Montelukast Inhibits Pentylenetetrazol-Induced Seizures in Rats

ABCEG 1 Betul Cevik  
ABEF 2 Volkan Solmaz  
CDF 1 Durdane Aksoy  
ABDFG 3 Oytun Erbas

1 Department of Neurology, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey  
2 Department of Neurology, Turhal State Hospital, Tokat, Turkey  
3 Department of Physiology, Faculty of Medicine, Istanbul Bilim University, Istanbul, Turkey

Background: Montelukast is an antiinflammatory drug with an antioxidant property. In this study, we aimed to reveal whether montelukast has a preventive effect against seizures and post-seizure oxidative stress in pentylenetetrazol (PTZ)-induced seizures in rats.

Material/Methods: Of the 48 male Sprague-Dawley rats used in the study, 24 were assigned to EEG recordings (group A) and 24 were assigned to behavioral studies (group B). In group A, the electrodes were implanted on dura over the left frontal cortex for EEG recording. After 10 days, in group A, i.p. saline, 25, 50, or 100 mg/kg montelukast+35 mg/kg PTZ was administered to the rats. EEG was recorded and spike percentage was evaluated. In group B, i.p. saline, 25, 50, or 100 mg/kg montelukast+70 mg/kg PTZ was administered to the rats. Racine’s Convulsion Scale (RCS) and onset times of first myoclonic jerk (FMJ) was used to evaluate the seizures. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels were determined in the brain tissue of animals.

Results: Animals treated with 50 or 100 mg/kg montelukast had significantly lower RCS and significantly increased FMJ onset time compared to the saline-treated animals. Moreover, groups given 25, 50, or 100 mg/kg montelukast had significantly lower MDA and higher SOD levels compared to the saline-treated group. The differences were more pronounced in the 100 mg/kg montelukast-pretreated group (p<0.001).

Conclusions: Montelukast showed anticonvulsant action and led to amelioration of oxidative stress markers in PTZ-induced seizures in rats.

MeSH Keywords: Malondialdehyde • Oxidative Stress • Pentylenetetrazole • Seizures • Superoxide Dismutase

Abbreviations: CysLTs – cysteinyl leukotrienes; CysLTR1A – cysteinyl leukotriene receptor 1 antagonist; LT – leukotriene; EEG – electroencephalography; FMJ – first myoclonic jerk; GABA – gama-aminobutyric acid; MDA – malondialdehyde; NBT – nitroblue tetrazolium; PTZ – pentylenetetrazole; RCS – Racine’s Convulsion Scale; ROS – reactive oxygen species; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances

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Background

Epilepsy is one of the most common neurodegenerative diseases and is characterized by recurrent spontaneous seizures arising from abnormal electrical activity in the brain. Despite the increasing number and variety of antiepileptic drugs, nearly 30% of epilepsy patients who receive the appropriate medical treatment have seizures that persist [1,2]. Increasing data from experimental and clinical reports suggest that oxidative stress is involved in the pathophysiology of epilepsy and that antioxidants or radical scavengers ameliorate the seizure activity and postseizure neuronal damage in various epileptic models [2]. These data indicate that there is a great need to develop novel antiepileptic drugs with broad spectrum of activity such as neuroprotective and antioxidant actions [2].

Antileukotriene drugs (leukotriene receptor antagonists and synthesis inhibitors) are antiinflammatory drugs with antioxidant and neuroprotective properties [3–5]. Montelukast is a potent, highly selective, and orally active leukotriene (LT) D4/E4 receptor antagonist (cysteinyl leukotriene receptor 1 antagonist (CysLTR1A)), used to treat bronchial asthma and allergic rhinitis with high safety/tolerability profiles [6]. The cysteinyl leukotrienes (CysLTs) – LTC4, LTD4, and LTE4 – are potent inflammatory lipid mediators derived from the 5-lipoxygenase pathway of arachidonic acid metabolism. The CysLTs and their receptors (CysLTR1 and CysLTR2 receptors) are closely associated with neuronal injury after various neurological diseases such as cerebral ischemia, subarachnoid hemorrhage, brain tumors, and epilepsy in animal models [7]. A number of reports have demonstrated a markedly enhanced transcription and activation of CysLTs synthesis during seizures both in experimental animals suffering from spontaneous epilepsy or chemically induced epilepsy as well as in clinical subjects [8,9].

The aim of the present study was to investigate the anticonvulsant potential of different dosages of montelukast and its action on markers of oxidative stress on the brain in PTZ-induced seizure in Sprague-Dawley rats.

Material and Methods

Animals and laboratory

Of the 48 male Sprague-Dawley rats weighing 200–250 g, 24 were assigned to EEG recordings (group A) and 24 were assigned to the behavioral studies (group B). The rats were kept on a 12 hour–12 hour light–dark cycle (light from 07:00 to 19:00) in quiet rooms, with 22–24°C ambient temperature. They were provided standard laboratory food and tap water ad libitum.

The experimental procedures used in the present study were approved by the Gaziosmanpasa University Animal Ethics Committee. All experiments were carried out according to the rules listed in the Guide for the Care and Use of Laboratory Animals, as confirmed by National Institutes of Health (U.S.).

Experimental procedures

Forty-eight rats were randomly divided into 2 groups: Animals in Group A were used for EEG recordings and those in the Group B for behavioral assessment. A single dose of 10–30 mg/kg intraperitoneal or subcutaneous injection of PTZ leads to synchronous bilateral spike-and slower waves in electroencephalography (EEG) at 7–9 Hz. When the dose increases over 40 mg/kg, clinically obvious behavioral seizures at various stages are seen within the 20 min after the injection [10,11]. In Group A, animals were deeply anesthetized by intraperitoneal (i.p.) injection of ketamine (80 mg/kg) (Alfamine®, Ege Vet, Alfasan International B.V. Holland) and xylazine (4 mg/kg) (Alfaxyne®, Ege Vet, Alfasan International B.V. Holland). Then, a small hole was opened with a drill stereotaxically. The electrodes (Polyamide-coated stainless steel wires 0.1 mm in diameter with an electrical resistance of <1Ω/10 mm) were implanted on dura over the left frontal cortex (2.0 mm lateral to the midline, 1.5 mm anterior to the bregma) and the reference electrode was implanted over the cerebellum (1.5 mm posterior to the lambda, on midline) for EEG recording [12]. Then, the electrodes were fixed by using a dental acrylic (a mixture of numerous alloys used for dental restoration). All animals were treated by single-injection of crystallized penicillin (intramuscular) to prevent postsurgical infections. Ten days after the electrodes were implanted, the 24 rats in Group A were divided randomly into 4 groups (n=6 in each): Group A1, A2, A3, and A4.

Group A1 was treated with i.p. saline, while Groups A2, A3, and A4 were treated with 25, 50, and 100 mg/kg i.p. montelukast (Singulair, Merck Sharp & Dohme BV Waarderweg 39, Haarlem – The Netherlands), respectively. The drugs were administered 30 min prior to the PTZ injection (35 mg/kg, i.p.) (Sigma-Aldrich). All these 4 groups received 35 mg/kg PTZ i.p. and EEG was recorded. EEG recordings were taken in conscious rats in a special container 5 min after PTZ administration. The EEG recording was made during a 60-min period (Figure 1) [13]. The EEG signals were amplified 10 000 times and filtered with a range of 1–60 Hz. EEG records were taken by using the Biopac MP 150 amplifier system and 2 clinical neurophysiologists (BC and DA) scored the EEG data for spike percentage.

We defined “spike percentage” as a reproducible way of quantifying epileptiform activity that quantifies the percentage of 1-s bins with at least 1 spike-wave in them, also termed as spike-wave percentage [14]. Following the euthanization, the location of the electrode was confirmed histologically.
The other 24 rats from Group B were then divided into 4 groups (n=6 in each): Group B1, B2, B3, and B4. Group B1 treated with i.p. saline, while Group B2, B3, and B4 were treated with 25, 50, and 100 mg/kg i.p. montelukast, respectively. The drugs were administered 30 min prior to the PTZ injection (70 mg/kg, i.p.). Racine's Convulsion Scale (RCS) and the onset times of ‘first myoclonic jerk’ (FMJ) was used to evaluate the seizures (only in animals treated with PTZ 70 mg/kg) as follows: 0= no convulsion; 1=twitching of vibrissae and pinnae; 2= motor arrest with more pronounced twitching; 3= motor arrest with generalized myoclonic jerks; 4=tonic-clonic seizure while the animal remains on its feed; 5=tonic-clonic seizure with loss of the righting reflex; 6=lethal seizure [15]. Rats were observed for onset times of FMJ as previously described. The onset times were recorded as seconds. Almost all animals showing tonic generalized extension died. The observation period for PTZ-induced seizures were limited to 30 min in duration [16], after which, the animals were euthanized.

**Measurement of brain lipid peroxidation (malondialdehyde)**

Lipid peroxidation was determined in brain samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS) [17]. Briefly, trichloroacetic acid and TBARS reagent were added to the brain samples, then mixed and incubated at 100°C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve using tetraethoxypropene and expressed as nmol/g protein.

**Determination of brain superoxide dismutase activity**

Total superoxide dismutase (SOD) activity was determined according to the method of [18]. The principle of the method is the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was expressed as U/mg protein.

**Statistical analysis**

Data were analyzed by using SPSS version 15.0 for Windows. The spike percentage, RCS, and the FMJ onset time were evaluated by one-way analysis of variance (ANOVA). Post hoc Bonferroni test was used to identify the differences between the experimental groups. MDA and SOD levels were analyzed by using the Mann-Whitney U-test. Results are expressed as mean ± standard error of mean (SEM). The value of p<0.05 was accepted as statistically significant.
Results

Comparison of the groups in terms of the spike percentage

The spike percentage was significantly different between the saline- and montelukast-pretreated groups (F=12.65, p<0.001). In the Post hoc Bonferroni test, spike percentage was significantly lower in groups A3 and A4 (50 and 100 mg/kg montelukast-pretreated groups, respectively) compared to Group A1 (the saline-pretreated group), which became more pronounced as the montelukast dose increases (p<0.05 and p<0.001, respectively) (Figure 1) (Table 1).

Assessment of the groups by using Racine’s Convulsion Scale

ANOVA test revealed significant differences in RCS between the groups (F=19.34, p<0.000). In the Post hoc Bonferroni test, RCS was significantly lower in groups B3 and B4 (50 and 100 mg/kg montelukast-pretreated groups, respectively) compared to the Group B1 (saline-pretreated group), which became more pronounced as the montelukast dose increases (p<0.05 and p<0.001, respectively) (Figure 2) (Table 2).

Comparison of the brain MDA level and SOD activity between the groups

There were significant differences in brain MDA level and SOD activity between the groups (F=13.73, p<0.01, and F=12.59, p<0.05, respectively). The brain MDA level was significantly lower in the groups pretreated with 25, 50, and 100 mg/kg montelukast compared to the saline-pretreated group (p<0.05, p<0.01, and p<0.000, respectively), while brain SOD activity was significantly higher in the groups pretreated with 25, 50, and 100 mg/kg montelukast compared to the saline-pretreated group (p<0.05, p<0.05, and p<0.01, respectively), becoming more pronounced with increasing doses of montelukast for both parameters (Table 3).

Discussion

In this study, montelukast showed anticonvulsant action and ameliorated the oxidative stress markers in PTZ-induced seizures in rats.
seizures in rats. For the rats treated with 35 mg of PTZ, pretreatment with 50 mg/kg and 100 mg/kg of montelukast reduced the spike percentages compared with the saline group (Table 1). In the rats treated with 70 mg of PTZ, a significant difference in the RCS and the FMJ onset time was observed with pretreatment with 50 or 100 mg/kg montelukast (Figure 2, Table 2). To the best of our knowledge, no previous study has evaluated the anticonvulsant and antioxidative potential of acute administration montelukast in PTZ-induced seizure in rats.

There are very limited studies on the anticonvulsant effect of Montelukast. In one of these studies, Rehni and Singh showed that montelukast sodium, as well as 1, 2, 3, 4, tetrahydroisquinoline, a leukotriene D4 synthetic pathway inhibitor, markedly and dose-dependently suppressed the development of kindled (with chronic administration of PTZ) seizures and spontaneous recurrent seizures induced by pilocarpine status epilepticus in mice [19], in contrast to the anticonvulsant effects induced by single-dose montelukast shown in our study. Takahashi et al. reported that pranlukast add-on therapy reduces seizure frequencies in patients with intractable partial epilepsy and that this effect possibly results from the pleiotropic effects, including normalization of matrix metalloproteinase-9 in sera, reduced leakage of pro-inflammatory cytokines into central nervous system, and inhibition of extravasation of leucocytes from brain capillaries [20]. Because montelukast is structurally different from pranlukast and it is the most prescribed CysLT1 receptor antagonist in Europe and the USA, we used montelukast in our study, whereas pranlukast is only marketed in Japan and other Asian countries [5].

We believe that in the present study, the anticonvulsant feature of montelukast is due to the decrease of lipid peroxidation and oxidative stress.

Oxidative stress resulting from enhanced production of reactive oxygen species (ROS) is known both as the cause and the consequence of epileptic seizures. Oxidative stress may disturb the balance of electrical activity in the brain, leading to seizures. The increased free radicals can induce seizure activity by direct inactivation of glutamine synthase and glutamate decarboxylase, thereby permitting an abnormal build-up of excitatory (glutamate/glutamic acid) and inhibitory (GABA) neurotransmitters [21]. On the other hand, recurrent or prolonged seizures can increase ROS and superoxide generation in the brain [22]. These increased ROS not only cause long-lasting seizure formation, but if not arrested, can also lead to neuronal degeneration/death through damage to cellular proteins, lipids, and DNA, and can decrease the enzymatic (glutathione) and non-enzymatic (SOD, glutathione peroxidase, and catalase) antioxidants in the epileptic focus [22]. The high rate of oxidative metabolism, coupled with the low antioxidant defenses, low repair mechanism, and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to the free radical damage [2]. SOD rapidly removes superoxide anions and protects cells from its direct toxic effect. Lipid peroxidation causes membrane structure alterations that affect membrane fluidity and permeability and membrane protein activity; this might be the proconvulsant effect of lipid peroxidation. As an end-product of lipid peroxidation, MDA is a well-known parameter for determining free radical formation in tissues [23]. In this study, pretreatment with montelukast (25, 50, and 100 mg/kg, i.p.) dose-dependently decreased the MDA levels and increased SOD activity compared to saline-pretreated group in the brain homogenate of PTZ-induced rats (Table 3), indicating the attenuation of lipid peroxidation and increase in antioxidant defense mechanisms. This theory is supported by the low MDA and high SOD levels in the montelukast groups found in our study.

Several experimental reports have also demonstrated the neuroprotective effects for montelukast and other members of the same class of drugs in various neurodegenerative conditions such as acute and chronic ischemic brain injury [5], traumatic brain injury [24], Huntington disease-like symptoms [25], ischemia-reperfusion-induced vasculitic neuropathic pain [26], spinal cord injury [3], and experimental autoimmune encephalomyelitis [4].

Table 3. Effect of montelukast on changes in MDA level and SOD activity in brain tissues of rats.

| Drugs group                        | Brain MDA level (nmol/g protein) | Brain SOD activity (U/mg protein) |
|------------------------------------|----------------------------------|----------------------------------|
| PTZ and saline (Group B1)          | 86.8±5.4                         | 0.038±0.007                      |
| PTZ and 25 mg/kg montelukast (Group B2) | 58.1±6.3*                      | 0.063±0.010*                     |
| PTZ and 50 mg/kg montelukast (Group B3) | 49.8±7.5**                      | 0.067±0.010*                     |
| PTZ and 100 mg/kg montelukast (Group B4) | 37.8±2.3***                     | 0.080±0.009**                    |

Data were expressed as mean ± SEM. Statistical analyses were performed by one-way ANOVA test. * p<0.05, ** p<0.01, *** p<0.000 (different from saline-treated PTZ group).
Conclusions

In conclusion, the results of the present study demonstrate the neuroprotective potential of montelukast via antioxidant (attenuated lipid peroxidation and increased SOD activity) and a dose-dependent anticonvulsive effect on the PTZ-induced seizures in rats. The results of the present study may add to the evidence that LTD4 plays a role in the pathogenesis of epilepsy and that LTD4 antagonist may be an effective alternative treatment for epilepsy. Further studies are needed to determine these effects of montelukast.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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