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Research Article

COMBINATION OF VENLAFAXINE AND DIAZEPAM ATTENUATES MARBLE-BURYING BEHAVIOR OF MICE

Parle Milind* and Gaikwad Uday Tukaram
Pharmacology Division, Dept. Pharm. Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

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*Milind Parle, Pharmacology Division, Dept. Pharm. Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125 001, Haryana, India

ABSTRACT
Obsessive-Compulsive Disorder (OCD) is an anxiety disorder featuring intrusive and troubling symptoms, which are perceived as the products of one's own mind. This disorder is characterized by absurd, recurrent and persistent thoughts (obsessions) followed by certain stereotyped actions (compulsions). The OCD patients realize the irrational nature of thoughts and rituals, but feel helpless and hopeless about controlling them. Numerous genes modulating the serotonin and dopaminergic systems are thought to participate in the pathophysiology of OCD. Marble-burying behavior of mice has been employed to study anxiety disorders including obsessive–compulsive disorder (OCD). The aim of this study was to test the efficacy of venlafaxine and diazepam *per se* and in combination on marble-burying behavior of mice. In the present project a total of 126 male Swiss mice divided in 21 groups were employed. Venlafaxine (1 mg kg^{-1}i.p.) per se as well as diazepam (0.25 mg kg^{-1}i.p.) *per se* did not show any anti-compulsive activity. But, the combination comprising of ineffective doses of venlafaxine (1 mg kg^{-1}i.p.) and diazepam (0.25 mg kg^{-1}i.p.) showed significant anti-compulsive activity as reflected by inhibition of marble-burying behavior.
KEYWORDS: Venlafaxine, Diazepam, Marble-Burying Behavior

INTRODUCTION

Obsessive-Compulsive Disorder (OCD) is characterized by absurd, recurrent and persistent thoughts (obsessions) followed by certain stereotyped actions (compulsions). The most common types of obsessions are related to contamination, pathological doubts, somatic dysfunctions, need for symmetry, aggression and hyper sexual drive. In OCD, senseless, repetitive rituals (such as counting, washing etc.) serve to counteract the anxiety precipitated by obsessive thoughts e.g. Symmetry and exactness preoccupations. Obsessive-Compulsive disorder can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying of mice has been employed to study anxiety disorders including obsessive–compulsive disorder[1,2]. Only potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive–compulsive disorder[3]. The aim of this study was to test the efficacy of venlafaxine and diazepam per se and in combination on marble-burying behavior of mice.

MATERIALS AND METHODS

Animals

All the experiments were carried out in adult male Swiss mice (22–25 g), housed under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25± 2 °C, 55± 2%). They received standard rodent chow (Goldmohar brand, Lipton India Ltd.) and water ad libitum. Separate groups of mice were used for each set of experiments and each animal was used only once. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC). The care of animal was taken as per the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India, New Delhi, India.

Drugs

Venlafaxine was gifted by Cipla Ltd., India. Diazepam was purchased from Sigma-Aldrich Ltd., USA. Venlafaxine was dissolved in 0.9% saline. Diazepam was dissolved in a 5% concentration of Tween-80.

Experimental Design

Mice were divided in 21 groups and each group consisted of a minimum of six animals. Separate animals were used for each experiment.

Group I: It represented the control group for young mice (n=6).

Groups II, III, IV, V, VI and VII: Venlafaxine (1, 3 and 5 mg kg-^{1}i.p.) was injected into young male mice. Marble-burying behavior/locomotor activity of mice was measured 30 min. after the drug administration.

Groups VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX: Diazepam (0.25, 0.3, 0.5, 2, 4 and 8 mg kg-^{1}i.p.) was injected to young male mice 30 min prior to the assessment of marble-burying behavior/locomotor activity

Group XX, XXI: Venlafaxine (1mg kg-^{1}i.p.) was given 30 min prior to the administration of diazepam (0.25 mg kg-^{1}i.p.) and the effect of this combination was studied on the marble-burying behavior/locomotor activity of mice after the passage of another 30 min.

In first set of experiments, Venlafaxine (1, 3 & 5 mg kg-^{1}i.p.) and Diazepam (0.25, 0.3, 0.5, 2, 4 and 8 mg kg-^{1}i.p.) were administered to separate groups of mice, 30 min prior to the assessment of marble-burying behavior or locomotor activity. In second set of experiments, Venlafaxine (1mg kg-^{1}i.p.) was given 30 min prior to the administration of diazepam (0.25 mg kg-^{1}i.p.). Thirty minutes after the administration of diazepam, mice were subjected to above behavioral tests.

Marble-burying Behavior Model

The Marble-burying behavior model as described earlier was employed in the present study[4]. In this model, mice were individually placed in separate plastic cages (21×38×14 cm) containing 5 cm thick sawdust bedding. Twenty clean glass marbles (diameter ~10 mm), were arranged evenly on the bedding. After 30 min exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered buried, if its two-third size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive–compulsive behavior.

Actophotometer

Motor activity was assessed in separate groups of mice using Actophotometer (Techno, Lukhnow), which had a circular arena of 40 cm, equipped with three infrared beams and photo-cells connected to digital counter. Motor activity was assessed in terms of total number of counts of light beam interruptions in 30 min.

Statistical Analysis

The data were analyzed with one-way ANOVA followed by Tukey test for multiple comparisons. The values were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant in all the cases.

RESULTS

Effect of Venlafaxine on marble-burying behavior and locomotor activity of mice

Low dose of venlafaxine (1 mg kg-^{1}i.p.) did not produce any significant effect on marble-burying behavior of mice but, high doses of venlafaxine (3, 5 mg kg-^{1}i.p.) reduced significantly ($P<0.001$) the number of marbles buried by mice (Fig. 1). Furthermore, venlafaxine (1 mg kg-^{1}i.p.) did not show any significant effect on locomotor activity of mice, when measured using actophotometer (Fig. 2).

Effect of Diazepam on marble-burying behavior and locomotor activity of mice

Low dose of diazepam (0.25 mg kg-^{1}i.p.) did not produce any effect on the marble-burying behavior of mice (Fig. 3). This dose of diazepam (0.25 mg kg-^{1}i.p.) showed significant enhancement ($P<0.01$) in locomotor function of mice (Fig. 4). However, diazepam in higher doses (2, 4, 8 mg kg-^{1}i.p.) not only reduced (Fig. 4) the locomotor activity of mice but also inhibited significantly ($P<0.001$) the marble-burying behavior of mice (Fig. 3).

Effect of venlafaxine 1 mg kg-^{1}i.p. plus diazepam 0.25 mg kg-^{1}i.p. on marble-burying behavior and locomotor activity of mice

The combination of low doses of venlafaxine (1 mg kg-^{1}i.p.) and diazepam (0.25 mg kg-^{1}i.p.) significantly ($P<0.001$) inhibited the number of marbles buried by mice (Fig. 5). Furthermore, this combination did not exhibit any significant effect on the locomotor function of mice (Fig. 6).

DISCUSSION

Marble-burying behavior is a well-accepted paradigm to screen anti-compulsive activity in mice. An acute administration of certain classes of antidepressants like selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) has been shown to dose-dependently inhibit marble-burying behavior of mice[5,6,7]. The marble-burying behavior is also reduced by other classes of compounds such as classical antipsychotics[8]. Chronic treatment with leuprolide prevented the increase in marble-burying behavior evoked by ethanol-withdrawal[9]. LHRH antagonist attenuated the effect of fluoxetine on marble-burying behavior of mice[4].

In the present study, venlafaxine (1 mg kg-^{1}i.p.) and diazepam (0.25 mg kg-^{1}i.p.) independently did not produce any significant effect on marble-burying behavior of mice but, the combination of venlafaxine (1 mg kg-^{1}i.p.) and diazepam (0.25 mg kg-^{1}i.p.) attenuated marble-burying behavior of mice. Venlafaxine per se at higher dose (5 mg kg-^{1}i.p.) however, showed inhibition of marble-burying behavior of mice. On the other hand, diazepam at higher doses (2, 4 & 8 mg kg-^{1}i.p.) produced

profound sedation of mice resulting in obvious diminished marble-burying behavior. However, this inhibition of marble-burying behavior of mice can be attributed to the sedative effect of diazepam at higher doses and probably has no correlation with compulsive behavior (i.e. marble-burying behavior) of mice. Diazepam exhibited differential effects on locomotor activity at different doses. The anti-compulsive effect observed with the combination is not dependent on the sedative action of diazepam, since their was increase in locomotor activity of animals at this particular dose of diazepam (0.25 mg kg-^{1}i.p.).

In view of above, we had selected such low doses of diazepam (0.25 mg kg-^{1}i.p.) and venlafaxine (1 mg kg-^{1}i.p.), which did not produce any effect on marble-burying behavior on their own. Thus, it appears that at these low doses, diazepam and venlafaxine, when administered in combination may be acting synergistically to produce anti-compulsive effect. This net response of the combination can be exploited therapeutically in the management of obsessive-compulsive disorder. Another advantage of this combination would be absence of any toxicity observed with the high doses of these drugs. Venlafaxine, the bicyclic antidepressant, is usually categorized as a serotonin-norepinephrine reuptake inhibitor and is therapeutically used against obsessive-compulsive disorder[10]. Diazepam appears to act on the areas of the limbic system, thalamus and hypothalamus, inducing anxiolytic effects due to the enhancement of GABA activity. Diazepam increased the inhibitory processes in the cerebral cortex[11] and in this way it, probably helped to control the compulsive behavior of mice.

CONCLUSION

From the present investigations, it can be concluded that the combination of venlafaxine (1 mg kg-^{1}i.p.) and diazepam (0.25 mg kg-^{1}i.p.) attenuates the marble-burying behavior of mice, thereby suggesting that the combination had anti-compulsive effect due to the synergistic action, which resulted in strong potentiation of serotonin reuptake inhibitory mechanism and weak antagonism of inhibitory processes at the level of cerebral cortex.

REFERENCES

1. Rapoport JL. The neurobiology of obsessive– compulsive disorder. J Am Med Assoc 1988; 260: 2888– 2890.
2. Njung'e K, Handley SL. Effects of 5-HAT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice: a putative test for anxiolytic agents. Brit J Pharmacol 1991; 104:105– 112.
3. El Mansari M, Blier P. Mechanisms of action of current and potential pharmacotherapies of obsessive–compulsive disorder. Prog Neuro-psychopharmacol Biol Psychiat 2006; 30:362–373.
4. Gaikwad U, Bhutada P, Wanjari M, Umathe S. LHRH antagonist attenuates the effect of fluoxetine on marble-burying behavior of mice. Eur J Pharmacol 2007; 563:155-159.
5. Martin JR, Bos M, Jenck F, Moreau J, Mutel V, Sleight AJ, Wichmann J, Andrews JS, Berendsen HH, Broekkamp CL, Ruigt GS, Kohler C, Delft AM. 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. J Pharmacol Exp Ther 1998; 286:913–924.
6. Millan MJ, Dekeyne A, Papp M, La Rochelle CD, MacSweeny C, Peglion JL, Brocco M. S33005, a novel ligand at both serotonin and norepinephrine transporters: II.Behavioral profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. J Pharmacol Exp Ther 2001; 298:581–591.
7. Ichimaru Y, Egawa T, Sawa A. 5-HT1A-receptor subtype mediates the effect of fluvoxamine, a selective serotonin reuptake inhibitor, on marble burying behavior of mice. Jpn J Pharmacol 1995; 68:65–70.
8. Broekkamp CL, Rijk HW, Joly-Gelouin D, Lloyd KL. Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming of mice. Eur J Pharmacol 1986; 126:223–229.
9. Umathe S, Bhutada P, Dixit P, Shende V. Increased marble-burying behavior in ethanol withdrawl state: Modulation by gonadotropin-releasing hormone agonist. Eur J Pharmacol 2008; 587:175–180.
10. Goeringer K, McIntyre I, Drummer O. Postmortem tissue concentrations of venlafaxine. Forensic Sci Int 2001; 121:70–75.
11. Zakusov VV, Ostrovskaya RU, Kozhechkin SN, Markovich VV, Molodavkin GM, Voronina TA. Further evidence for GABA-ergic mechanisms in the action of benzodiazepines. Arch Int Pharmacodyn Ther 1977; 229 suppl 2:313-26.

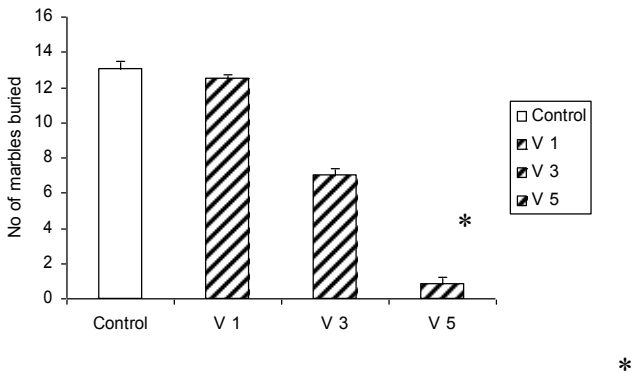

Fig. 1: Effect of Venlafaxine on marble-burying behavior of mice

Marble-burying behavior was tested in separate groups of mice. Each bar represents mean±S.E.M. * denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons)

V1=Venlafaxine 1 mg kg-^{1}i.p., V3= Venlafaxine 3 mg kg-^{1}i.p., V5= Venlafaxine 5 mg kg-^{1}i.p.

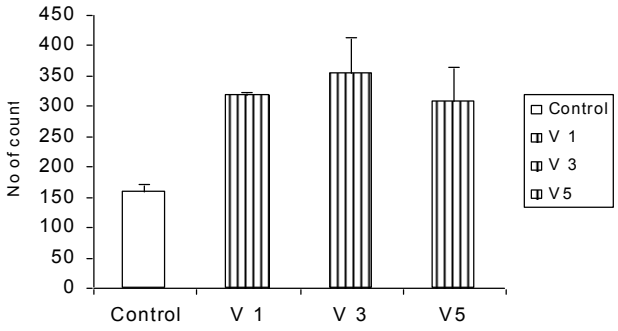

Fig. 2: Effect of Venlafaxine (1, 3 & 5 mg kg-^{1}i.p.) on locomotor activity of mice using actophotometer

Motor activity was tested in separate groups of mice. Each bar represents mean±S.E.M. V1=Venlafaxine 1 mg kg-^{1}i.p., V3= Venlafaxine 3 mg kg-^{1}i.p., V5= Venlafaxine 5 mg kg-^{1}i.p

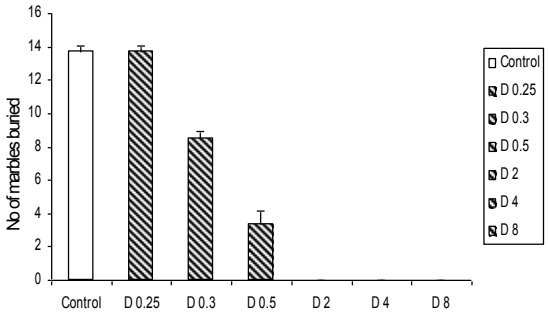

Fig. 3: Effect of Diazepam on marble-burying behavior of mice

Marble-burying behavior was tested in separate groups of mice. Each bar represents mean±S.E.M. * denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons)

D 0.25= Diazepam 0.25 mg kg-^{1}i.p., D 0.3= Diazepam 0.3 mg kg-^{1}i.p., D 0.5= Diazepam 0.5 mg kg-^{1}i.p., D 2= Diazepam 2 mg kg-^{1}i.p., D 4= Diazepam 4 mg kg-^{1}i.p., D 8= Diazepam 8 mg kg-^{1}i.p.

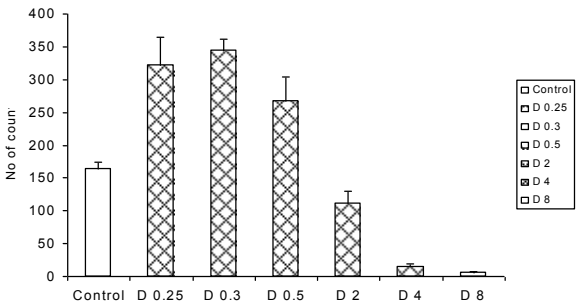

Fig. 4: Effect of Diazepam (0.25, 0.3, 0.5, 2, 4 & 8 mg kg-^{1}i.p.) on locomotor activity of mice

Motor activity was tested in separate groups of mice. Each bar represents mean±S.E.M. ♦denotes P<0.01 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons); *denotes P<0.001 as compared to control group

D 0.25= Diazepam 0.25 mg kg-^{1} i.p., D 0.3= Diazepam 0.3 mg kg-^{1} i.p., D 0.5= Diazepam 0.5 mg kg-^{1} i.p.,

D 2= Diazepam 2 mg kg-1i.p., D 4= Diazepam 4 mg kg-1i.p., D 8= Diazepam 8 mg kg-1i.p.

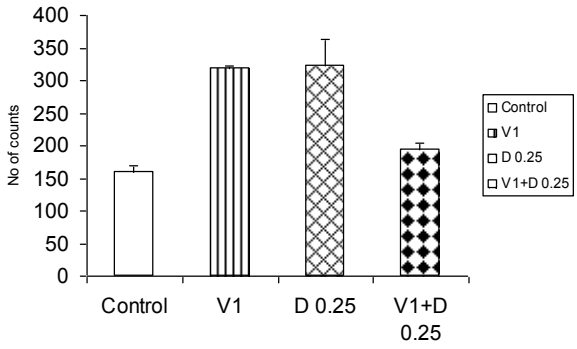

Fig. 5: Effect of Venlafaxine plus Diazepam on marble-burying behavior

Marble-burying behavior was tested in separate groups of mice. Each bar represents mean±S.E.M. * denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons)

V1= Venlafaxine 1 mg kg-^{1}i.p., D 0.25= Diazepam 0.25 mg kg-^{1}i.p.

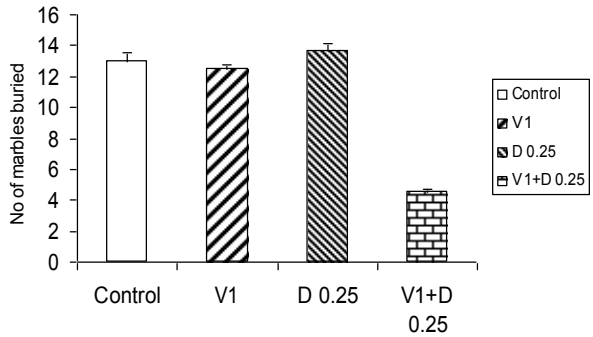

Fig. 6: Effect of Venlafaxine plus Diazepam on locomotor activity

Motor activity was tested in separate groups of mice. Each bar represents mean±S.E.M.

♦denotes P<0.01 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons). V1= Venlafaxine 1 mg kg-^{1}i.p., D 0.25= Diazepam 0.25 mg kg-^{1}i.p.