ZOLPIDEM POTENTIATE ANTICONVULSANT ACTIVITY OF PHENYTOIN, SODIUM VALPROATE AND LAMOTRIGINE IN WISTAR RATS.

Gurudeva C[1], Patil PA[1*], Hiremath SV[1], Vivek V[1]

[1]Dept of Pharmacology and Pharmacotherapeutics, JN Medical College, Belgaum, India.

Summary

Zolpidem, a non-benzodiazepine sedative-hypnotic devoid of adverse effects like tolerance and dependence has been reported to be a weak anticonvulsant. The present study was planned to confirm its anticonvulsant activity and to explore its interactions with commonly used anticonvulsants like phenytoin, sodium valproate and lamotrigine in maximum electroshock seizure (MES), minimum electroshock threshold (MET) and phenylenetetrazol (PTZ) seizure. For interaction studies sub anticonvulsant dose (SACD) of all the drugs were established in each model. Zolpidem (10 mg/kg) protected significantly ($p<0.01$) from MES, MET and PTZ induced seizures. SACD of zolpidem (7.5mg/kg) potentiated anticonvulsant activity of phenytoin, sodium valproate and lamotrigine significantly ($p<0.01$) in MES, while in MET it potentiated anti seizure activity of sodium valproate and phenytoin but failed to do so in lamotrigine treated group. In PTZ seizures zolpidem significantly ($p< 0.05$) reduced the duration as well as recovery time of seizure, in sodium valproate treated group, while it significantly ($p<0.01$) reduced only the recovery time in lamotrigine treated group. Results of the present study show that zolpidem has synergistic anticonvulsant activity, with phenytoin, sodium valproate and lamotrigine.

Keywords: interaction, lamotrigine, phenytoin, sodium valproate, zolpidem.

***Corresponding author:**
Patil P. A.,
Professor,
Dept of Pharmacology and Pharmacotherapeutics,
J N Medical College, Belgaum-590010, Karnataka, India.
Phone: 0831-24091828, Fax: 08312470759, e-mail: drpapatil@yahoo.co.in

Introduction

Epilepsies are common and frequently devastating neurological disorders, affecting about 57 individuals in every 10000 people in one or the other form of 40 and more distinct varieties identified[1]. The treatment for such a common disorder is often not satisfactory due to several reasons and compels the use of multiple drugs to control the seizure. The currently used antiepileptic drugs (AEDs) act by multiple mechanisms that include modulation of central neurotransmission (enhance GABA/ inhibit glutamate) and ion channel functions. The drugs acting through GABA mechanisms may be GABA receptor agonists or its synthesis enhancers or reuptake and/ degradation inhibitors. The major limitations of AEDs such as their inefficacy in some individuals and their dose dependent adverse effects necessitate the search for effective and safe alternate drugs. The drugs like aspirin[2], hormone like progestin [3] though have been shown to possess anticonvulsant activity, several adverse effects limit their routine use as anticonvulsants.

Benzodiazepines (BZD), safest of all AED are effectively used to treat only acute seizures and status epilepticus and not recommended for routine use to treat epilepsies due to development of dependence and tolerance to their anticonvulsant activity.

A novel non-benzodiazepine like zolpidem, an established sedative-hypnotic[4] acts through BZD receptors, but in contrast to other BZDs rarely develops tolerance, dependence in humans[5]. In mice Dapoortere et al. (1986) have also shown that pharmacological profile of zolpidem differs substantially from that of BZD hypnotics[6]. Because of these unique features and low incidence of adverse effects, zolpidem is routinely used to treat sleep disorders. Since zolpidem selectively binds to α_1 subunit of $GABA_A$ receptor similar to BZD, it can be expected to share the anticonvulsant activities of BZDs and in this regard studies have reported its very weak anticonvulsant activity in mice subjected to PTZ, MES[6] as well as in INH [7] induced convulsions. In clinical practice its use is limited to treat insomnia, but by virtue of its GABA mimetic activity co-administration with standard AEDs *viz*. phenytoin, sodium valproate, lamotrigine, it could be expected to exert synergistic anti-convulsant action. There is paucity of information regarding such interactions, therefore the present study was planned to establish the anticonvulsant activity of zolpidem and to explore its interactions with above mentioned AEDs in male Wistar rats subjected to MES,MET and PTZ seizures.

Materials and methods

Animals

Healthy male Wistar rats weighing 175±25 g maintained on food pellets (Amrut Brand) and water *ad lib.*, were acclimatized to the laboratory for about a week in 12:12 hr light and dark cycle. The room temperature and humidity were fairly constant during the experimental procedures. The animals were starved over night with free access to water prior to the day of experimental procedures. All the drugs were administered orally in the volume of 10ml/Kg. The study was approved by Institutional Animal Ethical Committee constituted as per CPCSEA guidelines.

Experimental Protocol

Zolpidem, phenytoin and lamotrigine were suspended in 1% tween 40, while sodium valproate was dissolved in water to obtain appropriate concentration. Zolpidem and sodium valproate were administered one hour before, while phenytoin and lamotrigine were administered two hour before in order to achieve their effective concentrations in blood at the time of induction of seizures. Control animals were administered the vehicle (1% tween 40) orally.

1. Maximum Electricshock Seizures (MES)[8]*:* MES were induced as described by Toman et al. (1946) with an alternating current of 150 mA delivered through the ear clip electrode for 0.2 sec with help of convulsiometer. Absence of HLE was taken as protection against seizures. Only the animals which showed HLE during screening procedure on the earlier day were included in the study.

2. Minimum Electroshock Threshold (MET[9]*):* The experiments were carried out as per the method described earlier by Swinyard et,al. (1952) with slight modification in initial stimulus. The METs were determined by giving the shock with 0.25 mA current through ear electrodes, with an increment of 0.01mA for a duration of 0.2 sec and the appearance of head jerk was taken as indication of convulsions. The animals with baseline threshold (appearance of headjerk) ranging between 0.25 mA and 0.5 mA were included in the study.

3. Pentylenetetrazol (PTZ) induced seizures[10] *:* in the present study the chemical convulsant, PTZ was used in the dose of 50 mg/kg to induce convulsions in as described earlier by Louis et al. (1982). Control animals within 30 min developed a sequence of excitement, myoclonic jerks, clonic seizures and sometimes leading

to death. The anti-convulsant efficacy is determined by noting number of animals protected from seizures, number of seizures, duration of seizure and time for onset and recovery from seizures.

For interaction studies SACD of zolpidem was co-administered with that of phenytoin; sodium valproate; lamotrigine in separate group (n=6, in each) and the animals were subjected for MES, MET and PTZ induced seizures in the same way as in the previous experiments.

Statistical analysis: The significant protection against sezures in various treated groups in contrast to control was assessed by Fisher's exact test in MES and by ANOVA followed by Dunnet's post-hoc test in case of MET & PTZ induced seizures. $p \leq 0.05$ was taken as significant.

Results

Dose determination

In the present study, the calculated anticonvulsant dose 7mg/kg of zolpidem taking reported dose of 10 mg/kg for mice in the earlier studies[6] with the help of conversion table[11] (Paget and Barnes, 1964) failed to exert anticonvulsant activity in MES and MET seizures. Incremental doses to different groups of rats revealed that 7.5 mg/kg was also ineffective to provide significant protection, while 10 mg/kg completely prevented the seizure in MES and MET groups. Therefore 10 mg/kg was taken as effective dose and 7.5 mg/kg as SACD for MES and MET, however in PTZ induced seizures SACD of zolpidem was found to be 5 mg/kg. The therapeutic equivalent dose of 18 mg/kg of phenytoin, 300 mg/kg of sodium valproate and 5 mg/kg of lamotrigine were found to be effective anticonvulsant doses and their SACDs were determined to be 13.5 mg/kg for phenytoin, 150 mg/kg for sodium valproate and 4 mg/kg for lamotrigine in MES and MET. However , the SACD of sodium valproate in PTZ model was found to be 200 mg/kg.

MES studies: In interaction studies SACD of zolpidem coadministered with SACD of phenytoin; sodium valproate & lamotrigine in different groups, a significant ($p<0.01$) protection (83.33%) was observed in all the treated groups subjected to MES. (Table I)

MET studies: Interaction studies in MET model, co-administration of SACD of zolpidem with that of sodium valproate or with phenytoin significant ($p<0.01$)

protection was observed (Table I), where as it failed to show significant protection in lamotrigine group.

PTZ induced seizures: Co-administration of SACDs of zolpidem and sodium valproate showed a significant ($p< 0.05$ & 0.01) reduction in duration of seizures as well as recovery time without altering the onset time, where as SACDs of zolpidem and lamotrigne showed a significant ($p < 0.01$) reduction in the recovery time without altering the onset time and duration of seizures. Since phenytoin alone (effective dose) worsened PTZ seizures, interaction studies were not carried out. (Table II.)

Table I: Interaction of Zolpidem with Phenytoin, Sodium valproate and Lamotrigine in MES & MET groups

Group (n=6 in each)	Drug dose (mg/kg) Oral	MES		MET (Mean±SEM) mA
		Number of animals protected	% Protection	
Control	**--**	**0**	**0**	**0.25 ± 0.0**
Zolpidem	**10**	**6****	**100**	**5.17 ± 0.17†**
Phenytoin	**18**	**6****	**100**	**0.25 ± 0**
Sodium valproate	**200**	**6****	**100**	**3.92 ± 0.44†**
Lamotrigine	**5**	**4***	**66.7**	**0.25 ± 0**
Zolpidem@ + Phenytoin@	**7.5+ 13.5**	**5****	**83.33**	**4.17 ± 0.53†**
Zolpidem@ + Sodium valproate@	**7.5 + 150**	**5****	**83.33**	**1.75 ± 0.40†**
Zolpidem@ + Lamotrigine@	**7.5 + 4**	**5****	**83.33**	**0.47 ± 0.11**

@ subanticonvulant doses; *p<0.05, **p<0.01 – Fisher's exact test; †p<0.01 ANOVA followed by Dunnet's post-hoc test,

Table II: Interaction of Zolpidem with Sodium valproate and Lamotrigine in PTZ treated animals

Group (n=6) in each	Drug dose (mg/kg) Oral	No. of animals protected	Onset of Myoclonic jerks in minutes	Number of Seizures	Seizure Duration in Seconds	Recovery Time in minutes
			(Mean±SEM)			
Control	--	0	2.42 ± 0.74	1.17 ± 0.17	82.17 ± 20.57	56.67 ± 0.88
Zolpidem	7.5	6**	24.2 ± 9.1**	0 ± 0**	0 ± 0**	33.4 ± 9.8**
Phenytoin	18	0	4.83 ± 1.4	1.83 ± 0.4	84.67 ± 16.81	55.17 ± 1.38
Sodium valproate	300	0	1.17 ± 0.17	1 ± 0	27.83 ± 3.3*	8.67 ± 0.88**
Lamotrigine	5	0	2.3 ± 0.8	1.0 ± 0.26	61.6 ± 8.55	21.83 ± 2.87**
Zolpidem[@] + Sodium valproate[@]	5 + 200	2	1.38 ± 0.13	0.67 ± 0.21	14.33 ± 4.57*	13.67 ± 4.43**
Zolipdem[@] + Lamotrigine[@]	5+ 4	0	1.75 ± 0.11	1.83 ± 0.40	101.7 ± 6.6	24.67 ± 2.01**
One-way ANOVA df =6,35	F		0.9788	1.377	3.906	8.983
	P		0.45	0.26	<0.001	<0.001

[@] Sub-anticonvulsant doses; *p<0.05, *p<0.01 – Dunnet's post-hoc test

Discussion

In the present study zolpidem in the dose of 10 mg/kg significantly protected the animals subjected to MES as well as PTZ seizures and these findings are in agreement with the earlier reports[6,7]. Zolpidem also significantly elevated MET and there is paucity of information regarding the effect of zolpidem on MET. The dose of zolpidem (10 mg/kg) effective in all models (MES, MET and PTZ) was five times its therapeutic equivalent dose (1.8 mg/kg) corroborate reported week anti-convulsant activity by Dapoortere et al. (1986). Findings of the present study clearly indicate that zolpidem appears to be more effective against PTZ seizures than MES and MET, since the dose of 7.5 mg/kg could completely abolish PTZ seizures in contrast to 10 mg/kg required to abolish MES and raise MET.

Zolpidem potentiated the activity of all the three anti-convulsants studied in MES, while in PTZ it potentiated sodium valproate and lamotrigine action. In MET, zolpidem potentiated sodium valproate and phenytoin activity, but no significant interaction with lamotrigine was observed. There are no reports of zolpidem interaction with the anticonvulsants used in the present study. Considering the earlier reports on zolpidem the synergistc interactions observed in the present study appear to be pharmacodynamic rather than pharmacokinetic. According to report of Von Moltke Lisa (2002) zolpidem approximately at 200 times maximum therapeutic concentrations produced negligible or weak inhibition of human CYP1A2, 2B6, 2C9, 2C19, 2D6 as well as 3A and also reported that zolpidem is very unlikely to cause clinical drug interactions attributable to impairment of CYP activity or P-glycoprotein mediated transport[12]. More over there are no reports on interference of zolpidem with absorption and excretion of anticonvulsants used in the present study. Phenytoin a substrate for CYP2C9/10, also a known inducer of CYP3A4[1] which is responsible for biotransformation of zolpidem [13]. In case of such an interaction the outcome should have been antagonism rather than synergism. Conjenctural explanation for pharmacodynamic mechanism of interaction involves the membrane stabilizing activity through sodium channel blockade by phenytoin, sodium valproate and lamotrigine which reinforced with GABA mimetic action of zolpidem leading to hyper polarization and eventual anticonvulsant activity. The present study does not probe into mechanism involved in the interactions and therefore is equally difficult to explain failure of zolpidem to potentiate lamotrigine activity in MET test. Zolpidem, a newer hypnotic devoid of major adverse effects such as tolerance and dependence in contrast to BZDs. Its anticonvulsant activity as observed in the present study, if could be extrapolated to clinical situation, would have important clinical implications. Treatment of epilepsy with any one of the existing drugs may be neither totally safe nor effective at times. Such situations call for drug combinations, which are often accompanied by added adverse effects. A safer drug like zolpidem therefore, could be useful to reduce the dose requirement of these potentially toxic antiepileptics without compromising their efficacy for the treatment of epilepsies. However persistence of this type of favorable interaction between zolpidem and these anticonvulsants on chronic treatment needs to be confirmed clinically.

Acknowledgement

The authors are grateful to the Principal, J. N. Medical College, Belgaum for providing facilities. Thanks to Mr. Mallapur, Biostastician, Mr. A. V. Karvekar and Mr. M. D. Kankanwadi for their skilful assistance.

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