Experimental Evaluation of Newer Non-Benzodiazepine (BZD) Hypno-Sedatives Using Different Animal Models

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ABSTRACT

Objectives: To evaluate hypno-sedative action of ‘Z’-drugs; Zopiclone and Zolpidem by using animal models for hypnosis and sedation (anxiolysis); and to further evaluate their effect on memory-learning in albino mice.

Methods: Pharmacological assays used for evaluating hypno-sedative action are righting reflex test, Pentobarbitone sleeping time potentiation test, Open field apparatus (OFT) and Elevated plus maze (EPM). While EPM retention test was used to study effect on memory-learning in albino mice. Data analyzed by student’s ‘t’-test and Chi-square (X²) test; p<0.05 considered significant.

Results: Zopiclone (7.5mg/kg) and Zolpidem (10mg/kg) p.o. did not inhibit the Righting reflex however cause significant (p<0.001) potentiation of Pentobarbitone sleeping time. Both the drugs significantly (P<0.001) increased ambulation and exploration in OFT as well as performance in EPM suggestive of anxiolysis. Whereas significant (p<0.05) decrease in transfer latency (TL) on Day II in EPM retention test was observed only with Zopiclone treated group of animals.

Conclusions: Zopiclone (7.5mg/kg) and Zolpidem (10mg/kg) both have promising selective hypno-sedative activity compared to BZDs. Zopiclone however scores over Zolpidem with respect to lack of memory-learning impairment.

Key Words: Righting reflex test, Zopiclone, Zolpidem, OFT, EPM

INTRODUCTION

Insomnia is a highly prevalent (10% to 30% of the general population) sleep disorder which is characterized by difficulties in initiating and/or maintaining sleep.[1] Benzodiazepines (BZDs) discovered in 1950’s have been mainstay of therapy for insomnia and anxiety, since then a large number of BZD analogues have been synthesized to obtain superior risk/benefit ratio. Most of the BZDs have similar pharmacological actions e.g. Hypnosedative-anticonvulsant-muscle relaxant etc. however they lack in specificity and associated with the problem of dependence, tolerance and impaired memory learning which have necessitated the search for alternatives.[2] Hence Novel, safe, and efficient hypnotic compounds capable of enhancing physiological sleep are still in great demand in the therapy of insomnia. [3] Zopiclone (Cyclopyrrolone) and Zolpidem (Imidazopyridine) i.e. ‘Z’-drugs, are newer Anxiolytic-hypnotic with chemical structure completely different from BZDs (see Fig1). Qualitatively they possess similar pharmacological profile as BZDs. Act at GABA-BZD-Cl--ionophore-receptor even then relatively more specific in action i.e. more potent Hypnosedative and weak anticonvulsant-muscle relaxant at hypno-sedative doses. This specificity is due to its distinctive binding mechanism at the site close to rather than identical to the site occupied by BZDs (see Fig 2). [3-7]

Zopiclone and Zolpidem have a low dependence liability. Thus, with their short duration of action and good tolerability profile compared to BZDs, ‘Z’-drugs could be a good alternative to the benzodiazepine hypnotics and may be particularly beneficial in those patients unable or unwilling to tolerate the residual effects associated with many other hypnotic agents.[9, 10]

Present study is a single dose pharmacodynamic study designed to evaluate hypno-sedative action of ‘Z’-drugs; Zopiclone and Zolpidem by using animal models for hypnosis and sedation (anxiolysis), and to further evaluate whether the hypno-sedation caused by these drugs impairs memory-learning in albino mice like conventional Hypnosedatives.
MATERIALS AND METHODS

Present study has been conducted at Government Medical College, Miraj. The experimental protocol was approved by Institutional Animal Ethics Committee.

Experimental animals: Albino mice of either sex weighing 20-25gms, bred in Central Animal House (CAH) facility of the Government Medical College, Miraj were used for the study. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to food and water. They were acclimatized to laboratory conditions before the experiment. Pre-experiment screening for righting reflex was done 1 day prior to rule out CNS disco-ordination. The animals that show positive righting reflex were selected for study. Each animal was used once in every experiment except in experiments for testing memory and learning. All experiments were carried out in daylight.

Drugs and doses: Doses were selected from earlier studies.\textsuperscript{6-8} Lorazepam (Ativan 2mg tablet obtained
from Wyeth Lederle Ltd.) dissolved in DW given orally in the dose of 5mg/kg. Zopiclone (Zopiclon 7.5mg tablet; obtained from Intas Pharmaceuticals Ltd.) suspended in 0.25% CMC, given orally in the dose of 7.5mg/kg, Zolpidem (Ambien 10mg tablet; obtained from Sanoﬁ Aventis Ltd.) suspended in a 0.3% Tween 80/saline solution, given orally in the dose of 10mg/kg and Pentobarbitone sodium dissolved in DW; given IP in the dose of 40mg/kg.

Test methods: Animals were divided into various groups in such way that 6 animals were there in each group. Group A received 0.1ml NS orally served as control for all experiments except righting reflex test where animals received Pentobarbitone (40mg/kg) IP as control, Group B received Lorazepam (5mg/kg) served as standard, test Groups C & D received Zopiclone (7.5mg/kg) and Zolpidem (10mg/kg) respectively. Each animal was treated with respective drug 30 min before experimentation. Following are the details of experiments performed,

Righting reflex test: Drugs like barbiturates induce hypnosis by CNS depression easily determined by loss of righting reflex. In righting reflex test animal is are kept gently on its back over an undulated surface, normally it corrects immediately; if retained on back for 30secs or more it is recorded as loss of righting reflex. Loss of righting reflex is taken as index of CNS depression[9].

Pentobarbitone sleeping time potentiation: Pentobarbitone (barbiturate) produces quick onset of sleep as indicated by loss of righting reflex and recovery is also easily detected as the animal regain righting reflex. Animals in all three groups received the respective drugs and 30 min later treated with Pentobarbitone (40mg/kg) IP. The time interval between loss of righting reflex and reappearance of righting reflex is recorded as duration of sleep. The animal that corrects itself 3 times in 1 min is considered to have recovered from drug effect[11].

Open field apparatus behavior (OFT): Open field apparatus (OFA) is designed as described by Gray and Lalji (1971) with little modifications. Dimensions are 100cm x 100cm x 40cm made up of themacol open from top and bottom kept on white table top; surface is divided into 25 equal squares i.e. 9 central and 16 peripheral. The animals were pretreated with the samples (Zopiclone7.5mg/kg, Zolpidem 10mg/kg and reference drugs) 30min before. During 5 min session of observation, each animal is placed in the corner of open field apparatus & behavior of animal as determined by ambulation (number of squares entered with both forelimbs) and, exploration (number of central squares entered) was recorded.[2]

Elevated plus maze performance: The apparatus consisted of two open arms (16 cm x 5 cm) and two closed arms (16 cm x 5 cm x 12 cm). The closed arms were painted black. The arms extended from a central platform (5 cm x 5 cm), and maze was elevated to a height of 25 cm from the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 12 cm in height. The drugs and treatments were same as mentioned under OFT. The animal was placed at the center of the maze, facing one of the closed arms. During 5 min test period the following parameters were recorded, 1. The number of entries into open arms 2.Time spent in the open arms 3. Number of animal giving preference to open arm as first arm entry. Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room[12-15].

Elevated plus maze retention test: The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the closed arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the closed arm within 90 sec., it was gently pushed into one of the two closed arms and the TL was assigned as 90 sec. Following entry into the closed arm the animals were allowed to explore the apparatus for 30 sec and then returned to their home cages. Memory retention was examined 24 h after the first day trial on the second day[13].

Statistical analysis: Data analyzed by Student’s ‘t’-test and Chi-square(X²) test. All the results were expressed as mean (±SEM). P <0.05 was considered significant.

RESULTS

Righting reflex test: Zopiclone(7.5 mg/kg), Zolpidem(10mg/kg) did not inhibit righting reflex like Pentobarbitone (Table 1).

Pentobarbitone sleeping time potentiation: In the Pentobarbitone sleep potentiation test, Zopiclone and Zolpidem significantly increased the sleeping time in mice at dose of 7.5 mg/kg and 10 mg/kg respectively compared to control (P<0.001). Wherein Zopiclone was found to be more potent in
this regard compared to Lorazepam (5mg/kg) and Zolpidem (10mg/kg); P<0.05 respectively, indicating potent hypnotic activity (Table 1). **Open field apparatus behavior (OFT):** In OFT both the ‘Z’-drugs significantly increased ambulation and exploration (P<0.001). This activity was comparable to Lorazepam (Table 2).

**Elevated plus maze performance:** Zopiclone (7.5 mg/kg) showed significant Anxiolytic activity in Elevated plus maze performance test compared to Lorazepam as indicated by increased number of entries into open arms, time spent in the open arms and number of animal giving preference to open arm as first arm entry while this activity of Zolpidem (10mg/kg) was comparable to Lorazepam (Table 2).

**Elevated plus maze retention test:** Zopiclone (7.5mg/kg) showed lower transfer latency (TL) values on second day retention test (after 24 h) similar to control group indicating lack of impairment in learning and memory. Zolpidem (10mg/kg) and Lorazepam (5mg/kg) however showed insignificant shortening of TL on next day indicating impairment in learning and memory (Table 3).

Table 1: Observations in Righting reflex test & Pentobarbitone sleeping time potentiation test:

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Treatment</th>
<th>Righting reflex</th>
<th>Treatment</th>
<th>Duration of sleep(min) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control (Pentobarbitone 40mg/kg)</td>
<td>Absent</td>
<td>Control NS(0.1ml)</td>
<td>63.3±1.2</td>
</tr>
<tr>
<td>B</td>
<td>Lorazepam (5 mg/Kg)</td>
<td>Present</td>
<td>Lorazepam (5 mg/Kg)</td>
<td>85.8 ± 2.2*</td>
</tr>
<tr>
<td>C</td>
<td>Zopiclone (7.5 mg/Kg)</td>
<td>Present</td>
<td>Zopiclone (7.5 mg/Kg)</td>
<td>a b 98.5 ± 4.8*</td>
</tr>
<tr>
<td>D</td>
<td>Zolpidem</td>
<td>Present</td>
<td>Zolpidem</td>
<td>86.5 ± 2.8*</td>
</tr>
</tbody>
</table>

Each group consists of 6 animals. Values are Mean ± SEM, data analyzed by student’s ‘t’ test. (*P<0.001 compared to control; a P<0.05 compared to Lorazepam and b P<0.05 compared to Zolpidem)

Table 2: Observations in Elevated plus maze performance & (OFT):

<table>
<thead>
<tr>
<th>Treatment Groups (n=6)</th>
<th>Dose mg/Kg</th>
<th>Number of animals giving preference to open arm Mean ± SEM</th>
<th>Number of entries to open arm Mean ± SEM</th>
<th>Total time spent in open arm Mean ± SEM</th>
<th>Number of central squares entered Mean ± SEM</th>
<th>Number of squares entered Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>NS(0.1ml)</td>
<td>1</td>
<td>4.5±0.43</td>
<td>2.7±0.25</td>
<td>4.5±0.43</td>
<td>22.4±1.24</td>
</tr>
<tr>
<td>Lorazepam (B)</td>
<td>5</td>
<td>4*</td>
<td>6±0.58</td>
<td>3.7±0.25*</td>
<td>13±1.5 ***</td>
<td>43.4±1.79***</td>
</tr>
<tr>
<td>Zopiclone (C)</td>
<td>7.5</td>
<td>5**</td>
<td>6.8±0.48**</td>
<td>3.8±0.31*</td>
<td>10.3±0.67 ***</td>
<td>43.4±1.79***</td>
</tr>
<tr>
<td>Zolpidem (D)</td>
<td>10</td>
<td>4*</td>
<td>6.1±0.26</td>
<td>3.7±0.28*</td>
<td>12±0.5 ***</td>
<td>42.3±1.24***</td>
</tr>
</tbody>
</table>

Each group consists of 6 animals. Values are Mean ± SEM, data analyzed by Chi-square(X²) and student’s ‘t’-test. (*P<0.05, **P<0.01, ***P<0.001 compared to control)

Table 3: Observations in Elevated plus maze retention test:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose mg/Kg</th>
<th>Transfer latency(secs) 1st day (Mean ± SEM)</th>
<th>Transfer latency (secs) 2nd day (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>NS(0.1ml)</td>
<td>50 ± 9.2</td>
<td>39.1±6.8*</td>
</tr>
<tr>
<td>B</td>
<td>Lorazepam</td>
<td>5</td>
<td>75 ± 6.7</td>
<td>71.7 ± 7.4</td>
</tr>
<tr>
<td>C</td>
<td>Zopiclone</td>
<td>7.5</td>
<td>90 ± 0</td>
<td>35.8 ± 11.9*</td>
</tr>
<tr>
<td>D</td>
<td>Zolpidem</td>
<td>10</td>
<td>88 ± 5.8</td>
<td>82.6 ± 6.2</td>
</tr>
</tbody>
</table>
DISCUSSION

Righting reflex and Pentobarbitone sleeping time potentiation test: Barbiturate like drugs produce hypnosedation by CNS depression determined by absence of righting reflex; however hypnosedation produced by BZDs did not inhibit righting reflex suggestive of more selective actions and lack of neuronal depression. Present study clearly demonstrated that Zopiclone (7.5mg/kg) and Zolpidem (10mg/kg) didn’t cause neuronal depression. Furthermore Zopiclone showed potent hypnotic activity determined by significant potentiation of Pentobarbitone sleeping time compared to Lorazepam while Zolpidem showed potentiation comparable to Lorazepam. This potent hypno-sedative activity of Zopiclone is thought to be due to its potent agonistic activity at omega-1 receptor subtype of central BZD-receptor[16]. Earlier few studies concluded that Zolpidem impart hypnosedation comparable to BZDs suggestive of similar or overlapping site of action on GABA-A receptor. However unlike BZDs its hypno-sedative action was observed at much lower doses than myorelaxant and anticonvulsant actions. [6,7].

Though these actions are antagonized by BZDs antagonist flumazenil its significant selectivity for hypno-sedation is suggestive of specific interaction with omega-1 receptor subtype of central BZD-receptor like Zopiclone[10].

Open field apparatus behavior (OFT): Exploration in a new environment is an essential part of normal behavior it is determined by ambulation in OFA[17]. Animals show lower ambulation values in new environment due to anxiety and fear. Disinhibitory actions of anxiolytics increase ambulation in new environment by releasing novelty induced suppression of behavior[2]. Present study clearly demonstrated that Zopiclone (7.5mg/kg) and Zolpidem (10mg/kg) had significant anxiolytic activity and it was comparable to Lorazepam (5 mg/kg).

Elevated plus maze performance: Animal dislike the open arm and high spaces hence spent more time in closed arm this natural aversion quality become apparent when it enters them. This is the basis for its use in the measurement of anxiety[12]. In present study, Zopiclone (7.5 mg/kg) showed significant anxiolytic activity in Elevated plus maze performance test compared to Lorazepam as indicated by increased number of entries into open arms, time spent in the open arms and number of animal giving preference to open arm as first arm entry. Zolpidem (10mg/kg) however showed effects comparable to Lorazepam this could be due to inadequate dose of Zolpidem.

Elevated plus maze retention test: If the animal has previous experience of entering open arm the TL might be shortened. Shortened TL on second day of experiment is then related to memory and learning. Increased TL is taken as index of impaired memory and learning[13]. Present study clearly showed that Zopiclone (7.5mg/kg) induced hypnosedation did not impair memory and learning (Shortened TL). Central cholinergic system plays an important role in learning and memory; its facilitatory effect on retention of acquired learning also suggestive of cholinomimetic activity[13]. However in contrast to our study; some studies reported that BZDs and ‘Z’- drugs did show effect on short term memory[18,19]. Lack of memory and learning impairment might be due to short duration of action of Zopiclone (5-6hrs) compared to Lorazepam (10-18hrs) and Zolpidem (8-12hrs). [6,7,10].

CONCLUSION

In the light of results of present study it can be concluded that both the ‘Z’-drugs have better and more selective hypno-sedative action compared to conventional hypno-sedatives used in insomnia. However Zopiclone appears to be superior to Zolpidem in this regard as it exhibited effective hypno-sedation with no impairment of memory-learning. To confirm the findings of present study further long term studies with multiple dose ranges need to be taken up as it’s a single dose pharmacodynamic study. Results of this study could be a noteworthy accumulation in the data on newer emerging hypno-sedative class of drugs.

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