



ANXIOGENIC EFFECT OF LANSOPRAZOLE IN WISTAR RATS

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ABSTRACT

Lansoprazole, a proton pump inhibitor, increases plasma levels of substance P. Substance P is known to play a role in anxiety. The objective of the study was to evaluate whether lansoprazole can cause anxiety in rats. Elevated plus maze and Open field test were used to assess anxiogenic activity. Four groups of rats were treated orally with gum acacia 10 ml/kg, lansoprazole 1 mg/kg, 2 mg/kg and 3 mg/kg doses respectively. The drugs were administered once daily for ten days. The time spent, number of entries, rears in the arms of the elevated plus maze and central and peripheral squares in the open field test were observed. In the elevated plus maze, the time spent by rats treated with lansoprazole (1, 2 and 3 mg/kg) was reduced in the open arm but increased in the closed arm as compared to control. The number of rearings in the closed arm in the lansoprazole treated rats was decreased as compared to control. In the open field, the time spent by rats treated with lansoprazole (2 mg/kg) was decreased in the centre but increased in the periphery as compared to control. The number of rearings in the periphery in rats treated with lansoprazole (2 mg/kg) was decreased as compared to control. Lansoprazole was found to induce anxiety in rats.

Keywords: Lansoprazole, anxiogenic, elevated plus maze, open field test, substance P.

INTRODUCTION

There has been a focus on the relationship between anxiety and peptic ulcer disease in clinical and research areas over the past few years¹. Lansoprazole, a proton-pump inhibitor, is used to treat gastric ulcers, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome². It is also used in the therapy of *Helicobacter pylori*, a bacterium that causes peptic ulcer². Lansoprazole can increase plasma levels of substance P³. Substance P can cross the blood brain barrier⁴. An injection of substance P into the central nervous system produced aversive effects in rats in the elevated plus maze⁵. This reflects anxiogenic effect of substance P⁵. Hence, this study was undertaken to evaluate anxiogenic property of lansoprazole in wistar albino male rats using two models - elevated plus maze and open field test.

MATERIALS AND METHODS

Animals

Adult male albino rats (150 ± 50 g) were used in this study. The rats were maintained under standard conditions in the Central Animal House, Manipal, Karnataka approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The rats were kept in polypropylene cages (U.N. Shah manufacturers, Mumbai, India) under standard environmental conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra, India) and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle. Experiments were performed during the dark cycle.

Drugs

Lansoprazole, oral disintegrating tablets 15 mg (Cipla, Solan, India); gum acacia (Nice Chemicals, Kochi, Kerala, India).

Experimental Design

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

The treatment schedule was as follows

Group 1- control, received 4% gum acacia in a dose of 10 ml/kg.

Group 2, 3 and 4 - received lansoprazole in a dose of 1 mg/kg, 2mg/kg and 3mg/kg respectively.

The drugs were administered orally, once daily, for ten days. The test drug lansoprazole was administered in doses based on a previous study⁶. The test was carried out 45 minutes after the last dose of the drugs on the 10th day. Two models were used in this study to assess anxiety in rats – elevated plus maze (EPM) and open field test (OFT). The apparatus in each model was wiped with 10% ethanol after the test with each rat to eliminate possible bias due to odour of previous animal.

Elevated plus maze (EPM)

The Elevated plus maze is widely used to assess anxiety⁷. The apparatus has two open arms (50×10 cm) and two closed arms (50×10×40 cm) with an open roof, around a central square (10×10 cm), such that arms of the same type are opposite to each other⁸. The entire maze is raised 50 cm above the ground. The drugs were



administered for ten days to four groups of rats. On the 10th day, 45 minutes after drug administration, each rat was placed in the central square of the maze facing one of the closed arms⁹. The number of entries, time spent and the number of rears in each type of arm (open/closed) was recorded for 5 min¹⁰. An entry was defined as the presence of all four paws in the arm.

Open field test

In this test, exploratory pattern of the rat was studied to assess anxiety. The apparatus consists of a square arena 96 x 96 cm² with 60 cm high walls. The floor is divided into 25 squares. Nine squares in the middle were defined as the centre and sixteen squares along the walls as the periphery¹¹. The open field was illuminated with 40W bulb from a height of about 100 cm. The experimental room was sound attenuated, dark room. Four groups of rats were administered drugs for ten days. On the 10th day, 45 minutes after drug administration, the rat was placed in the centre of the open field. The time spent, number of squares crossed and rearing in the central and peripheral areas was observed during a 5 minute exposure period. An entry into peripheral or central area was considered when all four paws were present in the area.

Statistical Analysis

All values are expressed as mean \pm standard error of mean (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 Elevated plus maze model, the time spent by lansoprazole treated rats (1, 2 and 3 mg/kg doses) in the open arm was significantly ($p < 0.05$) decreased (37.16 \pm 5.71, 23.00 \pm 8.69, 46.00 \pm 7.07 s, respectively versus 147.83 \pm 12.58 s of control group) but increased in the closed arm (262.83 \pm 5.72, 277.00 \pm 8.69, 254.00 \pm 7.07 s versus 157.16 \pm 17.11s of control group). The number of rearing in the closed arm was decreased in rats treated with lansoprazole (all three doses) as compared to the control group as shown in Table 1.

Open field test

In the open field test, rats treated with lansoprazole (2 mg/kg) showed a significant ($p < 0.05$) decrease in the number of entries (2.83 \pm 0.48 versus 9.00 \pm 0.68 of control) and time spent (7.33 \pm 1.12 s as compared to 35.16 \pm 6.51s of control) in the central area (Table 2). There was an increase in the time spent in the periphery (292.66 \pm 1.11 s versus 264.83 \pm 6.51 s). Also, the number of rearing in the periphery was decreased in lansoprazole (2 mg/kg) treated rats as shown in Table 2.

Table 1: Effect of lansoprazole on the behavior of rats in elevated plus maze model

Group/drug (dose)	Number of entries		Time spent in seconds (s)		Number of rears	
	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm
1/Gum acacia (10 ml/kg)	5.50 \pm 0.43	5.33 \pm 0.33	147.83 \pm 12.58	157.16 \pm 17.11	1.83 \pm 0.65	8.33 \pm 0.42
2/Lansoprazole (1 mg/kg)	4.50 \pm 0.72	4.66 \pm 0.88	37.16 \pm 5.71 *	262.83 \pm 5.72 *	1.50 \pm 0.62	4.50 \pm 0.56 *
3/Lansoprazole (2 mg/kg)	5.50 \pm 1.11	5.66 \pm 0.88	23.00 \pm 8.69 *	277.00 \pm 8.69 *	1.16 \pm 0.98	3.33 \pm 0.42 *
4/Lansoprazole (3 mg/kg)	5.33 \pm 1.43	5.16 \pm 1.45	46.00 \pm 7.07 *	254.00 \pm 7.07 *	0.83 \pm 0.31	3.50 \pm 0.42 *

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

* $p < 0.05$ as compared to control (ANOVA followed by Bonferroni's test)

Table 2: Effect of lansoprazole on the behavior of rats in the open field model

Group	Number of squares crossed in the		Number of entries in the	Time spent in seconds (s)		Number of rears	
	centre	periphery		centre	periphery	centre	periphery
1	18.00 \pm 2.48	68.00 \pm 4.58	9.00 \pm 0.68	35.16 \pm 6.51	264.83 \pm 6.51	0.66 \pm 0.42	11.83 \pm 1.72
2	15.50 \pm 3.97	77.50 \pm 11.93	5.50 \pm 1.11 *	13.83 \pm 3.44 *	276.00 \pm 5.76	0.33 \pm 0.33	7.50 \pm 1.63
3	6.50 \pm 1.87 *	67.83 \pm 9.24	2.83 \pm 0.48 *	7.33 \pm 1.12 *	292.66 \pm 1.11 *	0.33 \pm 0.23	3.50 \pm 1.06 *
4	15.16 \pm 1.30	63.00 \pm 6.27	5.16 \pm 0.87 *	18.83 \pm 3.60	281.16 \pm 3.60	1.33 \pm 0.84	3.33 \pm 0.71 *

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

* $p < 0.05$ as compared to control (ANOVA followed by Bonferroni's test)

Group 1- gum acacia; 2, 3 and 4 – lansoprazole 1, 2, 3 mg/kg respectively.



DISCUSSION

In the elevated plus maze, rats show preference towards closed arms and avoid open arms due to fear of open spaces^{7, 12}. Avoidance of the open arms by the animal indicates anxiety whereas increase in the time spent and/or entries into the open arms is a result of lack of anxiety¹⁰. In this study, the decrease in time spent in the open arms, increase in time spent in closed arms and decrease in rearing in the closed arm in lansoprazole treated rats is the result of its anxiogenic effect.

In the open field test, rodents show preference to the periphery of the apparatus¹³. A decrease in squares crossed, entries and time spent in the central area of the open field indicates anxiety¹⁴. A decrease in rearing indicates anxiety¹⁵. In our study, a decrease in activity in the centre and a decrease in rearing in the periphery in rats treated with 2mg/kg of lansoprazole is a result of anxiogenic effect of the drug.

Substance P acts as a neurotransmitter in the central nervous system and the enteric neurons in the gastrointestinal tract¹⁶. Lansoprazole can stimulate capsaicin-sensitive afferent nerves in the gastrointestinal mucosa. Stimulation of these nerves results in the release of substance P from the nerve endings and an increase in its plasma levels³. It is also known that substance P crosses the blood brain barrier⁴. Studies show that the substance P–preferring neurokinin receptor is found in parts of the brain that play an important role in responses to anxiety-related behavior^{16, 17}. Moreover, central injections of substance P resulted in an anxiogenic effect in rats in the elevated plus maze⁵.

Our study demonstrated anxiogenic activity of lansoprazole in male Wistar rats. The limitation of this study has been non measurement of substance P in the brain and periphery. Clinical studies are required to assess whether lansoprazole can produce anxiety as side effect.

REFERENCES

1. Levenstein S. Peptic ulcer at the end of the 20th century: Biological and psychological risk factors. *Can J Gastroenterol* 1999; 13:753–9.
2. McQuaid RK. Drugs used in treatment of gastrointestinal disease. In: Bertram G Katzung, ed. *Basic and Clinical pharmacology*. McGraw Hill Companies, 2009: 1067-1130.
3. Fumihiko K, Shin I, Yuhki S, et al. 2005. Lansoprazole raises somatostatin, calcitonin gene related peptide, and substance P levels in healthy human plasma. *J Health Sci* 2005; 51(3): 294-9.
4. Chappa AK, Audus KL, Lunte SM. Characteristics of substance P transport across the blood-brain barrier. *Pharm Res* 2006; 23(6):1201-8.
5. Aguiar MS and Brandão ML. Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal gray on the behaviour of rats in the plus-maze test. *Physiol Behav* 1996; 60(4):1183-6.
6. Ichikawa H, Yoshida N, Takagi T, Tomatsuri N, Katada K, Isozaki Y, et al. Lansoprazole ameliorates intestinal mucosal damage induced by ischemia-reperfusion in rats. *World J Gastroenterol* 2004; 10(19): 2814-7.
7. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in elevated plus maze as a measure of anxiety in the rat. *J Neurosci Meth* 1985;14:149-67.
8. Handley SL and Mithani S. Effects of α -adrenoreceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmeideberg's Arch Pharmacol* 1984; 327(1): 1–5.
9. Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus maze. *Neurosci Biobehav Rev* 1997; 1: 801– 10.
10. Walf AA and Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc* 2007; 2(2): 322 – 8.
11. Fernandez F. N/OFC exerts anxiogenic actions in an open field. *J Undergrad Res* 2002; 3:10.
12. Fernandes C and File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacol Biochem Behav* 1996; 54: 31–40.
13. Bhattacharya SK and Satyan KS. Experimental methods for the evaluation of psychotropic agents. *Ind J Exp Biol* 1997; 35(6): 565-75.
14. Ambavade SD, Mhetre NA, Patil KM, Bodhankar SL, et al. Anxiolytic activity of root extract of *Saussurea lappa* Clark. in mice. *J Nat Rem* 2006; 6: 103–8.
15. Imrana T, Zeba NS, Shamim JR. Effects of *Ocimum sanctum* and *Camellia sinensis* on stress induced anxiety and depression in male albino *Rattus norvegicus*. *Indian J Pharmacol* 2010; 42(5): 283-8.
16. Ebner K and Singewald N. The role of substance P in stress and anxiety responses. *Amino Acids* 2006; 31(3): 251-72.
17. Hokfelt T, Pernow B, Wahren J. Substance P: A pioneer amongst neuropeptides. *J Intern Med* 2001; 249: 27 - 40.

