assay, with results expressed in units per gram of protein.

Statistical analysis

Each experimental group comprised 8 specimens, necessitating non-parametric analyses as per established guidelines (16). Data are presented as median ± IQR (25th–75th percentiles). Intergroup comparisons were first evaluated with the Kruskal-Wallis test; where significance was reached ($p < 0.05$), post hoc Mann-Whitney U tests with Bonferroni-adjusted p-values were applied for subgroup contrasts. Statistical procedures were executed in IBM SPSS software (Version 23, Armonk, NY).

RESULTS

Pathological examination

Pathological assessment revealed pronounced perivascular and interstitial edema in injured brain regions, with no such changes detected in the sham group (Figure 1a). In contrast, the trauma group exhibited extensive edema formation (Figure 1b). However, rats administered pregabalin or MPSS showed a significant attenuation of perivascular edema relative to untreated controls (Figure 1c). Furthermore, the efficacy of pregabalin in reducing edema was comparable to that of MPSS (Figure 1d).

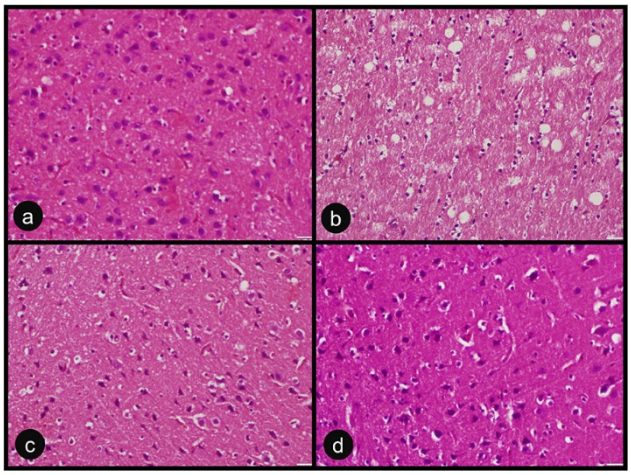

Figure 1. This figure displays tissue sections stained with H&E staining, highlighting clear differences in pathology among the various study groups. Image (a), representing the Sham group, shows undamaged brain tissue, serving as a normal reference point. Conversely, image (b), from the trauma group, demonstrates pronounced swelling around blood vessels and within the interstitial spaces, indicating substantial pathological alterations. Image (c), depicting the MPSS group, reveals a moderate degree of perivascular fluid accumulation, implying a limited beneficial impact from the treatment. Finally, image (d), from the pregabalin group, shows only slight perivascular edema, suggesting a more positive outcome with this therapeutic approach.

MDA Activity

There was a notable statistical discrepancy in MDA levels when comparing the trauma group with the others (P=0.000). It was observed that trauma caused a significant increase in MDA activity. In addition, the evaluation indicated that there was a significant difference between the pregabalin group and the trauma group when considering the MDA level (P=0.001). Furthermore, while there was a significant variation in MDA levels between the MPSS group and the trauma group, this incompatibility was not evident when comparing the MPSS group with the pregabalin group (P=0.001 for the trauma vs. MPSS; P=0.161 for the pregabalin vs. MPSS). These findings are illustrated in Figure 2.

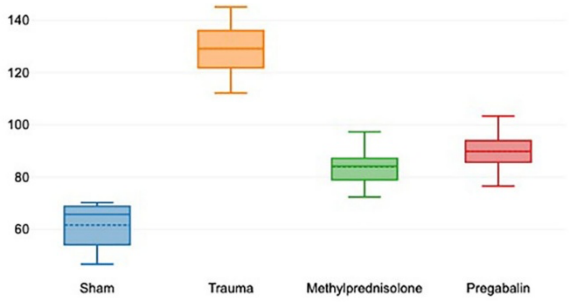

Figure 2. Box plot showing median MDA levels in brain tissue. Lipid peroxidation content is expressed as micromoles per gram protein.

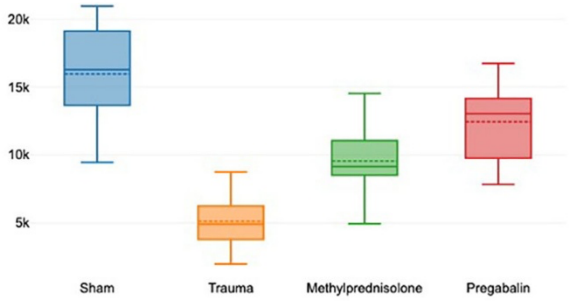

Figure 3. Box plot showing median SOD levels in brain tissue. SOD content of the brain is expressed as milliunits per gram protein.

GPx Activity

In the study, the levels of GPx was found to be significantly lower in trauma group tissue compared to others (P=0.000). Both the Pregabalin and MPSS groups exhibited elevated GPx levels relative to the trauma group, with the differences being statistically significant (P=0.002). However, no statistically significant difference was observed in GPx levels when comparing the Pregabalin group to the MPSS group (P=0.6; see Figure 3).

SOD Activity

A significant reduction in SOD levels was found in the trauma group when comparison done to the others (P=0.000). The group receiving pregabalin demonstrated a statistically significant difference in SOD activity relative to the trauma group; however, the difference was non-significant between the pregabalin group and the MPSS group (P=0.001 for the trauma comparison, and P=0.074 for the MPSS comparison) as illustrated in Figure 4.

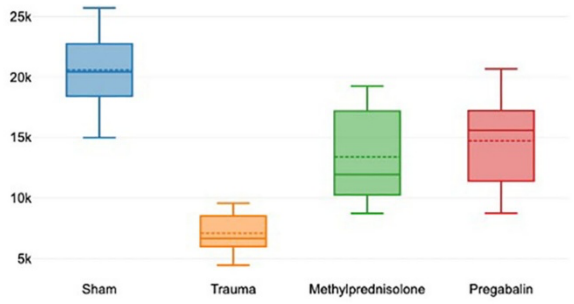

Figure 4. Box plot showing median GPx levels in brain tissue. GPx content of the brain is expressed as units per gram of protein.

DISCUSSION

Immediately after trauma to the central nervous system, primary damage is followed by a series of auto destructive mechanisms that lead to secondary damage. The resulting secondary damage causes progressive degeneration of the parenchyma, leading to chronic neurodegeneration (17). One of the main secondary mechanisms after traumatic injury is the attack of free radicals on the cell membrane (18), by the process of lipid peroxidation all cellular components, including unsaturated fatty acids, are affected and damaged (19). A frequently used biomarker that is an indicator of the overall level of lipid peroxidation is the plasma MDA concentration (20). In our study, higher MDA activity was detected in the trauma group exposed to trauma than the others.

Metabolic surges can lead to the reduction of oxygen into reactive species such as superoxide, hydrogen peroxide, and hydroxyl radicals, triggered by various stimuli (20). The enzyme SOD plays a crucial role in neutralizing superoxides by converting them into hydrogen peroxide. Subsequently, hydrogen peroxide is further reduced to water through the action of cytosolic antioxidants, specifically catalase and GPx (21). Brain tissue is very vulnerable to oxidative damage due to its fast metabolism, high content of polyunsaturated fatty acids and relative lack of antioxidant defenses (22). In our research, we observed a notable reduction in tissue levels of SOD and GPx in the pregabalin and MPSS group compared to non-drug the traumatic animals. Pathological examination revealed that traumatic brain injury induced pronounced perivascular edema, which was substantially attenuated by pregabalin treatment.

MPSS has been identified as a noteworthy antioxidant and anti-inflammatory compound that mitigates secondary injury following trauma, as noted by Kahraman S (23). Its efficacy in enhancing blood circulation and subsequently lowering lactate buildup in damaged tissues is contingent upon elevated glucocorticoid levels in those tissues, according to Braughler JM (24). Additionally, MPSS is effective in inhibiting lipid peroxidation and the formation of free radicals in the aftermath of traumatic events, leading to improved neurological outcomes, as highlighted by Bracken MB (25). Prior paper written by Kalayci M and Yang YB demonstrated that spinal cord injury markedly elevated levels of MDA in spinal cord tissues, while concurrently decreasing the antioxidant enzymes levels such as SOD and GPx. Their findings also indicated that treatment with MPSS not only reduced tissue MDA levels but also prevented the decline in SOD and GPx enzyme activities in affected tissues. tissues (25). Our research corroborated these findings, revealing that in the group treated with MPSS, there was a notable elevation in the tissue levels of GPx and SOD, alongside a decrease in MDA levels. These results align with the previous observations made by Kalayci M (26).

Oxidative stress following head trauma can be characterized as a disturbance in the delicate balance between the heightened generation of reactive

oxygen species and the inadequacy of the body's own antioxidant defense mechanisms (27). While ROS are typically produced naturally during metabolic processes within living cells, an overproduction can lead to detrimental cellular effects by harming essential cellular components, including proteins, lipids, and nucleic acids (28). The buildup of ROS is associated with lipid peroxidation in cell membranes, culminating in the generation of significant levels of MDA. Key antioxidant enzymes such as SOD, catalase, and GPx serve as primary protectants against cellular damage induced by reactive oxygen species (29). However, under conditions of pronounced oxidative stress, the activity of these enzymes may become diminished due to molecular injury.

Numerous studies have indicated that pregabalin offers protective effects on tissues exposed to oxidative stress by augmenting the activities of SOD, GPx, and catalase, as well as reducing MDA levels in a dose-dependent manner (11,12). In line with these findings, our research illustrated that traumatic injury led to a reduction in the activity of endogenous antioxidant enzymes alongside an increase in MDA levels within brain tissue. Notably, these alterations were substantially mitigated through the administration of pregabalin. Furthermore, the findings from our biochemical analysis corroborated the pathological observations of our study. To more comprehensively elucidate the mechanisms by which pregabalin affects TBI, additional investigations are warranted.

CONCLUSION

Pregabalin and MPSS protected the brain against traumatic injury by attenuating lipid peroxidation and inflammatory process. Moreover, the pathological evaluations were supported the biochemical analysis results. The lack of ultrastructural findings in our study is a shortcoming in terms of understanding the effective mechanism. Further studies may be needed to determine the neuroprotective activity of pregabalin in a time- and dose-dependent manner.

Acknowledgments No.

Author contribution Ömer Şahin: Idea-Concept-Design-Analysis-Literature review-Writing. Fatma Karaca Kara: Research-Data processing-Analysis-Literature review-Writing.

Conflict of interests: The authors have no conflict of interest to declare.

Ethical approval: This study was approved by Institutional Animal Experiments Ethics Committee of Ankara Training and Research Hospital, University of Health Sciences (Date: 01.11.2024, Approval No: 0086).

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