Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol.4 (4), Sept-Dec, 2015

Comparative Study of Agomelatine and Fluoxetine for the Reduction of Marble Buying Behavior in Obsessive Compulsive Disorder

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Abstract

Obsessive-Compulsive Disorder (OCD) is a debilitating disease which is characterized by persistent thoughts (obsessions), which are associated with seemingly purposeful ritualistic behavior (compulsions). Agomelatine, a novel melatonin agonist and selective serotonin antagonist (MASSA) antidepressant approved for major depressive disorder (MDD) has recently been additionally proposed as a treatment for anxiety disorders such as social anxiety disorder (SAD) and panic disorder (PD). The effect of acute and chronic administration of agomelatine on the marble-burying behavior (MBB) of mice, which is reported to be an index of anticompulsive behavior, was performed. In addition, to rule out the role of enhanced serotonerigic neurotransmission, studies were carried out in p-chlorophenylamine (PCPA). Results indicated a potent and dose dependent influence of agomelatine on MBB of mice, which was maintained after its chronic administration. However, the higher doses (40 and 50 mg/kg) were found to be locomotor depressant. Treatment with PCPA was not able to inhibit the effect of agomelatine on marble-burying behavior. Further the dunnett multiple comparison test revealed that fluoxetine had a significant effect at 10 mg/Kg ($P<0.01$). However, the lower dose found to be non-significant at 5 mg/Kg ($P>0.05$). In conclusion, agomelatine and fluoxetine administration reduces the MBB in mice, which should be explored for its potential use in the treatment of OCD.

Key words: *Obsessive Compulsive Disorder (OCD), Agomelatine, Fluoxetine, Marble, Buying Behavior (MBB), Locomotor Count (LC).*

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1. Introduction:

Obsessive compulsive disorder (OCD) involves severe alterations in thought processes and behavior. Its core features are intrusive and persistent thoughts that cause distress (obsessions), and compulsions, which are performed in order to alleviate this distress. Obsessions and compulsions are typically egodystonic, that is, the person is consciously aware that they are abnormal, yet cannot control them. Many people experience obsessive-like thoughts and/or compulsive-like behavior, but what distinguishes obsessions and compulsions of OCD is their frequency and intensity.[1]

OCD is characterized by high rates of partial and/or absent response to standard, recommended treatments, often prompting pharmacological and non-pharmacological augmentation or switching of strategies. Functional neuroimaging of subjects with OCD has revealed abnormalities in corticostriatal-thalamo-cortical circuits, involving the orbitofrontal cortex (OFC), anterior cingulated gyrus, insula, striatum and thalamus. These studies are consistent with the existence of distinct OCD symptom dimensions, as contamination/washing, checking and hoarding subgroups show different patterns of brain activity[2,3].

Agomelatine, a novel antidepressant with an innovative pharmacological profile, had received a marketing authorization by the European Medicines Agency for the treatment of major depressive disorder. One of the recent drugs used for the treatment of depression is agomelatine which happen to be a nonselective melatonin agonist. It has high-affinity for MT1/MT2 melatonin receptors and is also a 5-HT2C serotonin receptor antagonist. Agomelatine does not directly affect the uptake of serotonin, norepinephrine,or dopamine. By inhibiting 5HT-2C receptors, however, it secondarily increases norepinephrine and dopamine in the frontal cortex of the brain. This effect might contribute to its antidepressant activity. In view of these evidences, it appears that compulsive behavior might be modulated by OCD melatonergic system and hence this study aims to investigate the role of melatonergic system on the modulation of compulsive behavior in rodents.[4]

Fluoxetine also known by trade names Prozac and Sarafem among others is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It is used for the treatment of major depressive disorder, obsessive compulsive disorder (OCD), bulimia nervosa, panic disorder, and premenstrual dysphoric disorder. It may decrease the risk of suicide in those over the age of 65. Fluoxetine has also been used to treat premature ejaculation.[4]

Material and methods[6,7]

Adult male albino Swiss mice (22-25 g), were used for the present study. The animals were breed from an original stock purchased from Veterinary College, Mhow, India. The animals were group housed [mice (n=6)] under a standard 12h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). All animals

were acclimatized to laboratory conditions for at least seven days before carrying out the experiments, which were carried at 08.00 to 15.00 h daily. Separate group of mice (n=6/12) was used for each set of experiments.

Agomelatine and DL-4-chloro-phenylalanine (PCPA, a selective serotonin depletor) were purchased from Sigma-Aldrich; fluoxetine was gifted by Sun Pharmaceuticals, Baroda, India. Agomelatine was dissolved in 1% hydroxyl ethyl cellulose while fluoxetine was dissolved in 0.9% saline solution and pcpa was dissolved in propylene glycol.

Volume of drug administration

The volume of administration of drug vehicle was calculated based upon the body weight of mice i.e 10 ml/kg body weight of mice.

Apparatus: Marble-burying behaviour test apparatus

It consisted of plastic cages (40 × 28 × 14 cm) containing 5 cm thick wood dust bedding. Twenty small glass marbles (~10 mm), were arranged on the bedding evenly spaced in four rows of five each. The cage was covered by transparent plastic lead with line markings (2 × 2) and the apparatus is placed 1.5-2.0 m below a video camera in the experiment room with bright light (100 lux).

Figure 1. Marble burying behaviour of mice

Experimental methods

Assessment of marble-burying behavior and motor activity in mice

The marble-burying behavior and locomotor of mice was recorded as reported by Umathe et al., earlier with slight modifications (Umathe et al., 2008, Nicolas et al., 2006). In brief, mice were individually placed in marble-burying behavior apparatus with 20 glass marbles for 30 min. The behavior of the mice during the test session was recorded by a video camera. At the end 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. The video recording was analyzed to determine the number of line crossings made by the animal during a test session. Total number of line crossings measured during 30 min was considered as locomotor counts for the animals.

Treatments

Experiment 1: Acute study

Mice were randomly assigned to treatment conditions (n=6/12) in which Agomelatine (10,20,30,40,50mg/kg,i.p.) and Luzindole (10, 3, 5 mg/Kg, i.p.) were administered. Marble-burying behaviour was tested for 30 min after the administration of drugs.

Experiment 2: Combined drug study

Mice were randomly assigned to treatment conditions (n=6/12) in which:

1. ***Pre-treated with PCPA (300 mg/kg, i.p.) for 3 consecutive days and 24 hr thereafter 0.9% saline (10 ml/kg, i.p.) was administered 30 min prior to testing.***
2. ***Pre-treated with PCPA (300 mg/kg, i.p.) for 3 consecutive days, and 24 hr thereafter agomelatine (20, 30 mg/kg, i.p.) was administered 30 min prior to testing.***
3. ***Pre-treated with PCPA (300 mg/kg, i.p.) for 3 consecutive days, and 24 hr thereafter fluoxtine (10 mg/kg, i.p.) was administered 30 min prior to testing.***

Influence of acute drug treatment on MBB and locomotor count

Agomelatine

One-way ANOVA revealed that acute administration of agomelatine in different doses had a significant effect on the MBB of male mice [$F(5,53)=6.835, P<0.0001$] .

Post hoc analysis

Further the dunnett multiple comparison test revealed that agomelatine had a significant effect at 20mg/Kg ($P<0.01$), 30mg/Kg ($P<0.05$) and at 50mg/kg ($P<0.001$) however the lower dose found to be non significant at 10mg/Kg($P>0.05$). On studying locomotor activity, agomelatine has shown decrease in locomotor count in the dose dependent manner. At following doses of 20 and 30 mg/kg locomotor count has shown insignificant.

Fluoxetine

One-way ANOVA revealed that acute administration of Fluoxetine in different doses had a significant effect on the MBB of male mice [$F(2,17)=5.729, P=0.0412$].

Post hoc analysis

Further the dunnett multiple comparison test revealed that fluoxetine had a significant effect at 10 mg/Kg ($P<0.01$). However, the lower dose found to be non-significant at 5 mg/Kg ($P>0.05$). On studying locomotor activity fluoxetine showed insignificant effect on the following doses.

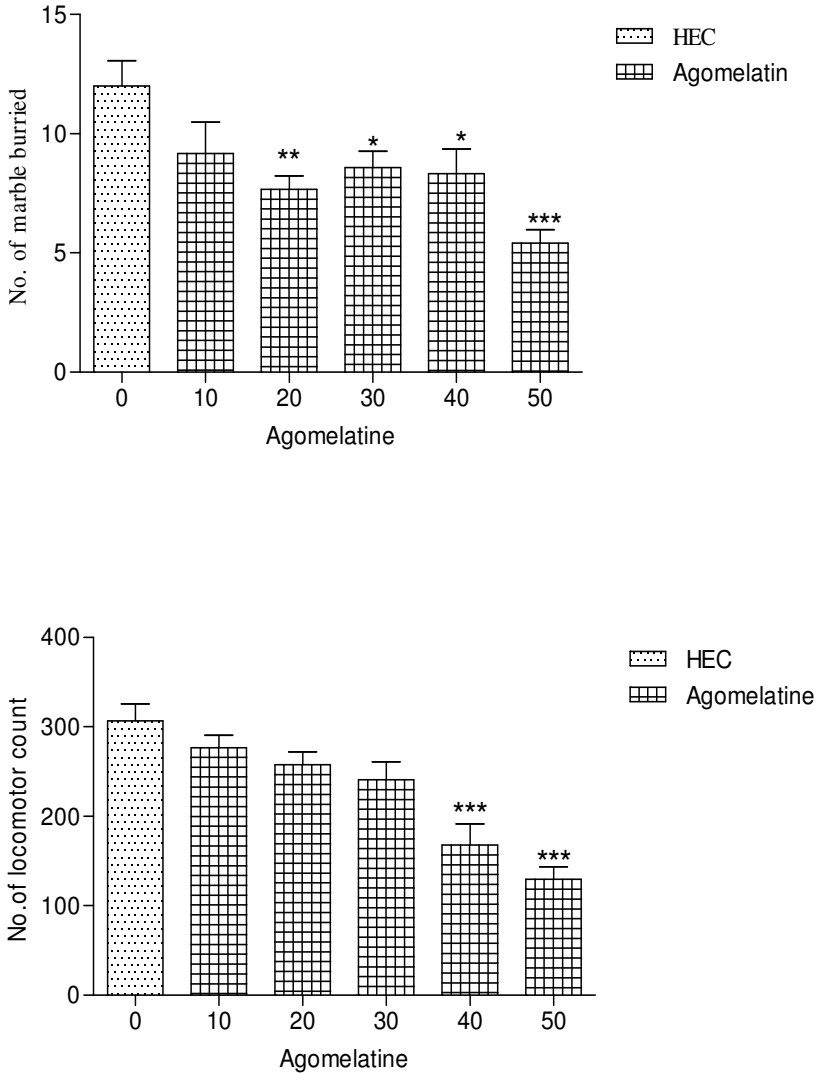

Figure 2. Influence of agomelatine on anticompulsive activity

 ISSN 2231 – 4423

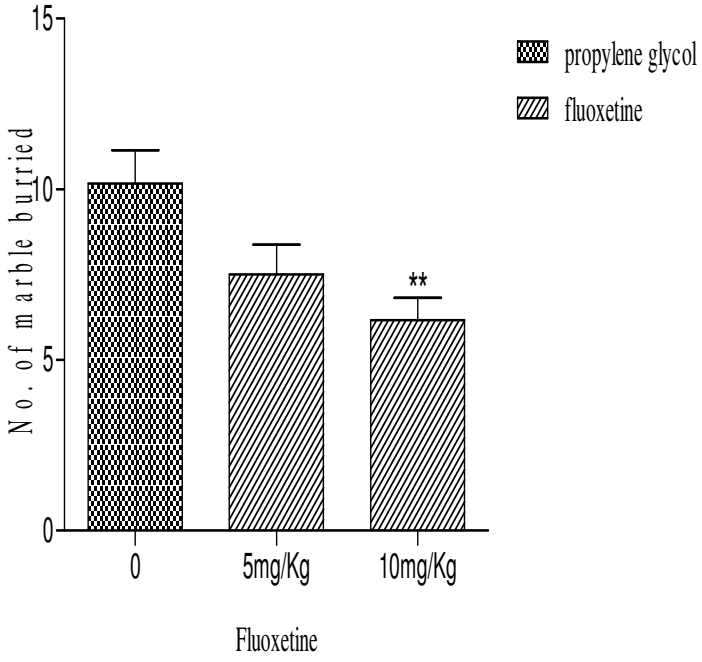

Figure 3. Influence of fluoxetine on marble buried

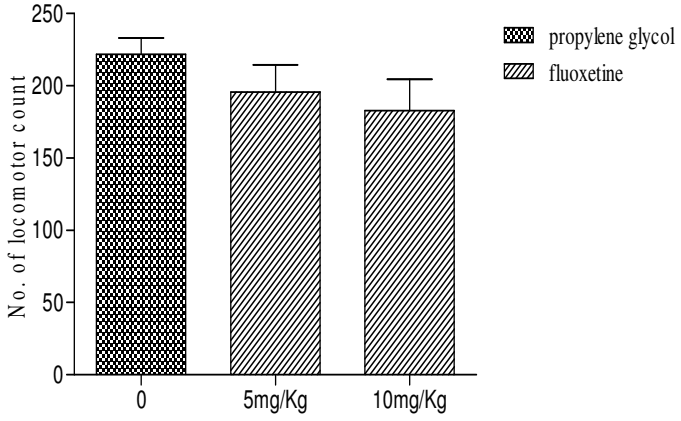

Figure 4. Influence of fluoxetine on locomotor count

Combined Studies

Influence of pCPA pre-treatment on the anticompulsive effect of

Separate groups of mice were injected with pCPA (300 mg/kg, i.p., ×3 days) or vehicle (10 ml/kg, i.p., × 3 days), and 24 h after the last dose, vehicle (10 ml/kg, i.p.), agomelatine (20 mg/kg, i.p.), or fluoxetine (10 mg/kg, i.p.) was

administered; 30 min thereafter, each mouse was tested for both marble-burying behavior and locomotor activity. Each bar represents mean±SD of data from 6-12 mice.

***P*<0.01 versus respective control (Two-way ANOVA, followed by the Bonferroni post-hoc test)

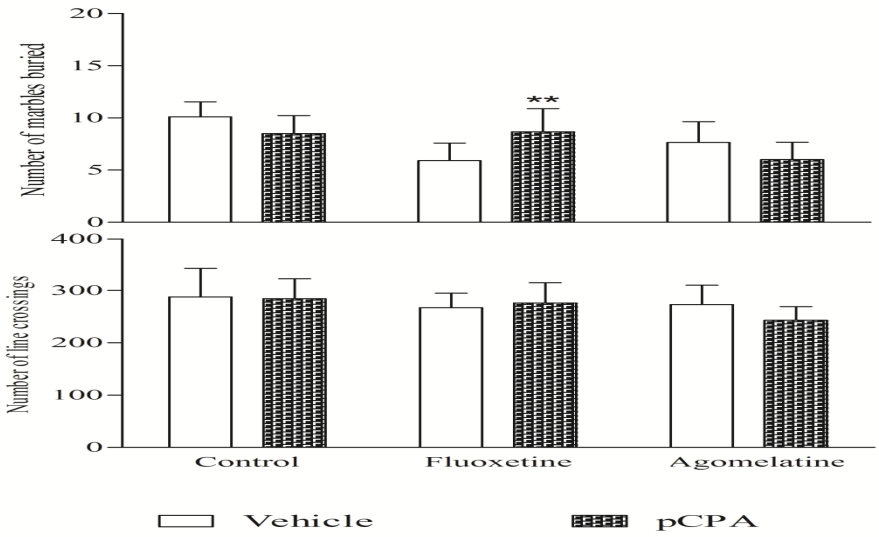

Figure 5. Influence of pCPA pretreatment on the anticompulsive effect of agomelatine

Conclusion

On studying the comparative study of agomelatine and fluoxetine on the rodents, pre treated with pCPA (Selective serotonin depletor) **,** agomelatine shows the less no. of locomotor count and marble buried count, which indicate the decrement in stress and anxiety level which is related to OCD. In the present work agomelatine a novel melatonergic analogue dose-dependently attenuated marble-burying behavior in mice, an effect that was comparable with that of fluoxetine. In addition, this effect of agomelatine was maintained after its administration for 10 days. Pretreatment with pCPA, which blocked the effects of fluoxetine, failed to reverse the influence of agomelatine on marble-burying behaviour in male mice.

It is observed that mice do not avoid marbles when given the opportunity to do so, indicating their non-aversive property. In addition, repeated exposure to marbles does not induce habituation, suggesting that this behaviour is not related to novelty or fear however, because the marbles are nonreactive, they cannot provide the animal with the necessary stimuli to a natural ending of the investigation, and this 'frustrated' investigation leads to compulsive burying.

Hence, although inhibition of object burying was originally suggested as a screening test for anxiolytic activity, the above findings and the reduction in burying behavior by serotonin reuptake inhibitors suggest that this behavior may be related to obsessive–compulsive disorder. For these reasons, we selected marble-burying behavior as a paradigm to screen the anticompulsive effect of agomelatine. It is a still a matter of debate whether marble burying measures anxiety or compulsivity; however, it is clear that marble burying is decreased by both anxiolytic drugs and anticompulsive drugs. Therefore, our results suggest that agomelatine may have anti-anxiety or anti-compulsive activity.

Finally, although the marble-burying model may at times fail to discriminate between anxiolytic and anticompulsive agents after acute administration, all drugs that modulate marble-burying behavior after repeated exposure without altering locomotor activity have been found to be clinically useful in the treatment of obsessive–compulsive disorder The anxiolytic actions of agomelatine are well documented; hence, the observed effect of agomelatine on marble burying observed in the present study indicates its anticompulsive potential and prompts further evaluation in other animal models of compulsivity.

Obsessive-compulsive disorder has a major impact on quality of life and affects all aspects of the individual's daily life. The etiology and pathophysiology of OCD is still far from being clear. Also the treatment of OCD is viewed as difficult and unsatisfactory, as almost half of the OCD patients do not respond to pharmacotherapies established so far. Available data indicates that agomelatine are involved in numerous neuropsychiatric disorders, and have modulatory role on neurotransmitters. Accordingly, the present study was designed to test the hypothesis of modulatory role of agomelatine in obsessive-compulsive disorder.

Major findings of the present investigations are that agomelatine reduced marble-burying behavior in mice, which was comparable to control. These studies were carried out by employing marble-burying behavior test in mice, which is widely employed to screen the effects of various agents on compulsive behavior. Marble-burying behavior is an unconditioned species-specific defensive reaction in rodents, which is not associated with physical danger, and does not habituate upon repeated testing). In male mice it is markedly attenuated by acute administration of SSRI and tricyclic antidepressants despite the acute anxiogenic properties of these drugs .These observations suggest that the burying behavior in male mice models the compulsive behavior rather than anxiety.

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