RESEARCH ARTICLE

COMBINATION OF VENLAFAXINE AND CHLORPROMAZINE ATTENUATES MARBLE-BURYING BEHAVIOR IN MICE

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Abstract: Obsessive–compulsive disorder is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviors (compulsions). Obsessive-compulsive disorder can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity of mice. The aim of present study was to test the efficacy of venlafaxine and chlorpromazine *per se* and in combination on marble-burying behavior of mice. In the present project, a total of 90 male swiss mice divided in 15 groups were employed. Venlafaxine (1 mg kg^{-1} i.p.) per se as well as chlorpromazine (1 mg kg^{-1} i.p.) *per se* did not show any anti-compulsive activity. However, at higher doses, both of these drugs, venlafaxine (3 mg kg^{-1} i.p. and 5 mg kg^{-1} i.p.) and chlorpromazine (2.5 mg kg^{-1} i.p. and 5 mg kg^{-1} i.p.) showed anti-compulsive effect, causing statistically significant inhibition of marble-burying behavior of mice. The combination comprising of ineffective doses of venlafaxine (1 mg kg^{-1} i.p.) and chlorpromazine (1 mg kg^{-1} i.p.) showed significant anti-compulsive activity as reflected by inhibition of marble-burying behavior.

Keywords: Venlafaxine, chlorpromazine, marble-burying behavior, motor activity.

INTRODUCTION

Marble burying in mice has been used to model anxiety disorders including obsessive–compulsive disorder (OCD) due to the excessive nature of the behavior and due to the pharmacological effects of clinical standards [1, 2]. Obsessive–compulsive disorder is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviors (compulsions) [3]. Only potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive–compulsive disorder [4]. The noxious and fearful stimuli associated with electrified prod, food of unpleasant tasting and predators such as scorpions activates defensive behavior of animal [5, 6, 7]. The rats and mice bury the unpleasant object able to cause aversion stimuli and fearful thoughts [8, 9]. An acute administration of certain classes of antidepressants like selective serotonin reuptake inhibitors (SSRIs), serotonin and tricyclic antidepressants (TCAs) has been shown to dose-dependently inhibit marble-burying in mice [10, 11, 12]. Chronic treatment with leuprolide prevented increase in marble-burying behavior evident in ethanol-withdrawal state [13]. LHRH antagonist attenuated the effect of fluoxetine on marble-burying behavior of mice [14]. The aim of present study was to test the efficacy of venlafaxine and chlorpromazine *per se* and in combination on marble-burying behavior of mice.

MATERIALS AND METHODS

Materials

Venlafaxine was obtained as a gift sample by Cipla Ltd., India. Chlorpromazine hydrochloride was purchased from Sigma-Aldrich Ltd., USA. All other ingredients used were of analytical grades.

Animals

The studies were carried out in adult male albino swiss mice (22–25 g), group housed (n=6), 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., India) and water ad libitum. Separate groups (n=6) of mice were used for each set of experiments and each animal was used only once. The animal studies were approved by Institutional Animal Ethics Committee (IAEC) vide sanction number 15 dated 23/01/2008 and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India, New Delhi, India.

Experimental design

Mice were divided in 15 groups and each group consisted of 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 were measured after 30 minutes of drug administration.

Groups VIII, IX, X, XI, XII and XIII: Chlorpromazine (1, 2.5 and 5 mg kg^{-1} i.p.) was injected to young male mice 30 minutes prior to the assessment of marble-burying behavior/locomotor activity.

Groups XIV, XV: Venlafaxine (1mg kg^{-1} i.p.) was given 30 minutes prior to the administration of chlorpromazine (1mg kg^{-1} i.p.). The effect of this combination was studied on the marble-burying behavior/locomotor activity of mice, after the passage of another 30 minutes.

In first set of experiments, Venlafaxine (1, 3 and 5 mg kg^{-1} i.p.) and Chlorpromazine (1, 2.5 and 5 mg kg^{-1} i.p.) were administered to separate groups of mice 30 minutes 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 minutes prior to the administration of chlorpromazine (1mg kg^{-1} i.p.). 30 minutes after the administration of diazepam, mice were subjected to above behavioral tests.

Marble burying behavior model

The Marble burying behavior model was studied as described previously by Gaikwad et al. [14]. 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 minutes 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 group 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 beams interruptions in 30 minutes.

Statistical analysis

The data were analyzed with One-way ANOVA followed by Tukey test for multiple comparisons. The results are expressed as mean ± SEM of six observations. P<0.05 was considered to be statistically significant in all the cases.

RESULTS

Effect of venlafaxine on marble-burying behavior and motor activity in mice

Venlafaxine (3 and 5 mg kg-1 i.p.) [*F* (3, 20) =212.00, *P*<0.001] (Figure 1) reduced marble-burying behavior in mice but venlafaxine (1 mg kg-1 i.p.) [*F* (3, 20) =212.00, *P*>0.05] (Figure 1) did not reduce marble-burying behavior in mice. In another method, venlafaxine (3 mg kg-1i.p.) [*F* (3, 20) =4.490, *P*<0.05] (Figure 2) produced effect on motor activity. Venlafaxine (1and 5 mg kg-1 i.p.) [*F* (3, 20) =4.490, *P*>0.05] (Figure 2) did not affect motor activity.

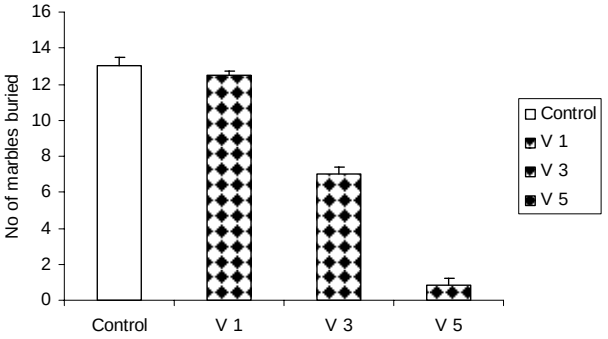

Figure 1. Effect of venlafaxine on marble-burying behavior of mice [Marble-burying behavior was tested in separate groups of mice. Each bar presents mean ± SEM * denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons) V1=Venlafaxine 1 mg kg-1 i.p., V3= Venlafaxine 3 mg kg-1 i.p., V5= Venlafaxine 5 mg kg-1 i.p.]

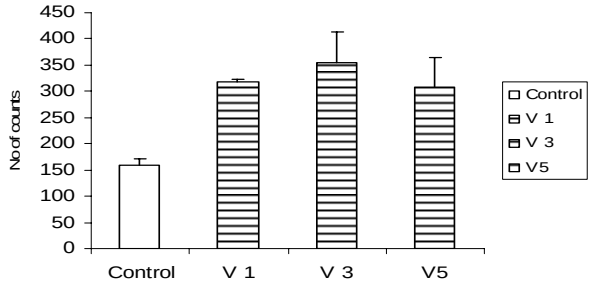

Figure 2. Effect of venlafaxine on locomotor activity of mice using actophotometer [Motor activity was tested in separate groups of mice. Each bar presents mean ± SEM * denotes P<0.05 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons) V1=Venlafaxine 1 mg kg-1 i.p., V3= Venlafaxine 3 mg kg-1 i.p., V5= Venlafaxine 5 mg kg-1 i.p.]

Effect of chlorpromazine on marble-burying behavior and motor activity in mice

Chlorpromazine (2.5 and 5 mg kg-1 i.p.) [*F* (3, 20) =270.00, *P*<0.001] (Figure 3) reduced marble-burying behavior in mice but Chlorpromazine (1 mg kg-1 i.p.) [*F* (3, 20) =270.00, *P*>0.05] (Figure 3) did not reduce marble-burying behavior in mice. In another method, chlorpromazine (5 mg kg-1 i.p.) [*F* (3, 20) =29.401, *P*<0.001] (Figure 4) produced significant effect on motor activity but chlorpromazine (1 and 2.5 mg kg-1 i.p.) [*F* (3, 20) =29.401, *P*>0.05] (Figure 4) did not affect motor activity.

Effect of venlafaxine 1 mg kg-1 i.p. in combination with chlorpromazine 1 mg kg-1 i.p.

Venlafaxine (1 mg kg-1 i.p.) [*F* (3, 20) =212.00, *P*>0.05] and chlorpromazine (1 mg kg-1i.p.) [*F* (3, 20) =270.00, *P*>0.05] did not reduce marble-burying behavior in mice but, venlafaxine 1 mg kg-1i.p. in combination with chlorpromazine (1 mg kg-1 i.p.) [*F* (3, 20) =307.33, *P*<0.001] (Figure 5) significantly reduced marble-burying behavior in mice. In another method, venlafaxine (1 mg kg-1 i.p.) [*F* (3, 20) =4.490, *P*>0.05], chlorpromazine (1 mg kg-1 i.p.) [*F* (3, 20) =29.401, *P*>0.05] did not produce effect on motor activity. The combination of venlafaxine (1 mg kg-1 i.p). and chlorpromazine (1 mg kg-1i.p.) [*F* (3, 20)=29.978, *P*>0.05] (Figure 6) did not affect motor activity.

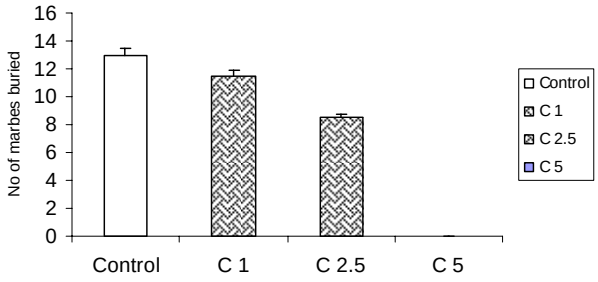

Figure 3. Effect of chlorpromazine on marble-burying behavior of mice [Marble-burying behavior was tested in separate groups of mice. Each bar presents mean ± SEM * denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons) C1= Chlorpromazine 1 mg kg-1 i.p., C 2.5= Chlorpromazine 2.5 mg kg-1 i.p., C 5= Chlorpromazine 5 mg kg-1 i.p.]

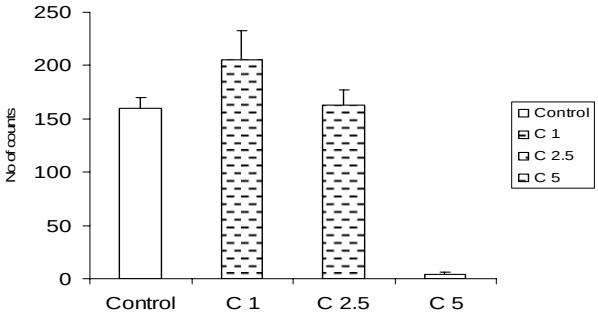

Figure 4. Effect of chlorpromazine on motor activity of mice **[**Motor activity was tested in separate groups of mice. Each bar presents mean ± SEM * denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons) C1= Chlorpromazine 1 mg kg-1 i.p., C 2.5= Chlorpromazine 2.5 mg kg-1 i.p., C 5= Chlorpromazine 5 mg kg-1 i.p.]

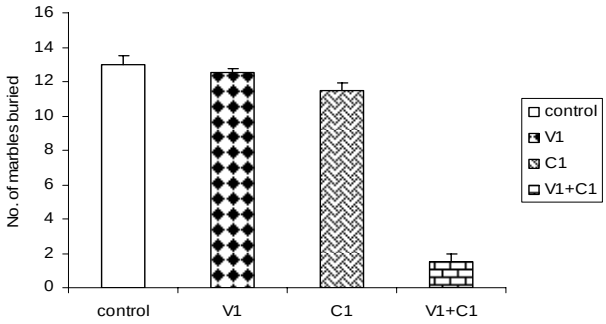

Figure 5. Effect of venlafaxine plus chlorpromazine on marble-burying behavior of mice [Marble-burying behavior was tested in separate groups of mice. Each bar presents mean ± SEM *denotes P<0.001 as compared to control group (One-way ANOVA followed by Tukey test for multiple comparisons) V1= Venlafaxine 1 mg kg-1 i.p., C 1= Chlorpromazine 1 mg

kg-1 i.p., V1+C1= Venlafaxine (1 mg kg-1 i.p.) plus Chlorpromazine (1 mg kg-1 i.p.)]

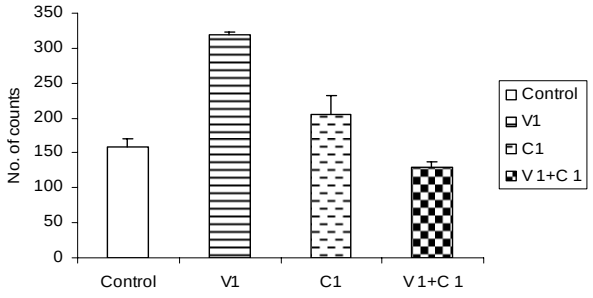

Figure 6. Effect of venlafaxine plus chlorpromazine on motor activity of mice [Motor activity was tested in separate groups of mice. Each bar presents mean±SEM. V1= Venlafaxine 1 mg kg-1 i.p.; C1= chlorpromazine 1mg kg-1 i.p.; V1+C1= Venlafaxine (1 mg kg-1 i.p.) plus Chlorpromazine (1 mg kg-1 i.p.)]

DISCUSSION

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. The rats and mice bury the unpleasant object able to cause aversion stimuli and fearful thoughts [15, 16]. Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity. 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 in mice [10, 11, 12].

In the present study, venlafaxine (1 mg kg-1 i.p.) and chlorpromazine (1 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 chlorpromazine (1 mg kg-1 i.p.) attenuated marble-burying behavior of mice. According to our experience, venlafaxine (1 mg kg-1 i.p.) *per se* did not show anti-compulsive effect and did not affect motor activity. Venlafaxine at higher dose (5 mg kg-1 i.p.) however, showed inhibition of marble-burying behavior of mice. However, chlorpromazine (1 mg kg-1 i.p.) did not produce significant anti-compulsive effect and did not affect motor activity. Chlorpromazine at higher dose (2.5 and 5 mg kg-1 i.p.) produced significant anti-compulsive effect and did not affect motor activity. In view of above, we had selected such low doses of venlafaxine (1 mg kg-1 i.p.) and chlorpromazine (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, venlafaxine and chlorpromazine, when administered in combination may be acting synergistically to produce anti-compulsive effect.

According to serotonin hypothesis, patients with OCD have a dysregulation in the serotonergic system, with a hypersensitivity of postsynaptic 5-HT receptors, which could account for a different mechanism of action of SSRI in OCD [17]. The reduced serotonin function has been shown to increase locomotor activity and aggressive behavior in animals [18]. Venlafaxine, the bicyclic antidepressant, is usually categorized as a serotonin-norepinephrine reuptake inhibitor and is therapeutically used against obsessive-compulsive disorder [19]. Chlorpromazine antagonises the dopamine receptors (D_1, D_2, D_3 and D_4) and able to show antipsychotic properties as well as blocks the serotonin receptors (5-HT_1 and 5-HT_2) and show anxiolytic and antiaggressive properties. The combination of venlafaxine (1 mg kg-1 i.p.) and chlorpromazine (1 mg kg-1 i.p.) attenuated the marble-burying behavior of mice, thereby suggesting that the combination had anti-compulsive effect due to the synergistic action, which probably resulted in weak antagonism of serotonin receptors (5-HT_1 and 5-HT_2) and strong potentiation of serotonin reuptake inhibitory mechanism.

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