Pentylenetetrazol-induced kindling in Animals: Protective Effect of BR-16A

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SUMMARY
Repeated administration of pentylenetetrazol (PTZ 30 mg/kg, thrice a week) to mice for 9 weeks and subsequent challenge of the animals with the same dose of PTZ on the last chronic dose produced chemical kindling. The mice showed myoclonic jerks, clonus, Straub’s tail, falling back response and full-blown convulsions. The anticonvulsant profile of chronic administration of BR-16A (100 and 500 mg/kg) was studied in this model of epilepsy. BR-16A offered protection against PTZ-induced chemical kindling. The per cent animals showing myoclonic jerks and clonus was reduced to 40 and 34 with 100 mg/kg and 54 and 27 with 500 mg/kg of BR-16A, respectively. The protective effect was compared with diazepam (1 mg/kg ip). The results demonstrated GABA<sub>A</sub> receptor mediated PTZ-induced kindling and protective effect of BR-16A therein.

Kindling is a model of epilepsy and epileptogenesis. Kindled seizures in animals after daily electrical stimulation of the amygdaloid complex through low-intensity electric current have been described. Repeated application of sub-convulsive doses of CNS stimulants like pentylenetetrazol (PTZ), cocaine, picrotoxin, strychnine and bicuculline once every 24 or 48 hr over a period of time is also known to induce a permanent change in the epileptogenic sensitivity of forebrain structures, such that initially subconvulsive stimuli become capable of evoking fully developed seizures. Pentylenetetrazol-induced kindling is related to the down regulation of GABA-ergic transmission the CNS. Pentylenetetrazol is reported to inhibit GABA<sub>A</sub>-induced chloride conductance in cultured mouse spinal cord neurons and also <sup>35</sup>S[TBPS binding in rat brain homogenates in a concentration-dependent manner.

BR-16A a herbal psychotropic preparation contains various ingredients reputed in the ancient system of Ayurvedic medicine to be useful in the management of nervous disorders. These include: Brahmi (Hydrocotyl asiatica) Shatavari (Asparagus racemosus) Buch (Acorus calamus), Jatamansi (Nardostachys jatamansi), Ashwagandha (Withania somnifera), Shankpushpi (Evolvulus alsinoides), Kuth (Sausurea lappa) and Triphala (Terminalia chebula). Some of the CNS activities of BR-16A have been related to its modulatory action on GABA<sub>A</sub>-ergic transmission. BR-16A has been recently tried as an adjuvant to the commonly used antiepileptic drugs in clinical trials and was found to significantly reduce seizure frequency (personal communication from The Himalaya Drug Co.). In the present study therefore, an
attempt has been made to study the protective effect of BR-16A in a laboratory model of epilepsy i.e. PTZ-induced kindling in mice.

MATERIALS AND METHODS

Animals – Laka mice of either sex, weighing between 20 and 25 g (bred in the Central Animal House facility of the Punjab University) were used. Animals were housed under standard laboratory conditions and were supplied with food and water ad libitum. The animals were maintained at 12-hr day/night cycle and all experiments were carried out between 0900 and 1700 hrs.

Chemical kindling in mice – Pentylenetetrazol (PTZ) was given in a subconvulsive dose of 30 mg/kg ip, thrice a week for 9 weeks in control mice. After each injection of PTZ, occurrence of CNS excitation was noted over 10-15 minutes by observing the mice in a Plexiglas chamber (30x24x22 cm) with partitions in between. The intensity of behavioral seizures was evaluated using a six point scoring system: 0=no effect; 1=jerks; 2=Staub’s tail; 3=clonus. The degree of behavioral response increased progressively over the weeks until the animals exhibited full motor seizures. Cumulative kindling score (calculated by taking the average of all the individual behavioral scores and then dividing them with the number of subjects) was plotted against duration of treatment.

These chronically-treated mice were then challenged with the same subconvulsive dose of PTZ (30 mg kg, ip) on the 3rd and 10th day of the last chronic dose. These mice showed different phases of CNS excitation and convulsions, which were recorded. The onset and the percentage of animals showing jerks and clonus were also recorded.

In order to study the involvement of GABA/benzodiazepine receptors in PTZ-induced kindling on the 3rd day after the last chronic dose a group of these animals were challenged with FG-7142 (20 mg/kg, ip) an inverse agonist of BZ-receptor, given 30 min prior to PTZ (30 mg/kg, ip). Cumulative kindling score was compared with the control group. Separate groups of mice were pretreated with BR-16A (100 and 500 mg/kg, po), given 30 min prior to chronic treatment with PTZ for 9 weeks. Behavioral seizure were scored as described earlier. These chronically pretreated animals were again challenged with PTZ (30 mg/kg) on the 3rd and 10th day of the last PTZ challenge dose.

Drugs – Pentylenetrazol (PTZ) – Sigma Chemicals, St. Louis, USA; diazepam –Ranbaxy, New Delhi, and FG-7142 – Schering, Germany were used. BR-16A (Mentat)® was a gift from Himalaya Drug Co., Bangalore.

Statistical analysis – The kindling scores expressed as the arithmetic mean ±SD were analysed by Student’s ‘t’ test (independent). One way analysis of variance (ANOVA) was performed on
the cumulative kindling score at (i) different weeks of treatment (3rd, 6th and 9th week), and (ii) the last day of 9th week, and the 3rd and 10th days after the last challenge dose. Posthoc comparisons were made using Duncan’s new multiple range test. Probability levels <5% were considered significant.

RESULTS

PTZ-induced kindling in mice – PTZ (30 mg/kg ip thrice a week) when given for 9 weeks produced different phases of CNS excitation and convulsions. These episodes included Straub’s tail, jerks, clonus, and circling. When these chronically treated animals were further challenged with the same subconvulsive dose of PTZ on the 3rd and 10th day of the last chronic dose (9 weeks), they exhibited frank convulsions (jerks, clonus, extensor and falling back) (Figs 1-3). The per cent animals showing jerks and clonus were 100 and 64.3 respectively on both 3rd and 10th day of challenge (Tables 1 and 2). These kindled effects were blocked by diazepam (1 mg/kg, ip) when give prior to PTZ challenge (Figs 1-3).

<p>| Table 1: Protective effect of BR-16A on PTZ-induced kindling in mice, on 3rd day of last PTZ challenge dose | (Values expressed in sec are mean ± SD. Figures in parentheses are no. of mice, out total used in that particular treatment, showing jerks/clonus) |</p>
<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>N</th>
<th>Onset</th>
<th>Severity of clonus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control PTZ, 30 ip</td>
<td>14</td>
<td>173.1 ± 100.91 (14)</td>
<td>331.2 ± 95.4 (9)</td>
</tr>
<tr>
<td>FG-7142, 20ip</td>
<td>3</td>
<td>130 ± 17.32 (3)</td>
<td>485.5 ± 495.68 (2)</td>
</tr>
<tr>
<td>BR-16A, 100 po</td>
<td>15</td>
<td>210.4 ± 80.53 (6)</td>
<td>422.0 ± 238.36 (5)</td>
</tr>
<tr>
<td>BR-16A, 500 po</td>
<td>22</td>
<td>222.9 ± 35.95 (12)</td>
<td>259.2 ± 59.47 (6)</td>
</tr>
<tr>
<td>Diazepam, 1 ip</td>
<td>9</td>
<td>435 (1)</td>
<td>–</td>
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Time of onset was taken as the time when the animal first starts showing jerks/clonus.

<p>| Table 2: Protective effect of BR-16A on PTZ-induced kindling in mice, on 10th day of last PTZ challenge dose | (Values expressed in sec are mean ± SD. Figures in parentheses are no. of mice, out total used in that particular treatment, showing jerks/clonus) |</p>
<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>N</th>
<th>Onset</th>
<th>Severity of clonus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control PTZ, 30 ip</td>
<td>14</td>
<td>194.1 ± 96.68 (14)</td>
<td>324.8 ± 177.84 (9)</td>
</tr>
<tr>
<td>BR-16A, 100 po</td>
<td>15</td>
<td>295.6 ± 122.18 (8)</td>
<td>275 (1)</td>
</tr>
<tr>
<td>BR-16A, 500 po</td>
<td>14</td>
<td>235.3 ± 47.71 (8)</td>
<td>266.7 ± 20.19 (3)</td>
</tr>
<tr>
<td>Diazepam, 1 ip</td>
<td>8</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Details are same as in Table 1.
Pretreatment of BR-16A on PTZ induced kindling in mice – BR-16A (100 and 500 mg/kg po) was given 30 min prior to each (chronic) treatment with PTZ for 9 weeks. These animals showed a milder degree of CNS excitation all throughout 9 weeks as compared to control group which received only PTZ (Fig. 1). Further, these BR-16A pretreated animals, when challenged with PTZ (30 mg/kg) on the 3rd and 10th day of the last challenge dose of PTZ, showed a significant attenuation of seizures to PTZ (Fig. 3). The percentage of animals showing jerks and clonus was decreased to 40 and 33 for the 100 mg/kg dose and 54 and 27 for the 500 mg/kg dose respectively on the 3rd day of challenge (Table 1). Similarly on the 10th day it was reduced to 53 and 7 for 100 mg/kg and 57 and 21 for 500 mg/kg dose of BR-16A respectively (Table 2).

DISCUSSION
The kindling phenomenon described by Doddard is now generally accepted as an experimental model of epilepsy and epileptogenesis. In kindling, repeated administration of an initially subconvulsive stimulus causes a progressive intensification of seizure activity leading to a distinct generalized seizure. Kindled seizures have been induced in rats, cats, monkeys, dogs, rabbits and frogs.

The CNS stimulant and convulsant actions of PTZ are reported to be mediated through specific interaction with GABA_a-gated chloride ionophore. PTZ inhibited
the binding of $[^{35}\text{S}]$ TBPS in rat brain homogenates in a concentration dependent manner\textsuperscript{17,18}. It also inhibited GABA-induced chloride conductance in functional assays in cultured spinal cord neurons. The role of GABA receptors in kindling is further substantiated by the observation that FG-7142, a beta-carboline potentiated the convulsive response of subconvulsive dose of PTZ. Repeated administration of this negative ligand of bezodiazepine receptor is known to produce generalized seizures in rats and mice. Concurrent administration of diazepam inhibited PTZ-induced kindling. Moreover Rol5-1788 a benzodiazepine receptor antagonist when given concurrently with FG-7142 prevented kindling\textsuperscript{20}; suggesting that the beta-carboline-induced kindling is mediated by the benzodiazepine-receptors\textsuperscript{21}. The inverse agonist FG-7142 (20 mg/kg, ip) potentiated the convulsive response of subconvulsive dose of PTZ as these animals showed quick onset of jerks.

BR-16A, a herbal psychotropic preparation contains besides many other indigenous ingredients, Brahmi (\textit{Hydrocotyl asiatica}) and Aswagandha (\textit{W. somnifera}). In Ayurvedic medicine, Brahmi has been extensively used in insanity and epilepsy\textsuperscript{22}. The extract of brahmi has also been found to enhance pentobarbitone-induced sleep time and protect animals from maximal electroshock seizures\textsuperscript{23}. The methanolic extract of the roots of \textit{W. somnifera} has been shown to potentiate pentobarbitone-induced narcosis and increase PTZ-induced seizure latency following chronic administration\textsuperscript{24}. Pretreatment with BR-16A offered a significant protection both during the development of kindling and also once kindling was established in these animals. The protection against kindling-induced epilepsy draws further support from the observation that BR-16A reversed ethanol withdrawal-induced anxiety and the pro-convulsive action of PTZ in rats and mice\textsuperscript{25}, a behavioural phenomenon known to involve GABA/BZ receptors\textsuperscript{26}. In this study non-convulsive doses of PTZ were able to produce full blown convulsions and increased mortality in ethanol withdrawn animals, a phenomenon known to involve GABA$_A$-ergic mechanism and susceptible to blockade by both acute and chronic treatment of BR-16A.
The present study demonstrated that PTZ-induced chemical kindling is a reliable and reproducible model of chronic epilepsy, the phenomenon being demonstrable for several weeks. Further, a decreased GAB\textsubscript{A}-ergic tone caused by PTZ is responsible for the epileptogenesis. Chronic treatment with BR-16A offered a significant protection against PTZ-induced kindling. The preparation may find an useful application as an adjunct in the management of epilepsy.

**ACKNOWLEDGEMENT**

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**REFERENCES**


