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ASPIRIN MODULATES THE ANTICONVULSANT EFFECT OF DIAZEPAM AND SODIUM VALPROATE IN PENTYLENETETRAZOLE AND MAXIMAL ELECTROSHOCK INDUCED SEIZURES IN MICE

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Abstract : Release of prostaglandins in brain after spontaneous and experimentally induced seizures, has been demonstrated. The possible role of prostaglandins in modulation of seizure activity is still inconclusive. In the present study, the effects of aspirin and its interaction with the anticonvulsants (diazepam and sodium valproate) were studied in pentylenetetrazole (PTZ) and maximal electroshock (MES) induced seizures in mice. Aspirin 50, 100, and 500 mg/kg, i.p. was administered 45 min before the pentylenetetrazole (60 mg/kg, i.p.) and MES (60 mA, 0.2 s duration via car clip electrodes) challenge. In MES seizures significant protection was seen with aspirin 100 mg/kg where as higher dose of aspirin 500 mg/kg was required to elicit maximum protection against PTZ seizures. Sub anticonvulsant dose of sodium valproate 150 mg/kg, i.p. and aspirin 50 mg/kg i.p. showed complete protection in MES seizures and the same dose of sodium valproate offered superior protection in PTZ seizures than either drug used alone

When mice were pretreated with combination of diazepam 0.5 mg/kg and aspirin 50 mg/kg protection was significantly enhanced in PTZ seizures. However, aspirin did not show any significant protection with subanticonvulsant dose of diazepam against MES seizures. The present study suggests that prostaglandins may have anticonvulsant potential and also may have modulatory effect on anticonvulsant effect of conventional antiepileptic drugs.

Key words : seizure prostaglandin PTZ MES, sodium valproate diazepam

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INTRODUCTION

Epilepsy is one of the most common neurological disorder and affects approximately 0.5–1% of population world over (1). Many reports consider the role of prostaglandins and their inhibitors in convulsive phenomenon. The levels of prostaglandins have been shown to increase both during experimentally induced and spontaneous seizures (2, 3). However, various studies using different chemoconvulsants (PTZ, picrotoxin) and different non-steroidal anti-inflammatory drugs (NSAIDs) have reported contradictory results. Findings of one group of researchers indicate that prostaglandins may have proconvulsant effect, as number of prostaglandins synthesis inhibitors were found to delay the onset of PTZ seizures (4). Wallenstien and Mauss also, reported that prostaglandin inhibitors as a group displayed anticonvulsive properties when used as a pretreatment for either fluroethyl or PTZ induced convulsions (5). Furthermore, potentiation of protective activity of antiepileptic drugs in MES seizures by NSAIDS have also been reported (6).

Contradictory to above findings, studies also suggest that prostaglandins have anticonvulsant action Prostaglandin synthesis inhibitors (indomethacin, flurbiprofen and diclofenec) have shown to lower the convulsive seizure threshold of chemoconvulsant PTZ (7).

Therefore, we investigated the effect of a commonly prescribed NSAID (aspirin) against experimental model of seizures and their interaction with conventional AEDs diazepam and sodium valproate as the prevalence and incidence of adverse drug interactions, involving NSAIDs remain largely unknown and may be crucial for patients treated with anti epileptic drugs.

METHODS

Animals

Male albino mice weighing 30/40 g were used. The animals were group housed in polypropylene cages and maintained under standard laboratory conditions with a natural- light dark cycle. Each treatment group comprised of 7–8 animals. The animals were used only once in the study. Drugs were prepared freshly and injected intraperitoneally in a volume not exceeding 0.1 ml/10 gm using a 26 gauge needle.

Pentylenetetrazole (PTZ) induced seizures

PTZ (Sigma, St Loius, USA) was dissolved in normal saline. The dose of 80 mg/kg, i.p, consistently produced seizures in 100% of the mice with minimum mortality and was used in the entire study to see the effect of different drug treatments. Animals were observed for a period of 30 min following PTZ challenge. The number of animals showing generalized clonic seizures with falling was recorded and have been expressed as percent incidence.

Maximal electroshock (MES) seizures

The mice were placed in a Perspex monitoring box for observation. MES were

induced by using electroconvulsiometer (Techno India Ltd). The current of 60 mA for 0.2 s, duration was delivered through car clip electrodes by attaching them to the pinnae. Electrodes were dipped in normal saline before application. The mice were observed for the presence or absence of hind limb tonic extension.

Drugs used

PTZ and sodium valproate (Courtesy, Intas India) were dissolved in normal saline. Aspirin was dissolved in propylene glycol. Diazepam (Courtesy, Intas, India) was suspended in 0.3% Tween 80. Pretreatment time for aspirin was 45 min. The pretreatment times for diazepam and sodium valproate were 60 and 15 min respectively.

Statistical analysis

The results were calculated as mean ± S.E.M and statistically analyzed by ANOVA and Chi square test.

RESULTS

Effect of aspirin on PTZ induced seizures

Aspirin pretreatment (45 min) dose dependently reduced the incidence of generalized clonic seizures in PTZ treated rats. At the dose of 50, 100 and 500 mg/kg, the percent incidence reduced to 85.7, 50 ($P<0.05$) and 28.5% ($P<0.05$) respectively. And the doses of 50 and 100 mg/kg were used for further experiments (Table I).

TABLE I: Effect of aspirin against PTZ induced seizures in mice.

Drug	*Dose mg/kg i.p*	*Number of animals convulsed/ no of animals tested*	*Percent incidence of generalized tonic clonic convulsions*
Control		8/8	100
Aspirin	50	6/7	85.7
	100	4/8	50*
	500	2/7	28.5*

*P<0.05 vs control

Effect of aspirin on maximal electroshock induced seizures

Aspirin 50, i.p. 45 minutes before MES challenges, showed reduction in percent incidence of hind limb tonic extension to 57.1% ($P<0.05$). The 100 mg/kg dose showed greater reduction in incidence of tonic extension to 16.6% ($P<0.05$) as it was observed in only one out of eight animals (Table II).

TABLE II: Effect of aspirin against MES seizures in mice.

Drug	*Dose mg/kg*	*Number of animals convulsed/ no of animals tested*	*Percent incidence of tonic extension of hind limb*
Control		8/8	100
Aspirin	50	4/7	57.1*
	100	1/7	14.1*

*P<0.05 vs control

Interaction of aspirin with subanticonvulsant dose of diazepam against PTZ seizures

Aspirin in the dose of 50 mg/kg,

potentiated the anticonvulsant effect of sub anticonvulsant dose of diazepam 0.5 mg/kg, in PTZ induced seizures. With aspirin 50 mg/kg along with diazepam 0.5 mg/kg treatment, the incidence of generalized clonic seizure was 14.2% (P<0.05). In only one animal generalized clonic were observed with increased latency i.e. 371 s. Interestingly, combination of diazepam 0.5 mg/kg with 100 mg/kg dose of aspirin showed less protection as compared to 50 mg/kg aspirin treatment (Table III).

TABLE III: Effect of aspirin in PTZ seizures in mice pretreated with diazepam and sodium valproate.

Drug	*Dose mg/kg*	*Number of animals convulsed/ no of animals tested*	*Percent incidence of generalized tonic clonic convulsions*
Control		8/8	100
Aspirin[a]	50	6/7	85.7
	100	4/8	50
Diazepam[b]	0.5	4/8	50
	2.0	2/7	28.5
	3.0	0/8	0
Valproate[c]	75	8/8	100
	150	4/8	50
	300	2/7	28.5
Diazepam+Aspirin	0.5+100	2/7	28.5
	0.5+150	1/7	14.2*
Aspirin+Valproate	100+1150	2/7	28.5**

*P<0.05 [a] and [b]
**P<0.05 [a] and [c]

Interaction of aspirin with subanticonvulsant dose of diazepam against MES seizures.

Aspirin did not show any significant protection with sub anticonvulsant dose of diazepam in MES seizure. In animals pretreated with aspirin 50 mg/kg along with 0.5 mg/kg diazepam, the incidence of tonic extension was 57.1% as compared to diazepam 0.5 mg/kg dose where the percent incidence of tonic extension was 71.4%. (Table IV)

TABLE IV: Effect of aspirin in MES seizures in mice pretreated with diazepam and sodium valproate.

Pretreatment drug	*Dose mg/kg*	*Number of animals convulsed/ no of animals tested*	*Percent incidence of hind limb extension*
Control		8/8	100
Aspirin[a]	50	4/7	57.1
	100	1/7	14.2
Diazepam[b]	0.5	5/7	71.4
	2.0	1/7	14.2
	3.0	0/7	0
Valproate[c]	75	4/8	50
	150	3/8	37.5
	300	0/8	0
Diazepam+Aspirin	0.5+50	4/7	57.1[NS]
Aspirin+Valproate	50+150	0/7	0

**P<0.05
Diazepam+Asprin vs [a] and [b] NS = Non significant

Interaction of aspirin with subanticonvulsant dose of sodium valproate against PTZ seizures

Sodium valproate in doses of 75 mg/kg and 150 mg/kg and 300 mg/kg i.p was administered to mice 15 min before the PTZ challenge to obtain the anticonvulsant and subanticonvulsant doses. Sodium valproate in doses of 150 mg/kg showed subanticonvulsant effect against PTZ induced seizures. The incidence of generalized clonus was 50%. Aspirin 100 mg/kg along with sodium valproate 150 mg/kg showed reduction in incidence of generalized clonic seizure to 28.5%. (P<0.05) (Table III).

Interaction of aspirin with subanticonvulsant dose of sodium valproate against MES seizures

Aspirin 50 mg/kg along with sodium valproate 150 mg/kg showed complete protection against MES seizure. In none of the animals, hind limb tonic extension was observed. Sodium Valproate per se at 150 mg/kg showed reduction in hind limb tonic extension to 37.5%. (Table IV).

DISCUSSION

Studies indicate that prostaglandin levels increase after experimental and spontaneous seizures and $PGF_{2\alpha}$ is the predominant prostaglandin identified (8). However the results are contradictory that whether PGs have convulsant or anticonvulsant activity. Therefore, the study was carried out to see the role of PGs in convulsions.

Our data suggests that aspirin inhibits the PTZ as well as MES induced seizures,. Wallenstien found that PG synthase inhibitors mefenamic acid, meclofenamic acid, indomethacin and ibuprofen delayed the onset latency of PTZ induced convulsions (4). Wali and Nair also suggested that aspirin has anticonvulsant in PTZ and MES models (9). Recently Czuczwar et al 1998 also reported that NSAIDs potentiates the protective activity of anticonvulsants in MES seizures (6). Our study showed greater inhibition of MES induced seizures than PTZ induced seizures. With the present study it is difficult to explain in the two models. In a study conducted by Zath and Roth it was observed that electroconvulsive shock raises prostaglandin F in rat cerebral cortex (8). Also, there is strong evidenced that PGF_s and PGE_s have antagonistic action (11, 12). Prostaglandin F_2 decreases the PGE_1 level. Prostaglandin PGF_1 has been shown to have inhibitory action and seizures activity (10). Based on these it is hypothesized that aspirin may inhibit electroshock induced rise $PGF_{2\alpha}$. Thus uninhibited PGF_1 effect would result in inhibition of seizures. In order to investigate if aspirin modifies the anticonvulsant activity of commonly used anticonvulsants, we studied effect of aspirin pretreatment with sodium valproate and diazepam against PTZ and MES. It was observed that when aspirin 50 mg/kg, i.p. was administered along with sub anticonvulsant dose of sodium valproate and diazepam, the anti convulsant effect was greater than observed with these drugs per se. The potentiating interaction of aspirin on subanticonvulsant doses of diazepam and sodium valproate could also be on a pharmacokinetic level. Salicylates are highly protein bound drugs and are known to displace other drug protein binding sites, thus increasing their free drug concentration.

Aspirin is commonly used as a prophylactic agent in myocardial infarction. If these experimental findings in mice could be confirmed in human subjects, aspirin will have added benefit in patients of myocardial infarction with coexistent epilepsy.

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