

Meloxicam, a selective COX-2 inhibitor, displays anticonvulsive effects in pentylenetetrazole-induced acute seizures in mice through GABA and glutamate mediated mechanism

Bilal Sahin¹, Recep Akkaya², Sebahattin Karabulut³

¹Department of Physiology, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey

²Department of Biophysics, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey

³Department of Medical Services and Techniques, Vocational School of Health Services, Sivas Cumhuriyet University, Sivas, Turkey

ABSTRACT

Aim: To investigate the possible anticonvulsive effect of the selective COX-2 inhibitor meloxicam in pentylenetetrazole (PTZ)-induced epileptic seizures in mice and to examine its possible role on inhibition and excitation balance in the brain.

Method: 30 BALB-c albino mice (16-18 weeks old) weighing 30-33 gr were used. Animals were randomly divided into five groups (n = 6 for each group). Group 1: control, group 2: received saline (10 ml/kg, i.p.) 30 minutes before PTZ (60 mg/kg i.p.) administration, group 3: received saline (10 ml/kg, i.p.) 30 minutes after PTZ (60 mg/kg i.p.) injection, group 4: received 60 mg/kg meloxicam i.p., 30 minutes before PTZ (60 mg/kg i.p.) administration. Group 5: received meloxicam (60 mg/kg i.p.) 30 minutes after PTZ injection (60 mg/kg, i.p.). The animals were observed for 30 minutes and the seizure stages and first myoclonic jerk times (FMJ) were recorded. After 24 hours, brain tissues were removed and the cortex and hippocampus were separated for biochemical assessments. ELISA method was used to measure GABA and glutamate levels.

Results: Administration of meloxicam before PTZ induced seizure, reduced seizure stages and prolonged FMJ duration ($p < 0.05$). Pre-treatment with meloxicam increased GABA levels in the cortex and decreased glutamate levels in the hippocampus ($p < 0.05$). Post-treatment of meloxicam after PTZ-induced seizure increased GABA levels in the hippocampus ($p < 0.05$).

Conclusion: The results of our experimental study suggest that meloxicam has anti-convulsive effects and these effects may be mediated by GABA and glutamate, which are the main indicators of inhibition and excitation balance in the brain.

Key words: Meloxicam, COX-2, epilepsy, GABA, glutamate, mice.

✉ Bilal Sahin

Department of Physiology, Sivas Cumhuriyet University,
School of Medicine, Sivas, Turkey

E-mail: bilalsahin@cumhuriyet.edu.tr

Received: 2021-06-23 / Revisions: 2021-08-13

Accepted: 2021-08-28 / Published online: 2022-01-01

Introduction

Epilepsy is a short-term paroxysmal disorder of brain function characterized by sudden, abnormal and hypersynchronous discharges and seizures observed in a group of neurons in

the central nervous system [1]. Although many studies have been conducted to understand the underlying mechanism and to develop pharmacological treatment, our knowledge about the biological disorders that cause epilepsy is limited [2]. Because of this deficit, currently available anti-epilepsy treatment is symptomatic and ineffective in 30% of cases [3]. Therefore, more effective therapies should be developed to target epileptogenesis. Various molecular and cellular changes accompanying

epilepsy include inflammatory processes in the brain as well as inhibitory-stimulatory processes and an imbalance in the antioxidant system [4]. Studies aiming to elucidate the processes that lead to epilepsy have suggested that inflammation plays an important role both as a cause and a consequence of seizure development [5]. Additionally, several anti-inflammatory drugs have been reported for antiepileptic activities [6,7]. In contrast, there is inverse evidence on the relationship between inflammation and epilepsy [2]. Consequently, further research is needed to understand this relationship.

Cyclooxygenase-2 (COX-2) has received much attention for its important role in the development of various inflammatory processes over the past two decades, and therefore it involves in the formation of seizures and the development of epilepsy. The COX enzyme group consists of oxygenases that convert arachidonic acid to prostaglandins (PGs), which are pro-inflammatory mediators as COX-1 and COX-2 [8]. While COX-1 is mainly expressed in almost all tissue types, COX-2 is an inducible isoform-inducible enzyme that is primarily localized in immune cells such as macrophages and leukocytes, and it is upregulated in pathological conditions like neuronal death and neuronal hyper-excitability and is predominantly expressed in the brain [9]. In addition, COX-1 is thought to be involved in the homeostatic production of PG while COX-2 generally produces PGs related to pathophysiological processes [10]. In this context, COX-2 inhibitor drugs are used for treatments to reduce inflammation in both acute and chronic conditions [11]. In the past few years, a serious research has been done on the applicability of COX-2 as a treatment target in various neuro-inflammatory diseases, including epilepsy.

The animal models for epilepsy have been frequently used to examine the potentiality of anti-convulsing effects of COX-2 inhibition and its effect on seizure activity and development. It has been observed that the findings differ in different seizure and treatment conditions. Meloxicam, a selective COX-2 inhibitor, is used clinically to ease inflammation, swelling, stiffness, and joint pain that is associated with juvenile rheumatoid arthritis, osteoarthritis, and rheumatoid arthritis as part of class drugs called nonsteroidal anti-inflammatory (NSAID)[2]. Meloxicam inhibits prostaglandin biosynthesis in inflammation through COX-2 inhibiting effect [12]. In previous studies, meloxicam was observed to increase the first myoclonic jerk (FMJ) time in acute pentylenetetrazole (PTZ) epilepsy model [13] and to reduce the levels of myeloperoxidase and malondialdehyde and to reintegrate the brain glutathione content [14]. Finally, in a study of kindling model epilepsy in mice, meloxicam reduced inflammation and oxidative stress, and thus it showed anti-epileptic effects [15]. Therefore, for the first time in this study, in PTZ-induced acute seizure mouse model, we examined whether pre- and post-treatment of meloxicam would affect seizure susceptibility through GABAergic and glutamatergic balance in the hippocampus and cortex.

Materials and methods

Animals

Male adult BALB-c Albino 30-33 g mice (16-18 weeks old) purchased from Sivas Cumhuriyet University were used for experiments. The animals were placed in a room with an ambient temperature of $22\pm 3^{\circ}\text{C}$, with a stable humidity between 35–60% and with water and food ad libitum. All experimental procedures were performed under

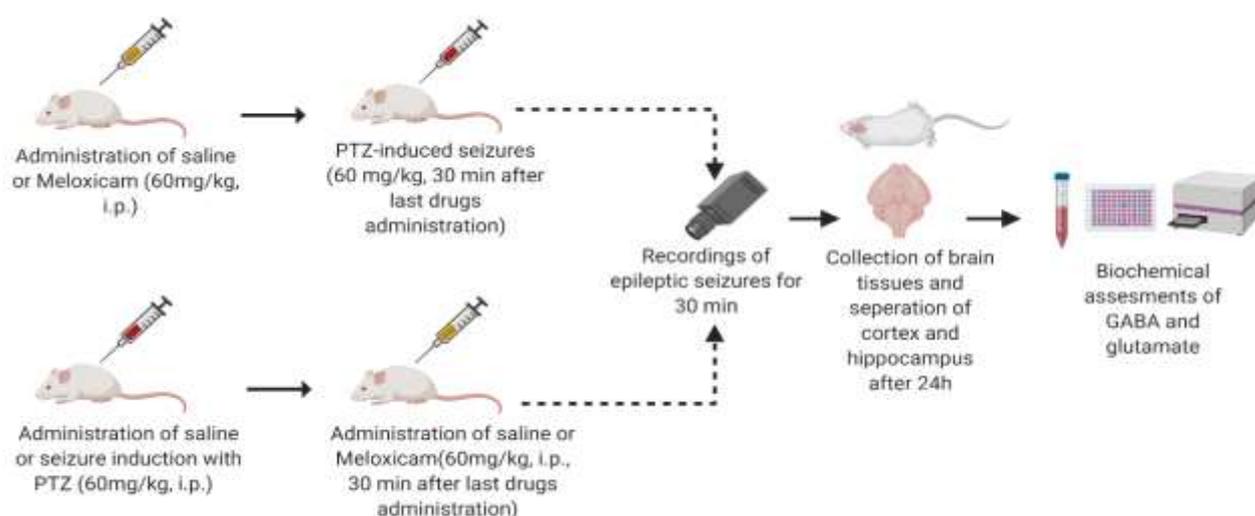


Figure 1. Experimental design of the study (created with BioRender.com).

the guidelines of the Local Ethics Committee of Sivas Cumhuriyet University (Registry Number: 65202830-050.04.04 dated 24.02.2020). The animals were acclimated to laboratory conditions prior to the assay.

Chemicals

Pentylenetetrazole and meloxicam were dissolved in physiological saline. Solutions were freshly prepared for the experiment days. All chemicals used in the studies were of analytical purity. All drugs were purchased from Sigma-Aldrich Co., St Louis, MO, USA.

Experimental protocols

Thirty mice were randomly divided into five clusters for behavioral and biochemical evaluations ($n = 6$ for each groups). Group 1 was control, group 2 was given intraperitoneal saline (10 ml/kg, i.p.) before 30 min PTZ (60 mg/kg i.p.) [16,17], group 3 was given intraperitoneal saline (10 ml/kg, i.p.) after 30 min PTZ injection (60 mg/kg i.p.), group 4 was injected 60 mg/kg meloxicam i.p. before 30 min PTZ (60 mg/kg i.p.), and group 5 was administered 60 mg/kg meloxicam i.p. after 30

min PTZ injection (60 mg/kg i.p.). To determine seizure stages, the Racine convulsion scale (RCS) was used as follows:

- Phase 0 = no response after PTZ administration
- Phase 1 = short or long-term ear and facial twitching
- Phase 2 = myoclonic body jerks and severe myoclonic reflexes
- Phase 3 = clonic forelimb convulsions, severe rearing-up on hind-legs and transition to clonic seizure
- Phase 4 = short or long-term tonic clonic seizures
- Phase 5 = Severe recurrent generalized tonic-clonic seizures
- Phase 6 = lethal seizure/death

For 30 min., mice were observed both for assessing seizure stage and recording the time of the first myoclonic jerk (FMJ) with Phase 3 seizures [18]. Animals were sacrificed and the hippocampus and cortex tissues were removed for the assessment of biochemical parameters. The experimental design is shown in detail in Figure 1.

Biochemical analysis of glutamate and gamma amino butyric acid (GABA)

The cortical and hippocampal regions of brain tissues were homogenized at (pH 7.4) in ice-cold Phosphate Buffered Saline (PBS) solution by using a manual homogenizer, and then they were centrifuged at 12.000g for 10 min at 4°C. The supernatants of the centrifuged homogenates were removed. Mice ELISA commercial kits (YL Biont, Shanghai, China, detection range: 24.69-2000pg/mL) were used to measure glutamate and GABA levels from cortical and hippocampal supernatants according to the manufacturer's instructions. In summary, standard and tissue samples were added to the plate and were incubated for 60 minutes at 37°C. Following the wash stage, staining solutions were added and were

incubated for 15 minutes at 37°C. Stop solution was added and it was read as 450 nm. To determine the total protein content of samples, Bradford method was used to optimize outcomes from the hippocampus and cortex[19].

Statistical analysis

The data were presented as mean ± SEM (standard error of the mean). For all data, the one-way analysis of variance (ANOVA) with Tukey post hoc comparisons test was used. The criterion for the statistical significance less than 5% was accepted.

Results

The effect of Meloxicam on epileptic behaviour

The epileptic seizure scores were significantly

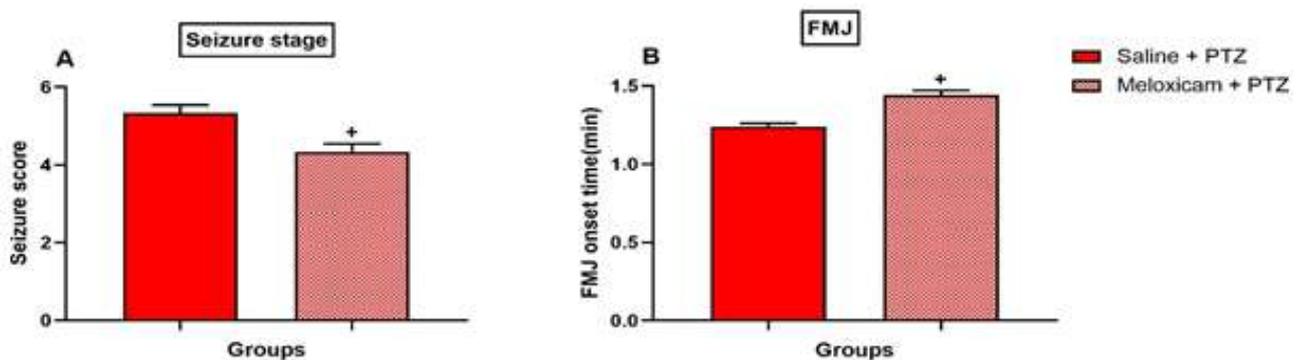


Figure 2A, B. The effect of Meloxicam on seizure stage and FMJ time

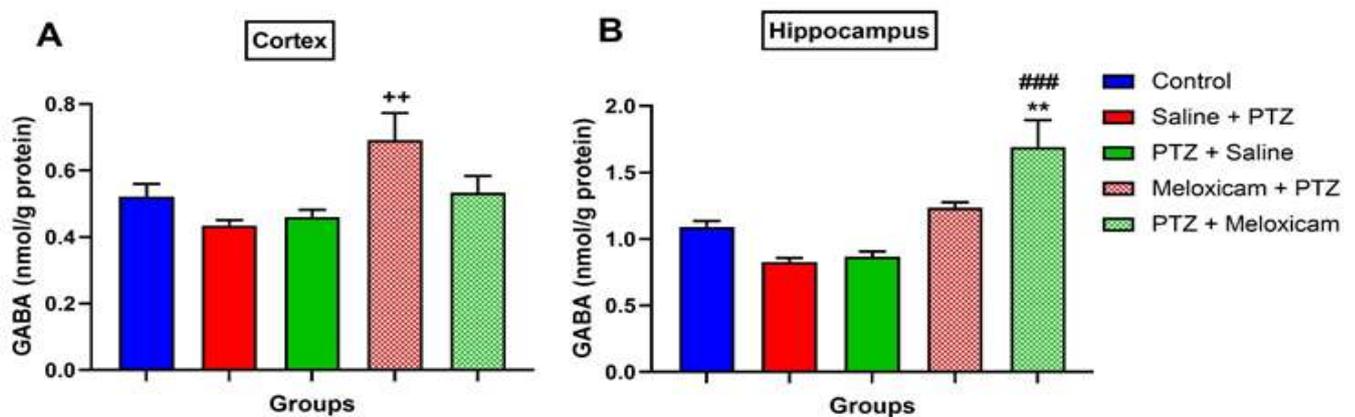


Figure 3A, B. Effect of meloxicam on GABA levels. (A), Cortex (B), Hippocampus. Values are presented as mean ± SEM. ** $p < 0.01$ compared with control group. ++ $p < 0.01$, +++ $p < 0.001$ compared with saline+PTZ group. ### $p < 0.01$, #### $p < 0.001$ compared with PTZ+saline group.

lower in meloxicam+PTZ group by comparison with the saline+PTZ group ($p < 0.05$). (Figure 2A).

The FMJ time was significantly higher in meloxicam+PTZ group (1.44 ± 0.03 min) by comparison with saline+PTZ group (1.24 ± 0.02 min) ($p < 0.05$). (Figure 2B). No seizure-related death was observed in mice during PTZ - induced acute seizure experiments.

The effect of Meloxicam on GABA levels in cortex and hippocampus

In the cortex, there was no statistically significant difference between saline+PTZ and PTZ+saline group by comparison with control group (0.52 ± 0.04 8 nmol/g protein) ($p > 0.05$). On the other hand, there was a significant difference between meloxicam+PTZ group (0.69 ± 0.8 nmol/g protein) by comparison with the saline+PTZ group (0.43 ± 0.01 nmol/g protein) ($p < 0.05$). Otherwise, there was no statistically significant difference between PTZ+meloxicam and PTZ+saline group ($p > 0.05$) (Figure 3A). In the hippocampus, GABA levels were significantly higher in

PTZ+meloxicam group (1.69 ± 0.20 nmol/g protein) by comparison with the control (1.08 ± 0.04 nmol/g protein) ($p < 0.05$) and PTZ+saline (0.86 ± 0.03 nmol/g protein) ($p < 0.001$) groups (1.23 ± 0.04 nmol/g protein) ($p < 0.01$).

Furthermore, GABA levels were higher in meloxicam+PTZ group (1.22 ± 0.04 nmol/g protein) by comparison with saline+PTZ group (0.83 ± 0.03 nmol/g protein) ($p < 0.05$). However, no statistically significant difference was found between PTZ+meloxicam (1.62 ± 0.16 nmol/g protein) and PTZ+saline group in the hippocampus ($p > 0.05$) (Figure 3B).

No seizure-related death was observed in mice during PTZ-induced acute seizure experiments.

The effect of Meloxicam on glutamate levels in cortex and hippocampus

In the cortex, no statistically significant difference was found between saline+PTZ and meloxicam+PTZ groups ($p > 0.05$). Similarly, there was no statistically significant difference between PTZ + saline and PTZ + meloxicam

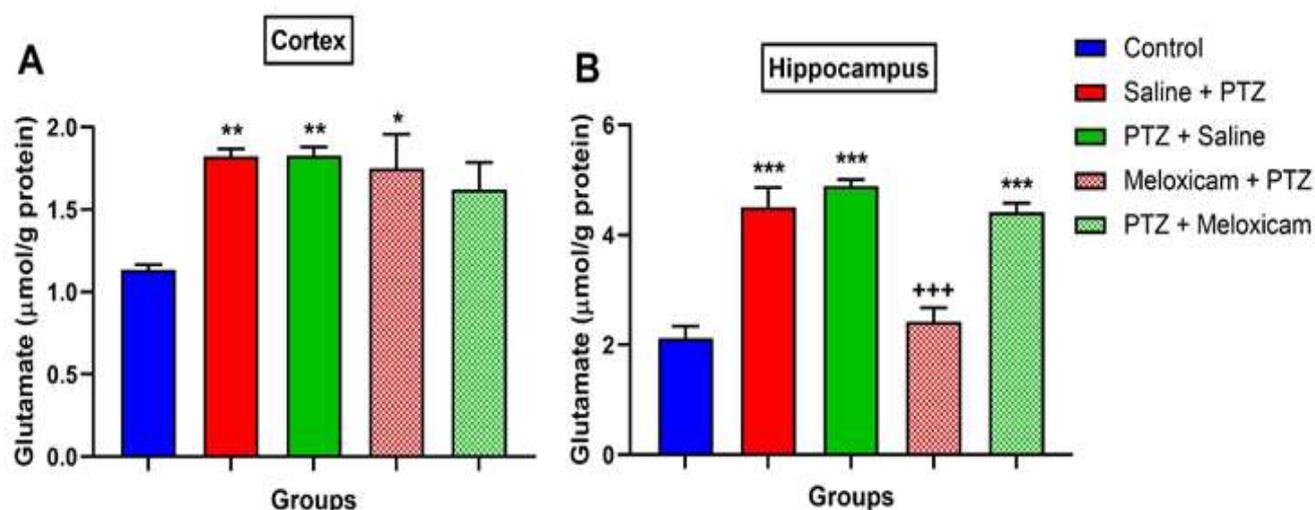


Figure 4. Effect of meloxicam on glutamate levels. (A), Cortex (B), Hippocampus. Values are presented as mean \pm SEM. ** $p < 0.01$ compared with control group. ++ $p < 0.01$, +++ $p < 0.001$ compared with saline+PTZ group. ### $p < 0.01$, #### $p < 0.001$ compared with PTZ+saline group.

groups ($p > 0.05$). Contrarily, glutamate levels were significantly higher in the saline+PTZ (1.82 ± 0.04 nmol/g protein) and PTZ+saline group (1.82 ± 0.05 nmol/g protein) by comparison with the control group (1.13 ± 0.03 nmol/g protein) ($p < 0.01$). Moreover, glutamate levels were higher in the meloxicam+PTZ group (1.75 ± 0.20 nmol/g protein) by comparison with the control group ($p < 0.05$). However, in the cortex, no significant difference was found in PTZ+meloxicam group (1.62 ± 0.16 nmol/g protein) by comparison with the control group ($p > 0.05$) (Figure 4A). In the Hippocampus, glutamate levels in saline+PTZ (4.50 ± 0.35 nmol/g protein), PTZ+saline (4.88 ± 0.12 nmol/g protein) and PTZ+meloxicam groups (4.41 ± 0.16 nmol/g protein) were higher than the control group (2.12 ± 0.22 nmol/g protein) ($p = 0.0001$). However, glutamate levels in meloxicam+PTZ group (2.42 ± 0.25 nmol/g protein) were significantly lower than saline+PTZ group ($p < 0.001$) (Figure 4B). In addition, there was no statistically significant difference between PTZ+saline and PTZ+meloxicam groups ($p > 0.05$).

Discussion

In the present study, it is shown that administration of meloxicam, a COX-2 inhibitor, before and after PTZ-induced acute seizures in mice reduces seizure stages and increases FMJ, and therefore it shows an anticonvulsant activity. Preliminary administration of meloxicam has increased GABA levels both in the cortex and hippocampus, and it has decreased glutamate levels in the hippocampus. On the other hand, administration of meloxicam after seizure induction has no effect on glutamate levels, but it has increased GABA levels in the hippocampus. These findings suggest that the

anticonvulsant activity of meloxicam in acute PTZ-induced seizures in mice may be mediated through the levels of GABA and glutamate, two of the most important molecules in the inhibition and excitation processes in brain.

In brain, COX-2 is expressed in discrete neuronal populations, particularly in the cortex and hippocampus [20]. In many reports, it has been stated that COX-2 plays a significant role in some neurological disorders such as Alzheimer's disease [21], traumatic brain injury [22], cerebral ischemia [23], and epilepsy [24]. The effect of COX-2 inhibitors on seizure type and seizure activity may vary according to different seizure and treatment conditions. Pre-treatment with selective COX-2 inhibitors nimesulide and rofecoxib for 45 minutes prior to seizure induction has demonstrated the diverse efficacy of COX-2 inhibitors in 3 different types of seizure models in mice. These inhibitors have showed anti-convulsing activities by increasing the mean onset time of seizures and by reducing seizure duration in bicuculline- and picrotoxin-induced seizures; on the other hand, they have revealed no effect in maximal electroshock-induced seizures [7]. It shows that the administration of the COX-2 inhibitor rofecoxib at 2 mg/kg and 4 mg/kg increases the seizure threshold, but it does not show that there are any anticonvulsive effects at a low dose, and consequently, there is a dose-dependent effect [25]. Pre-treatment with the selective COX-2 inhibitor, celecoxib, also shows the anticonvulsant effects in 60 minutes before seizure induction in the PTZ-induced rat model [26]. Similarly, in our study, pre-treatment with meloxicam shows the anticonvulsant effect in 30 minutes before seizure induction. Moreover, selective COX-2 inhibitor, etoricoxib, shows an anticonvulsant effect both in PTZ-induced rat model and genetic Wag/Rij absence epilepsy rat model.

However, a number of studies suggest that pre-treatment with COX-2 inhibitors could have a proconvulsive effect [27,28].

Animal models of epilepsy, including PTZ-induced acute seizures, are widely used to identify molecules with anticonvulsant potential and to investigate their efficacy. PTZ exerts its convulsive effect by inhibiting the GABA_A receptor. Decreased GABAergic activity and increased glutamatergic system activity are shown as the most frequent causes of seizures [29]. GABA_A receptor and a ligand-gated chloride channel mediate inhibitory transmission at synapses. In the present work, we have found that PTZ-induced epileptic seizure activity decreases due to the pre- and post-treatment with the COX-2 inhibitor meloxicam. COX-2 mRNA and protein induction, for the first time, have been revealed in the hippocampus and cerebral cortex tissues of rats in a seizure model induced by a maximum electroconvulsive shock [20]. In the same study, it is also shown that COX-2 induction is regulated by glutamatergic N-methyl-D-aspartate (NMDA) receptor-dependent synaptic activity. Several studies have demonstrated that meloxicam prevents ischemia-induced excitotoxicity by changing the transcript levels of different genes of the glutamatergic system, especially NMDA and AMPA receptor subunits [30–32].

PGE₂, the major product of COX-2, binds EP1 receptor with a higher affinity which is a subfamily of G protein-coupled receptors (GPCRs). It has been suggested that COX-2 inhibitors exhibit an anticonvulsant activity by reducing PGE₂ production and lead to a decrease in EP receptor activation, and as a result, they reduce calcium ion entry and the release of the excitatory neurotransmitter glutamate, thereby blocking seizures [33]. These findings are consistent with this current

study in which meloxicam decreases glutamate levels in the hippocampus. Activation of COX-2 also increases oxidative stress by increasing the production of free radicals. Increased oxidative stress causes continuous apoptosis of GABAergic neurons, leading to an increase in glutamate-mediated excitation in neuronal network [34]. Selective COX-2 inhibitor, NS-398, causes up-regulation in the expression of GABA_A receptors, thus, it prevents epileptic seizures by reducing neuronal excitability via MAPK/ERK pathway in hippocampus in pilocarpine-induced status epilepticus in rats [35]. Similarly, in our study, both pre- and post-treatments with meloxicam have increased GABA levels in the cortex and hippocampus. The data suggest that meloxicam shows its anti-convulsive effect by increasing GABAergic activity with pre- and post- treatments and by reducing glutamatergic activity in hippocampus via pre- treatment.

Conclusion

In conclusion, meloxicam shows an anti-epileptic effect on mice both in pre- and post-treatments through GABA and glutamate in PTZ-induced acute seizure models. However, there is a need for further research to understand the underlying mechanisms.

Funding: *The author(s) received no financial support for the research, authorship, and/or publication of this article.*

Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *All experimental procedures were performed under the guidelines of the Local Ethics Committee of Sivas Cumhuriyet University (Registry Number: 65202830-050.04.04 dated 24.02.2020).*

Open Access Statement

This is an open access journal which means that all content is freely available without charge to the user or his/her institution under the terms of the Creative Commons Attribution Non-Commercial License

(<https://creativecommons.org/licenses/by/4.0/>).

Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, without asking prior permission from the publisher or the author.

Copyright (c) 2021: Author (s).

References

- [1]Taskiran AS, Ergul M, Gunes H, et al. The Effects of Proton Pump Inhibitors (Pantoprazole) on Pentylentetrazole-Induced Epileptic Seizures in Rats and Neurotoxicity in the SH-SY5Y Human Neuroblastoma Cell Line. *Cell Mol. Neurobiol.* 2021;41(1):173–83.
- [2]Rawat C, Kukal S, Dahiya UR, et al. Cyclooxygenase-2 (COX-2) inhibitors: future therapeutic strategies for epilepsy management. *J Neuroinflammation.* 2019;16(1):197.
- [3]Chen Z, Brodie MJ, Liew D, et al. Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs a 30-year longitudinal cohort study. *JAMA Neurol.* 2018;75(3):279–86.
- [4]Borowicz-Reutt KK, Czuczwar SJ. Role of oxidative stress in epileptogenesis and potential implications for therapy. *Pharmacol Reports.* 2020;72(5):1218–26.
- [5]Vezzani A, French J, Bartfai T, et al. The role of inflammation in epilepsy. *Nat Rev Neurol.* 2011;7(1):31–40.
- [6]Dhir A, Naidu PS, Kulkarni SK. Neuroprotective effect of nimesulide, a preferential COX-2 inhibitor, against pentylentetrazol (PTZ)-induced chemical kindling and associated biochemical parameters in mice. *Seizure.* 2007;16(8):691–97.
- [7]Dhir A, Naidu PS, Kulkarni SK. Effect of cyclooxygenase-2 (COX-2) inhibitors in various animal models (bicuculline, picrotoxin, maximal electroshock-induced convulsions) of epilepsy with possible mechanism of action. *Indian J Exp Biol.* 2006;44(4):286–91.
- [8]Takemiya T, Suzuki K, Sugiura H, et al. Inducible brain COX-2 facilitates the recurrence of hippocampal seizures in mouse rapid kindling. *Prostaglandins Other Lipid Mediat.* 2003;71(3–4):205–16.
- [9]Zhang J, Goorha S, Raghov R, et al. The tissue-specific, compensatory expression of cyclooxygenase-1 and -2 in transgenic mice. *Prostaglandins Other Lipid Mediat.* 2002;67(2):121–35.
- [10]Kulkarni SK, Dhir A. Cyclooxygenase in epilepsy: From perception to application. *Drugs of Today.* 2009;45(2):135–54.
- [11]Löscher W, Klitgaard H, Twyman RE, et al. New avenues for anti-epileptic drug discovery and development. *Nat Rev Drug Discov.* 2013;12(10):757–76.
- [12]Edfawy M, Hassan MH, Mansour A, et al. Meloxicam modulates oxidative stress status, inhibits prostaglandin E2, and abrogates apoptosis in carbon tetrachloride-induced rat hepatic injury. *Int J Toxicol.* 2012;31(3):276–86.
- [13]Darvishi H, Rezaei M, Khodayar MJ, et al. Differential effects of meloxicam on pentylentetrazole- and maximal electroshock-induced convulsions in mice. *Jundishapur J Nat Pharm Prod.* 2017;12(2). e.36412.
- [14]Hakan T, Toklu HZ, Biber N, et al. Effect of COX-2 inhibitor meloxicam against

- traumatic brain injury-induced biochemical, histopathological changes and blood-brain barrier permeability. *Neurol Res.* 2010;32(6):629–35.
- [15] Elgarhi R, Shehata MM, Abdelsameea AA, et al. Effects of Diclofenac Versus Meloxicam in Pentylentetrazol-Kindled Mice. *Neurochem Res.* 2020;45:1913–19.
- [16] Akkaya R, Karabulut S, Taskiran AS. Investigation of the effect of REM sleep deprivation on epileptic seizures caused by pentylentetrazole in mice. *Exp Biomed Res.* 2020;3(3):149–56.
- [17] Li B, Wang L, Sun Z, et al. The Anticonvulsant Effects of SR 57227 on Pentylentetrazole-Induced Seizure in Mice. *PLoS One.* 2014;9(4):93158.
- [18] Racine RJ. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. *Electroencephalogr Clin Neurophysiol.* 1972;32(3):269-79.
- [19] Ernst O, Zor T. Linearization of the Bradford protein assay. *J Vis Exp.* 2010;(38):1918.
- [20] Yamagata K, Andreasson KI, Kaufmann WE, et al. Expression of a mitogen-inducible cyclooxygenase in brain neurons: Regulation by synaptic activity and glucocorticoids. *Neuron.* 1993;11(2):371–86.
- [21] Melnikova T, Savonenko A, Wang Q, et al. Cyclooxygenase-2 activity promotes cognitive deficits but not increased amyloid burden in a model of Alzheimer's disease in a sex-dimorphic pattern. *Neuroscience.* 2006;141(3):1149-62.
- [22] Cernak I, O'Connor C, Vink R. Activation of cyclo-oxygenase-2 contributes to motor and cognitive dysfunction following diffuse traumatic brain injury in rats. *Clin Exp Pharmacol Physiol.* 2001;28(11):922-25.
- [23] Nakayama M, Uchimura K, Zhu RL, et al. Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. *Proc Natl Acad Sci U. S. A.* 1998;95(18):10954–59.
- [24] Vezzani A, Granata T. Brain inflammation in epilepsy: Experimental and clinical evidence. *Epilepsia.* 2005;46(11):1724–43.
- [25] Akula KK, Dhir A, Kulkarni SK. Rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor increases pentylentetrazol seizure threshold in mice: Possible involvement of adenosinergic mechanism. *Epilepsy Res.* 2008;78(1):60–70.
- [26] Oliveira MS, Furian AF, Royes LFF, et al. Cyclooxygenase-2/PGE2 pathway facilitates pentylentetrazol-induced seizures. *Epilepsy Res.* 2008;79(1):14–21.
- [27] Jeong KH, Kim JY, Choi YS, et al. Influence of aspirin on pilocarpine-induced epilepsy in mice. *Korean J Physiol Pharmacol.* 2013;17(1):15–21.
- [28] KunzNote T, Oliw EH. Nimesulide Aggravates Kainic Acid-Induced Seizures in the Rat. *Pharmacol Toxicol.* 2001;88(5):271–76.
- [29] Li HH, Lin PJ, Wang WH, et al. Treatment effects of the combination of ceftriaxone and valproic acid on neuronal and behavioural functions in a rat model of epilepsy. *Exp Physiol.* 2021;106(8):1814-28.
- [30] Montori S, Dos-Anjos S, Martínez-Villayandre B, et al. Age and meloxicam attenuate the ischemia/reperfusion-induced down-regulation in the NMDA receptor genes. *Neurochem Int.* 2010;56(8):878–85.
- [31] Llorente IL, Burgin TC, Pérez-Rodríguez D, et al. Unfolded protein response to global ischemia following 48 h of reperfusion in the rat brain: The effect of age and meloxicam. *J Neurochem.* 2013;127(5):701–10.
- [32] Montori S, Dosanjós S, Ríos-Granja MA, et al. AMPA receptor downregulation induced by ischaemia/reperfusion is attenuated by

age and blocked by meloxicam. *Neuropathol. Appl Neurobiol.* 2010;36(5):436–47.

[33] Lin TY, Lu CW, Wang CC, et al. Cyclooxygenase 2 inhibitor celecoxib inhibits glutamate release by attenuating the PGE2/EP2 pathway in rat cerebral cortex endings. *J Pharmacol Exp Ther.* 2014;351(1):134–45.

[34] Dhir A, Naidu PS, Kulkarni SK. Effect of naproxen, a non-selective cyclo-oxygenase inhibitor, on pentylenetetrazol-induced kindling in mice. *Clin Exp Pharmacol Physiol.* 2005;32(7):574–84.

[35] Zhang HJ, Sun RP, Lei GF, et al. Cyclooxygenase-2 inhibitor inhibits hippocampal synaptic reorganization in pilocarpine-induced status epilepticus rats. *J Zhejiang Univ Sci B.* 2008;9(11):903–915.