



**A COMPARATIVE STUDY OF THE EFFECT OF ONDANSETRON,
GRANISETRON AND ALPRAZOLAM ON ANXIETY IN WISTAR RATS.**

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ABSTRACT : Objective: The study was undertaken to compare the effect of ondansetron, granisetron and alprazolam on anxiety in rats.

Materials and Methods: Elevated plus maze and Open field test were used to compare the effect of drugs on anxiety in rats. Six groups of rats were treated with 2% gum acacia orally, alprazolam 0.08mg/kg body weight of the rat orally, ondansetron (0.01mg/kg, 1mg/kg intraperitoneally) and granisetron (0.01mg/kg and 1mg/kg intraperitoneally) respectively. The time spent, number of entries

and rears in the arms of the elevated plus maze and central and peripheral areas in the open field test

Results: Alprazolam and ondansetron significantly increased ($P < 0.05$) the time spent in open arm of elevated plus maze and central squares in the open field and decreased ($P < 0.05$) the time spent in the closed arm of elevated maze and peripheral squares in the open field as compared to control. There was no significant difference between the effects of alprazolam and ondansetron. Granisetron did not produce any significant effect in any of the models.

Conclusion: Ondansetron, but not granisetron, produced anxiolytic activity in rats which was comparable to alprazolam.

Key words: anxiolytic, ondansetron, granisetron, alprazolam, elevated plus maze, open field.

INTRODUCTION

Anxiety is a psychological and physiological disorder characterized by fear, apprehension, poor concentration, etc. Somatic manifestations of anxiety include palpitation, tachycardia, tremor, sweating and hyperventilation. Complaints involving various organ system is common (Eisendrath SJ, 2009). The commonly used classes of drugs for the treatment of anxiety are benzodiazepines, azapirones, selective serotonin reuptake inhibitors (SSRIs) and beta blockers (Eisendrath SJ, 2009).

The benzodiazepines are the drugs of choice for the treatment of generalized anxiety as they provide immediate relief. But, there is a potential for abuse and dependence, which limits the usefulness of these drugs (Baldessarini RJ, 2006). SSRIs are perhaps more effective than benzodiazepines in the long-term treatment of generalized anxiety disorders (DeBattista, 2009). Patients treated with SSRIs report loss of libido, delayed orgasm, diminished arousal, insomnia, hypersomnia, weight gain, etc (Baldessarini RJ, 2006). Azapirones are effective in mild to moderate generalized anxiety, but are ineffective in severe cases (Eisendrath SJ, 2009). Beta blockers block autonomic symptoms but do not affect psychological symptoms (Baldessarini RJ, 2006).

Both adrenergic and serotonergic systems in the CNS play a role in the pathophysiology of anxiety (Baldessarini RJ, 2006). An earlier study demonstrated that ondansetron and granisetron decreased fear potentiated startle response in rats (Nevins ME and Anthony EW, 1994). Hence, this study was undertaken to compare the effect of ondansetron, granisetron and alprazolam on anxiety in elevated plus maze and open field test in rats.

MATERIALS AND METHODS

Drugs

Alprazolam, 1mg tab (Torrent Pharmaceuticals Ltd., Ahmedabad), Ondansetron 2 mg/ml vial (Cipla Pharmaceuticals Ltd., Mumbai), Granisetron 1 mg/ml vial (Sun Pharmaceutical Industries Ltd., Mumbai), were purchased from the pharmacy of Kasturba Hospital, Manipal.

Animals

Adult male, wistar albino rats weighing 150-200 g were used in the study. The rats were maintained under standard conditions in Central Animal House, Manipal, approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The rats were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard environmental conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle. Experiments were performed during the dark cycle.

Experimental Design

The study was carried out after obtaining approval by the Institutional Animals Ethics Committee. Thirty six rats were used in this study. They were divided into six groups of six animals each.

The treatment schedule was as follows –

Group I- control, received gum acacia 10 ml/kg body weight of the rat orally.

Group II- standard, received alprazolam orally, in a dose of 0.08mg/kg body weight of the rat.

Group III & IV- test group, received ondansetron intraperitoneally (i.p.), in a dose of 0.01 mg/kg and 1mg/kg body weight of the rat respectively.

Group V & VI- test group, received granisetron intraperitoneally (i.p.), in a dose of 0.01 mg/kg and 1mg/kg body weight of the rat respectively.

All the drugs were administered daily for 10 days in doses based on a previous study (Jung ME, et al.,

2000; Wright IK, et al., 1992). Two models were used in this study to compare the effect of the drugs on anxiety in rats – elevated plus maze (EPM) and open field test (OFT). The test was carried out 45

minutes after the last dose of the drugs on the 10th day. The apparatus in each model was wiped with 10% ethanol after trial with each rat to eliminate possible bias due to odor of previous animal.

Elevated plus maze (EPM)

The Elevated plus maze apparatus has two closed arms (50×10×40 cm) and two open arms (50×10 cm) with an open roof, arranged around a central square (10×10 cm). The arms of the same type are opposite to each other. The entire maze is raised 50 cm above the ground. The drugs were administered to the rats as mentioned above. On the 10th day, 45 minutes after drug administration, the rat was placed in the central square of the maze facing one of the closed arms (Rodgers RJ and Dalvi A, 1997). The number of entries and time spent in each type of arm (open/closed) was recorded for 5 min (Walf AA and Frye CA, 2007). An entry was defined as the presence of all four paws in the arm. The total number of rears was also noted.

Open field test (OFT)

The apparatus consists of a square arena 96 x 96 cm² with 60 cm high walls. The walls and the floor are painted white. The floor is divided into 25 squares. Nine squares in the middle were defined as the center and sixteen squares along the walls as the periphery. On the 10th day, 45 minutes after drug administration, the rat was placed in a corner square of the open field and the time spent in the central

and peripheral areas was observed during a 5 min exposure period (Fernandez F, 2002). The number of entries in the central area and total number of rears was also noted during this time period. The experimental room was a sound attenuated, dark room. The open field was illuminated with 40W bulb from a height of about 100 cm.

Statistical analysis

All values are expressed as mean \pm SEM. Data was analyzed using one-way ANOVA. Post-hoc comparisons were performed by applying Bonferroni test. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out by using SPSS for Windows (SPSS 17.0).

RESULTS

Elevated plus maze

In the EPM model, the time spent in the open arm by the rats treated with alprazolam (210.83 \pm 4.21s) and 0.01mg/kg and 1mg/kg of ondansetron (198.00 \pm 7.40s, 227.66 \pm 7.77s respectively) was significantly ($P < 0.05$) increased with respect to control (124.33 \pm 1.58s). The time spent in closed arm by alprazolam and ondansetron treated rats was significantly ($P < 0.05$) decreased with respect to control (Table 1). There was no significant difference between alprazolam and ondansetron treated rats in the time spent in the open and closed arms. Rats treated with granisetron did not show any significant change in any of the parameters tested. There was no significant change in the number of

| Group/Drug (dose) | Number of entries | | Time spent in second(s) | | Total number of rears |
|--------------------------|-------------------|-----------------|--------------------------------------|--------------------------------------|-----------------------|
| | Open arm | Closed | Open arm | Closed arm | |
| 1/Gum acacia(10 ml/kg) | 6.66 \pm 0.95 | 6.50 \pm 1.08 | 124.33 \pm 1.58 | 175.66 \pm 1.58 | 9.00 \pm 0.77 |
| 2/Alprazolam (0.08mg/kg) | 6.83 \pm 0.30 | 6.66 \pm 0.33 | 210.83 \pm 4.21 ^{a, b, c} | 89.16 \pm 4.10 ^{a, b, c} | 11.66 \pm 0.88 |
| 3/Ondansetron(0.01mg/kg) | 6.67 \pm 0.76 | 6.26 \pm 0.76 | 198.00 \pm 7.40 ^{a, b, c} | 102.00 \pm 1.40 ^{a, b, c} | 10.12 \pm 1.07 |
| 4/Ondansetron(1mg/kg) | 6.60 \pm 0.68 | 6.00 \pm 0.68 | 227.66 \pm 7.77 ^{a, b, c} | 72.33 \pm 9.77 ^{a, b, c} | 11.09 \pm 0.73 |
| 5/Granisetron(0.01mg/kg) | 6.83 \pm 0.83 | 6.83 \pm 1.02 | 133.83 \pm 6.40 | 166.17 \pm 0.04 | 9.73 \pm 1.92 |
| 6/Granisetron(1mg/kg) | 6.03 \pm 1.02 | 6.33 \pm 1.02 | 137.66 \pm 9.53 | 162.33 \pm 9.53 | 10.66 \pm 0.66 |

entries into the arms and rears in any of the drug treated groups (Table 1).

Table 1: Effect of ondansetron, granisetron and alprazolam on the behavior of rats in elevated plus maze model

Values are expressed as mean \pm SEM, n = 6 in each group.

a - $P < 0.05$ vs control, b - $P < 0.05$ vs granisetron 0.01mg/kg, c- $P < 0.05$ vs granisetron 1mg/kg (ANOVA followed by Bonferroni's test)

Open field test

In OFT model, the time spent in peripheral area by the rats treated with alprazolam (241.83 ± 5.36 s) and 0.01 mg/kg and 1 mg/kg ondansetron (251.66 ± 2.70 s, 238.72 ± 2.74 s respectively) was significantly ($P < 0.05$) decreased as compared to control (288.66 ± 2.10 s). The time spent in central area by the rats treated with alprazolam and ondansetron was significantly ($P < 0.05$) increased as compared to control (Table 2). However, there was no significant difference between alprazolam and ondansetron (1 mg/kg) treated rats in the time spent in the central and peripheral areas. Rats treated with granisetron did not show any significant change in the time spent in peripheral and central areas. There was no significant change in the number of rears in any of the drug treated groups (Table 2).

Table 2: Effect of ondansetron, granisetron and alprazolam on the behavior of

| Group | Number of entries in central area | Time spent in seconds (s) | | Total number of rears |
|-------|-----------------------------------|-----------------------------|----------------------------|-----------------------|
| | | In periphery | In central area | |
| 1 | 2.66 ± 0.56 | 288.66 ± 2.10 | 11.33 ± 2.10 | 5.33 ± 0.21 |
| 2 | $7.50 \pm 1.43^{***}$ | $241.83 \pm 5.36^{a,b,c,d}$ | $66.50 \pm 3.78^{a,b,c,d}$ | 10.83 ± 1.53 |
| 3 | 4.33 ± 0.33 | $251.66 \pm 2.70^{a,c,d}$ | $28.33 \pm 2.70^{a,c,d}$ | 8.16 ± 0.70 |
| 4 | 5.66 ± 0.71 | $238.72 \pm 2.74^{a,b,c,d}$ | $62.00 \pm 2.74^{a,b,c,d}$ | 7.66 ± 1.08 |
| 5 | 3.50 ± 0.50 | 282.50 ± 2.45 | 15.83 ± 1.35 | 8.83 ± 1.57 |
| 6 | 4.50 ± 0.80 | 288.00 ± 1.86 | 12.00 ± 1.86 | 6.83 ± 0.74 |

rats in open field test

Values are expressed as mean \pm SEM, n = 6 in each group. *, a- $P < 0.05$ vs control; b- $P < 0.05$ vs ondansetron 0.01 mg/kg; **, c- $P < 0.05$ vs granisetron 0.01 mg/kg; d - $P < 0.05$ vs granisetron 1 mg/kg; (ANOVA followed by Bonferroni's test)

Group 1- gum acacia 10 ml/kg; 2 – alprazolam 0.08 mg/kg; 3, 4 – ondansetron $0.01, 1$ mg/kg respectively; 5,6 – granisetron $0.01, 1$ mg/kg respectively.

DISCUSSION

In the elevated plus maze, avoidance of open arms, preference for closed arms and a decrease in rears by the animal indicates anxiety (Pellow S, et al.,1985; Fernandes C and File SE,1996).The elevated plus maze has also been used to demonstrate anxiolytic activity of non-benzodiazepines (Wright IK, et al.,1992). In this study, the decrease in time spent in the closed arms and an increase in time spent in the open arms indicates anxiolytic activity of alprazolam and ondansetron. Alprazolam, a benzodiazepine, is used for the treatment of anxiety. Ondansetron is a 5HT₃ receptor antagonist.

Ondansetron and granisetron have been found to decrease fear potentiated startle response in rats. In an earlier study, ondansetron in a dose of 0.01 mg/kg twice daily for 14 days was found to increase the time spent by the rats in the open arm of the elevated plus maze (Wright IK, et al., 1992).In our study, there was a similar finding with ondansetron 0.01mg/kg and 1mg/kg, intraperitoneally, but given once daily for 10 days.

In the open field test, rats prefer the peripheral part of the apparatus (Bhattacharya SK and Satyan KS,

1997).In our study, both alprazolam and ondansetron demonstrated anxiolytic activity which was evident from the increase in time spent in the centre as compared to the periphery by the rats. Granisetron did not produce anxiolytic effect in any of the models.

In both the models, the anxiolytic effect of higher dose of ondansetron was comparable to alprazolam. Since there is a concern about the risk of dependence and abuse of benzodiazepines on long term use, ondansetron could be a potential alternative for chronic treatment of anxiety.

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