

LETTER TO THE EDITOR

DIFFERENTIAL EFFECT OF CYCLOOXYGENASE-2 PREFERING
INHIBITORS ON ELECTRICALLY- AND CHEMICALLY –
INDUCED SEIZURES IN MICE

Sir,

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Based on the findings that levels of prostaglandins (PGs), the cyclooxygenase (COX) metabolites of arachidonic acid are increased in brain during experimentally-induced seizures in mice (1), a role for PGs and their synthesis inhibitors in convulsive behaviour have been suggested. However, the obtained data have provided contradictory results. Different classes of non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin, flurbiprofen and diclofenac were shown to decrease the LD50 of, and threshold for the pentylenetetrazole (PTZ)-induced convulsion in mice (2). Based on these observations and the evidence that peripheral (3) or intracerebral administration of PGs (4) antagonized chemically- and electrically-induced convulsions, it was suggested that endogenous PGs may exert anticonvulsant effect (3, 4). 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 (5, 6). Confirming this line of thinking, some recent studies have reported a potentiating effect of certain NSAIDs on concomitantly administered antiepileptic drugs in MES and PTZ seizure tests (7, 8).

The COX enzyme activity is associated with two distinct isozymes, namely COX-1 (constitutive) and COX-2 (inducible) (9). However, recent observations have suggested that COX-2 is expressed constitutively in certain parts of the central nervous system (CNS) notably the cerebral cortex, hippocampus, hypothalamus and spinal cord and is further induced as a result of seizurogenic activity (10–11).

Some new NSAIDs, which have shown preferential COX-2 inhibitory activity, are increasingly being used clinically due to their proclaimed better safety profile compared to the older non-selective agents. In view of the constitutive nature of COX-2 enzyme in the CNS and its reported upregulation during seizures and consequent exaggerated role in postsynaptic signaling in the excitatory neurons and the contradictory results obtained in previous studies on the role of PGs in convulsive phenomenon using older non-selective NSAIDs, in the present study an attempt has been made to investigate the effects of three COX-2 preferring inhibitors, namely, nimesulide, celecoxib and rofecoxib (9) in two experimental models of convulsion, maximal electroshock (MES)- 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 (12), 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.

Locally in-bred Swiss albino mice of either sex (20–25 g) were group housed in polypropylene cages at an ambient temperature ($22 \pm 2^\circ\text{C}$) and 45–55% relative humidity and maintained on natural light: dark cycle. Food and water were given ad libitum except for the period of experimentation. Experiments were conducted in groups of 8 mice/group and were treated intraperitoneally (ip) with either nimesulide (10–20 mg/kg), celecoxib (5–10 mg/kg), rofecoxib (2.5–5 mg/kg), nimodipine (20–40 mg/kg) or diazepam (0.5 and 5 mg/kg) or in a combination with latter two drugs. All compounds, except diazepam, which was used as commercial solution diluted with 0.9% saline, were dispersed with 2–3 drops Tween 80 in saline. PTZ was dissolved in distilled water. Drugs or vehicle were injected in a volume of 10 ml/kg, 40 min before inducing seizures by PTZ or MES. Doses of drugs used in the study were selected on the basis of pilot experiments. MES seizures were induced by an electroconvulsimeter (Technoelectronics, Ltd., Lucknow, India) using a 60 mA current for 0.2 sec via small alligator pinnal clips. A dose of PTZ (70 mg/kg, ip), which produced convulsions with minimum mortality, was selected and the onset to preclonic seizures and duration of clonus

were recorded. Each animal was observed for 30 min and the animals, which were still convulsing were injected diazepam, 5 mg/kg ip to terminate the seizure. The Institutional Research and Ethics Review Committee approved all the experimental protocols.

In the MES test, the duration of tonic hind limb extension (THLE) in the control group was 12.64 ± 0.49 sec (mean \pm SEM) (Table I) and all the three studied drugs: celecoxib, rofecoxib and nimesulide in a higher dose produced a significant decrease in the duration of THLE ($P < 0.001$). Nimodipine also displayed a statistically significant decrease in the duration of THLE in a dose-dependent manner. A less effective dose of nimodipine (20 mg/kg) when given in combination with a subeffective dose of nimesulide (10 mg/kg) produced a significant

TABLE I: Effect of the preferential COX-2 inhibitors and nimodipine on the duration of tonic hind limb extension (THLE) in maximal electroshock seizures (MES).

<i>Treatment (ml or mg/kg)</i>	<i>THLE (sec)</i>
Control (Vehicle) (10)	12.64 \pm 0.49
Celecoxib (5)	11.53 \pm 0.52
Celecoxib (10)	6.14 \pm 1.08****
Rofecoxib (2.5)	8.62 \pm 1.2
Rofecoxib (5)	4.36 \pm 0.86****
Nimesulide (10)	10.14 \pm 0.74
Nimesulide (20)	7.57 \pm 0.66 ****
Nimodipine (20)	8.70 \pm 0.67 ***
Nimodipine (40)	2.43 \pm 1.15 ****
Nimodipine (20) + Nimesulide (10)	4.57 \pm 1.42**** a,b,c

Values are given as mean \pm SEM (n=8).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's unpaired 't' test).

a- vs. control group; b- vs. nimesulide (10)-treated group; c- vs. nimodipine (20)-treated group.

decrease in the duration of THLE, which was more than the effect of either drug alone. In PTZ test, the vehicle treated control mice showed a preclonic seizure latency of 75.28 ± 6.29 sec (Table II). Nimesulide and both the coxib compounds in doses effective in MES test produced a decrease in the latency to onset of preclonic seizures with an increase in the duration of

effect of both the latter agents was significantly reduced. This reduction was less prominent with diazepam as this drug was still effective in completely attenuating the clonic phase of PTZ convulsions.

TABLE II: Effect of preferential COX-2 inhibitors, nimodipine and diazepam on onset to preclonic seizures and duration of clonus in pentylenetetrazole (PTZ)-induced seizures in mice.

<i>Treatment (ml or mg/kg)</i>	<i>Onset to preclonic seizures (sec)</i>	<i>Duration of clonus (sec)</i>
Control (vehicle)	75.28 ± 6.29	32.50 ± 2.87
Celecoxib (10)	57.67 ± 3.16	$73.00 \pm 1.98^{**a}$
Rofecoxib (5)	$49.50 \pm 2.78^{*a}$	$80.83 \pm 2.62^{**a}$
Nimesulide (10)	55.25 ± 5.49	$72.50 \pm 8.49^{**a}$
Nimodipine (40)	$128.66 \pm 13.77^{*a}$	26.66 ± 4.49
Diazepam (0.5)	$166.25 \pm 5.24^{****a}$	$00.00 \pm 00^{****a}$
Nimodipine (40) + Nimesulide (10)	$76.16 \pm 6.02^{*b}$	$70.83 \pm 8.96^{**b}$
Diazepam (0.5) + Nimesulide (10)	$121.88 \pm 12.81^{*d} **c$	$0.00 \pm 00^{****c}$

Values are given as mean \pm SEM (n=8).

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (Student's unpaired 't' test).

a-vs. control; b-vs. nimodipine; c-vs. nimesulide; d-vs. diazepam.

clonic phase. Nimodipine, only in the higher dose (40 mg/kg), significantly increased the latency to onset of seizures by 70.6% (P<0.05) but diazepam in a low dose (0.5 mg/kg) completely protected animals from getting full clonus and significantly increased the latency to preclonic head and forelimb jerks. When nimesulide was given in combination with effective doses of nimodipine and diazepam, the combined

The results show 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, whereas 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 (1-2). Out of various PGs, while $\text{PGF}_{2\alpha}$ and TxA_2 are implicated in promoting, the PGEs, PGI_2 and PGD_2 have been suggested to suppress seizure activity (1-4). Further $\text{PGF}_{2\alpha}$ has been reported to antagonize the seizure suppressing activity of PGE_1 (3), which, like PGE_2 also inhibits PTZ-induced seizures (4). 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., $\text{PGF}_{2\alpha}$ and TxA_2 as a result of blockade of COX-2 isoform which is not only constitutively expressed in the brain but also gets upregulated during MES (1-3) and other seizurogenic stimuli (1-4, 10-11).

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 explanation 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 (13). 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 (11–12). The latter is mainly responsible for prolonged/continuous phases of clonic or even clonic-tonic seizure activity (14–15). 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 ($\text{PGF}_{2\alpha}/\text{TxA}_2$)

or anticonvulsant (PGE_2), 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 GABA-gated chloride channel (16). Besides an absence of PGs may enhance the excitatory amino acid activity as the latter has been reported to be inhibited by PGE_2 (17). Thus, in the present study, both of these effects may be contributing to the observed exaggerated PTZ response in animals pretreated with COX-2 preferring inhibitory agents.

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