On the sleep promoting effects of BR-16A: Interaction with GABAergic modulators

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Received 15 September 2003; revised 26 February 2004

Pentobarbitone-induced hypnosis test was used as an animal model to explore the role of BR-16A, a polyherbal formulation in sleep. Pentobarbitone produces quick sleep latency (onset) and prolongation of total sleep time (duration). Sleep latency and total sleep time were used as parameters for the evaluation. BR-16A potentiated the effect of triazolam (0.1 mg/kg, ip) and alprazolam (0.25 mg/kg, ip). Melatonin (5.0 mg/kg, ip) and zolpidem (0.5 mg/kg, ip) did not produce any significant effect on sleep parameters. However, alprazolam (0.25 mg/kg, ip) potentiated the effect of BR-16A (100 mg/kg, po) in higher dose only. Sleep promoting effect of BR-16A in combination with GABAergic drugs (triazolam and alprazolam) suggested that these drugs have common mechanism in sleep promoting effect of pentobarbitone and could be used along with other GABAergic hypnotics for the treatment of insomnia. This may reduce the dose of the latter drug(s). BR-16A can be used for the treatment of sleep and sleep-related disorders.

Keywords: Pentobarbitone-induced hypnosis, GABAergic, Sleep, BR-16A, Insomnia

IPC Code: Int.Cl./Abi K 38/00

Insomnia is one of the major problems of modern times. A number of hypnotics are used for the treatment of insomnia with varying success rates1-5. Herbal drugs or their preparations can be considered as an alternative for the treatment of sleep and related disorders: BR-16A (Mentat®), a herbal psychotropic preparation contains the following indigenous ingredients: Brahmi (Hydrocotyle asiatica), Shatawari (Asparagus racemosus), Buchh (Acorus calamus), ashwagandha (Withania somnifera), giloi (Tinospora cordifolia), anila (Emblica officinalis), Shankhpushpi (Evolvulus alsinoides), Kuth (Saussarea lappa) and Triphala. Preliminary toxicity studies have shown it to be a safe herbal preparation and no adverse effects followed the chronic use of Mentat®. LD50 value of Mentat® has been reported to be 2400 mg/kg by oral route of administration (personal communication).

Studies conducted on BR-16A have shown to be a safe preparation, having a wide spectrum of CNS profile particularly, anticonvulsant, nootropic, adaptogenic, and sedative and anti-addictive activities6-9. Since most of the hypnotic and sedative drugs act through GABAergic modulation10-11, in the present study attempt has been made to explore the action of BR-16A in sleep pattern i.e. pentobarbitone-induced hypnosis and its interactions with GABAergic mechanisms in animals.

Materials and Methods

Animals—Laca mice of either sex (weighing 20-25 g), bred in Central Animal House facility of Panjab University, were used. The animals were housed under standard light/dark cycle with food and water provided ad libitum. Animals were acclimatized to laboratory condition before test. The animals were divided into 18 groups each consisting of five animals and were used once in the experiment. The experiments were performed between 0900 and 1700 hrs. The experimental protocols were approved by the Institutional Ethics Animal Committee and were conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs treatment—Following drugs and their dosages were used: BR-16A (25,50,100 mg/kg, po), melatonin (2.5, 5.0 mg/kg, ip), triazolam (0.1 mg/kg, ip), alprazolam (0.25 mg/kg, ip), zolpidem (0.5 mg/kg, ip), and pentobarbitone sod (45 mg/kg, ip). Melatonin was dissolved in a few drops of dimethyl-sulfoxide (DMSO) and the volume made up with distilled water. Triazolam was dissolved in one drop of dilute
hydrochloric acid and the volume made up with distilled water (pH 7.5). Other drugs were dissolved in distilled water. Drugs were administered 30 min before pentobarbitone administration to overnight-fasted animals. Doses were selected on the basis of previous studies conducted in our laboratory and reported in the literature.

Statistical analysis—The data were expressed as mean ± SE and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett test. In all the tests, the criterion for statistical significance was *P*<0.05.

Results

**Effect of BR-16A on pentobarbitone-induced hypnosis**—BR-16A (25-100 mg/kg, po) dose dependently shortened the sleep latency (onset) and increased the total sleep time (duration of sleep) due to pentobarbitone. The effect of BR-16A on total sleep time was found to be significant (*P*<0.05; Fig. 1).

**Effect of melatonin, triazolam, alprazolam, or zolpidem on pentobarbitone-induced sleep**—Melatonin (2.5, 5 mg/kg, ip) and triazolam (0.1 mg/kg, ip), alprazolam (0.25 mg/kg, ip), or zolpidem (0.5 mg/kg, ip), decreased the sleep latency (onset of sleep) and increased total sleep time (duration of sleep) significantly due to pentobarbitone. (*P*<0.05; Table 1)

When BR-16A (25, 50, 100 mg/kg, po) was administered in combination with triazolam (0.1 mg/kg, ip), potentiation of effect (total sleep time) was observed (*P*< 0.05; Fig. 2). However, the combination of BR-16A (25, 50, 100 mg/kg, po) and triazolam (0.1 mg/kg, ip) only shortened the sleep latency up to 2.19, 2.15 and 2.1 min, respectively as compared to control.

<table>
<thead>
<tr>
<th>Drug treatment (mg/kg, ip route)</th>
<th>Onset of action (min ± SE)</th>
<th>Sleep time (min ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.50±0.27</td>
<td>44.40±2.19</td>
</tr>
<tr>
<td>Mel 2.5</td>
<td>2.51±0.19</td>
<td>183.07±8.7</td>
</tr>
<tr>
<td>Mel 5.0</td>
<td>2.20±0.3</td>
<td>201.4±5.1</td>
</tr>
<tr>
<td>Tria 0.1</td>
<td>1.94 ±0.39</td>
<td>113.3±6.4</td>
</tr>
<tr>
<td>Alp 0.25</td>
<td>2.03±0.19</td>
<td>95.76±6.4</td>
</tr>
<tr>
<td>Zol 0.5</td>
<td>2.07±0.17</td>
<td>65.53±3.8</td>
</tr>
</tbody>
</table>

All values are significant at *P*<0.05 (Control, Pentobarbitone 45 mg/kg).

Mel= Melatonin, Tria=Triazolam, Alp= Alprazolam, Zol= Zolpidem.
The proportion of BR-16A was noticed (sleep latency: 3.31±0.56 min and total sleep time: 113.3±7.3 min) as compared to control (3.59±0.30 min and total sleep time: 116.0±5.0 min as sleep latency: 3.27±0.50 min and total sleep time: 115.2±5.1 min, respectively and melatonin 5 mg/kg, ip (2.36±0.17 min and total sleep time: 65.53±0.38 min).

Discussion

Pentobarbitone-induced hypnosis test has been used widely as an animal model in sleep studies and CNS depressant effects. GABAergic system is known to play a role in sleep and its related problems. In the present study, BR-16A shortened the sleep latency and prolonged the total sleep time as compared to control groups. This suggested for a CNS depressant effect of BR-16A. Melatonin, triazolam, alprazolam and zolpidem are well-known agents recommended for sleep induction and modulation of sleep in various sleep problems particularly in the management of insomnia in human beings. Reports are available that clearly indicate that these agents act through GABAergic mechanism or modulation. It has also been earlier reported that BR-16A, may act through GABAergic system.

In combination studies with triazolam (0.1mg/kg, ip) and alprazolam (0.25mg/kg, ip) and BR 16A, a significant potentiation of total sleep time (duration) of BR 16A was observed. This may suggest a modulatory action of BR 16A on the action of other hypnotics. However, BR-16A did not produce any significant effect at lower doses (25, 50 mg/kg, ip) when combined with alprazolam (0.25 mg/kg, ip). Further BR-16A (25, 50 mg/kg, po) did not potentiate the effects of alprazolam (5 mg/kg, ip) or zolpidem (0.5 mg/kg, ip). These agents may have other mechanisms besides GABAergic modulation.

These observations suggest that BR-16A may be used as sleep promoting herbal preparation and could be used along with other GABAergic hypnotics for the treatment of insomnia. This may reduce the dose of the latter drug(s).

References

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