Research Journal of Biological Sciences 6 (9): 421-431, 2011
ISSN: 1815-8846

Anticonvulsant Effect of Rofecoxib in Mice Induced by PTZ

Khayatnouri Mirhadi
Department of Pharmacology and Toxicology, Faculty of Veterinary,
Tabriz Branch, Islamic Azad University, Tabriz, Iran

Abstract: The Cyclooxygenases (COXs), the key enzymes that convert arachidonic acid to Prostaglandins (PGs) have been implicated in physiological and pathophysiological functions in the CNS. NSAIDs (Non-Stesoidal Anti-Inflammatory Drugs), COX inhibitors are used largely to treat febrile condition, pain state and for prevention of and therapeutics of many diseases. However, the role of PGs and NSAIDs in the seizure activity has been disputed. The aim of this study was to evaluation the effect of intraperitoneally injection of different doses of Rofecoxib on PTZ-induced seizure threshold in mice. Mice were divided into 9 groups randomly, the 1st group received saline normal (ip) (control group); the 2nd group received Carboxymethylcellulose (CMC) 0.25% (ip) (vehicle group) and the next groups received respectively different doses of Rofecoxib (1, 2, 4, 8, 10, 15 and 20 mg kg^{-1} ip) 45 min before determination of seizure threshold induced by PTZ. Results showed that PTZ-induced seizure threshold in control mice was 34.75±1.54 mg kg^{-1}. Intraperitoneal injection of Rofecoxib showed significant ($p<0.05$) increase of PTZ-induced seizure threshold in a dose dependently manner compared with control group. According to the results, Rofecoxib has anticonvulsant effects on mice. Nevertheless, new studies must be carried out in order to determine the beneficial effects of NSAIDs in treatment of epilepsy.

Key words: Rofecoxib, seizure, PTZ, threshold, mice, Iran

INTRODUCTION

Epilepsy is one of the major neurological diseases in humans and about 1% of the population is involved. Neuroinflammation is an important mechanism in the defense response to pathogenic events, traumatic injury and environmental toxins but it is also recognized as a major contributor to various neurological and neurodegenerative diseases such as seizure. An innate immune response is mediated by microglia that contributes to the progression of the diseases. Activated microglia produce several proinflammatory and neurotoxic mediators including complement, cytokines, chemokines, acid arashidonic and its metabolites and reactive oxygen and nitrogen species, several of which contribute directly to neuronal injury (Perrone *et al*., 2010).

Alterations in the microenvironment such as microglial inflammation and the release of proinflammatory cytokines may affect normal cell proliferation and differentiation which could cause ectopic neurogenesis, astrogliosis and ectopic synaptic reorganization (Zhang *et al*., 2009). Inflammatory processes have been implicated in both acute and chronic neurodegenerative conditions such as epilepsy (Dhir *et al*., 2008). The Cyclooxygenases (COX) are the principle and obligatory enzymes in the synthesis of Prostaglandins (PGs) and other prostanoids and also the key targets for anti-inflammatory drugs (Dhir *et al*., 2008). Although, cyclooxygenases, the key enzymes that convert arachidonic acid to prostaglandins (including PGE2, PGD2, PGF2α, PGI2 and thromboxane A2) have been implicated in physiological and pathophysiological functions in the CNS, the cellular mechanisms by which COX reaction products are involved have yet to be elucidated (Chen and Bazan, 2005). These enzymes have shown to be expressed in different areas of brain besides peripheral organs and hence speculated to play an important role in neurological disorders (Dhir *et al*., 2008). NSAIDs (Non-Stesoidal Anti-Inflammatory Drugs), COX inhibitors are used largely to treat febrile condition, pain state and for prevention of and therapeutics of many diseases. However, the role of PGs and NSAIDs in the seizure activity has been disputed (Kim *et al*., 2008). The enzyme exists as Constitutive (COX-1) and inducible (COX-2) isoforms, being the latter a major player in inflammation (Minghetti and Pocchiari, 2007). In the brain, COX-2 expression has been associated with inflammatory and neurodegenerative processes of several human neurological diseases (Minghetti and Pocchiari, 2007). Reports indicated the up-regulation of cyclooxygenase enzyme following seizure activity (Takemiya *et al*., 2003). The potential role of COX isoforms and PGs in brain diseases has been extensively reviewed in the past years (Minghetti and Pocchiari, 2007). Interestingly, COX-2 induction appears to be characteristic of forebrain seizures, since COX-2 was not induced in midbrain

seizures (Dhir *et al*., 2008). Transcription of COX-2, a proinflammatory mediator encoded in an early-response gene is induced by synaptic activity; therefore, COX-2 activity could contribute to epileptic neuronal injury (Zhang *et al*., 2009). Also, some trials have been performed to find out clinical and pre-clinical evidence of COX-1-mediated proinflammatory effects in neuro-degenerative disorders and in models of neuro-inflammation.

Besides, COX-1 plays a previously unrecognized part in the neuro-inflammation, a key stage in the development of several neuro-degenerative diseases including Alzheimer's disease, Parkinson's disease, traumatic brain injury, HIV-associated dementia ischemic stroke and epilepsy, whereas COX-2, mainly localized in pyramidal neurons is expected to predominantly contribute to increase PG synthesis in response to insults that directly challenge neurons such as ischemia and exitotoxicity. Under these circumstances, COX-2 inhibition seems to afford protection without altering the inflammatory response.

Thus the type of insult, the cellular target of the stimulus and whether neuroinflammation is a primary or a secondary response could determine whether COX-2 activity mediates neurotoxicity or neuroprotection. In particular, genetic ablation or pharmacological inhibition of COX-1 activity attenuates the inflammatory response and neuronal loss. This indicates that NSAIDs with higher selectivity for COX-1 rather than COX-2 are more likely to reduce neuroinflammation and should be further investigated as a potential therapeutic approach in neurodegenerative diseases with a marked inflammatory component (Choi *et al*., 2009).

In the CNS, COX-2 is mainly expressed in glutamatergic neurons particularly with in the hippocampus and cerebral cortex, the areas that demonstrate prominent role in the onset of seizures (Choi *et al*., 2009). It was found that COX-2 regulates cell membrane excitability and long term synaptic plasticity in the hippocampus (Chen and Bazan, 2005) suggesting that COX-2 may play a critical role in convulsive states. However, results of previous studies about the role of COX-2 in the genesis and maintenance of convulsion are controversial for instance, both proconvulsant and anticonvulsant role for COX-2 has been reported in kainic acid-induced seizure (Kim *et al*., 2008). Also, overexpression of COX-2 has been associated with neurotoxiticy in acute conditions such as hypoxia/ischemia and seizures as well as in chronic neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease (Minghetti and Pocchiari, 2007). Studies have been shown that COX-2 which is expressed in postsynaptic dendritic spines, regulates PGE2 signaling in activity-dependent long-term synaptic plasticity at hippocampal perforant path-dentate granule cell synapses suggests an important role for COX-2-generated PGE2 in synaptic signaling (Zhang *et al*., 2009). Increased levels of PGD2 and PGE2 following PTZ-induced seizures were observed (Takemiya *et al*., 2003).

PGE1 and 2 have excitatory effects on the cerebral cortex, the area that plays an important role in the onset of seizure activity. PGF2α is the predominant prostaglandin identified in the experimentally induced as well as spontaneous seizure activity (Dhir *et al*., 2008). Animal models of CNS injury have described a pivotal role for COX-2 in promoting neuropathology (Kyrkanides *et al*., 2002). Furthermore, the induction of astrocytic COX-2 was observed in epilepsy patients with hippocampal sclerosis and the concentrations of PGs increased in the cerebrospinal fluid of these patients (Desjardins *et al*., 2003).

In one model of lithium chloride and tacrine induced status epilepticus seizures there was an increased expression of COX-2 enzyme protein particularly indorsal hippocampus, further resulting in elevated brain PGE2 levels (Dhir and Kulkarni, 2006). In another study, COX-2 induction was found to be responsible for epileptic neuronal injury and that selective COX-2 inhibitors are neuroprotective (Kawaguchi *et al*., 2005). Studies of seizure activity in animal models indicate that PGD2 and PGE1 have seizure-inhibiting properties incontrast, intracerebroventricular administration of PGF2α promotes chemical and electronic induced seizures while intra-amygdaloid administration of PGs showed no significant effects on electrically kindled seizures. Effect of COX inhibitors which are suggested to prevent the production of PGs during seizure activity were inconsistent according to seizure models types of COX inhibitors or time of administration.

Thus, the role of COX isozymes or PGs in seizure activity is not clear (Kim *et al*., 2008). During kainic acid-induced seizure, the PGF2α, PGE2 and PGD2 was increased in the brain after a few minutes. Especially, PGF2α was the highest concentration in hippocampus following systemic kainic acid administration and the highest level of PGF2α reaches 30 min (Yoshikawa *et al*., 2006). In convulsion-prone gerbil, the increase of PGD2 in the brain is significant and exogenous PGD2 inhibits pentylenetetrazole-induced convulsions. PGE2 activated on the electroencephalogram while prostaglandin E1 inhibit pentylenetetrazole-induced convulsions in rats (Kim *et al*., 2008). The drugs that inhibit COX-2 activity such as indomethacin and selective COX-2 inhibitors

could reduce hippocampal cell death and seizure frequencies in several animal models of epilepsy (Jung *et al*., 2006; Kunz and Oliw, 2001a; Shafiq *et al*., 2003). Thus, drugs that reduce the production of PGs may have useful therapeutic effects in epilepsy. Most of the reports point to the anticonvulsant effects of NSAIDs focused on about orally administration of these drugs. With this background, the aim of this study was investigated the anticonvulsant effect of intraperitoneally administration of Rofecoxib, a COX inhibitor, against PTZ-induced seizure threshold in mice.

MATERIALS AND METHODS

Animals: Experiments were performed on 22-25 g adult NMRI male mice in their 8-9 weeks (n = 8 for each group), purchased from Razi Institute (Iran). Animals were housed 8 per cage in the Animal House of Faculty of Veterinary, Tabriz Branch, Islamic Azad University in a temperature (20-22°C) and humidity (50±10%) controlled environment under a 12 h light/dark cycle (lights on at 7 a.m.). Food and water were available *ad libitum*.

This study was performed in accordance with the guide for the Care and Use of Laboratory Animals of Research affairs of Tabriz University of Medical Sciences, Tabriz, Iran. All efforts were made to minimize the number of animals which were used and their suffering degree. Animals were divided into 9 groups randomly; the 1st group received saline normal (ip) (control group); the 2nd group received Carboxymethylcellulose (CMC) 0.25% (ip) (vehicle group) and the next groups received, respectively different doses of Rofecoxib (1, 2, 4, 8, 10, 15 and 20 mg kg^{-1} ip) 45 min before PTZ-induced seizure threshold.

Chemicals: PTZ (Pentylenetetrazole) was purchased from Sigma-Aldrich. Rofecoxib was provided from Merck (Germany). All other reagents were of analytical grade. Rofecoxib was prepared by being suspended in 0.25% Carboxymethylcellulose (CMC) and the vehicle group was given an equal volume of vehicle.

PTZ-induced clonic seizure threshold: Behavioral experiments were done in a quiet, temperature-controlled (20-22°C) room between 10 a.m. and 4 p.m. PTZ-induced clonic seizure threshold was determined by inserting a 30-gauge needle into the tail vein of mice and infusion of 0.5% PTZ solution at a constant rate of 1 mL min^{-1} to unrestrained freely moving animals. Minimal dose of PTZ (mg kg^{-1}) needed to induce forelimb clonus followed by full clonus of the body was recorded as an index of clonic seizure threshold (Samini *et al*., 2005; Shafaroodi *et al*., 2004).

Data analysis: Group data are presented as mean±SEM and analyzed statistically using student test. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. The level for statistical significance was set at a $p<0.05$.

RESULTS AND DISCUSSION

PTZ-induced seizure threshold in control mice was 34.75±1.54 mg kg^{-1}. In vehicle group, CMC have not shown significant change on PTZ-induced seizure threshold compared with control group (Fig. 1). Intraperitoneal injection of Rofecoxib showed significant ($p<0.05$) increase of PTZ-induced seizure threshold in a dose dependently manner compared with control and vehicle groups (Fig. 1).

Pentylenetetrazole (PTZ) has been used widely to produce the animal model of chemically induced seizure because this model is highly sensitivity for comparing different chemical under standardized conditions (Samini *et al*., 2005; Shafaroodi *et al*., 2004). In this study, PTZ-induced seizure threshold in control mice was 34.75±1.54 mg kg^{-1} and pretreatment with Rofecoxib significantly increased the PTZ-induced seizure threshold in a dose dependently manner in mice.

Previous studies have been demonstrated the protective effect of COX-inhibitors in various models of epilepsy in animals (Dhir and Kulkarni, 2006; Dhir *et al*., 2005, 2006a-c) but effects of intraperitoneally injection of different doses of NSAIDs have not shown. The effect of COX inhibitors on neuronal death also is disputed. It has been reported that the pretreatment of COX-2 inhibitors

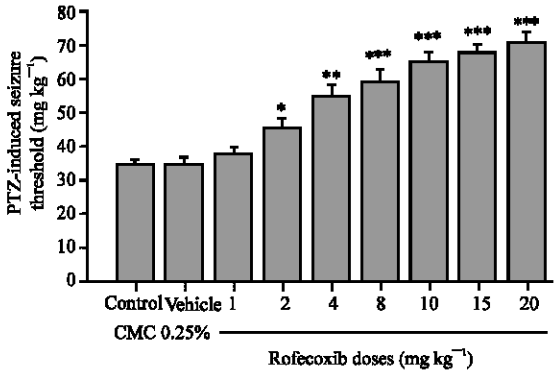

Fig. 1: PTZ-induced seizure threshold in mice (mg kg^{-1}). Effect of intraperitoneally injection of different doses of Rofecoxib on seizure threshold. Each column represents mean±SEM of 8 mice. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared with control and vehicle groups

including celecoxib aggravated kainic acid-induced seizure activity in rodents and aggravated kainic acid-induced neuronal death in the hippocampus (Baik *et al*., 1999). The broad COX inhibitor such as ibuprofen caused deficits in spatial learning in a water maze (Shaw *et al*., 2003) whereas several reports showed the post-treatment of COX-2 inhibitors restored the memory deficit and learning behaviors and prevented seizure-induced neuronal death (Gobbo and O'Mara, 2004; Kunz and Oliw, 2001a, b). The effects of COX-2 inhibitors on the PTZ-induced seizures are also controversial; other researchers reported the anticonvulsant effect of COX-2 inhibitors (Dhir and Kulkarni, 2006; Dhir *et al*., 2006b) while Akarsu *et al*. (2006) showed that COX-2 inhibitors have neither anticonvulsant nor proconvulsant effects on PTZ-induced seizures.

Treatment with indomethacin, a COX-inhibitor offered full recovery in the E1 mouse, a genetically prone mouse (Okada *et al*., 2001). In one other report, non-selective COX-inhibitor such as indomethacin, aggravated kainic acid-induced seizure activity and the following hippocampal neuronal death (Baik *et al*., 1999). Dhir *et al*. (2006a) also reported that COX inhibitors, viz. nimesulide and rofecoxib, administered 45 min prior to an epileptic challenge prolonged mean onset time of convulsions, decreased duration of clonus and decreased mortality rate against bicuculline- and picrotoxin-induced convulsions in mice (Dhir *et al*., 2006a, c).

Different classes of NSAIDs like indomethacin, flurbiprofen and diclofenac were shown to decrease the LD50 and threshold for the PTZ-induced convulsion in mice. Based on these observations and the evidence that peripheral or intracerebral administration of PGs antagonized chemically and electrically induced convulsions, it was suggested that endogenous PGs may exert anticonvulsant effect. In contrast, findings from other studies indicated that PGs may have proconvulsant effect as some NSAIDs, like paracetamol and diclofenac were found to increase the latency to onset of PTZ-induced seizures in mice as a result of blockade of PGs synthesis. Confirming this line of thinking some studies have reported a potentiating effect of certain NSAIDs on concomitantly administered antiepileptic drugs in MES and PTZ seizure tests (Reeta *et al*., 2000).

COX-2 inhibitors such NS-398, indomethacin, diclofenac and celecoxib aggravated significantly seizure activity in animal model, especially Seizure Model (Baik *et al*., 1999). There were some evidences in which Shafiq *et al*. (2003) showed that there was an increase in percentage protection when celecoxib was combined with standard antiepileptic drug such as phenytoin against electroshock-induced convulsions (Samini *et al*., 2005). Tu and Bazan (2003) also demonstrated that the COX-2-selective inhibitor nimesulide attenuated kindling development. In other studies, effects of COX2 inhibitor on seizure activity are different according to seizure models types of NSAIDs and methods of administration (Dhir *et al*., 2005; Yuhas *et al*., 2005). In electroshock convulsion, celecoxib showed anticonvulsant action (Shafiq *et al*., 2003) and also posttreatment of celecoxib improved learning and memory deficit (Gobbo and O'Mara, 2004). Nimesulide and rofecoxib were reported to aggravate kainic acid-induced seizures and reduce the cell loss. However, those researchers reported rofecoxib did not reduce memory deficit and delayed neuronal death (Kunz *et al*., 2005). In associated with febrile seizure, the potent COX inhibitor has more disadvantage of seizure activity than weak COX inhibitor (Rantala *et al*., 2001). However, the preventive use of ibuprofen as a safe NSAID used largely worldwide for recurrent febrile seizure was not recommended. Ibuprofen also induced the learning and memory deficit. These cumulated data shows that NSAID including COX-2 inhibitors might be harmful, especially in seizure conditions (Kim *et al*., 2008). Protective effect of COX-1 inhibitors have been shown in other neuro-inflammation condition in human and animal models. Alzheimer's Disease (AD), the most common cause of dementia associated with neuro-degeneration in the elderly is clinically characterized by a progressive memory loss and other cognitive impairments.

The prevalence of AD increases exponentially with age. In aged rats, COX-1 mRNA expression is selectively increased in the hippocampus, possibly causing an increased susceptibility to neuroinflammation and COX-1-expressing microglia are found surrounding amyloid plaques in the AD brain indicating a role of this isoform in the pathophysiology of the disease. Supporting this concept, a 6 months, double-blind, placebocontrolled study with indomethacin, a preferential COX-1 inhibitor, seemed to protect AD patients from cognitive decline and reduced levels of β-Amyloid (Aβ) in the hippocampus and cortex in a Transgenic Mouse Model of AD. The use of aspirin, a non-selective Aβ42-lowering agent that preferentially inhibits COX-1 has been associated with a reduced risk of AD in humans (Perrone *et al*., 2010). In the 1-Methyl-4-Phenyl-1, 2, 3, 6-Tetrahydropyridine (MPTP) model of Parkinson's Disease (PD) in which dopaminergic neurons in the substantia nigra are selectively injured, >65% of ventral midbrain PGE2 originated from COX-1 without any change in COX-1 expression; this indicates a predominant role of this isoform in PGE2 synthesis during MPTP-induced neurodegeneration (Perrone *et al*., 2010). In Traumatic Brain Injury (TBI), COX-1 involvement has not been extensively studied. There are evidences of

accumulation of COX-1-expressing microglia and/or macrophages at peri-lesional areas and in the developing core in patients with TBI indicating that further investigation of the potential beneficial effects of COX-1 inhibition is necessary (Perrone *et al*., 2010). Neuronal degeneration in patients with prion diseases is well documented, little is known about downstream signaling cascades to enable targeted therapeutic strategies. Coexpression of CD68 (phagocytic macrophages and microglial cells) in COX-1-expressing cells is found in areas of severe tissue damage and also around vacuoles in post-mortem brains from patients with Creutzfeldt-Jacob Disease (CJD). Therefore, activated COX-1-expressing microglia in CJD patients could account for increased production of PGE2 and cytokines and might contribute to the complex process of neurodegeneration (Perrone *et al*., 2010).

Elevated levels of PGE2, PGF2α and TXB2 have been found in the cerebrospinal fluid of patient with HIV associated dementia and have been linked with the severity of cognitive impairment. Because COX-1 mRNA is upregulated approximately 2-fold in HIV-demented compared with the non-demented patients whereas COX-2 mRNA expression is unchanged, the increase in the PG levels is likely to be selectively mediated by COX-1. Thus, the roles of microglia and COX-1 in the host defense system against viral pathogens deserve further investigation (Perrone *et al*., 2010).

In humans and in animals subjected to transient global cerebral ischemia, specific neurons degenerate following the ischemic episode. In determining brain injury there is the primary involvement of COX-2-expressing neurons and the secondary involvement of COX-1-expressing microglia (Perrone *et al*., 2010). Although, mice lacking COX-1 are more vulnerable to focal cerebral ischemia, probably owing to a more severe cerebral blood-flow reduction in vulnerable regions, pharmacological inhibition of COX-1 potently reduced neuronal injury and oxidative stress in the hippo-campus during transient global ischemia. Interestingly in human focal ischemic brains, COX-1-positive microglia accumulation was observed in peri-infarctional regions and in the developing necrotic core early after infarction. Therefore, the potential therapeutic effects of COX-1 inhibition should be further investigated, particularly in the secondary post-ischemic neuroinflammatory phase (Perrone *et al*., 2010). COX-1 inhibition also seems to attenuate neuro-inflammation-induced Blood-Brain Barrier (BBB) disruption. Selective pharmacological inhibition of COX-1 but not of COX-2, reduced TNF-α-induced BBB disruption and free radical production and indomethacin completely blocked LPS induced permeability changes in cultured monolayers of brain microvessel endothelial cells (Perrone *et al*., 2010). In stroke, neurons which express COX-2 are directly injured whereas COX-1-expressing microglia only contribute to the secondary neuro-inflammatory response. In a study on electrical amygdala kindling mouse, an animal model of human temporal lobe epilepsy, it was demonstrated the involvement of activate microglial cells in hippocampus during the epileptogenesis, the kindling development and concurrent COX-1 enhancement in this region. Stage and region specific cyclooxygenase expression were identified in the kindled brain and changes in COX-1 immunoreactive cells were found. Treatment with SC-560 dose-dependently delayed the advancement of seizure stage with its anti-seizure effect. Indomethacin at lower doses than SC-560 is more potent but at higher doses all animals died. Furthermore, *in vitro* experiment demonstrated that SC-560 reduced also PGE2 and 8-isoPGF2α productions by LPS-activated microglia leading to suppress the kindling development. This result implies that COX-1 and -2 are involved in manifestation of epilepsy and occurs a cooperation between the two isoforms during the kindling process (Perrone *et al*., 2010). The molecular mechanisms of the anticonvulsant and neuroprotective effects of NSAIDs (COX-inhibitors) have not been fully clarified but evidences tend to suggest the possible involvement of γ-Amino Butyric Acid (GABA) because rofecoxib and nimesulide (both COX-2 inhibitors) potentiated the anticonvulsant effects of subprotective dose of diazepam and muscimol both GABAergic modulators (Dhir *et al*., 2008).

Similarly the effect of tiagabine, a GABA reuptake inhibitor and a newer antiepileptic was also potentiated by rofecoxib (Dhir and Kulkarni, 2006). Such similar studies have been reported by Tandon *et al*. (2003) against electro and chemoconvulsions, respectively. In other study, the combined effect of rofecoxib and topiramate had a synergistic action in protecting against PTZ-induced convulsions has been reported. Topiramate is a antiepileptic agent and is reported to enhance GABA-evoked whole cell Cl^- currents in mouse cerebral cortical neurons in culture. Besides enhancement of GABA-mediated Cl^- fluxes into neurons, topiramate is also considered to produce its antiepileptic effect through several other distinct mechanisms including modification of Na^+ and/or Ca^{2+} dependent action potentials and inhibition of kainate-mediated conductance at glutamate receptors of the AMPA/kainate type. Therefore, rofecoxib when administered concurrently with topiramate displayed synergistic effect indicating that both the drugs acted through the same mechanism (Dhir *et al*., 2008). In study of Zhang *et al*. (2009), NS398, a selective COX-2

inhibitor increased the frequency of mIPSCs and the decay-time constant. Bicuculline completely blocked mIPSCs indicating that these synaptic events were mediated by GABAA receptors. These results indicate that NS398 clearly enhances GABAergic transmission in the hippocampus. Also, the Western blotting analysis results showed that celecoxib upregulated the expression of GABAA receptors protein. COX inhibitors might be acting through GABAergic neurons, thus increasing the inhibitory neurotransmitter and the expression of GABAA receptor protein (Zhang *et al*., 2009).

Furthermore, arachidonic acid which is metabolized to prostaglandins by cyclooxygenases and has been proposed to be a diffusible second messenger in the CNS with a pathophysiological role in epilepsy. This is possibly due to the ability of arachidonic acid to enhance extra-neuronal glutamate concentration. Cyclooxygenase induction lead to the increase in prostaglandins levels particularly PGE2 which may facilitates glutamate release from the nerve terminal and astrocytes. Glutamate is an excitatory neuro-transmitter that can leads to decrease in GABA input, results in convulsions. Therefore, it may be conceived that COX inhibitors is acting through glutamate and GABAergic modulation (Dhir and Kulkarni, 2006). Other mechanisms by which PGE2 could indirectly contribute to synaptic plasticity include modulation of adrenergic, noradrenegic and glutamatergic neuro-transmission and regulation of membrane excitability (Minghetti and Pocchiari, 2007).

The possibility is that activation of cyclooxygenases causes increases in free-radical production, leading to oxidative stress and apoptosis of GABAergic neurons, thus increasing glutamate and causing epileptic discharges. Also, COX-2 may play a role in excitatory synapses as excitatory amino acid agonists such as NMDA induce strong COX-2 immunostaining in many regions of the limbic cortex and isocortex, hippocampus and amygdale (Zhang *et al*., 2009). Furthermore, NMDA-receptor-induced c-fos expression is prostaglandin-dependent and the calcium-dependent activation of cyclooxygenases results in superoxide production (Bidmon *et al*., 2009).

For PTZ Models, it is known that COX inhibitors attenuate seizure activity. Although not as strong as in rat models for cerebral ischaemia, COX-2 expression is induced in human patients with epilepsy and in the model of repeated PTZ induced seizures. COX-2-expressing neurons are shown to be closely associated with the processes of neurons expressing nNOS, thus revealing a direct topographical link between COX-2-related superoxide and NOS-I-related NO production. As the normal constitutive neuronal expression of COX-2 and the seizure-induced induction of COX-2 take place in a region-specific manner, COX-2-related production of oxygen radicals may represent the basis for region-specific pathological changes attributed to seizure-induced oxidative stress. Some of the most affected cortical regions are the piriform and entorhinal cortices which exhibit not only high COX-2 expression but also one of the highest packing densities of NOSI-expressing cortical neurons.

This association of neurons responding with the production of radicals such as superoxide and nitric oxide which results in the formation of peroxynitrite, may be one reason for the fact that seizure-induced protein nitration and glutamine synthetase inhibition are much more easily detectable in piriform and entorhinal cortices than in other regions (Bidmon *et al*., 2009). The results of the Zandieh *et al*. (2010) study indicated that the selective COX-2 inhibitor, celecoxib attenuates seizure induced by PTZ. Co-administration of sub-effective doses of L-NAME and celecoxib protected the animals against PTZ. In addition, L-NAME improved the anticonvulsant activity of celecoxib significantly. L-arginine at the dose which was not able to influence PTZ-induced convulsion, blunted the anticonvulsant effect of celecoxib. In the CNS, NO behaves as a multifunctional messenger and neuro-transmitter, influences various physiological and pathological functions.

The main intracellular action of NO is activation of the soluble guanylate cyclase which leads to the formation of cycline Guanine Monophosphate (cGMP) in the CNS. An increase in cGMP follows stimulation of L-glutamate receptors mainly of the NMDA type. It has been shown that NO as a retrograde messenger that is synthesized postsynaptically and acts on presynaptic terminals, plays an important role in hippocampal LTP. Further, there is direct evidence from hippocampal cultures that NO can potentiate synaptic transmission. In addition, it has been reported that PTZ kindling in mice is associated with an increase in the amount of neuronal NOS. In concordance with aforementioned findings, NO is considered to be involved in the pathophysiology of epilepsy, although the results of experiments carried out by several researchers are often conflicting. Different findings may arise as a consequence of discrepancies in the kinds of drugs, the model of seizures and the species of animals used in experiments. In study of Zandieh *et al*. (2010) showed that L-NAME, a drug that inhibits all sub-types of NOS non-specifically, attenuates PTZ-induced convulsion dose-dependently which is consistent with previous studies. There are evidences implying the possible interaction between NO and COX pathways in some pathophysiological states including

osteoarthritis, angiogenesis, renal perfusion and endotoxin-induced cardiomyopathy (Zandieh *et al*., 2010). Salvemini (1997) reported that COX activity is regulated by NO. They found that NO directly increases COX-1 activity which leads to increase in prostaglandin E2 production. Similarly, it has been reported that inhibition of NOS inhibits not only NO but also prostaglandin production, suggesting that COX enzymes are targets for pathophysiological roles of NO (Salvemini, 1997). It has been reported that apart from activation of COX-2 by free NO molecules, inducible NOS binds to COX-2 and activates it. There are evidences regarding that the interaction between NO and prostaglandin production pathways occurs at multilevels. For example, COX-2 expression is also modulated and up-regulated by NO. In addition, it has been shown that inducible NOS inhibitors ameliorate the antihyperalgesic effect of COX-2 inhibitors. Most of these findings indicate the interaction between NOS and COX-2 or NO and prostaglandin biosynthetic pathways in inflammatory responses and seizure model (Zandieh *et al*., 2010). The latter may also explain why certain other changes affecting neuro-transmitter metabolism seen in other rodent models of epilepsy seem to occur in a most pronounced manner in these cortices. However, the question of why a neuronal induction of the Heat Shock Protein 70 (HSP-70) family does not occur in PTZ Models whereas it is a normal finding in other rodent models of epilepsy still has to be explained, especially as HSP-70-overexpressing mice seem to be partly protected against PTZ-induced seizure activity (Bidmon *et al*., 2009). As oxidative and nitrosative stress are not restricted to neurons that are functionally connected with glial cells and blood vessels, it may not be surprising that concomitant glial and endothelial changes do occur. The latter are seen by strong region-specific focal glial HSP-27 expression. HSP-27 is a well-established marker for oxidative stress. As glial cells are well equipped to cope with pathological alterations and ongoing processes such as during scar formation, it is understandable that glial cells respond in a more delayed manner. When focusing on currently used experimental models of epilepsy, glial responses have to be viewed as a secondary consequence of neuronal seizure activity rather than an initial cause of seizures as is sometimes suggested. Nevertheless, these secondary glial responses may subsequently contribute to a continuation or progression of epileptic seizures, for example during successful kindling (Bidmon *et al*., 2009). Rimoli *et al.* (2009) have been shown that two imidazo (1, 2-b] pyridazine derivatives, namely DM1 and DM2 are completely devoid of COX-1 and -2 inhibitory activity but are effective in suppressing Spike and Wave Discharges (SWDs) in WAG/Rij rats, a genetic rodent model of absence epilepsy, similar to what was described for their structural congener Indomethacin (IDM) which significantly decreased SWDs in these rats. As T-type channel blockade has been considered as an electrophysiological feature common to antiabsence drugs they also investigated whether DM1 and 2 COX-independent antiseizure effect could depend on T-type channel blockade and they found that these compounds are indeed powerful T-type channel blockers and that this property is displayed by IDM as well.

Rimoli *et al.* (2009)'s study showed that IDM suppresses SWDs *in vivo* in a rat model of absence epilepsy and blocks CaV3.1 channels *in vitro* (Rimoli *et al*., 2009). In addition, they report evidence suggesting that both these effects are at least in part independent from COX inhibition as they can be also observed with two indomethacin-like imidazopyridazines DM1 and 2 which are ineffective in blocking COXs. This suggests that COX inhibition is neither the only nor the major mechanism responsible for the antiseizure activity of NSAIDs. A large body of experimental data accumulated in the past show that COX inhibitors possess antiseizure activity in different forms of epilepsy including absence epilepsy. However, doubts still exist on the intimate mechanism responsible for these effects (Rimoli *et al*., 2009).

As the blockade of voltage gated T-type Ca^{2+} channels has been traditionally considered as a major mechanism of action of antiabsence drugs on the basis of classical electrophysiological evidence and of more research on CaV3.1 knock out mice in the study of Rimoli *et al.* (2009) investigated whether IDM was effective in blocking these ion channels. In particular, they investigated IDM effect on CaV3.1 channels which represent the main T-type isoform responsible for the firing of thalamic relay neurons and whose dysfunction is considered crucial in the genesis of absence epilepsy.

Their finding that IDM potently blocks CaV3.1 channels suggests that the antiabsence effect of this NSAID could be at least in part related to T-type channel blockade. To the best of their knowledge no previous study has specifically addressed the effect of PGs on T-type channel currents and thus the hypothesis that IDM and other NSAIDs could affect the activity of these channels by decreasing PG synthesis remains speculative and could represent an interesting subject of future investigation (Rimoli *et al*., 2009). A second possible mechanism that could account for IDM-induced decrease in CaV3.1 channel activity could be the accumulation of arachidonic acid that NSAIDs induce by blocking the

conversion of this fatty acid in PGs. Arachidonic acid has been shown indeed to potently inhibit CaV3.1 and 3.2 channel activity *in vitro*.

Interestingly, a significant increase in the expression and activity of PLA2 and in free fatty acid generation have been observed in epilepsy. On the basis of these considerations, Rimoli *et al.* (2009) cannot exclude that COX-dependent mechanisms such as a decrease in PG synthesis and an increase in plasma membrane arachidonic acid concentration could have a role in determining IDM-induced T-type channel blockade. However, the study of Rimoli *et al.* (2009) provides strong evidence suggesting that this effect is at least in part independent from COX inhibition. The main argument to support this conclusion is their finding that the ability to block CaV3.1 channels is preserved in two imidazopyridazines DM1 and 2, structurally related to IDM but unable to block COXs importantly, these compounds showed a relevant antiabsence activity in vivo as well (Rimoli *et al*., 2009). Also, as the structures of DM1 and 2 have significant similarities with the GABAA binding drug zolpidem which is known to suppress SWDs in WAG/Rij an interesting possibility is that these drugs could act on GABAA receptors. However, preliminary binding studies performed in their laboratories did not show any affinity of either DM1 or 2 for GABAergic receptors *in vitro* (Rimoli *et al*., 2009).

Also in the other study, an attempt has been made to investigate the effects of three COX-2 preferring inhibitors; namely, nimesulide, celecoxib and rofecoxib in two experimental models of convulsion, Maximal Electroshock (MES)-induced and PTZ-induced seizures in mice. Since, Benzodiazepine (BZD) GABA-ergic and Ca^{2+} channel neuronal activities have been intimately involved in the process of epileptogenesis an interaction of nimesulide, the most commonly used COX-2 preferring inhibitor with diazepam and nimodipine has also been studied in order to elucidate the nature of interaction of preferential COX-2 inhibitors with established antiepileptic drugs.

The results of this studies shown that preferential COX-2 inhibitors have opposite effects on MES and PTZ-induced convulsions in mice. In MES test, all the three agents protected against THLE where as in PTZ convulsions these drugs reduced the latency to preclonic jerks and increased the duration of clonus. There is evidence of an increase in levels of different PGs in brain during/after induction of seizure by MES and PTZ. Out of various PGs while PGF2α and TxA2 are implicated in promoting, the PGEs, PGI2 and PGD2 have been suggested to suppress seizure activity. Further PGF2α has been reported to antagonize the seizure suppressing activity of PGE1 which like PGE2 also inhibits PTZ-induced seizures. It thus appears that inhibitory effect on THLE in MES seizure of three studied preferential COX-2 inhibitors may be due to a decrease in synthesis of proconvulsive PGs, i.e., PGF2α and TxA2 as a result of blockade of COX-2 isoform which is not only constitutively expressed in the brain but also gets upregulated during MES and other seizurogenic stimuli (Reeta *et al*., 2000). In contrast to anticonvulsive effect produced by the three preferential COX-2 inhibitors in the MES test all of these compounds displayed a proconvulsive activity in PTZ seizure model.

Although, all drugs showed a decrease in the latency to preclonic jerk and increased the duration of clonic convulsions, this effect was more marked on the latter parameter since, it achieved the minimal level of significance with all the studied agents. The explaination for this differential effect in two models of seizure lies in the nature of convulsive sequences in the two test paradigms. In MES, THLE occurs first followed by clonic episodes whereas in the PTZ test, latter events precede the phase of THLE. In the PTZ test, besides a decrease in GABA activity due to PTZ there is evidence of an increased excitatory amino acid activity which is relative to GABA in the beginning but is further exaggerated as a result of nitric oxide-induced upregulated excitatory amino acid release.

The latter is mainly responsible for prolonged/ continuous phases of clonic or even clonic-tonic seizure activity. Due to blockade of upregulated COX-2 activity all the three COX-2 preferring drugs would inhibit the enhanced activity of PGs which may be proconvulsant (PGF2α/TxA2) or anticonvulsant (PGE2) but since these compounds had no effect on excitatory amino acid activity were observed to enhance the PTZ convulsive effect. An antagonism of anti-PTZ effect of nimodipine and diazepam also points to such a possibility of unabated excitatory amino acid activity as a result of blockade of anticonvulsive PG synthesis by the COX-2 preferring inhibitor during the PTZ seizure. In this context, it is pertinent to note that an accumulated arachidonic acid due to its inhibited metabolism to PGs have in earlier studies been shown to block GABAgated chloride channel. Besides an absence of PGs may enhance the excitatory amino acid activity as the latter has been reported to be inhibited by PGE2. Thus in this study, both of these effects may be contributing to the observed exaggerated PTZ response in animals pretreated with COX-2 preferring inhibitory agents (Reeta *et al*., 2000). Ion channel blockade is probably one of the best examples of such a COX-independent action of NSAIDs. Several published studies already demonstrated that other members of the NSAID superfamily do affect the activity of ion channels by a direct interaction with channel

subunits as it has been shown for instance for acid sensitive channels (Voilley *et al*., 2001) are directly blocked by aspirin, diclofenac and flurbiprofen; high voltage activated Ca^{2+} channels (Zhang *et al*., 2007), voltage gated Na^{+} channels (Park *et al*., 2007) activity is increased by celecoxib chloride channels (Liantonio *et al*., 2007), KCNQ channels (Peretz *et al*., 2005) and hERG channels (Malykhina *et al*., 2002) are activated by fenamates.

Therefore, COX inhibitors may have useful therapeutic effects in seizure. Nevertheless, new and completely studies must be carried out in order to determine in more detail the beneficial actions of NSAIDs regarding the reduction of epilepsy.

CONCLUSION

According to the results, Rofecoxib has anticonvulsant effects on mice. Probably any tendency to inhibit the COX-2 enzyme by NSAIDs to be more, neuro-protective and anticonvulsant effect of drugs will be higher. Nevertheless, new studies must be carried out in order to determine the beneficial effects of NSAIDs in treatment of epilepsy.

REFERENCES

Akarsu, E.S., S. Ozdayi, E. Algan and F. Ulupinar, 2006. The neuronal excitability time-dependently changes after lipopolysaccharide administration in mice: Possible role of cyclooxygenase-2 induction. Epilepsy Res., 71: 181-187.

Baik, E.J., E.K. Kim, S.H. Lee and C.H. Moon, 1999. Cyclooxygenase-2 selective inhibitors aggravate kainic acid induced seizures and neuronal cell death in the hippocampus. Brain Res., 843: 118-129.

Bidmon, H.J., E.J. Speckmann and K.J. Zilles, 2009. Seizure semiology, neurotransmitter receptors and cellular-stress responses in pentylenetetrazole models of epilepsy. European Neuro. Rev., 4: 76-80.

Chen, C. and N.G. Bazan, 2005. Endogenous PGE_2 regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. J. Neurophysiol., 93: 929-941.

Choi, S.H., S. Aid and F. Bosetti, 2009. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: Implications for translational research. Trends Pharmacol. Sci., 30: 174-181.

Desjardins, P., A. Sauvageau, A. Bouthillier, D. Navarro, A.S. Hazell, C. Rose and R.F. Butterworth, 2003. Induction of astrocytic cyclooxygenase-2 in epileptic patients with hippocampal sclerosis. Neurochem. Int., 42: 299-303.

Dhir, A. and S.K. Kulkarni, 2006. Rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor potentiates the anticonvulsant activity of tiagabine against pentylenetetrazol-induced convulsions in mice. Inflammopharmacology, 14: 222-225.

Dhir, A., K.K. Akula and S.K. Kulkarni, 2008. Rofecoxib potentiates the anticonvulsant effect of topiramate. Inflammopharmacology, 16: 83-86.

Dhir, A., P.S. Naidu and S.K. Kulkarni, 2005. Protective effect of naproxen, a non-selective COX-inhibitor on PTZ-induced kindling in mice. Clin. Exp. Pharmacol. Physiol., 32: 574-584.

Dhir, A., P.S. Naidu and S.K. Kulkarni, 2006a. Effect of rofecoxib, a cyclooxygenase-2 (COX-2) inhibitor, on various biochemical parameters of brain associated with pentylenetetrazol-induced chemical kindling in mice. Fundam. Clin. Pharmacol., 20: 255-261.

Dhir, A., P.S. Naidu and S.K. Kulkarni, 2006b. Effect of cyclooxygenase inhibitors on pentylenetetrazol (PTZ)- induced convulsions: Possible mechanism of action. Prog. Neuropsychopharmacol. Biol. Psychiatry, 30: 1478-1485.

Dhir, A., P.S. Naidu and S.K. Kulkarni, 2006c. Effect of cyclooxygenase inhibitors on various animal models (picrotoxin, bicuculine and maximal electroshock-induced convulsions, possible mechanism of action. Indian J. Exp. Biol., 44: 286-291.

Gobbo, O.L. and S.M. O'Mara, 2004. Post-treatment, but not pre-treatment, with the selective cyclooxygenase-2 inhibitor celecoxib markedly enhances functional recovery from kainic acid-induced neuro-degeneration. Neuroscience, 125: 317-327.

Jung, K.H., K. Chu, S.T. Lee, J. Kim and D.I. Sinn *et al*., 2006. Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. Neurobiol. Dis., 23: 237-246.

Kawaguchi, K., R.W. Hickey, M.E. Rose, L. Zhu, J. Chen and S.H. Graham, 2005. Cyclooxygenase-2 expression is induced in rat brain after kainate-induced seizures and promotes neuronal death in CA3 hippocampus. Brain Res., 1050: 130-137.

Kim, H.J., J.I. Chung, S.H. Lee, Y.S. Jung, C.H. Moon and E.J. Baik, 2008. Involvement of endogenous prostaglandin F2α on kainic acid-induced seizure activity through FP receptor: The mechanism of proconvulsant effects of COX-2 inhibitors. Brain Res., 1193: 153-161.

Kunz, T. and E.H. Oliw, 2001a. The selective cyclooxygenase-2 inhibitor rofecoxib reduces kainate-induced cell death in the rat hippocampus. Eur. J. Neurosci., 13: 569-575.

Kunz, T. and E.H. Oliw, 2001b. Nimesulide aggravates kainic acid-induced seizures in the rat. Pharmacol. Toxicol., 88: 271-276.

Kunz, T., N. Marklund, L. Hillered and E.H. Oliw, 2005. Assessment of the effects of the cyclooxygenase-2 inhibitor rofecoxib on visuospatial learning and hippocampal cell death following kainate-induced seizures in the rat. Cogn. Brain Res., 25: 826-832.

Kyrkanides, S., A.H. Moore, J.A. Olschowka, J.C. Daeschner, J.P. Williams, J.T. Hansen and M.K. O'Banion, 2002. Cyclooxygenase-2 modulates brain inflammation-related gene expression in central nervous system radiation injury. Mol. Brain Res., 104: 159-169.

Liantonio, A., V. Giannuzzi, A. Picollo, E. Babini, M. Pusch and D.C. Camerino, 2007. Niflumic acid inhibits chloride conductance of rat skeletal muscle by direct inhibiting the CLC-1 channel and by increasing intracellular calcium. Br. J. Pharmacol., 150: 235-247.

Malykhina, A.P., F. Shoeb and H.I. Akbarali, 2002. Fenamate-induced enhancement of heterologously expressed HERG currents in *Xenopus oocytes*. Eur. J. Pharmacol., 11: 269-277.

Minghetti, L. and M. Pocchiari, 2007. Cyclooxygenase-2, prostaglandin E2 and microglial activation in prion diseases. Int. Rev. Neurobiol., 82: 265-275.

Okada, K., T. Yuhi, S. Tsuji and U. Yamashita, 2001. Cyclooxygenase-2 expression in the hippocampus of genetically epilepsy susceptible El mice was increased after seizure. Brain Res., 894: 332-335.

Park, S.Y., T.H. Kim, H.I. Kim, Y.K. Shin, C.S. Lee, M. Park and J.H. Song, 2007. Celecoxib inhibits Nap currents in rat dorsal root ganglion neurons. Brain Res., 1148: 53-61.

Peretz, A., N. Degani, R. Nachman, Y. Uziyel, G. Gibor, D. Shabat and B. Attali, 2005. Meclofenamic acid and diclofenac, novel templates of KCNQ2/Q3 potassium channel openers, depress cortical neuron activity and exhibit anticonvulsant properties. Mol. Pharmacol., 67: 1053-1066.

Perrone, M.G., A. Scilimati, L. Simone and P. Vitale, 2010. Selective COX-1 inhibition: A therapeutic target to be reconsidered. Curr. Med. Chem., 17: 3769-3805.

Rantala, H., R. Tarkka and M. Uhari, 2001. Systematic review of the role of prostaglandins and their synthase inhibitors with respect to febrile seizures. Epilepsy Res., 46: 251-257.

Reeta, K., P.K. Mediratta, P. Mahajan and K.K. Sharma, 2000. Differential effect of cyclooxygenase-2 prefering inhibitors on electrically- and chemically-induced seizures in mice. Indian J. Physiol. Pharmacol., 44: 471-475.

Rimoli, M.G., E. Russo, M. Cataldi, R. Citraro and P. Ambrosino *et al*., 2009. T-type channel blocking properties and antiabsence activity of two imidazo [1,2-b]pyridazine derivatives structurally related to indomethacin. Neuropharmacology, 56: 1-10.

Salvemini, D., 1997. Regulation of cyclooxygenase enzymes by nitric oxide. Cell Mol. Life Sci., 53: 576-582.

Samini, M., H. Shafaroodi, V. Sehat and S. Allahdini, 2005. Comparison of the effects of lindane and permethrin on pentylenetetrazole-induced clonic seizure in mice. Online J. Vet. Res., 9: 37-47.

Shafaroodi, H., M. Samini, L. Moezi, H. Homayoun and H. Sadeghipour *et al*., 2004. The interaction of cannabinoids and opioids on pentylenetetrazole-induced seizure threshold in mice. Neuro-pharmacology, 47: 390-400.

Shafiq, N., S. Malhotra and P. Pandhi, 2003. Anticonvulsant action of celecoxib (alone and in combination with sub-threshold dose of phenytoin) in electroshock induced convulsion. Methods Find Exp. Clin. Pharmacol., 25: 87-90.

Shaw, K.N., S. Commins and S.M. O'Mara, 2003. Deficits in spatial learning and synaptic plasticity induced by the rapid and competitive broad-spectrum cyclooxygenase inhibitor ibuprofen are reversed by increasing endogenous brain-derived neurotrophic factor. Eur. J. Neurosci., 17: 2438-2446.

Takemiya, T., K. Suzuki, H. Sugiura, S. Yasuda, K. Yamagata, Y. Kawakami and E. Maru, 2003. Inducible brain COX-2 facilitates the recurrence of hippocampal seizures in mouse rapid kindling. Prostaglandins Lipid Mediators, 71: 205-216.

Tandon, M., K. Anuradha and P. Pandhi, 2003. Evaluation of antiepileptic activity of aspirin in combination with newer antiepileptic lamotrigine in mice. Method Find Exp. Clin. Pharmacol., 25: 607-610.

Tu, B. and N.G. Bazan, 2003. Hippocampal kindling epileptogenesis upregulates neuronal cyclooxygenase-2 expression in neocortex. Exp. Neurol., 179: 167-175.

Voilley, N., J. de Weille, J. Mamet and M. Lazdunski, 2001. Nonsteroid anti-inflammatory drugs inhibit both the activity and the inflammation-induced expression of acid-sensing ion channels in nociceptors. J. Neurosci., 21: 8026-8033.

Yoshikawa, K., Y. Kita, K. Kishimoto and T. Shimizu, 2006. Profiling of eicosanoid production in the rat hippocampus during kainic acid-induced seizure: Dual phase regulation and differential involvement of cox-1 and cox. J. Biol. Chem., 281: 14663-14669.

Yuhas, Y., A. Weizman, A. Vanichkin and S. Ashkenazi, 2005. Involvement of prostaglandins in an animal model of Shigella-related seizures. J. Neuroimmunol., 168: 34-39.

Zandieh, A., F. Maleki, A. Hajimirzabeigi, B. Zandieh, O. Khalilzadeh and A.R. Dehpour, 2010. Anticonvulsant effect of celecoxib on pentylenetetrazoleinduced convulsion: Modulation by NO pathway. Acta Neurobiol. Exp., 70: 390-397.

Zhang, H., R. Sun, G. Lei, L. Yang and C. Liu, 2009. Cyclooxygenase-2 inhibitor inhibits the hippocampal synaptic reorganization by inhibiting MAPK/ERK activity and modulating GABAergic transmission in pilocarpine-induced status epilepticus rats. Med. Chem. Res., 18: 71-90.

Zhang, Y., J. Tao, H. Huang, G. Ding, Y. Cheng and W. Sun, 2007. Effects of celecoxib on voltage-gated calcium channel currents in rat pheochromocytoma (PC12) cells. Pharmacol. Res., 56: 267-274.