Treatment of phenytoin and piracetam

To study the effect of the combined treatment of phenytoin and piracetam on seizure control, cognitive and motor functions in mice.

Material and Methods:

Increasing current electroshock seizure test was used to evaluate the effect of the combination of phenytoin and piracetam on convulsions. Cognitive functions in mice were assessed by spontaneous alternation in behavior on a plus maze while motor functions were screened using rolling roller apparatus and by counting the number of arms entries on a plus maze. Brain acetylcholinesterase (AChE) activity was measured using the Ellman et al method.

Results: The study showed that piracetam when co-administered with phenytoin, significantly reversed phenytoin-induced reduction in spontaneous alternation without altering the efficacy of phenytoin against ICES in both acute and chronic studies. Further, it also reversed phenytoin-induced increase in AChE activity.

Conclusion: Piracetam alleviated the phenytoin-induced cognitive impairment without compromising its antiepileptic efficacy.

Introduction

Phenytoin (PHT) is one of the low-cost and widely prescribed antiepileptic drugs (AED) known to cause cognitive impairment. Both acute and chronic administration of PHT has been shown to significantly impair learning and memory.

For an optimum antiepileptic therapy, it is desirable to have complete seizure control without interfering cognitive effects. A combination of antiepileptic drugs (AEDs) with known nootropic agents appears to be a promising research area for desirable seizure control with minimal/no

memory deficit. A better approach would be to use an agent that not only corrects the cognitive disturbances but also provides seizure protection. In this regard, one of the promising agents is piracetam (PIM).
PIM (2-oxo-1-pyrolidone acetamide)—a nootropic—has been shown to be an effective antimyoclonic agent. It has been shown to have a specific anti-amnesic activity in many experimental paradigms. In addition, it has demonstrated a protective effect against pentylenetetrazol (PTZ) kindling-induced neuronal loss and learning deficit.
However, it lacks anticonvulsant activity in the MES model. Convincing neuroprotective functions have also been shown experimentally. Thus it would be worthwhile to assess the use of PIM along with PHT on seizure and cognitive functions.

The central cholinergic system plays an important role in learning and memory. PHT is known to reduce hippocampal ACh concentration. In view of this we also studied the effect of this combination on the brain cholinergic system. Since the majority of AEDs including PHT are known to impair motor performance, the study also evaluated this combination on motor function.

Material and Methods
Animals
Scope of study: Combined effect of phenytoin and piracetam on cognition

Drugs and dosing schedules

The following drugs were used: Phenytoin ('Dilantin' suspension, Parke Davis) was given p.o. in a volume of 10 ml/ kg body weight in doses of 8, 12 and 22 mg/kg body weight 2 h prior to each observation.
Piracetam ('Nootropil' syrup, UCB) was given p.o. in a volume of 10 ml/kg body weight in doses of 125, 250 and 500 mg/kg body weight 1 h prior to each experiment.
Control groups were given distilled water in a volume of 10 ml/kg body weight. Chronic studies were done for 21 days. All observations were made

on day 21 after 2 h of phenytoin and 1 h of piracetam administration. In chronic studies, drugs were administered between 10-12 A.M.

Increasing Current Electroshock Seizures (ICES)

The ICES as proposed by Kitano et al and modified by Marwah et al was used to evaluate the anticonvulsant effect of the drugs.
To start with a current of 2 mA electroshock to each mouse via ear electrodes as a single train of pulses (for 0.2 sec) was given with linearly increasing intensity of 2 mA / 2 sec using an electroconvulsometer. The current at which tonic Hind Limb Extension (HLE) occurred was recorded as the seizure threshold current (STC). When no tonic HLE was observed by a current of 30 mA, electroshock was terminated.

Spontaneous Alteral Behavior (SAB) on a plus maze

Rodents have a natural tendency to alternate. An amnesic drug impairs this behavior and vice versa with nootropics. Hence, an increase in alternation implies improved cognition and vice versa. Cognitive functions were assessed using a plus maze proposed by Itoh et al and SAB was noted following the method of Ragozzino et al. The number of arm entries was also recorded separately to determine the motor influence on the observed effects.
Rolling roller apparatus
This method as devised and proposed by Dunham et al was used to screen the neurological deficit caused by the drugs. The speed selector was set so that the roller made revolutions/minute. The animals were placed on the roller for one minute as a testing time. A normal animal can maintain its equilibrium throughout the period. Neurological deficit was indicated by the inability of the animal to remain on the roller for a one-minute test period.
Estimation of brain acetylcholinesterase (AChE) activity
The whole brain AChE activity was measured using the Ellman et al method. This was measured on the basis of the formation of yellow color

due to the reaction of thiocholine with dithiobisnitrobenzoate ions. P values <0.05 were considered significant.

Results

Increasing current electroshock seizures (ICES)
Acute studies: PHT (22 mg/kg, p.o.) showed 100% protection against ICES as evidenced by a complete abolition of HLE. PHT (12 mg/kg, p.o.) produced 50% protection while a low dose of PHT (8 mg/kg, p.o.) afforded 0% protection.

Table 1
Effect of acute phenytoin (PHT), acute piracetam (PIM) and its combination on ICES and SAB in mice

			ICES		SAB	
Group	Treatment	Dose (mg/kg, p.o.)	Seizure threshold current (mA)	% Protection	% alternation	No. of arm entries
I (Control)	Distilled water	10 ml/kg	15.2 ± 0.33 (10)	0	69.3 + 4.14 (9)	16.2 + 1.75
II	PHT	8	19.3 ± 1.33 (6)	0	73.8 + 4.40 (5)	18.6 + 1.05
III	PHT	12	25.7 + 2.03* (6)	50	52.6 + 4.60* (5)	20.0 + 1.23
IV	PHT	22	30 + 0.0* (6)	100	49.3 + 0.7*(5)	21.2 + 1.74
			F 41.3486 df 3, 24 P <0.01		H 13.33 df 3 P <0.01	
I (Control)	Distilled water	10 ml/kg	15.2 + 0.33 (10)	0	69.3 + 4.14 (9)	16.2 + 1.75
V	PIM	125	15.0 + 0.85	0	69.0 ± 6.01	20.0 ±

			(6)			(5)	2.860
VI	PIM	250	15.3 + 0.42 (6)	0		84.7 ± 4.72* (5)	16.8 ± 2.420
VII	PIM	500	16.3 + 1.74 (6)	0		86.9 ± 3.91f (5)	20.1 ± 1.900
					H	8.64	
					df	3	
					P	<0.05	
III	PHT	12	25.7 + 2.03t (6)	50		52.6 + 4.60t (5)	20.0 + 1.23
VI	PIM	250	15.3 + 0.42 (6)	0		84.7 ± 4.72* (5)	16.8 ± 2.420
VIII	PHT+PIM	12+250	27.4 + 1.05t (8)	50		73.0 + 7.88* (5)	22.2 + 1.85
			F	41. 70	H	9.42	
			df	2, 21	df	3	
			P	< 0.01	P	<0.05	

Values are mean + SEM, Values within parentheses are number of animals, ICES- Increasing current electroshock seizure, SAB-Spontaneous alternation behaviour. Seizure threshold current values were analyzed using one-way ANOVA followed by Dunnett's test and alternation values by Kruskal-Wallis H test followed by a multiple range test, *P<0.05, t P<0.01 Vs control, * P< 0.05 Vs Group III.

on ICES (Table 1).

Chronic studies

PHT (12 mg/kg, p.o. X 21 days) caused a significant reduction in the percentage alternation i.e. markedly impaired memory. Combination of PIM (125 mg/kg, p.o. X 21 days) with PHT (125 mg/kg, p.o. X 21 days) reversed such impairment.

Rolling roller apparatus:

No dose of PHT and PIM in both acute and chronic studies, as well as in combination, produced any motor deficit.

Whole brain AChE activity

The whole brain AChE activity with PHT (8 mg/kg, p.o.) did not differ from the control. However, PHT (12 mg/kg, p.o.) demonstrated a significant rise in AChE activity as compared to control. PIM at a dose of 125 mg/kg, p.o. did not alter brain AChE activity significantly but at a dose of 250 mg/kg, p.o. lowered AChE levels significantly. A combination of PHT (12 mg/kg, p.o.) and PIM (250 mg/kg, p.o.) exhibited AChE levels similar to control.

Discussion

The results of the present study show that PHT in doses 12-22 mg/kg, p.o., adversely affected the cognitive function in both acute and chronic studies.
PIM, a well-known nootropic agent as well as an antimyoclonic agent exhibited significant nootropic effect on spontaneous alternation behavior, a model specific for measuring spatial memory in rodents. At the nootropic doses used, it was found to be ineffective against ICES thus consistent with the earlier reports on the MES model.

Effect of chronic phenytoin (PHT) and piracetam (PIM) on SAB

Treatment	Dose (mg/kg, p.o.)	% Alternation		No. of arms entries	
Control (distilled water)	10 ml/kg	69.32	+4.14 (9)	16.25	+1.75
PHT	8	73.21	+1.35 (7)	22.0	+2.15
PHT	12	50.22	+4.52 * (5)	17.4	+2.27
PIM	125	76.05	+2.59 (7)	20.0	+2.86
PHT+PIM	12+125	82.12	+5.69t (5)	17.4	+1.36

All observations were made on 21st day starting from treatment, Values are mean + SEM, Values within parantheses are no. of animals, SAB-

Spontaneous alternation behaviour. H=13.11, df=4, P<0.05(Kruskal-Wallis H test followed by multiple range test). * P<0.05 Vs control, t P<0.01 Vs PHT (12).

Combined effect of phenytoin and piracetam on cognition

Effect of acute phenytoin (PHT), acute piracetam (PIM) and its combination on AChE activity in mice

Treatment	Dose (mg/kg, p.o.)	AChE (X moles)
Control (distilled water)	10 ml/kg	117.4 + 6.19 (5)
PHT	8	109.0 + 6.67 (5)
PHT	12	191.7 + 11.83* (5)
PIM	125	101.0 + 10.44 (5)
PIM	250	89.5 + 8.67* (5)
PHT+PIM	12+250	114.7 + 5.13 (5)

H 16.67
df 5
P <0.01

Values are mean + SEM, Values within parentheses are number of animals, AChE-whole brain AChE activity. *P<0.05 Vs control (multiple range test)
PIM, however, were found to have significant antiepileptic effect against ICES (data not shown).

The present study was based on the assumption that coadministration of PIM with clinically used AED might be useful in reducing some of the cognitive adverse effects of antiepileptic therapy. Our study showed that

PIM, when co-administered with PHT, significantly reversed PHT-induced cognitive impairment without altering the efficacy of PHT against ICES.

In the chronic study, PIM at lower dose of 125 mg/kg enhanced the percentage alternation but it was not statistically significant. This dose, however, could reverse the PHT-induced impairment of SAB. This is in agreement with another report where PIM was shown to prevent PTZ kindling-induced neuronal loss and learning deficits.
To study the effect of motor influences on observed effects, the rolling roller apparatus was used but PIM alone, as well as in combination with PHT, did not exhibit any significant effect on motor functions.

Further, the number of arm entries remained unaffected in SAB, thus ruling out the involvement of motor functions in the observed cognitive effects.

The precise mechanism by which PIM exerts its nootropic effect is not known. Multiple mechanisms have been suggested such as an enhancement of oxidative glycolysis, an effect on the Ca2+ channels and an effect on the cholinergic system. The latter is known to have an important role in the learning and memory processes.

In our study, PHT, per se (12 mg/kg, p.o.) significantly elevated the 'brain AChE activity'. PIM (250 mg/kg, p.o.) on the other hand significantly lowered this activity indicating the counteracting action of the two drugs on the cholinergic system.

The impairing effects of PHT on learning and memory have been attributed to alternations in the cholinergic system. It has been reported that PHT lowers brain ACh levels. Our results on AChE are thus consistent with these reports. It is worth noting that PHT at 8 mg/kg, did not show an impairment and did not affect AChE levels.
PIM is a member of the pyrrolidones group. Most of the pyrrolidones are known to influence cholinergic functions. ACh production and turnover

are stimulated by most pyrrolidones but with varying actions at muscarinic and nicotinic receptors.

In our study PIM reduced the AChE activity of the brain. But, more importantly, in this context, it is interesting to note that when co-administered with an effective dose of PHT, PIM significantly alleviated the PHT-induced sharp rise in total brain AChE level, indicating the counteracting action of PIM and PHT on the cholinergic system.

To conclude, PIM when co-administered with therapeutic doses of PHT, significantly alleviated the adverse effects of PHT on cognitive function without compromising its antiepileptic efficacy, the effect possibly mediated by an action on the cholinergic system. However, clinical studies are required to explore the full potential of PIM in correcting PHT-induced cognitive deficits and finding a place in the current AED therapy.