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Research Paper

Influence of etoricoxib on anticonvulsant activity of phenytoin and diazepam in experimental seizure models in mice

R. Jayaraman[a], K.T. Manisenthil[b], T. Anitha[c], Vishal D. Joshi[a], Narahari N. Palei[a], Kalpesh Gajera[a] and Krupali Ladani[a]

[a]K.N.V. Pharmacy College - Metoda, Rajkot, Gujarat, [b]KMCH College of Pharmacy, Coimbatore, Tamilnadu and [c]Vivekananda College of Arts and Science for Women, Tiruchengodu, India

Abstract

Objectives Our aim was to investigate the effect of etoricoxib on the anticonvulsant activity of phenytoin and diazepam against seizure models in mice. In addition the acute adverse effect of etoricoxib was assessed with a chimney test.

Methods The maximal seizure pattern was induced in mice by giving an alternating current of 50 mA for 0.2 s, while chemical seizures were induced by intraperitoneal injection of pentylenetetrazole at its CD97 dose (97% convulsive dose for the clonic phase). Test drug was administered 45 min before the electrical or chemical induction of seizures in combination with conventional antiepileptics. The ability of the test drug to reduce or abolish the extensor phase of maximal electroshock and clonic-type seizures in the chemical induction method was selected as anti-seizure criteria.

Key findings Concurrent treatment with etoricoxib at an oral dose of 10 mg/kg reduced the anticonvulsant potency of phenytoin. The protective effects of diazepam against pentylenetetrazole-induced convulsions was significantly increased and the mortality rate was reduced by concurrent treatment with etoricoxib (10 mg/kg p.o.) when compared with diazepam groups. No neurotoxic effect was observed with etoricoxib (10 mg/kg p.o.) and it had no impact on motor coordination in the chimney test in mice. Etoricoxib applied at its highest dose (10 mg/kg) significantly enhanced the free plasma levels of diazepam whereas the free plasma levels of phenytoin were significantly reduced.

Conclusions The obtained results suggest that the preferential cyclooxygenase-2 inhibitor etoricoxib significantly reduced the anticonvulsant action of phenytoin and significantly increased the beneficial action of diazepam against maximal electroshock and pentylenetetrazole-induced convulsions in a mouse model.

Keywords diazepam; etoricoxib; maximal electroshock seizures; pentylenetetrazole-induced seizures; phenytoin

Introduction

Epilepsy is one of the most common afflictions in humans with a prevalence of approximately 1% of the total population.[1] Epileptic seizures refer to a transient alteration of behaviour due to disordered, synchronous and rhythmic firing of populations of brain neurons. Seizures can be non-epileptic when evoked in a normal brain by treatments such as electric shock or chemical convulsants or epileptic when occurring without evident provocation.[2] Cyclocoxygenase (COX) is a rate-limiting enzyme which catalyses the metabolism of arachidonic acid to prostaglandins and exists as two isoforms (COX-1 and COX-2). Recent evidence has demonstrated the up-regulation of COX, particularly COX-2 isoforms, in various neurological disorders, including stroke, Alzheimer's disease and epilepsy.[3]

Studies have suggested increased expression of the COX-2 isoform occurs in mouse brain after electrical kindling. The expression of COX-2 is known to increase the granules and the pyramidal cells of the hippocampus following kindling.[3] Studies with celecoxib, a highly selective COX-2 inhibitor, have shown beneficial effect when given in combination with phenytoin.[4] Moreover, many studies indicate that in 20–30% of epilepsy patients, seizures are not sufficiently controlled with monotherapy.[5–7] In addition about 20% of epilepsy patients

Correspondence: Mr. R. Jayaraman, K.N.V. Pharmacy College - Metoda, Rajkot, Gujarat, India.
E-mail: jayaraam81@gmail.com

cannot be effectively cured with existing conventional antiepileptic drugs or their combinations so there is a need for either new antiepileptic drugs, or better combinations, or changes in treatment strategies. However, for this the experimental background has not been sufficient. Considering this we made an attempt to study the effect of etoricoxib with conventional antiepileptics.

Experimental studies based on a number of recognized models of epilepsy may provide good clues for clinicians about efficient combination therapy that would exert potent anticonvulsant action with minimal, or even no, adverse effects.[8] With this background, this study was designed to expound the effect of etoricoxib, a preferential COX-2 inhibitor, in combination with conventional antiepileptics (phenytoin and diazepam) against maximal electroshock and pentylenetetrazole-induced convulsions in mice.

Materials and Methods

Drugs

The following drugs were used in this study: pentylenetetrazole (Sigma, India), etoricoxib, phenytoin and diazepam (Sun Pharmaceuticals, India). Etoricoxib was suspended in 0.25% w/v carboxymethyl cellulose and administered orally 45 min before conventional antiepileptics. Phenytoin was suspended in 1% solution of Tween 80[9] and diazepam was suspended in 1% w/v gum acacia; an appropriate amount of the corresponding vehicle was given to the control mice. The dose used for phenytoin and diazepam has been used successfully in similar experimental models.[10–14] The dose of etoricoxib was based on recommended human treatment regimes.[15,16] All the drugs except pentylenetetrazole, which was administered intraperitoneally, were given by the oral route by using oral feeding needles.

Animals

The experiments were carried out on adult albino male mice, 25–30 g. They were housed at a temperature of 25 ± 1°C and relative humidity of 45–55%. A 12-h light–dark cycle was maintained during the experiment. They were given free access to food and water, except during the test period. All experiments were performed at the same time of the day and during the light period. Each group consists of six mice per dose and the experimental protocols were approved by the institutional animal ethics committee (IAEC) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

Maximal electroshock seizure model in mice

A total of 36 albino male mice, 25–30 g, were used in this experiment. The mice were divided into six groups ($n = 6$). The maximal seizure pattern was induced by using an electro convulsiometer (Techno, India) with an alternating current (0.2 s stimulus duration and 50 mA) delivered via ear-clip electrodes and the duration of tonic flexion and extensor phase was noted.[17–21] A drop of 0.9% saline solution was poured into each ear before placing the electrodes. Etoricoxib alone and in combination with phenytoin was administered orally 45 min before mice were subjected to an electroshock.[22] The mice were observed closely. The ability of etoricoxib to abolish or reduce the duration of tonic flexion and extensor phase was noted, relative to control groups.

Pentylenetetrazole-induced seizures in mice

A total of 36 mice were used in this experiment. The mice were divided into six groups ($n = 6$). Mice were injected intraperitoneally with pentylenetetrazole at a dose of 105 mg/kg, which was its CD97 (97% convulsive dose for the clonic phase) for the induction of chemo-convulsions.[23,24] The test drug etoricoxib, either alone or in combination with diazepam, was administered orally 45 min before each pentylenetetrazole treatment. The mice were observed for the next 30 min for the development of clonic seizures. Clonic seizure activity was defined as clonus of the whole body lasting more than 3 s with an accompanying loss of righting reflex. The ability of etoricoxib to abolish or reduce such movements was selected as the criterion to establish anticonvulsant activity of the drug.

Neurotoxicity test

The neurotoxicity of etoricoxib was assessed by means of a chimney test.[25,26] This test was carried out over a period of 45 min and the mice were subjected to prior training. The mice were placed in a 25-cm long and 3-cm diameter, horizontally located, tube which was reversed in such a way that the mice were able to leave only by climbing up backwards as soon as they reached the other end. The ability of mice to leave the tube within 1 min was considered to indicate a lack of neurotoxic properties of the test compound.

Estimation of free plasma levels of antiepileptic drugs

Mice were decapitated at times scheduled for the convulsive test and blood samples of approximately 1 ml were collected from phenytoin- and phenytoin/etoricoxib-treated groups. Similarly, blood sample were collected from diazepam- and diazepam/etoricoxib-treated groups and transferred into Eppendorf tubes. Collected blood samples were centrifuged at 10 000 rev/min (REMI, INDIA) and then free plasma levels of antiepileptic drugs were determined after removing protein-bound molecules by an immunofluorescence method using an analyser (Technicon Systems, India). Control plasma samples of phenytoin and diazepam were used to verify the calibration. Plasma levels were expressed in μg/ml as the mean ± SD of at least eight determinations.

Statistical analysis

Results were expressed as mean ± SEM and the significance of difference in the response between treatment group and control was determined by one-way analysis of variance followed by Tukey–Kramer multiple comparisons test. $P < 0.05$ was considered as statistically significant. Plasma levels of antiepileptic drugs were statistically analysed by the use of unpaired Student's *t*-test.

Results

Maximal electroshock seizures

The duration of tonic flexion and extensor phase was recorded in control mice and in mice treated with phenytoin or phenytoin plus etoricoxib both before and after the electroshock (Table 1). Phenytoin (25 mg/kg, p.o.) administered alone before electroshock lowered the convulsive threshold ($P < 0.001$) when compared with control groups. But concurrent oral treatment with etoricoxib at a dose of 10 mg/kg reduced the potency of phenytoin when compared with phenytoin-treated groups.

Pentylenetetrazole induced seizures

The onset and duration of action in clonus-type convulsions was recorded in control mice and in mice treated with diazepam or etoricoxib plus diazepam, after treatment with pentylenetetrazole. Pretreatment with diazepam showed a dose-dependent protective effect upon the mortality and the duration of seizures in mice (Table 2). The protective effect of diazepam against pentylenetetrazole-induced convulsions was significantly increased and the mortality rate was reduced by concurrent treatment with etoricoxib (10 mg/kg, p.o.) (Table 2) when compared with diazepam groups.

Chimney test

No neurotoxic effect was observed with the test compound etoricoxib (10 mg/kg, p.o.) and it had no impact on motor coordination in the chimney test in mice at the investigated doses and the mice were able to leave the tube within 1 min.

Influence of etoricoxib on free plasma levels of antiepileptic drugs

Etoricoxib applied at its highest dose of 10 mg/kg significantly enhanced the free plasma levels of diazepam from 0.39 ± 0.21 to 0.52 ± 0.21 μg/ml whereas the free plasma levels of phenytoin were significantly reduced by etoricoxib from 0.47 ± 0.23 to 0.31 ± 0.17 μg/ml (Table 3).

Discussion

The effects of COX-2 inhibitors in epileptic animal models have been contradictory. However, some data suggest that treatment with COX-2 inhibitors, like nimesulide and rofecoxib, before an epileptic challenge show an anticonvulsant action.[3] In addition, many previous studies indicate that COX-2 expression is induced after seizures in different animal models of epilepsy and epilepsy patients with hippocampal sclerosis.[23,27] In our study, concurrent treatment of pentylenetetrazole-seizure-induced mice with etoricoxib showed a dose-dependent protective effect upon the mortality and a reduction in seizure duration was observed (Table 2). Etoricoxib (at a dose of 10 mg/kg p.o.) diminished the anticonvulsant activity of phenytoin in the maximal electroshock seizure model in mice, which was supported by the decreased free plasma level in mice (from 0.47 ± 0.23 to 0.31 ± 0.17 μg/ml, Table 3). However, etoricoxib (10 mg/kg, p.o.) in combination with phenytoin or diazepam did not affect the motor coordination in mice, which showed the lack of neurotoxic properties of etoricoxib.

The observations emanated in this study show that etoricoxib (10 mg/kg p.o.) significantly altered the protection

Table 1 Effect of etoricoxib alone and in combination with phenytoin on maximal electroshock-induced seizures in mice

Treatment	Dose (mg/kg p.o.)	Duration of tonic flexion (s)	Duration of tonic extension (s)	Mortality
Control (normal saline)	0.5 ml	10.25 ± 0.37	10.25 ± 0.37	5
Phenytoin	25	$5.09 \pm 0.23^{***}$	$4.68 \pm 0.34^{***}$	0
Etoricoxib	6	$8.74 \pm 0.48^{*}$	$8.86 \pm 0.23^{*}$	2
Etoricoxib	10	$8.82 \pm 0.21^{*}$	$8.99 \pm 0.18^{*}$	2
Etoricoxib + phenytoin	6 + 25	$7.41 \pm 0.21^{**}$	$6.01 \pm 0.21^{**}$	0
Etoricoxib + phenytoin	10 + 25	$7.15 \pm 0.22^{**}$	$5.72 \pm 0.22^{***}$	1

Values are expressed as mean ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared with control groups.

Table 2 Effect of etoricoxib alone and in combination with diazepam on pentylenetetrazole-induced convulsions in mice

Treatment	Dose (mg/kg p.o.)	Clonic convulsions		Mortality
		Onset (s)	Duration (min)	
Vehicle + pentylenetetrazole	105 (i.p.)	97.38 ± 5.50	9.07 ± 0.25	4
Diazepam	2	338.33 ± 6.00	$3.77 \pm 0.22^{***}$	0
Etoricoxib	6	301.22 ± 3.19	$8.41 \pm 0.37^{*}$	2
Etoricoxib	10	309.32 ± 2.29	$8.12 \pm 0.23^{*}$	1
Etoricoxib + diazepam	6 + 2	364.16 ± 11.86	$2.57 \pm 0.15^{**}$	1
Etoricoxib + diazepam	10 + 2	429.16 ± 10.67	$2.60 \pm 0.17^{**}$	0

Values are expressed as mean ± SEM, $n = 6$. $^{**}P < 0.01$ when compared with diazepam treatment; $^{*}P < 0.05$, $^{***}P < 0.001$ when compared with control.

Table 3 Influence of etoricoxib on the free plasma levels of phenytoin and diazepam in mice

Treatment	Dose (mg/kg p.o.)	Free plasma levels (μg/ml)
Phenytoin	25	0.47 ± 0.21
Etoricoxib + phenytoin	10 + 25	0.31 ± 0.15
Diazepam	2	0.39 ± 0.21
Etoricoxib + diazepam	10 + 2	0.52 ± 0.23

Values are the mean ± SD of eight determinations. Blood samples were taken at times scheduled for the convulsive test. Unpaired Student's *t*-test was used for statistical evaluation of the data.

against seizures in the maximal electroshock and pentylenetetrazole seizure models in mice, which indicates the pharmacokinetic interaction of the drug when combined with conventional antiepileptics. Also, etoricoxib possesses good pharmacokinetic properties.[16] It is rapidly absorbed orally, is 92% bound to plasma proteins and crosses the placenta and blood–brain barrier in rats and rabbits. It is metabolized extensively by cytochrome P450 enzymes and is eliminated via renal excretion.

Conclusions

The results from these studies indicate that acute treatment with etoricoxib diminished the activity of phenytoin against maximal electroshock-induced convulsions in mice due to pharmacokinetic interaction. The enhanced anticonvulsant activity of diazepam against pentylenetetrazole-induced convulsions in mice may be due to pharmacokinetic interactions or it acting through GABAergic neurons. Because the available data support this, there may be a possibility of activation of COX an increase in free radical production leading to oxidative stress and apoptosis of GABAergic neurons, thus increasing the glutamate and causing epileptic discharges.[3] So COX-2 inhibitors might be acting through GABAergic neurons thus increasing the inhibitory neurotransmitter and the expression of GABA receptor protein.[3,27–29]

However, at this stage further experiments are necessary to elucidate the exact mechanism of COX-2 inhibitors such as etoricoxib when they are combined with conventional antiepileptics in humans to warrant a recommendation for altering the dosage of phenytoin in patients undergoing chronic therapy.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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