Effect of piperine on pentylenetetrazole induced seizures, cognition and oxidative stress in mice

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Cognitive impairment in epileptics may be a consequence of the epileptogenic process as well as antiepileptic medication. Thus, there is need for drugs, which can suppress epileptogenesis as well as prevent cognitive impairment. In the present study, the effect of piperine was evaluated on the course of pentylenetetrazole (PTZ) induced seizures, learning deficit and oxidative stress markers in mice. Male albino mice were injected with PTZ (65 mg/kg sc) on the 5th day of the treatment for the development of seizures. Spontaneous alternation behaviour (SAB) was carried out on the 1st and the 5th day of the treatment after PTZ administration, while the oxidative stress parameters (malondialdehyde and glutathione) were carried out in the whole brain upon the completion of the behavioural assessment. The administration of piperine, 2 mg/kg significantly decreased the PTZ induced seizures and showed improvement in the learning deficit induced by PTZ as evidenced by the increased latency time and frequency of jerks and improvement in spontaneous alternation behavior (SAB). The findings suggest the potential of piperine as adjuvant to antiepileptic drugs with an added advantage of preventing cognitive impairment.

Key words: Cognitive impairment, piperine, pentylenetetrazole.

INTRODUCTION

Epilepsy and antiepileptic drug therapy: Present status

Epilepsy is one of the oldest conditions known to mankind and still the most common neurological condition affecting individual of all ages. At any given time, it is estimated that 50 million individuals worldwide have a diagnosis of epilepsy. The prevalence is much higher in developing countries than in developed countries owing to low economic status and limited access to health care (Beghi and Hesdorffer, 2014; Banarjee et al., 2009). Our understanding of the pathophysiology of the epilepsies has advanced dramatically in the last 30 years, especially in terms of their cellular physiology and genetics. Drug treatment of epilepsy has made remarkable strides, with the introduction of many new antiepileptic drugs since 1978. Improvement in terms of clinical outcome however, has fallen short of expectations; with up to one third of patients continuing to experience seizures or unacceptable
side medication related side effects in spite of efforts to identify optimal treatment regimens with one or more drugs (Meldrum and Rogawski, 2007). There is an urgent need to identify the problems associated with drug therapy in epilepsy. Antiepileptic drug treatment may last a lifetime in many patients so the objective of treatment should be such so as to attain the best compromise between maximum seizure control and minimum side effects. The current vastly improved understanding of the molecular targets, coupled with advances in the pathophysiology of epilepsy which include a succession of breakthroughs in genetics will lead to improved therapies for epilepsy.

**Epilepsy, AEDs and cognition**

Cognitive impairments are commonly seen in patients taking antiepileptic drugs (AEDs). For many patients, there may be more debilitating than the actual seizures themselves and thus, contribute to a worse quality of life. Common cognitive deficits in people with epilepsy are intellectual decline, reduced information processing speed, reduced reaction time, attention deficits and memory impairments (Menlove and Reilly, 2015; Mc Cagh et al., 2009; Fischer et al., 2000). The origin of such cognitive impairments has been attributed to several factors: a) the underlying etiology of epilepsy, b) the central side effects of taking antiepileptic drugs (AEDs), c) the effects of the seizures themselves and d) mood (Eddy et al., 2011; Mula and Trimble, 2009; Motamedi and Meador, 2004; Drane and Meador, 2002). Thus, while the underlying brain pathology, type, frequency and severity of seizures and psychological factors play an important role, ironically the therapy used also add to the problem (Arif et al., 2009). Most often, these factors are related and contribute in varying degrees to the cognitive profile of the individual patients. Of these factors, side effects associated with AED therapy may be one of the few potentially preventable tolerability issues, so it is worthwhile to further explore ways to prevent or minimize them. Therefore, induced cognitive impairments need pharmacological intervention.

**Need of the hour: Drug therapy with maximum seizure control and minimum side effects**

Modern antiepileptic therapy is neither universally effective nor invariably safe. Advancement in understanding pathophysiology of epilepsies in term of cellular physiology and genetics would allow for more judicious therapeutic approaches to this complex neurological disorder (White and Loscher, 2014; Jacob et al., 2009). Current practice suggests that combining drug with different mechanisms is likely to optimize the therapeutic response. Monotherapy may not be effective in all cases. Therefore, the need of the hour is to search for combinations of AEDs with other drugs so as to achieve supra-additive efficacy and infra-additive toxicity. Combinations of AEDs with nootropic agents appear to be promising directions for research in this area for maximal seizure control with minimal cognitive deficits. The adjuncts chosen for reducing cognitive impairments should be free of additional risk of side effects.

On the background of these observations, the present study was conducted to investigate the effect of piperine on pentylenetetrazole (PTZ) induced seizures, cognition and oxidative stress in mice. Piperine, a nitrogenous pungent substance, is an alkaloid presents in the fruits of black pepper (Piper nigrum), long pepper (Piper longum) and other piper species (family: Piperaceae). It is used as an important ingredient for various medicinal purposes in traditional systems of medicine. piperine reported to exhibit cognition enhancer and anticonvulsant properties (Bukhari et al., 2013; Chen et al., 2013; Saraogi et al., 2013; Chonpathompikunlert et al., 2010; Wallanthorn et al., 2008), so an attempt was made to study its effect per se and in combination of SVP on PTZ induced seizures.

SVP was used as a reference drug for comparison and combination studies. This study was designed to look for a combination therapy for epilepsy that may help to achieve maximal efficacy with minimal side effects.

**MATERIALS AND METHODS**

**Animals**

Swiss strain adult male albino mice weighing between 18 to 25 g were used. The animals were housed in polypropylene cages in groups of 8 mice per cage and were kept under controlled environmental conditions (temperature: 22 to 28°C, natural light-dark cycle). The mice were maintained on a standard pellet diet and water ad libitum. Only active and apparently healthy animals with no visible lesions or gross abnormalities were selected for the experiments. All studies were conducted during the day time.

**Drugs and dosing schedule**

Pentylenetetrazole powder (Sigma, USA), sodium valproate powder (Sigma, USA) and piperine powder (Sigma, USA) were used in the study. All the drugs were dissolved in distilled water. The dose of sodium valproate (300 mg/kg) was selected on the basis of pilot experiments in our lab. This dose exhibited less than 50% protection against the chemoshock caused by PTZ (65 mg/kg, s.c.). Two doses of piperine (2 and 4 mg/kg) were used. All observations were made 90 min after sodium valproate (SVP) and 60 minutes piperine treatment. All drugs were given in a volume of 10 ml/kg. Control animals received the appropriate vehicle. The treatment schedule is given in Table 1. There were 6 groups, each having 6 mice. The animals were treated as per the given schedule.

**PTZ-induced seizures**

Pilot experiments were carried out to ascertain the dose of PTZ that produced convulsions in 100% of animals without mortality. This was found to be 65 mg/kg, s.c. The animals were observed...
Table 1. Treatment schedule.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dosage, route of administration and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NS</td>
<td>0.9% NaCl p.o., single dose for 5 days</td>
</tr>
<tr>
<td>B</td>
<td>PTZ</td>
<td>0.9% NaCl p.o., single dose for 4 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9% NaCl p.o.+ 65 mg/kg PTZ s.c. on 5th day</td>
</tr>
<tr>
<td>C</td>
<td>SVP</td>
<td>300 mg/kg S.V.P p.o., single dose for 4 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 mg/kg S.V.P p.o.+ 65 mg/kg PTZ s.c. on 5th day</td>
</tr>
<tr>
<td>D</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2 mg/kg p.o. single dose for 4 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mg/kg p.o.+ 65 mg/kg PTZ s.c. on 5th day</td>
</tr>
<tr>
<td>E</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4 mg/kg p.o. single dose for 4 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 mg/kg p.o.+ 65 mg/kg PTZ s.c. on 5th day</td>
</tr>
<tr>
<td>F</td>
<td>SVP + P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2 mg/kg Compound P1 + 300 mg/kg S.V.P p.o. single dose for 4 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mg/kg Compound P1+300 mg/kg S.V.P p.o.+ 65 mg/kg PTZ s.c. on 5th day</td>
</tr>
</tbody>
</table>

NS: Normal saline; PTZ: Pentylenetetrazole; SVP: Sodium valproate; P<sub>1</sub>: Piperine.

immediately after PTZ injection for a period of 30 min. The assessment was done following the method of Osonoe et al. (1994). The latency to jerks, myoclonus and clonic generalized seizures with the loss of righting reflex was observed. In the absence of seizures within 30 min, the latency was taken as 1800 s.

Assessment of cognitive function

**Spontaneous alternation behavior (SAB) on a cross maze**

The method described by McIntyre et al. (1998) was followed. A wooden cross maze was used. Mice were placed individually on the central platform of the maze and were allowed to traverse the maze freely. The number and sequence of entries was noted during an observation period of 6 min. An alternation was defined as entry into four different arms on overlapping quintuple sets. Five consecutive arm choices within the total set of arm choices made up a quintuple set. A quintuple set consisting of arm choices B, A, C, B, D comprised an alternation while the set with B, A, D, B, A did not. Percent alternation was calculated as follows:

\[
\text{Alternation} \% = \frac{\text{Actual number of alternations}}{\text{Possible number of alternations}} \times 100
\]

Where, possible alternations = number of arm entries minus 4. Memory was assessed on the cross maze before (1st day) and after (5th day) of the drug treatment.

Assessment of oxidative stress

At the end of the drug treatment schedule, the animals were killed under deep ether anaesthesia. Whole brain was removed and 10% tissue homogenates were prepared by separately homogenizing sufficient amounts of brain tissues in 0.15 M solution of potassium chloride (KCl). Homogenate was separated and used to determine protein content, malondialdehyde and glutathione. Protein content was estimated by the method described by Lowry et al. (1951). Malondialdehyde, an indicator of lipid peroxidation was estimated as described by Ohkawa et al. (1979) and glutathione was assessed by the method as described by Ellman (1959).

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM). The results were analysed by a one-way analysis of variance (ANOVA) followed by a Dunnett’s t test or Mann-Whitney test, wherever appropriate.

RESULTS

**PTZ induced seizures**

SVP (300 mg/kg p.o.) pre-treatment for 5 days significantly increased the latency for the onset of jerks and myoclonus and clonic generalised seizures. It also significantly decreased the frequency of jerks. Piperine (2 and 4 mg/kg p.o.) significantly increased the latency for the onset of jerks and myoclonus and clonic generalised seizures. There was also a significant decrease in the frequency of jerks with these two doses of P<sub>1</sub>. Concurrent administration of piperine (2 mg/kg p.o.) with SVP (300 mg/kg p.o.) significantly prolonged the latency to jerks and myoclonus and clonic generalised seizures. The frequency of jerks was also significantly decreased with this combination. All the comparisons were made with respect to the PTZ group (Table 2).

**Cognitive function**

**Spontaneous alternation behavior (SAB) on a cross maze**

The concomitant administration of P<sub>1</sub> (2 mg/kg p.o.) and
Table 2. Effect of sodium valproate, piperine and their combination on PTZ induced seizures in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency (sec) to.</th>
<th>Frequency of jerks within 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jerks</td>
<td>Myoclonus and clonic generalised seizures</td>
</tr>
<tr>
<td>A</td>
<td>PTZ</td>
<td>65</td>
<td>333.14±13.465</td>
<td>584.50±60.713</td>
</tr>
<tr>
<td>B</td>
<td>SVP</td>
<td>300</td>
<td>1184.4±275.34**</td>
<td>1475.1±160.58**</td>
</tr>
<tr>
<td>C</td>
<td>P1</td>
<td>2</td>
<td>502.80±19.873**</td>
<td>905.30±68.250*</td>
</tr>
<tr>
<td>D</td>
<td>P4</td>
<td>4</td>
<td>661.45±20.083**</td>
<td>931.11±20.028**</td>
</tr>
<tr>
<td>E</td>
<td>SVP+P1</td>
<td>300+2</td>
<td>1120.6±214.86**</td>
<td>1300.3±170.89**</td>
</tr>
</tbody>
</table>

Values are represented as Mean±SEM. Number of animals: 6; PTZ: Pentylenetetrazole; SVP: Sodium Valproate; P1: Piperine. Animals not showing seizures in 30 minutes were assigned a latency of 1800 seconds. Dose of PTZ: 65 mg/kg s.c. PTZ given on 5th day of treatment. The vehicle, standard drug and test drugs were given by oral route of administration. Treatment duration: 5 days. * p< 0.05 and ** p< 0.01 versus Group A. Significant by Mann-Whitney test (latencies) and one-way ANOVA followed by Dunnett’s t test (frequency of jerks).

Table 3. Effect of sodium valproate, piperine and their combination in the presence of PTZ on Spontaneous Alternation Behavior in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>% alternation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NS</td>
<td>10 ml/kg</td>
<td>65.236±1.666**</td>
</tr>
<tr>
<td>B</td>
<td>PTZ</td>
<td>65</td>
<td>49.296±3.008</td>
</tr>
<tr>
<td>C</td>
<td>SVP</td>
<td>300</td>
<td>43.026±1.737</td>
</tr>
<tr>
<td>D</td>
<td>P1</td>
<td>2</td>
<td>60.448±1.437**</td>
</tr>
<tr>
<td>E</td>
<td>P4</td>
<td>4</td>
<td>68.273±2.306**</td>
</tr>
<tr>
<td>F</td>
<td>SVP+P1</td>
<td>300+2</td>
<td>70.566±1.059**</td>
</tr>
</tbody>
</table>

Values are represented as Mean±SEM. Number of animals: 6; NS: Normal Saline (0.9% NaCl); PTZ: Pentylenetetrazole; SVP: Sodium Valproate; P1: Piperine; Dose of PTZ: 65 mg/kg s.c. PTZ given on 5th day of treatment except in Group A. Treatment duration: 5 days. The vehicle, standard drug and test drugs were given by oral route of administration. * p<0.05 and ** p<0.01 versus Group B. Significant by one-way ANOVA followed by Dunnett’s t test.

SVP (300 mg/kg p.o.) significantly increased the % alternation as compared to the toxic control group. Pretreatment with P1 (2 and 4 mg/kg p.o.) significantly increased the % alternation. However, % alternation with SVP (300 mg/kg p.o.) was found to be insignificant (Table 3).

Assessment of oxidative stress

Malondialdehyde estimation

A significant reduction in the whole brain MDA level by SVP (300 mg/kg p.o.) and piperine (2 and 4 mg/kg p.o.) was observed. The combination of SVP (300 mg/kg p.o.) with piperine (2 mg/kg p.o.) also reduced the MDA levels significantly (Table 4).

Glutathione estimation

A significant change in brain GSH level was observed. A significant increase in brain GSH level by SVP (300 mg/kg p.o.) and piperine (2 and 4 mg/kg p.o.) was observed. The combination of SVP (300 mg/kg p.o.) with piperine (2 mg/kg p.o.) significantly increased the GSH levels (Table 5).

DISCUSSION

Epilepsy continues to be a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic effectiveness. Epilepsy is associated with the alternation in psychological, emotional and educational parameters. More than half of the epileptics had some sort of cognitive problems with abnormal behavioral manifestations (Menlove and Reilly, 2015; Motamedi and Meador, 2004). These abnormalities are related to multiple factors including seizure type, age of onset, location of the focus, seizure frequency and the type of EEG pattern (Arif et al., 2009). Another factor that affects cognition is antiepileptic drug therapy (Eddy et
Table 4. Effect of sodium valproate, piperine and their combination in PTZ induced changes of brain MDA levels in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>MDA (nmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NS</td>
<td>10 ml/kg</td>
<td>0.706±0.023**</td>
</tr>
<tr>
<td>B</td>
<td>PTZ</td>
<td>65</td>
<td>0.998±0.074</td>
</tr>
<tr>
<td>C</td>
<td>SVP</td>
<td>300</td>
<td>0.716±0.041**</td>
</tr>
<tr>
<td>D</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2</td>
<td>0.823±0.041*</td>
</tr>
<tr>
<td>E</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4</td>
<td>0.678±0.030**</td>
</tr>
<tr>
<td>F</td>
<td>SVP+P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>300+2</td>
<td>0.618±0.021**</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SEM. Number of animals: 6; NS: Normal Saline (0.9% NaCl); PTZ: Pentylenetetrazole; SVP: Sodium Valproate; P: Piperine; MDA: Malondialdehyde; Dose of PTZ: 65 mg/kg s.c. PTZ given on 5<sup>th</sup> day of treatment except in Group A. Treatment duration: 5 days. The vehicle, standard drug and test drugs were given by oral route of administration. * p<0.05 and ** p<0.01 versus Group B. Significant by one- way ANOVA followed by Dunnett’s t test.

Table 5. Effect of sodium valproate, piperine and their combination in PTZ induced changes of brain GSH levels in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>GSH (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NS</td>
<td>10 ml/kg</td>
<td>0.408±0.034**</td>
</tr>
<tr>
<td>B</td>
<td>PTZ</td>
<td>65</td>
<td>0.125±0.024</td>
</tr>
<tr>
<td>C</td>
<td>SVP</td>
<td>300</td>
<td>0.298±0.037*</td>
</tr>
<tr>
<td>D</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2</td>
<td>0.301±0.022*</td>
</tr>
<tr>
<td>E</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4</td>
<td>0.378±0.030**</td>
</tr>
<tr>
<td>F</td>
<td>SVP+P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>300+2</td>
<td>0.395±0.030**</td>
</tr>
</tbody>
</table>

Values are represented as Mean±SEM. Number of animals: 6; NS: Normal Saline (0.9% NaCl); PTZ: Pentylenetetrazole; SVP: Sodium Valproate; P: Piperine; GSH: Glutathione; Dose of PTZ: 65 mg/kg s.c. PTZ given on 5<sup>th</sup> day of treatment except in Group A. Treatment duration: 5 days. The vehicle, standard drug and test drugs were given by oral route of administration. * p<0.05 and ** p<0.01 versus Group B. Significant by one- way ANOVA followed by Dunnett’s t test.

al., 2011; Mula and Trimble, 2009). Although it is understood that the beneficial results of seizure suppression are of great clinical importance, there are indications of cognitive side effects of the drugs, administered at therapeutic doses, especially with polytherapy. Thus, there is a need for drugs which can suppress epileptogenesis and contain cognitive improving property.

Many laboratory models simulate human epilepsy as well as provide a system for studying epileptogenesis (Temkin et al., 2001). In the present study, we used the PTZ model, as it is the most widely employed technique for studying seizure mechanisms and considered to be a useful experimental model for human epilepsy (Mason and Cooper, 1972). SVP was used in the present study since it is broad spectrum, first line drug used in the management of diverse seizure types (Davis et al., 1994; Brodie and French, 2000). It has been categorized as a drug with a narrow margin of safety and with well reported adverse effects on memory. There is need for a drug combination which could bring supra-additive beneficial effects and infra-additive toxicity.

Piperine is reported to exhibit cognition enhancing (Saraogi et al., 2013; Chanthapomphikunlert et al., 2010; Wallanthorn et al., 2008) and anticonvulsant properties (Bukhari et al., 2013; Chen et al., 2013), so an attempt was made to study its effect per se and in combination of SVP on PTZ induced seizures. In our study, piperine and SVP per se or in combination attenuated the PTZ induced seizures in mice as evident by the increased latency time and decreased frequency of jerks. These findings are consistent with earlier studies reporting inhibitory effect of piperine on PTZ induced seizures in mice (Bukhari et al., 2013). GABA is the major inhibitory neurotransmitter in the brain, and is widely implicated in epilepsy (Corda et al., 1990). GABAergic neurotransmission enhancement has been shown to attenuate seizures, while inhibition of GABAergic activity facilitates seizures (Okada et al., 1989). The anticonvulsant effect of piperine may be attributed to its effect on GABAergic neurotransmission but the precise mechanism is not known.

Following administration of PTZ we found out increase in oxidative stress as evident by a significant decline in...
GSH and an increase in MDA. Pre-treatment with piperine and SVP per se or in combination significantly reduce the PTZ induced oxidative stress as apparent by the increase in GSH and decrease in MDA. Our findings are consistent with the previous studies reporting the antioxidant potential of piperine (Saraogi et al., 2013; Selvendiran et al., 2003). PTZ produced a reduction in % alternation which might be due to its oxidative potential. These results are consistent with the findings of study conducted by Becker et al. (1995), which reported PTZ induced learning impairment in rats. It is well established that SVP causes cognitive impairment (Eddy et al., 2011; Mula and Trimble et al., 2009; Drane and Meador, 2002). We also observed impairment in SAB following administration of SVP which is seen from the decrease in % alternation. This impairment was successfully reversed when piperine was given in combination with SVP. Piperine has been reported to have cognitive enhancing effect (Saraogi et al., 2013; Chanpathompikunlert et al., 2010; Wallanthorn et al., 2008). Numerous transmitters have been reported to play roles in memory including glutamate, acetylcholine and serotonin (Myhrer, 2003).

Wattanthorn et al. (2008) suggested that the cognitive enhancing effect of piperine probably occur partly via the facilitation of acquisition and consolidation process induced by the alternation in serotonin level. It is also suggested that serotonin interact with acetylcholine to regulate spatial memory (Sirnio et al., 1994; Cassel and Jeltsch, 1995; Steckler and Sahgal, 1995). However the precise mechanism underlying the cognitive enhancing effect of piperine in PTZ model is still not known and requires further investigation.

Conclusion
This study presented a preliminary investigation demonstrating that piperine significantly prevented the cognitive impairment and attenuated the oxidative stress induced by the PTZ model. Therefore, it could be useful support to the basic antiepileptic therapy in preventing the development of cognitive impairment reported with several AEDs. However, further studies are required on this drug.

Conflict of interest
Authors have none to declare

REFERENCES


