IJBCP International Journal of Basic & Clinical Pharmacology

Research Article

Evaluation of anticonvulsant effect of celecoxib, a selective cyclooxygenase-2 inhibitor in experimentally induced convulsions in albino rats

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Received: 18 May 2016
Accepted: 13 June 2016

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ABSTRACT

Background: Cyclooxygenase-2 (COX-2) exists as the inducible form of the cyclooxygenase enzyme, the levels of which are elevated in inflammatory conditions. COX-2 is located in regions of brain like hippocampus and cerebral cortex. When induced, COX-2 forms prostaglandin E2 (PGE2), which is responsible for CNS excitation, in turn leading to generation of seizures. COX-2 inhibitors by preventing the formation of PGE2 may serve as effective anticonvulsants. Since none of the anti-epileptics in current use are able to cure the patient of the seizures, a search for newer anti-epileptics is warranted. The present study assesses the anti-seizure activity of celecoxib against experimentally induced convulsions in Albino rats.
Methods: Two models of experimentally induced convulsions in Albino rats were used: 1) maximum electroshock seizure (MES) test model and 2) pentylenetetrazole (PTZ) test model. 30 rats were taken for each method and randomly assigned to 5 groups (N=6). 1st group served as control group, which received normal saline intra-peritoneal. 2nd, 3rd and 4th groups received celecoxib in doses of 10, 20 and 40 mg respectively through intraperitoneal route. The 5th group received the standard drugs phenytoin sodium (12.5 mg/kg) and sodium valproate (100 mg/kg) through intraperitoneal route in MES and PTZ models respectively.
Results: Celecoxib showed significant anticonvulsant effect with all 3 doses in MES model and with 2 doses (20 and 40 mg/kg) with PTZ model.
Conclusions: The results of this study indicate that celecoxib has anticonvulsant effect in albino rats.

Keywords: Cycloxygenase-2, Celecoxib, MES test, Pentylenetetrazole test

INTRODUCTION

Epilepsy is a chronic neurological disorder with an estimated 50 million people affected worldwide. Nearly 75 % of people suffering from epilepsy belong to low and middle socioeconomic status with little or no access to medical treatment.[1] Epilepsy is characterized by a persisting tendency to generate seizures.

An epileptic seizure results from an electrical disruption in the brain, characterized by an imbalance in the synchrony between excitation and inhibition. Sizeable progress has been made in the pharmacological management of epilepsy with the introduction of novel anti-epileptics in recent times. One third of epileptic patients continue to present with refractory seizures, in spite of the availability and use of a number of antiepileptic drugs in use.[2] Existing anti-epileptics are however associated with a number of adverse effects, teratogenic effects and long term toxicities. None of the currently used anti-epileptics completely cure the patient of the disease nor prevent future episodes of convulsions, and thereby substantiates the need to develop new and more effective anti-epileptics.

Cyclooxygenases (COX) and prostaglandins (PGs) are known since long to play highly important roles in the functioning of the central nervous system. They are either directly or indirectly involved with neuronal activity in the brain.[3] Cyclooxygenases are responsible for the generation of PGs through metabolism of arachidonic acid. Of the two types of COX, COX-2 is the inducible type, the levels of which vary in response to stimuli to tissues. COX-2 is expressed in brain in certain inflammatory and neuro-degenerative disease states.[4] A rise in the levels of COX-2 and PGs is seen during and after seizure induction in animal models.[5] Both COX-2 and PGs were reported to have critical functions in both early and delayed phases after seizure.[6] Since COX-2 and consequently PG levels were found to raise in brain during or after seizure, it was hypothesized that COX-inhibitors could reduce or prevent seizures by inhibiting the synthesis of PGs.[6]

In vivo animal evaluation methods like maximum electroshock test and pentylenetetrazole test still remain the most ideal methods for evaluating the anticonvulsant effect of drugs.[7] The present study is undertaken to investigate the anticonvulsant activity of celecoxib, a selective COX-2 inhibitor in rat models of maximal electroshock induced and pentylenetetrazole induced seizures.

METHODS

In the present study, two experimental models of epilepsy were prepared in Albino rats. The anticonvulsant effects of celecoxib were evaluated on these rat models taking 3 graded doses of celecoxib and comparing it with standard anticonvulsant drugs. This study was approved by the institutional animal ethics committee. The two experimental models used in this study for inducing convulsions in rats are:

Maximal electro-shock (MES) method

Electro-convulsiometer used for inducing tonic-clonic seizures.[8]

Pentylenetetrazole (PTZ) method

PTZ is a chemical agent used for inducing clonic convulsions. PTZ induced seizures model is considered analogous to Petitmal (absence) seizures in humans.[9]

Wistar albino rats were taken for the experiment, weighing between 100 to 200 grams. Rats of either sex bred at national institute of nutrition, Hyderabad were obtained for the study and reared in the central animal house of the institute, where the study was done. In the animal house, rats were housed in polypropylene cages at temperature maintained in the range of 20-24°C and relative humidity maintained from 50 to 60 %. Light was set on a 12 hour light/12 hour dark cycle beginning at 6.00 AM. They received standard diet and water ad libitum. The chemicals and drugs used for both the test methods were celecoxib (Zydus Cadilla Pharmaceuticals), phenytoin (Zydus Neurosciences), sodium valproate (S. S. Pharmaceuticals), pentylenetetrazole (Himedia Laboratories), polyethylene glycol (SD Fine chem. Limited). Individual doses of the drugs to be administered were calculated for each rat according to their body weight and the drug was delivered to the rat through intra-peritoneal (IP) route.

Maximal electro-shock (MES) method

MES test is the most accepted preclinical evaluation method for predicting the anti-seizure effect of a drug against generalized tonic-clonic (grand mal) seizures. Rats were screened one day before the experiment by subjecting them to MES with an alternating current of 150 mA intensity for 0.2 sec through trans-auricular electrodes. 30 rats which screened positive for MES seizures, identified by the development of characteristic tonic-clonic seizures were selected and then randomly allocated to 5 groups with 6 rats in each group (Table 1). Each group of rats was kept in separate polypropylene cages in the laboratory, for conditioning them to the laboratory environment for 3 days and to avoid any possible kindling effect. The night before the experiment, food was withheld but water was allowed freely.

On the day of the MES test, the drug solutions to be injected were freshly prepared in the morning and administered to the rats via IP route. 40 minutes post drug administration, each rat was subjected to an electrical stimulus (alternating current of 150 mA intensity for 0.2 sec through trans-auricular electrodes, originating from the electro-convulsio meter) to induce maximal seizures of its hind limbs, with tonic extension as the endpoint of the test. Experimental procedure was performed at nearly the same time each day. The 3 main parameters observed were

- Onset of tonic hind limb extension (THLE)
- Duration of THLE and
- Duration of clonic convulsions (Table 1).

A decline in the length of time of duration of tonic hind limb extension by a drug was taken as the principle deciding parameter to determine the drug's anti-seizure effect. Alleviation in the above parameter by celecoxib was compared with that of control and standard drugs. Statistical analysis was done using ANOVA and post-hoc analysis using LSD test.

Pentylenetetrazole (PTZ) method

30 Wistar albino rats of either sex, weighing between 100-200gms were taken and randomly assigned to 5 groups with 6 rats in each group (Table 2). PTZ (80 mg/kg) was injected subcutaneously for inducing seizures in rats. After 30 minutes, the test drugs were administered via IP route. Each rat was observed for 60 minutes for the

development of seizures. Initially myoclonic jerks were seen, followed by clonic convulsions. Parameters observed were

- Onset of myoclonic jerks
- Onset of clonic convulsions and
- Duration of clonic convulsions (Table 2).

A delay in the onset of clonic convulsions and a decrease in the duration of clonic convulsions were taken as an indication of anticonvulsant activity. The above parameters were compared among the 5 groups. Statistical analysis was done using ANOVA and post-hoc analysis using LSD test.

Statistical analysis

Results were conveyed as mean±standard error of mean. One-way analysis of variance (ANOVA) was used for the statistical analysis of data followed by post-hoc analysis

done by least significant difference (LSD) test for multiple comparisons. A probability value of $p < 0.05$ was considered as significant.

RESULTS

Maximal electro-shock (MES) method

Phenytoin (12.5 mg/kg) and celecoxib (10, 20 and 40 mg/kg) pre-treatment significantly ($P < 0.05$) delayed the onset of THLE and reduced the duration of THLE and also clonic convulsions when compared to normal saline (NS) pre-treatment, indicating that both phenytoin and celecoxib has anticonvulsant effect. Phenytoin sodium however showed a significantly greater ($P < 0.05$) delay in onset of THLE and higher reduction in duration of THLE and clonic convulsions when compared to celecoxib, suggesting that the anticonvulsant effect of phenytoin is more than that of celecoxib (Table 1).

Table 1: Effect of drugs on MES induced seizures in albino rats.

Group No.	Drugs	Dose, mg/kg	Time in various phases of convulsions (seconds)			Recovered/ death
			Onset of THLE	Duration of THLE	Duration- clonic convulsions	
1	Normal saline	2.5 ml/rat	3.45±0.29	12.90±0.16	11.03±0.39	Recovered
2	Celecoxib	10	3.87±0.20*	11.44±0.26*	10.06±0.31*	Recovered
3	Celecoxib	20	5.52±0.26*	8.97±0.30*	9.74±0.45*	Recovered
4	Celecoxib	40	6.43±0.26*	5.10±0.18*	7.61±0.19*	Recovered
5	Phenytoin	12.5	7.62±0.26*	2.32±0.20*	4.09±0.40*	Recovered

Values are expressed as MEAN±SE of mean of 6 rats. One way ANOVA followed by LSD test for post-hoc analysis. (*$P < 0.05$ is taken as significant value).

Pentylenetetrazole (PTZ) method

Myoclonic jerks were observed initially which lasted for a relatively small period, with variation of duration in different groups. Significant ($P < 0.05$) delay in onset of myoclonic jerks was seen on pre-treatment with celecoxib (20 and 40 mg/kg) and sodium valproate (100 mg/kg) when compared to normal saline pre-treatment in the PTZ method. Sodium valproate (100 mg/kg) and celecoxib (20 and 40 mg/kg) significantly ($P < 0.05$) delayed the onset

of clonic convulsions and decreased the duration of clonic convulsions when compared to NS pre-treatment. However, the prolongation in onset time of PTZ induced clonic convulsions by sodium valproate 100 mg/kg was significantly more ($P < 0.05$) when compared to celecoxib. Duration of clonic convulsions with sodium valproate was significantly less, when compared to celecoxib, indicating that sodium valproate is a better anticonvulsant than celecoxib by providing a better protection against PTZ induced seizures (Table 2).

Table 2: Effect of drugs on PTZ induced seizures in albino rats.

Group No.	Drugs	Doses (mg/kg)	Time in various phases of convulsions (seconds)			Recovery/ death
			Onset mycoclonic jerks	Onset clonic convulsions	Duration clonic convulsions	
1	Normal saline	2.5 ml/ rat	41.2±2.17	127.9±3.69	746±0.41	Death
2	Celecoxib	10	55±1.65	141.9±2.71	659.4±0.51	Death
3	Celecoxib	20	84.9±2.27*	163.8±3.45*	569±0.45*	Death
4	Celecoxib	40	186.6±4.04*	240.7±4.67*	289.2±0.37*	Death
5	Sodium valproate	100	319.7±4.34*	368.3±3.03*	79.8±0.20*	Recovery

Values are expressed as MEAN±SE of mean of 6 rats. One Way ANOVA followed by LSD test for post-hoc analysis. (*$P < 0.05$ is taken as significant value).

DISCUSSION

Celecoxib produced significant anticonvulsant effect at 10, 20 and 40 mg/kg in MES model, indicating its effectiveness in controlling tonic-clonic seizures. In PTZ model, celecoxib produced significant anticonvulsant effect at 20 and 40 mg/kg, indicating its effectiveness in controlling absence seizures. In the present study, a reduction in COX-2 levels in brain by using a COX-2 inhibitor provided protection against seizures, suggesting that an increase in levels of COX-2 plays an important role in the induction of seizures.

Findings in the present study indicate that prostaglandins (PGs) and COX-2 may play an important role in MES and PTZ induced convulsions. This finding is also in concurrence with the reports of Srivastava and Gupta and Dhir et al.[10,11] Srivastava and Gupta reported that COX activity was increased in the rat brain after chemically and electrically induced convulsions. Yang and Chen suggested that COX-2 along with PGE2 regulate cell membrane excitability and long term synaptic plasticity in the hippocampus.[12] Oliveira et al reported that prostaglandin mediates induced inflammation in brain, which has epileptogenic properties.[13] Choi et al reported that glutamatergic neurons present in the hippocampus and cerebral cortex play a prominent role in onset of seizures, and also that COX-2 is mainly expressed within these regions.[14] PGE2 mediated inflammatory processes in brain modulates glutamatergic neurotransmission, contributing to excitotoxic neuronal damage in the brain.[15,16] PGE2 generated by induced brain COX-2 facilitates the recurrence of hippocampal seizure by stimulating neuronal excitability immediately after a seizure episode.[17] Chen et al also reported the regulatory role of PGE2 in membrane excitability and synaptic transmission in hippocampal neurons and suggested that the induced COX-2 accelerated the hyperexcitotoxicity immediately after seizure.[18]

Table 3: Comparing MES test results of this study with that of Shafiq et al.[22]

Drugs, doses (mg/kg)	Duration of THLE (second)	
	N Shafiq et al	Present study
NS (2.5 ml)	13.6	12.9
C 10	12.1	11.5
C 20	9.3	8.5
C 30	6.6	-
C 40	-	5.1
P 6	4.4	-
P 12.5	-	2.3

NS- Normal saline, C-celecoxib, P-Phenytoin

Apart from COX-2's role in hyperexcitation, reports also suggest a neurodegenerative role of COX-2 and its products. A delayed PGE2 production derived from induced COX-2 is involved in neuronal cell death after seizure, as reported by Mirjany et al.[19] They demonstrated

that COX-2 over-expression accelerated glutamate mediated apoptotic damage. Takadera et al proved that PGE2 induced caspase dependent apoptosis in hippocampal neurons.[20] Takemiya et al also reported that COX-2 expression caused an over excitation of hippocampal nerve cells in mouse brain following rapid kindling.[21] It was reported in earlier studies that COX-2 inhibitors like aspirin, paracetamol and diclofenac sodium antagonized the MES and PTZ induced convulsions in animal models of epilepsy.[10,11] A study done by Shafiq et al reported findings of MES test which were comparable to the present study (Table 3).[22]

Results from the current study were found to be in concordance with earlier reports of similar studies, most of them reporting anticonvulsant effects of COX-2 inhibitors on induced seizures in animals. Dhir et al reported that rofexocib, another COX-2 inhibitor has anticonvulsant effects on mice.[23] However, a study done by Akasura et al reported that COX-2 inhibitors have neither anticonvulsant nor pro-convulsant effects on PTZ-induced seizures which was in contrast to the findings of the present study.[24] Another study done by Bhaduri J et al, reported PGs to possess pro-convulsant effect rather than anticonvulsant effect with paracetamol on induced convulsions in mice, which is contradictory to our results.[25]

CONCLUSION

The present study suggests that celecoxib has an anticonvulsant effect in Albino rats. The anti-convulsant effect of celecoxib is relatively less when compared to that of the standard anti-epileptics in use. A limitation of this study is that it does not evaluate the potentiating effect of celecoxib when used in combination with standard anti-epileptics, for which further evaluation is needed.

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