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ADDITIVE ANTICONVULSANT EFFECT OF FLUNARIZINE AND SODIUM VALPROATE ON ELECTROSHOCK AND CHEMOSHOCK INDUCED SEIZURES IN MICE

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Abstract : The efficacy of Flunarizine (FLU), a calcium channel blocker, in combination with conventional antiepileptic drugs, phenytoin (PHT), carbamazepine (CBZ), sodium valproate (VPA), and ethosuximide (ESM), at ED_{50} doses, were examined for protective effects against maximal electroshock seizures (MES) and pentylenetetrazol (PTZ) induced seizures in mice. In both models, only VPA and FLU showed significantly enhanced protection, which was additive ie. 100% protection. In the MES test, though FLU combined with PHT did show a slightly enhanced protection (66.6%), with CBZ there was no enhancement as compared to either drug alone. In the PTZ test, FLU with ESM showed 83% protection this however was not statistically significant. The findings of this study in mice suggest that FLU would be a suitable candidate for add-on therapy with VPA for clinical epilepsy.

Key words : flunarizine antiepileptic drugs electro and chemoshock seizures

INTRODUCTION

Cortical evoked potentials accompanied by low amplitude depolarization followed by prolonged hyperpolarizaition, referred to as the "Paroxysmal depolarisation shift" (PDS) is the best cellular marker of an epileptic event. In neurons showing burst firing triggered by PDS, there is a massive influx of extracellular Ca^{++} into the neurons, which is one of the important features in initiating epileptic activity (1). Hence, Ca^{++} channel blockade may be important in preventing seizure spread. The above findings suggest that in refractory epilepsy, treatment with conventional antiepileptic drugs (AED) combined with agents which modify, Ca^{++} modulation (viz. Ca^{++} antagonists), as add-on therapy, may provide better seizure control.

Several Ca^{++} antagonists have been shown to possess anticonvulsant activity in experimental animals and flunarizine, in particular, has been shown to have potent activity against maximal electroshock seizures (MES) and pentylenetetrazol (PTZ) induced seizures in mice (2–5). In humans

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flunarizine as add-on therapy has been found to reduce seizures significantly at well tolerated doses (6). Therefore, flunarizine would probably be the preferred choice for add-on therapy in certain forms of epilepsy which prove refractory to treatment with the older classical AEDs (7).

The present study was undertaken in order to determine whether interactions between flunarizine and conventional AEDs viz: phenytoin (PHT), carbamazepine (CBZ), sodium valproate (VPA) and ethosuximide (ESM) would provide superior seizure control in MES and PTZ tests.

METHODS

Experimental Animals : Male albino mice in the weight range 20–30 gm were used. They were maintained under standard 12h light dark cycle and were fed *ad libitum* with food and water except during the time of the experiment. Six mice/drug dose/ vehicle were used and experiments carried out at around the same time each day.

Experimental models : Anticonvulsant activity was tested using the MES model and PTZ model. MES seizures were evoked through transauricular electrodes using the Techno electro-convulsiometer (serial no. 710088), with a current of 50 mA [acquired with the help of two dial adjustments in the above apparatus, with the help of the first dial the current can be adjusted anywhere between 1–30 mA, while the second dial helps to multiply the first selection by 1–12 times as required], frequency of 50 Hz [this is the line frequency since there is no dial setting for frequency in the old model of the Techno electro-convulsiometer] for a duration of 0.2 sec. The MES profile consists of two phases: a. The ictal phase-comprising tonic flexion of fore and hind limbs; Hind limb tonic extension (HLTE) and terminal clonus and b. the post ictal depression (PID) which is the time from end of HLTE to the time when the animals walk away after regaining forelimb righting reflex. Abolition of HLTE was taken as the index of anticonvulsant activity.

For PTZ induced chemoshock model, PTZ dissolved in saline in the dose 70 mg/kg ip was used. The following effects were sequentially observed in all mice tested, myoclonic jerks, clonic tonic seizures and death following tonic seizures. Failure to observe even a single episode of clonic spasms of atleast 5 sec duration for a period of 1h was the index of protection.

All mice for MES were pretested and those which did not show HLTE were rejected. A recovery period of 3–4 days was given before repeating the experiment with test drugs - hence each mouse served as its own control. No pretesting was done for PTZ induced seizures, hence separate groups of mice were used as controls.

Drugs : FLU [Torrent Laboratories] was weighed under subdued lighting and dissolved in 50% polyethylene glycol and 50% distilled water. PHT [Boots India Ltd.] was dissolved in distilled water and 1–2 drops of 1 N sodium hydroxide. CBZ [Hindustan Ciba Geigy Ltd.] was dissolved in 50% propyleneglycol and 50% saline, and the mixture warmed in a water bath till the solution turned clear. VPA [Reckitt and Colman India Ltd.] and ESM [Parke Davis

India Ltd.] was dissolved in distilled water. FLU, PHT, CBZ and ESM were given 45 mins prior to electro or chemoshock, while VPA was given 10 mins prior to shock. When combination of drugs were given, the schedule as mentioned above for the individual drugs was maintained.

To establish ED_{50} doses four doses were employed and the ED_{50} dose was determined graphically for all the drugs. FLU and PHT was tested in increasing doses of 10–20 mg/kg, CBZ 5–10 mg/kg, VPA 250–350 mg/kg and ESM 100–300 mg/kg. All drugs/vehicles were given ip in a volume of 0.5 ml/100 gm BW.

Statistical analysis: To analyse significance of % protection, the formula of critical ratio was used (8). Ratio >2 was considered significant.

RESULTS

Behavioural effects : With PHT no behavioural or neurological effects were observed in a dose of 10 mg/kg ip. Mild restriction of motor activity was seen after CBZ 10 mg/kg. Postdrug mild ataxia for about 15–20 mins was seen in all the mice, at all the doses of VPA tested. One significant finding following VPA was the presence of "wet dog" shakes beginning in about 1 min and lasting for about 5–10 mins with all the doses tested. No significant side effects were observed following ESM upto a dose of 300 mg/kg ip. A moderate degree of motor incoordination was observed with

TABLE I : Effect of combining ED_{50} doses of Flunarizine [FLU] with ED_{50} doses of Phenytoin [PHT], Carbamazepine [CBZ], Sodium valproate [VPA] and Ethosuccimide [ESM] on percentage protection in MES and PTZ induced seizures.

Drug (mg/kg, ip)	*Number protected against MES seizures*	*Percentage protection against MES seizures*	*Number protected against PTZ seizures*	*Percentage protection against PTZ seizures*
FLU (20)	3/6	50 %		
PHT (10)	3/6	50 %		
CBZ (6)	3/6	50 %		
VPA (300)	3/6	50 %		
FLU (20) + PHT (10)	4/6	66.66 %		
FLU (20) + CBZ (6)	3/6	50 %		
FLU (20) + VPA (300)	6/6	100 %*		
FLU (10)			3/6	50%
ESM (120)			3/6	50%
VPA (110)			3/6	50%
FLU (10) + ESM (120)			5/6	83.66%
FLU (10) + VPA (110)			6/6	100%

*Significant compared to individual drugs (P > 2).

FLU 20 mg/kg. No mortality was seen with any of the drugs upto 24h.

The combined administration of FLU with PHT showed no significant behavioral changes as compared to the individual drugs. When FLU was combined with CBZ there was greater quiescence as compared to the individual drugs. With the FLU plus VPA combination the restriction of motor activity, ataxia and hypotonia was slightly more apparent than with the individual drugs.

Anticonvulsant effects : MES induced seizures : Protective ED_{50} doses against MES for FLU, PHT, CBZ and VPA were 20, 10, 6 and 300 mg/kg, ip, respectively. ESM afforded no protection. The % protection afforded by the ED_{50} doses of FLU and the various AEDs given alone and in combination are shown in Table I. The combination of FLU plus VPA in the doses used produced additive effects affording 100% protection (P>2). Though the combination of FLU and PHT produced 66.7% protection as against 50% protection given by the individual drugs, this increase was not statistically significant. FLU and CBZ had the same protective effect as when the individual drugs were given alone i.e. only 50% protection.

PTZ induced seizures : For protection against PTZ induced seizures the ED_{50} doses for FLU, VPA and ESM were 10, 110 and 120 mg/kg respectively. PHT and CBZ were not effective. The protection afforded by FLU and the various AEDs alone and in combination are shown in Table I. While FLU did not alter the lack of protection given by PHT or CBZ, it increased protection of ESM from 50 to 83.33% and that of VPA to 100%, only the latter was significant (P>2).

The most significant finding, was that only the combination of FLU with VPA produced additive anticonvulsant effects as compared to the anticonvulsant effect of either drug given alone in both the MES and PTZ induced seizure models.

DISCUSSION

The results obtained in the present study are in accordance with established evidence that PHT and CBZ are effective primarily against MES induced seizures, whereas ESM is solely effective against PTZ induced seizures VPA with its wide spectrum of antiepileptic activity, is effective in both seizure models and in human partial, generalized and absence seizures (9, 10).

FLU exerts its effects primarily under conditions where calcium and/or sodium influx is pathologically increased following ischaemia, pharmacological stimulation or during seizure activity (11, 12). None of the other calcium channel blockers tested (4) show this unique characteristic of FLU in preventing pathological calcium overload without affecting the normal function of voltage dependent calcium/sodium channels and hence, synaptic transmission. Therefore FLU has been proposed as a promising candidate, particularly as add-on therapy in the treatment of refractory epilepsy (6).

The current investigation has demonstrated that the ED_{50} dose of FLU in the MES test was 20 mg/kg ip and in the PTZ test was 10 mg/kg ip. Our data confirms the anticonvulsant properties of FLU that has been reported in experimental models of epilepsy viz. MES and PTZ induced seizures in mice (2–5). FLU was shown to have an action profile similar to PHT and

CBZ in MES and PTZ induced seizures (5). In another study Rodger and Plevry (2) found that FLU was only effective in decreasing the latency and incidence of seizures in PTZ induced convulsions in mice, but had no effect on seizure threshold. In our study, it was found that FLU at its ED_{50} dose of 10 mg/kg ip, showed an abolition of the continuous clonic phase against PTZ induced seizures, but subsequently the mice showed intermittent myoclonic jerks.

The concept of rational polytherapy developed within recent years is based on the assumption that combining some AED's may result in supra-additive (synergistic) efficacy and infra-additive (antagonistic) toxicity, resulting in an enhanced efficacy/ toxicity profile (13). In order to provide evidence to support this assumption experimentally and because of the above reasons, together with the advantage of FLU, such as improvement of cognitive learning capacity, over the other calcium blockers, FLU was combined with established AED's and evaluated in MES and PTZ tests, to examine the potential of FLU as an add-on therapy. The results obtained in this study show that augmented effects were obtained when FLU was added to PHT in the MES test and to ESM in the PTZ test. Complete seizure control (100%) occurred only with the combination of FLU and VPA in both MES and PTZ tests, with minimal behavioural neurotoxicity. This screening procedure in mice has the inherent limitation that only additive, but not supra-additive (synergistic) effects can be demonstrated. In order to detect synergistic effects, comprehensive studies are contemplated.

The explanation for the additive anticonvulsant effect obtained when ED_{50} doses of FLU and VPA are combined, can be based on the assumption that the individual pharmacological mechanism of action of each drug summates at a common target. In the case of VPA, two general hypotheses have been proposed to elucidate its anticonvulsant action viz., blockade of voltage dependent Na^{++} channels and enhancing GABA mediated inhibition. On the other hand, FLU does not increase GABAergic inhibition, but has been claimed to have an effect on voltage sensitive sodium channels (10). The explanation for this effect is that the cerebrovascular effect of FLU could provide a direct neuroprotective effect, against the damaging influx of Ca^{++}, and could also prevent neuronal damage as a result of MES or PTZ induced seizures (14). There is a possibility that FLU may exert its anticonvulsant effect by interacting with voltage dependent Na^{++} channels (15), in addition to the selective blockade of Ca^{++} channels. Moreover, FLU exerts its anticonvulsant effects without producing adverse effects (16). For the present, the pharmacological mechanism underlying the additive interaction must await further elucidation. It is essential to draw attention to the observation that in human clinical trials, when FLU was used as add-on therapy with conventional AEDs, mean seizure frequency was decreased by about 35% in all patients (6).

The results obtained in this study provide supporting pharmacological evidence of the efficacy, safety and possible potential benefit of combining FLU with VPA in refractory epilepsy.

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