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PREDICTIVE VALIDITY OF BEHAVIORAL DESPAIR MODELS IN APPRAISING ANTIIMMOBILITY EFFECTS OF ONDANSETRON

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ABSTRACT

Experimental appraisal of antidepressants (ADs) in varied animal models is the need of time. There is a regular hunt for novel models with ease and quick screening of AD activity. As previous studies depict AD effect of ondansetron (OND) in animal models, the study was carried out to compare the efficacy of two behavioral despair models in delineating antidepressant-like effect of OND. OND in tail suspension test (TST) produced significant AD effect at a low dose of 0.25 mg/kg, as depicted by reduction in immobility period of drug-treated mice compared to control group ($p<0.05$), whereas OND in forced swim test (FST) produced insignificant AD effect at the dose of 0.25 mg/kg as compared to control ($p>0.05$). Results of our study highlight AD-like activity of OND in TST and FST models of depression, but TST in mice seems to be more sensitive in evaluating the AD like effect of OND.

KEYWORDS: **Antidepressant (AD), forced swim test (FST), 5-HT_3 antagonist, ondansetron (OND), tail suspension test (TST)**

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INTRODUCTION

Depression is a catastrophic psychiatric malady with lifetime prevalence of about 20 percent [1]. As per WHO, by the year 2020, depression will be the second largest cause of mortality [1,2]. It is a syndrome that embraces a gamut of symptoms such as feeling of despair, misery and low self-esteem which are not smoothly extrapolated in animal research [3]. Enduring research into vital pathophysiologic mechanism of depression and drug development is laborious but feasible [4]. Prevailing remedies for depression fall short to produce complete revival and are allied with unwanted side effects [5,6]. Thus, there is an immense degree of clinical demand for better drugs endowed with high efficacy and low toxicity. Nonetheless, evolution of new therapies need novel animal models or rational use of existing models [7,8]. However, there is a huge requirement for economical, simple, animal-friendly model with admirable predictive validity for quick screening. Forced swim test (FST) and tail suspension test (TST) are the two most popular behavioral despair models commonly used in acute settings. In both these models a state of immobility represents behavioral despair that clinically correlates with depression [9-11]. Ondansetron (OND) a 5-HT_3 receptor antagonist is a preferred drug for the management of cancer chemotherapy induced nausea and vomiting. The 5-HT_3 receptors has been tagged as a target for probable AD drugs [12]. Ondansetron and tropisetron show antidepressant-like effects in rodent models of depression [13]. Additionally, OND potentiated the AD effect of selective serotonin reuptake inhibitors (SSRIs) when SSRIs were used at sub-threshold doses in mouse FST [14]. Most of the studies conducted so far document AD-like effect of OND in FST. There are limited and diverse studies comparing antidepressant profile of OND in various models of depression based on behavioral despair (TST and FST). Hence, the present study was conducted to appraise the comparative efficacy of models in depicting antiimmobility (AD) effect of OND.

MATERIALS AND METHODS

Animals

Swiss albino mice weighing between 25-30 gm were used for this study. Mice were procured from central animal house of Narayana Medical College, Nellore. The animals were housed in cages in temperature-regulated rooms with air cooling and 12 h light and dark cycle, and had an access to food and water ad libitum. They were allowed to acclimatize to the laboratory conditions for a period of one week. The study was approved by the Institutional Animal Ethics Committee, (protocol number IAEC 16/2011/NMC)

Drugs

Test drug ondansetron was sourced from Sigma Aldrich, Bengaluru, India, while reference drug fluoxetine (FLX) was from Sun Pharma, Mumbai, India, dissolved in normal saline (NS). All the chemicals and reagents used in the study were of analytical grade and were prepared fresh before test.

Study design

The animals were randomly divided into six groups containing six animals in each group (n=6) for both the models (TST and FST). Group 1 was control group, pre-treated with NS (0.1 ml/10g), Group 2, 3, 4 and 5 were pre-treated with the doses of ondansetron (OND) as 0.25, 0.5, 1.0, and 2 mg/kg (i.p.) respectively for 7 days (Table 1 and 2), Group 6 was pre-treated with fluoxetine (FLX) 20 mg/kg (i.p) for 7 days. OND and FLX were dissolved in NS. A pre-test session was provided to all the animal groups 24 hour prior to the experimentation.

Behavioural despair models

(i) Tail suspension test (TST)[11]

This animal model is based on the principle that when an animal is suspended upside down it displays characteristic behaviour of immobility after an initial period of mobility. The period of immobility reflects a state of depression which can be reduced by antidepressant drugs [15,16].

Mice were suspended 50 cm above the floor by adhesive tape placed 1 cm from the tip of tail. Group 1 was treated with NS (0.1 ml/10g), Group 2, 3, 4 and 5 were given OND in the doses of 0.25, 0.5, 1.0, 2.0 mg/kg (i.p.) respectively, while Group 6 received FLX 20 mg/kg (i.p.) for 7 days. On the 7th day animals were subjected to TST after 30 minute (i.p.) treatment. Each mice was observed for a period of six minutes, initially mice showed vigorous struggling movement, then mice displayed alternating periods of immobility and vigorous struggling. The duration of immobility was recorded during the last 4 minute period, a mouse was considered immobile when it did not show any movement and hanged passively. Decrease in the duration of immobility period was considered as an index of antidepressant activity.

(ii) Forced swim test (FST)[9]

This animal model is based on the principle that forcing mice to swim in restricted space from which they cannot escape leads to a characteristic behaviour of immobility. Such behaviour reflects a state of hopelessness, which can be treated by various drugs that are therapeutically effective in human depression. Mice were placed individually in a glass chamber containing water. The test included, 6 min exposure of mice to the water container (25x12x25cm) containing water up to 15 cm and maintained at 23-25°C. Group 1 was treated with NS (0.1 ml/10g), Group 2, 3, 4 and 5 were given OND in the doses of 0.25, 0.5, 1.0 and 2.0 mg/kg (i.p.) respectively, while Group 6 received FLX 20 mg/kg (i.p.) for 7 days. On the 7th day animals were subjected to FST after 30 minute (i.p.) treatment. Each mice was observed for a period of six minutes, a mouse was considered to be immobile when it floated or made only small movements necessary to keep its head above water. Decrease in the duration of immobility period was considered as an index of antidepressant activity.

Statistical Analysis

The data was reported in case record forms and entered into excel spreadsheet 2007. Statistical analysis was carried out using Microsoft Excel-2007 and Sigma Graph pad prism version-5 USA. Data was described as Mean (Standard deviation) [17]. One way ANOVA followed by Post hoc Tukey's multiple Comparison tests was used for analysis of data between the six groups. For all inferential statistical tests a two tailed ($p<0.05$) was considered significant.

RESULTS

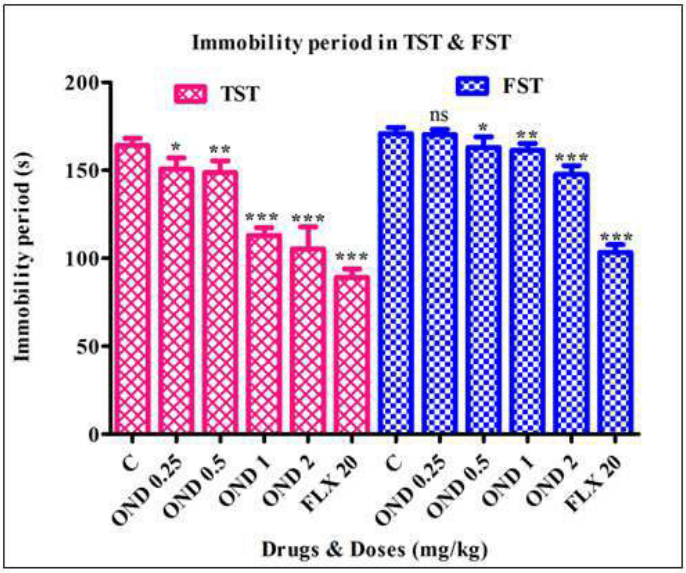
Table1

Effect of Ondansetron on immobility period in tail suspension test (TST)

Groups	Number of Mice	Duration of immobility time (sec) Mean(SD)
1. Control (NS)	6	164.2(4.2)
2. Ondansetron 0.25 mg/kg, i.p	6	150.7(6.4)*
3. Ondansetron 0.5 mg/kg, i.p	6	148.7(6.7)**
4. Ondansetron 1.0 mg/kg, i.p	6	113(4.5)***
5. Ondansetron 2.0 mg/kg, i.p	6	105.3(12.5)***
6. Fluoxetine 20 mg/kg, i.p	6	89(4.7)***
ns- p>0.05, *p<0.05, ** p<0.01, *** p<0.001		

In TST as per Table1 the immobility period with control (NS) was 164.2(4.2) sec whereas, it was 150.7(6.4) sec, 148.7(6.7) sec, 113(4.5) sec, 105.3(12.5) sec and 89(4.7) sec with OND (0.25, 0.5, 1.0, 2.0 mg/kg) and FLX 20 mg/kg respectively. OND 0.25 mg/kg significantly decreased the immobility period as compared to the control (p<0.05). On the other hand, OND at higher doses of 0.5 mg/kg (p<0.01), 1.0 mg/kg (p<0.001) and 2.0 mg/kg (p<0.001) significantly reduced the immobility period as compared to the control and at a higher dose of 1-2 mg/kg was of comparable efficacy to FLX. FLX 20 mg/kg (p<0.001) treated group showed significant reduction in immobility period as compared to control. Therefore, OND induced dose dependent reduction in immobility period in TST as compared to control (Graph 1).

Graph 1
Immobility period in Tail Suspension Test (TST) and Forced Swim Test (FST)



Bars represent the mean immobility period in seconds (SD) in TST and FST as compared to control (C). ns- not significant (P>0.05),*P<0.05, **P<0.01,P<0.001 one way ANOVA followed by Post hoc Tukey's multiple comparison tests.***

Table2
Effect of Ondansetron on immobility period in forced swim test (FST)

Groups	Number of Mice	Duration of immobility time (sec) Mean(SD)
1. Control (NS)	6	170.8(3.4)
2. Ondansetron 0.25 mg/kg, i.p	6	170.3(2.9)^{ns}
3. Ondansetron 0.5 mg/kg, i.p	6	163(6.0)*
4. Ondansetron 1.0 mg/kg, i.p	6	161.3(3.9)**
5. Ondansetron 2.0 mg/kg, i.p	6	147.7(4.9)***
6. Fluoxetine 20 mg/kg, i.p	6	103.3(4.3)***
ns- p>0.05, *p<0.05, ** p<0.01, *** p<0.001		

In FST as per Table 2 the immobility period with control (NS) was 170.8(3.4) sec whereas, it was 170.3(2.9) sec, 163(6.0) sec, 161.3(3.9) sec, 147.7(4.9) sec and 103.3(4.3) sec with OND (0.25, 0.5, 1.0, 2.0 mg/kg) and FLX 20 mg/kg respectively. Reduction in the immobility period with OND at 0.25 mg/kg was not significant as compared to control (p>0.05). Whereas, OND at higher doses of 0.5 (p<0.05), 1.0 (p<0.01), 2.0 mg/kg (p<0.001) significantly reduced the immobility period as compared to control. OND at the dose of 2.0 mg/kg was of comparable efficacy to FLX, while FLX treated groups showed significant reduction in immobility period as compared to control (p<0.001) (Graph 1).

DISCUSSION

The results of our study depict AD activity of OND in both the models of behavioral despair. OND in TST shows significant AD activity at a low dose of 0.25 mg/kg (p<0.05) as compared to control (Table 1, Graph 1), while in FST at the same dose (0.25 mg/kg) AD activity was not elicited (p>0.05) as compared to control (Table 2, Graph 1).The results of our study are in accordance with the study conducted by Radhakrishnan et al., [18]. They documented AD activity of OND in FST and reported that OND elicits significant AD-like effect only at the dose of 0.5, 1.0, 2.0 mg/kg showing no AD efficacy in low dose of 0.25 mg/kg as compared to control. The possible mechanism for antidepressant profile of OND may be due to blockade of 5-HT_3 receptors, thereby modulating neurotransmitter release [19]. Hence from the outcome of our study we infer that TST seems to have a better predictive validity in evaluating AD-like activity of OND as compared to FST. Our findings are further supported by the work carried out by

St´eru et al. They documented that TST has a higher sensitivity in depicting AD effect of drugs at comparatively lower doses and is more specific for drugs that predominantly modulate serotonergic neurotransmission like selective serotonin reuptake inhibitors (SSRIs), atypical antidepressant, tricyclic antidepressants (TCA) and monoamine oxidase inhibitor (MAOI) in comparison to other models of behavioral despair [11].Further, in our study we observed some remarkable merits of TST as compared to FST such as the dose requirement is comparatively less, it is a dry method, precious water is saved, protection of animal from hypothermic effects of cold water, animal resumes normal spontaneous motor activity on the spot once the animal is out of the experimental setup, no additional maintenance of animal in warm surrounding or rubbing is required. These merits make the model economical. Moreover, TST augments the ease of investigator. Observations of our study are in agreement with the study carried out by Castagn´e, et al., [20]. They additionally documented that automated version of TST enables researcher to investigate numerous animals simultaneously, subsequently boosting the productivity for screening of novel compounds with AD activity. Their findings highlight that TST is a better model to use transgenic mice which are highly efficacious in portraying AD activity of various pharmacologically active compounds mediating AD like activity through novel mechanisms. Cryan et al., reported that strains of mice suffering with motor deficits can be used for experimental purpose in TST model only as swimming is difficult in FST [21]. Thus our study emphasizes superior sensitivity of TST as compared to FST in portraying antiimmobility (AD) profile of ondansetron.

CONCLUSION

There are numerous models for predicting AD activity of drugs. They are broadly classified into acute and chronic models. TST model of behavioral despair (acute), with all its merits as stated above seems to be more precise, economical, congenial to animal and researcher. The model goes in harmony with good laboratory and animal handling guidelines. So, it should be ranked as a pinnacle of acute model for rapid screening of AD drugs. Nonetheless, these observations call for further affirmation by more innovative and exploratory studies.

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