Zopiclone (Cyclopyrrolone): A Novel Hypnosedative; Hypnosedation Caused by Zopiclone Does Not Impair Memory-Learning in Albino Mice

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Keywords

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SUMMARY

Objectives: To evaluate hypnosedative action of Zopiclone by using animal models for hypnosis and sedation (anxiolysis); and to further evaluate whether this hypnosedation impairs memory-learning in albino mice like conventional hypnosedatives. **Methods:** For evaluation of hypnosedation, following experiments were performed in albino mice: (1) righting reflex test, (2) pentobarbitone sleeping time potentiation, (3) open field apparatus behavior, and (4) elevated plus maze performance. For evaluation of effects on impairment of memory-learning, elevated plus maze retention test was performed in albino mice. **Results:** Zopiclone (7.5 mg/kg p.o.) did not inhibit the righting reflex. Significant ($P < 0.001$) potentiation of pentobarbitone sleeping time and increase in exploration in open field apparatus was observed. Elevated plus maze performance also showed significant ($P < 0.01$) increase in number of entries to open arm at the same time significant ($P < 0.02$) increase in time spent in open arm was observed. Elevated plus maze retention test showed significant ($P < 0.01$) increase in transfer latency on second day of experiment. **Conclusions:** Zopiclone (7.5 mg/kg p.o.) has selective hypnosedative activity but not CNS-depressant activity similar to BZDs. Hypnosedative action of Zopiclone does not impair memory-learning in albino mice like conventional hypnosedatives.

Introduction

Benzodiazepines (BZDs) discovered in 1950s 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, for example, 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 [1].

Zopiclone (Cyclopyrrolone) is a new anxiolytic-hypnotic with chemical structure completely different from BZDs (Figure 1). Qualitatively, it possesses similar pharmacological profile as BZDs. It acts at GABA-BZD-Cl⁻-ionophore receptor. Even then rela-

tively more specific in action, that is, more potent hypnosedative and weak anticonvulsant-muscle relaxant. This specificity is because of its distinctive binding mechanism at the site close to rather than identical to the site occupied by BZDs (Figure 2) [2–4].

Zopiclone has a low dependence liability. Thus, with its short duration of action and good tolerability profile, Zopiclone could be a good alternative to the benzodiazepine hypnotics and may be particularly beneficial in those patients who are unable or unwilling to tolerate the residual effects associated with many other hypnotic agents [5].

Aim and Objectives

This study is a single-dose pharmacodynamic study designed to evaluate hypnosedative action of Zopiclone by

Figure 1 Chemical structures of Zopiclone, Lorazepam, and Benzodiazepines.

Figure 2 Diagrammatic presentation of GABA-BZD-chloride-ionophore complex and site of action of cyclopyrrolones and other drugs.

using animal models for hypnosis and sedation (anxiolysis); and to further evaluate whether this hypnosedation impairs memory-learning in albino mice like conventional Hypnosedatives.

Materials and Methods

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

Experimental Animals

Albino mice of either sex weighing 20–25 g, 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. Preexperiment screening for righting reflex was done 1 day prior to rule out CNS discoordination. The animals those 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. Lorazepam (Ativan 2 mg tablet obtained from Wyeth Lederle Ltd., Goa, India) dissolved in DW given orally in the dose of 5mg/kg. Zopiclone (Zopicon 7.5 mg tablet; obtained from Intas Pharmaceuticals Ltd., Gujarat, India) suspended in 0.25% CMC, given orally in the dose of 7.5 mg/kg and pentobarbitone sodium dissolved in DW; given IP in the dose of 40 mg/kg.

Test Methods

Animals were divided into various groups in such a way that six animals were there in each group. Group A received 0.1 mL NS orally served as control for all experiments except righting reflex test where animals received pentobarbitone (40 mg/kg) i.p. as control, Group B received lorazepam (5 mg/kg) served as standard, and Group C received zopiclone (7.5 mg/kg). Each animal was treated with respective drug 30 min before experimentation. Following are the details of experiments performed.

Righting Reflex Test

Drugs such as barbiturates induce hypnosis by CNS depression easily determined by loss of righting reflex. In righting reflex test, animals are kept gently on its back over an undulated surface, normally it corrects immediately; if retained on back for 30 seconds or more it is recorded as loss of righting reflex. Loss of righting reflex is taken as index of CNS depression [6].

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 (40 mg/kg) i.p. The time interval between loss of righting reflex and

reappearance of righting reflex is recorded as duration of sleep. The animal that corrects itself three times in 1 min is considered to have recovered from drug effect [7].

Open Field Apparatus Behavior (OFT)

Open field apparatus (OFA) is designed as described by Gray and Lalji (1971) with little modifications. Dimensions are 100 cm × 100 cm × 40 cm, made up of themacol open from top and bottom kept on white table top; surface is divided into 25 equal squares, that is, 9 central and 16 peripheral. The animals were pretreated with the samples (Zopiclone 7.5 mg/kg and reference drugs) 30 min before. During 5 min session of observation, each animal is placed in the corner of open field apparatus and behavior of animal as determined by ambulation (number of squares entered with both forelimbs) and, exploration (number of central squares entered) was recorded [1].

Elevated Plus Maze Performance

The apparatus consisted of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 12 cm). The closed arms were painted black. The arms extended from a central platform (5 cm × 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, and (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 [8–11].

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 seconds, it was gently pushed into one of the two closed arms and the TL was assigned as 90 seconds. Following entry into the closed arm the animals were allowed to explore the apparatus for 30 seconds and then returned to their home cages. Memory retention was examined 24 h after the first day trial on the second day [9].

Statistical Analysis

Data analyzed by Student's unpaired t-test and Chi-square (χ^2) test. All the results were expressed as mean ($\pm$ SEM). $P < 0.05$ was considered significant.

Results

Righting Reflex Test

Zopiclone did not inhibit righting reflex at dose of 7.5 mg/kg like pentobarbitone (Table 1).

Pentobarbitone Sleeping Time Potentiation

In the potentiation of pentobarbitone sleep test, Zopiclone significantly increased the sleeping time in mice at dose of 7.5 mg/kg compared to controls ($P < 0.001$) and Lorazepam 5 mg/kg ($P < 0.05$), indicating potent hypnotic activity (Table 1).

Open Field Apparatus Behavior (OFT)

In OFT, Zopiclone significantly increased ambulation and exploration ($P < 0.001$). This activity was comparable to Lorazepam (Table 2).

Table 1 Observations in righting reflex test and pentobarbitone sleeping time potentiation test

Groups (n = 6)	Treatment	Righting reflex	Treatment	Duration of sleep (min) (mean $\pm$ SEM)
A	Control (pentobarbitone 40 mg/kg)	Absent	Control NS (0.1 mL)	63.3 $\pm$ 1.2
B	Lorazepam (5 mg/kg)	Present	Lorazepam (5 mg/kg)	85.8 $\pm$ 2.2*
C	Zopiclone (7.5 mg/kg)	Present	Zopiclone (7.5 mg/kg)	^a 98.5 $\pm$ 4.8*

Each group consists of six animals. Values are mean $\pm$ SEM, data analyzed by Student's unpaired t-test.

^a $P < 0.05$ compared to Lorazepam.

* $P < 0.001$ compared to control.

Table 2 Observations in elevated plus maze performance and OFT

Treatment groups (n = 6)	Dose (mg/kg)	Elevated plus maze performance			Open field apparatus behavior (OFT)	
		^a Number of animals giving preference to open arm	^b Number of entries to open arm (mean $\pm$ SEM)	^b Total time spent in open arm (mean $\pm$ SEM)	^b Number of central squares entered (mean $\pm$ SEM)	^b Number of squares entered (mean $\pm$ SEM)
Control (A)	NS (0.1 mL)	1	4.5 $\pm$ 0.43	2.7 $\pm$ 0.25	4.5 $\pm$ 0.43	22.4 $\pm$ 1.24
Lorazepam (B)	5	4**	6 $\pm$ 0.58	3.7 $\pm$ 0.25**	13 $\pm$ 1.5*****	43.4 $\pm$ 1.79*****
Zopiclone (C)	7.5	5****	6.8 $\pm$ 0.48***	3.8 $\pm$ 0.31**	10.3 $\pm$ 0.67*****	43.4 $\pm$ 1.79*****

Each group consists of six animals. Values are mean $\pm$ SEM.

^aData analyzed by Chi-square (χ^2).

^bStudent's unpaired t-test.

* $P < 0.05$ compared to control.

** $P < 0.02$ compared to control.

*** $P < 0.01$ compared to control.

**** $P < 0.005$ compared to control.

***** $P < 0.001$ compared to control.

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 (Table 2).

Elevated Plus Maze Retention Test

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

Discussion

Righting Reflex Test and Pentobarbitone Sleeping Time Potentiation

Barbiturate like drugs produce hypnosis by CNS depression determined by absence of righting reflex; however, hypnosis produced by BZDs did not inhibit

righting reflex suggestive of more selective actions and lack of neuronal depression. This study clearly demonstrated that Zopiclone (7.5 mg/kg) did not cause neuronal depression. Furthermore, it showed potent hypnotic activity determined by significant potentiation of Pentobarbitone sleeping time compared to Lorazepam. This potent hypnosedative activity is thought to be due to its potent agonistic activity at omega-1 receptor subtype of central BZD-receptor [12].

Open Field Apparatus Behavior (OFT)

Exploration in a new environment is an essential part of normal behavior it is determined by ambulation in OFA [13]. 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 [1]. This study clearly demonstrated that Zopiclone (7.5 mg/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

Table 3 Observations in elevated plus maze retention test

Groups	Treatment	Dose (mg/kg)	Transfer latency (seconds) first day (mean $\pm$ SEM)	Transfer latency (second) second day (mean $\pm$ SEM)
A	Control	NS (0.1 mL)	50 $\pm$ 9.2	39.1 $\pm$ 6.8*
B	Lorazepam	5	75 $\pm$ 6.7	71.7 $\pm$ 7.4
C	Zopiclone	7.5	90 $\pm$ 0	35.8 $\pm$ 11.9*

Each group consists of six animals. Values are mean $\pm$ SEM, data analyzed by Student's unpaired t-test.

* $P < 0.05$ compared to TL on Day 1.

quality become apparent when it enters them. This is the basis for its use in the measurement of anxiety [8]. In this 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.

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 [9]. This study clearly showed that Zopiclone (7.5 mg/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 [9]. However, in contrast to our study, some studies reported that BZDs and Zopiclone did show effect on short-term memory [14]. Lack of memory and learning impairment might be due to short duration of action of Zopiclone (5–6 h) compared to Lorazepam (10–18 h).

Authors' Contributions

All authors read and approved the final manuscript. UK as an investigator in the study drafted the manuscript and has made a substantial contribution to acquisition of data. AB contributed in data acquisition and analysis.

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