Herbal Pharmacotherapy for the Attenuation of Electroconvulsive Shock-Induced Anterograde and Retrograde Amnestic Deficits

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SUMMARY
BR-16A is a herbal (non-allopathic) medication used in India to enhance cognition. Sixty adult male; Sprague Dawley rats received either BR-16A (200mg/kg/day) or vehicle alone for 16 days. During the first 7 days the rats were trained in a spatial memory task using the Hebb Williams complex maze. Once a day for the next 2 days, rats in BR-16A and control groups received either true or sham electroconvulsive shock (ECS). During the last 7 days of the study, the rats were re-exposed to the maze to assess recall or pre-ECS training and to evaluate further improvement in learning scores. BR-16A treated rats performed better than controls both before and after ECS. It is concluded that BR-16A facilitates learning and that this effect extends to a protection against ECS-induced anterograde and retrograde amnesia. BR-16A may hence hold promise in the restriction of ECT-induced cognitive compromise. An unexpected observation in this study was that BR-16A attenuated seizure duration; implications and mechanisms are discussed.

Electroconvulsive therapy (ECT) induces memory and non-memory cognitive deficits. Memory deficits with ECT include both anterograde and retrograde amnesia. The former describes impaired new learning post-ECT. Many pharmacological and nonpharmacological methods have been proposed to reduce ECT-induced memory impairment (Andrade, 1990; Nobler and Sackeim, 1993). Recently BR-16A, a herbal preparation marketed in India for the treatment of cognitive deficit states, was found to attenuate anterograde amnestic deficits induced by electroconvulsive shock (ECS) in rats; BR-16A has been discussed in other articles (Andrade et al., 1994a; Joseph et al., 1994).

The present study sought to confirm the findings of earlier research and more important to assess (in the animal model) whether BR-16A attenuates ECS-induced retrograde amnesia as well. Specifically, the experiment comprised (in sequence) training of rats in a spatial memory task, administration of ECS, and assessment of recall and further learning.
MATERIALS AND METHODS
Sixty adult male Sprague Dawley rats (180-200 g) were housed four per cage under a 12-h light/dark cycle (lights on at 6 a.m.) during the period of the study. In each cage, the rats were randomised two by two to receive either BR-16A in a freshly prepared aqueous suspension (200 mg/ml) or water alone. Administration was effected using an intragastric tube, in a volume of 1 ml/kg, once a day for 16 days. During the first 7 days of the study, all rats were trained in a spatial memory task using the Hebb Williams complex animal maze (Techno Electronics, Lucknow, India). Each day the rats received five consecutive trials of training in the maze.

The maze comprises a completely enclosed hexagonal box with an entry and a reward chamber appended at opposite ends. The box is partitioned with wooden slats into blind passages, leaving just one twisting corridor leading from the entry to the reward chamber. In each trial, the rat is placed in the entry chamber; a timer is activated just as the rat leaves the chamber. A pressure-sensitive plate detects the entry of the rat into the reward chamber and the timer is immediately deactivated. The five-trial total of the times taken by the rat to reach the reward (food pellet) chamber was taken as its learning score for the day. Since animals showing more efficient learning reach the reward chamber faster, higher scores indicate poorer learning or in rats receiving ECS, greater ECS-induced amnesia (Rao et al., 1991).

Rats in BR-16A and vehicle groups were randomised to receive either true or sham ECS. One true/sham ECS was administered on each of days 8 and 9 of the study. True ECS was administered through saline-soaked ear-clip electrodes using the Electrocon MK II 50 Hz-sinusoidal wave ECT device (Associated Electronics Engineering, Bangalore, India) at stimulus settings of 140 V x 0.6 s. The resultant seizures were timed by a single observer using a stopwatch. The motor seizure was defined as extending from the commencement of passage of current to the cessation of motor activity or the onset of asymmetrical/asynchronous limb movements, whichever occurred earlier. Sham ECS involved an identical procedure sans passage of current. From days 10 to 16, rats were re-exposed to the maze and performance was assessed as before.

To ensure adequate motivation during learning assessments, the animals were exposed to (ad lib) standard laboratory diet for just 1 hour a day, after the maze exposure for the day was completed. Access to water was unrestricted. This being a food-motivated experiment, note was made of changes in the animals’ body weight during the course of the study. No differences were observed between the groups, suggesting that there were no motivational or physiological biases. All procedures were conducted between 8 p.m. and 11 p.m. A single, “zero-watt” dim red bulb was used so that disturbance of the rats’ nocturnal cycle would be minimal.
**DATA ANALYSIS**

The learning/recall data were examined using two-way analysis of variance (ANOVA) and two- or three-way repeated-measures ANOVAs as appropriate. In the various analyses, day of assessment was the within-subjects factor, while BR-16A/vehicle and ECS/sham ECS were between-subjects factors. Raw scores were log-transformed wherever necessary to reduce heterogeneity of dispersion. Huynh-Feldt epsilon correction of degrees of freedom was used when required to protect against deviations from sphericity. Covariates were used in the analyses as specified. Seizure duration data were examined using two-way repeated-measures analysis of variance with day of ECS as the within-subjects factor and BR-16A/vehicle as the between-subjects factor. Association between cumulative seizure duration and recall scores was examined using Pearson’s product-moment correlation. Alpha for all inferences was set at 0.05.

**RESULTS**

Table 1 presents the mean plus or minus standard deviation (M ± SD) learning scores in BR-16A- and vehicle-treated rats on days 1-7 of the study (the training phase). A two-way ANOVA applied to the day 1 data found no significant main effect either for BR-16A or for ECS; the BR-16A x ECS interaction was also nonsignificant. This finding indicates that the groups did not differ significantly (in the learning parameter) at intake.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR-16A/ECS</td>
<td>55.7 (10.8)</td>
<td>42.0 (8.6)</td>
<td>30.8 (8.7)</td>
<td>19.9 (3.6)</td>
<td>17.9 (2.8)</td>
<td>16.8 (2.2)</td>
<td>15.9 (1.9)</td>
</tr>
<tr>
<td>BR-16A/sham ECS</td>
<td>58.2 (11.2)</td>
<td>40.9 (9.2)</td>
<td>30.7 (7.2)</td>
<td>21.5 (4.0)</td>
<td>18.6 (4.2)</td>
<td>19.8 (3.0)</td>
<td>15.1 (2.1)</td>
</tr>
<tr>
<td>Vehicle/ECS</td>
<td>59.8 (8.2)</td>
<td>45.3 (7.6)</td>
<td>32.7 (8.3)</td>
<td>26.0 (6.5)</td>
<td>22.9 (4.2)</td>
<td>19.9 (3.1)</td>
<td>17.9 (2.1)</td>
</tr>
<tr>
<td>Vehicle/sham ECS</td>
<td>57.1 (11.1)</td>
<td>43.1 (8.3)</td>
<td>32.6 (8.6)</td>
<td>24.8 (6.6)</td>
<td>20.9 (4.2)</td>
<td>19.1 (3.7)</td>
<td>16.5 (2.6)</td>
</tr>
</tbody>
</table>

Learning scores are the five-trial totals of the times taken by the rats to reach the reward chamber in the Hebb Williams maze; n=15 per group. Days refer to days of the study. Statistical inferences are presented in the text.

A three-way repeated-measures ANOVA applied to the day 1-7 data confirmed a significant main effect for time (day of assessment; $F df 3,168=926.43; p<0.001$), a significant main effect for BR-16A ($F df 1,56=7.58; p=0.008$), and a significant BR-16A x time interaction ($F df 3,168=4.57; p<0.01$). This finding indicates that there was significant learning across time and that learning was greater as well as quicker in BR-16A-treated rats.

The M ± SD seizure duration data in ECS-treated rats are presented in Table 2. A two-way repeated-measures ANOVA found a significant main effect for BR-16A ($F df 1,28=20.21; p<0.001$), indicating that BR-16A attenuated seizure duration. There was also a significant main
effect for day of ECS (F df 1,28=28.90; p<0.001), indicating that seizure duration declined on the second day of ECS compared with the first. The Br-16A x ECS interaction was not significant, indicating that BR-16A did not influence the degree of decrease in seizure duration from ECS 1 to ECS 2.

<table>
<thead>
<tr>
<th>Table 2: Mean ± standard deviation (range) seizure durations (secs) in BR-16A-ECS and vehicle-ECS groups on days 1 and 2 of ECS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>BR-16A/ECS</td>
</tr>
<tr>
<td>Vehicle/ECS</td>
</tr>
</tbody>
</table>

Statistical inferences are presented in the text; n=15 per group.

The M ± SD learning scores on days 10-16 of the study (the assessment phase) are presented in Table 3. Day 10 scores, the learning scores on the first day after ECS represented the degree of recall of previous learning or, in ECS-treated rats, the degree of ECS-induced retrograde amnesia. Learning scores on days 11-16 represent not only the degree of recall of pre-ECS learning but also the degree of renewed learning post-ECS. Hence, separate analyses were conducted, as we will describe.

A three-way repeated-measures ANOVA was conducted on the day 10 versus day 7 data to determine whether BR-16A influenced recall in the ECS group. The BR-16A x ECS x day of assessment interaction was significant (F 1,56=31.04; p<0.001), indicating that day 10 (recall) scores after true ECS were significantly lower (i.e., implying less retrograde amnesia) in rats that had received BR-16A.

It is conceivable that the longer seizures experienced by vehicle-treated rats could have been responsible for the greater retrograde amnesia in the vehicle-ECS group. The correlation of recall (day 10) scores with cumulative seizure duration was, however, not significant in both BR-16A-ECS (r=–0.019) and vehicle-ECS (r=–0.35) groups. The very direction of the correlations (negative) suggested that increased seizure duration was unrelated to impairment of recall. The correlation was not assessed for both ECS groups together because of a confounding variable (BR-16A treatment) that could have affected the dependent variable (recall scores) in the analysis.

To evaluate improvement in learning scores post-ECS (a measure of anterograde amnesia) in the BR-16A-ECS group, the days 10-16 data of this group were compared with those of the vehicle-sham ECS group. A two-way repeated-measures ANOVA with the day 7 scores taken as a covariate uncovered a significant main effect for day of assessment (F df 4,112=30.72, p<0.001), indicating that the two groups showed significant learning across time. The main effects for groups and the group x day of assessment interaction were not significant (p<0.30). This finding
indicates that intake of BR-16A permitted ECS-treated rats to learn at a rate comparable to controls. Examining the data of these two groups in Table 3 underlines their comparability.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
<th>Day 15</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR-16A/ECS</td>
<td>17.1 (2.2)</td>
<td>16.0 (1.8)</td>
<td>15.9 (1.8)</td>
<td>16.1 (2.2)</td>
<td>15.5 (1.7)</td>
<td>15.4 (2.0)</td>
<td>15.2 (1.7)</td>
</tr>
<tr>
<td>BR-16A/sham ECS</td>
<td>16.1 (2.4)</td>
<td>15.8 (2.5)</td>
<td>15.7 (2.6)</td>
<td>15.5 (2.5)</td>
<td>15.4 (2.5)</td>
<td>14.9 (2.2)</td>
<td>14.6 (2.0)</td>
</tr>
<tr>
<td>Vehicle/ECS</td>
<td>33.4 (10.1)</td>
<td>23.6 (6.7)</td>
<td>19.3 (2.6)</td>
<td>18.4 (2.3)</td>
<td>17.7 (2.3)</td>
<td>17.6 (1.7)</td>
<td>17.3 (1.9)</td>
</tr>
<tr>
<td>Vehicle/sham ECS</td>
<td>17.4 (2.9)</td>
<td>16.9 (2.8)</td>
<td>16.6 (2.6)</td>
<td>16.5 (2.5)</td>
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<td>16.0 (2.5)</td>
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Learning scores are the five-trial totals of the times taken by the rats to reach the reward chamber in the Hebb Williams maze; n=15 per group. Days refer to the days of the study. Statistical inferences are presented in the text.

**DISCUSSION**

At the outset, it must be understood that although BR-16A has been commercially marketed by the pharmaceutical industry in India, it has not been subjected to detailed preclinical assessment, and Phase I, II and III clinical trials have not been formally conducted. This is in keeping with the policy of the Government of India to encourage indigenous traditional forms of medicine. An offshoot of such a policy is the potential discovery of new molecules—consider, for example, the isolation of reserpine from the root of *Rauwolfia serpentina* more than half a century ago. Research on BR-16A is beginning to burgeon because of the potential psychotropic applications of the preparation.

The first interesting observation in this study is that BR-16A enhanced learning in naïve rats (however, the data in Table 1 show that the advantage with BR-16A, though statistically significant, was small). There is some support in the literature for this result. Using an identical complex maze paradigm, we found that BR-16A accelerated learning in Wistar rats (Joseph, *et al.*, 1994). A preliminary study in normal volunteers found BR-16A to improve memory parameters after 12 weeks of administration at a dose of 350 mg twice daily (Agrawal *et al.*, 1990a, b).

The second interesting finding in this study is that BR-16A attenuated ECS-induced retrograde amnesia. In a differently designed experiment, studying trials to satisfactory learning and wrong-arm entries in rats exposed to a T-maze learning paradigm, we found that BR-16A (200 mg/kg/day) significantly lessened retrograde amnestic deficits induced by two ECS treatments spread 5 h apart (Andrade *et al.*, 1995).
The third interesting (though somewhat indirect) observation in this study is that BR-16A attenuated ECS-induced anterograde amnesia. To demonstrate the protective effect of BR-16A herein, no comparison of the BR-16A-ECS group data could be drawn against the vehicle-ECS group since the latter group had already suffered significant ECS-induced retrograde amnesia. Instead, the BR-16A-ECS group was compared with the vehicle-sham ECS group, which had no amnestic deficits. Scores in the two groups did not differ overall and changed in parallel across time, indicating a protection against ECS-induced learning deficits in the BR-16A-ECS group. In both T- and complex maze models. BR-16A has been shown to protect against anterograde amnesia induced in rats by six once-daily ECS treatments (Andrade et al., 1994a; Joseph et al., 1994).

The fourth interesting finding in this study is that BR-16A attenuated seizure duration. Such a finding has not been reported earlier. There is only one other study (Andrade et al., 1995) that has reported seizure duration data for BR-16A in ECS-treated animals; no differences were observed between BR-16A- and vehicle-treated rats. A possible explanation is that the earlier study used much lower stimulus doses (120 V x 0.5 s) than our study (140 V x 0.6 s). It is hypothetically conceivable that at lower stimulus doses a floor effect results in the lack of a perceivable BR-16A influence on seizure duration. Unpublished material, however, documents that BR-16A shortens chemically induced seizures and that BR-16A attenuates the duration of breakthrough seizures in epileptics on anticonvulsant therapy (The Himalaya Drug Company, personal communication).

The mechanism whereby BR-16A exerts this inhibitory effect on seizures is unknown; however, it may differ from the mechanism by which ECS exerts its anticonvulsant action. From the analysis of the seizure duration data, this conclusion is suggested by the finding of significant main effects for ECS and for BR-16A, along with the finding of an absence of a significant ECS-BR-16A interaction.

Cumulative seizure duration did not correlate positively or significantly with post-ECS maze scores, and assuming that seizure duration is a sufficiently representative index of seizure inhibition, this result suggests that the anti-amnestic action of BR-16A may be unrelated to its seizure inhibitory mechanisms.

The mechanism of action of BR-16A is unclear. Cholinergic facilitation (augmentation of cholinergic neurotransmission) may be involved, since the drug reverses scopolamine-induced memory deficits (Verma and Kulkarni, 1991; Kulkarni and Verma, 1992a). BR-16A may also influence opioid neurotransmission; Kulkarni and Verma (1992b) found that the drug prevented the development of tolerance and dependence to morphine in mice. Preliminary work indicates no effects on alpha-2 noradrenergic receptors and dopamine autoreceptors but a possible sensitization of dopamine postsynaptic receptors (Andrade et al., 1994b).
BR-16A has a high therapeutic index. No mortality has been observed in mice even at a dose of 1,600 mg/kg; compare this amount with the recommended human dose of 15-30 mg/kg/day. The drug does not influence the course or outcome of pregnancy, nor does it produce teratogenic effects in Wistar rats even at 2,000 mg/kg/day for 20 days. In humans, long-term use of BR-16A does not produce impairments in cardiophysiological, hematological, and biochemical indexes (The Himalaya Drug Company, 1991). More than 4 years of experience with the drug after marketing have not resulted in the recording of any adverse effects of note (The Himalaya Drug Company, data on file).

In conclusion, it appears from this study that BR-16A enhances learning and that this cognition-enhancing effect offers protection against ECS-induced anterograde and retrograde amnesia in rats. It is hoped that BR-16A may be of use in the attenuation of the cognitive deficits induced by electroconvulsive therapy. The possible anticonvulsant actions of BR-16A merit further investigation.

REFERENCES


