National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Behavioral assessment of antidepressant activity of Memantine - a NMDA receptor antagonist in animal models: An experimental study

Harish G Bagewadi, Rajeshwari, Banderao V Patil

Department of Pharmacology, Gulbarga Institute of Medical Sciences, Gulbarga, Karnataka, India

Correspondence to: Rajeshwari, E-mail: rajeshwarineela6@gmail.com

Received: February 14, 2018; **Accepted:** March 04, 2018

ABSTRACT

Background: Around 5–10% of patients on selective serotonin reuptake inhibitors discontinue therapy because of side effects. Therefore, research for newer antidepressants with greater effectiveness has to be explored. **Aims and Objectives:** The present study is undertaken to evaluate the antidepressant activity of Memantine in Swiss albino mice. **Materials and Methods:** They were divided into four groups containing six mice in each group. First group mice were given normal saline (control) 10 mL/kg, Fluoxetine (20 mg/kg, i.p.) as standard for the second group and for third group Memantine 3 mg/kg (test drug), and Memantine plus Fluoxetine (3 mg/kg + 20 mg/kg) for the fourth group intraperitoneally daily for 15 consecutive days. Duration of immobility was observed for 4 min in forced swimming test. Duration of locomotor activity was observed in photoactometer. Results were analyzed by ANOVA followed by *post hoc* Tukey's test. **Results:** Memantine significantly reduced the immobility time in forced swim test compared to control ($P < 0.001$). Memantine showed no significant effect on locomotor activity in photoactometer. Memantine showed synergistic antidepressant effect with Fluoxetine when combined together. **Conclusion:** N-methyl-D-aspartate antagonist, Memantine has showed significant antidepressant activity in experimental models of depression in mice.

KEY WORDS: Memantine; N-methyl-D-aspartate -antagonist; Forced Swim Test; Anti-depressant; Fluoxetine

INTRODUCTION

Depression is one of the major mental disorders. It is common in women, who have a lifetime prevalence for the major depressive disorder of 21.3% when compared to 12.7% in men.[1] Researchers have discovered associations between clinical depression and the function of three major neurotransmitters-serotonin, norepinephrine, and dopamine. Most antidepressant medications increase the levels of one or more of these neurotransmitters in the synaptic cleft.

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2018.8.0208004032018	

Approximately two-thirds of the patients with depression respond better to the currently available treatments, but the magnitude of improvement is still disappointing.[2] Around 5–10% of patients on SSRI's discontinue therapy because of adverse effects related to the gastrointestinal tract and central nervous system and weight gain.[3] Therefore, research for new antidepressants with greater effectiveness is still desirable. Over the past decade, interest has turned to a potential role of the glutaminergic system in depression, particularly with focus on N-methyl-D-aspartate (NMDA) receptor.[4] This is a departure from previous thinking, which had focused on serotonin and norepinephrine. It is evident that neurotransmission through NMDA receptors is deregulated in depression.

Neurotrophin brain-derived neurotrophic factor (BDNF) and its tropomyosin-related kinase B receptor have been connected to the mechanism of action of antidepressants

and the pathophysiology of major depressive disorder.[5,6] The neurotrophin hypothesis of depression presumes that stress-induced reduction of BDNF signaling and neuronal plasticity causes atrophy and weakening of synaptic connections in specific brain areas, finally leading to altered information processing and mood disorders.[6,7] Furthermore, antidepressant treatment enhances BDNF signaling and, in the long term, increases BDNF-mediated neuronal plasticity in the brain, facilitating patient recovery. Because BDNF-induced changes in plasticity take time to develop, the clinical relief of MDD symptoms is delayed. In animal studies, different stressors or corticoid injections decrease the expression of BDNF in the hippocampus and prefrontal cortex, brain areas related to mood disorders. Altered BDNF expression levels can be reversed by both chronic electroconvulsive therapy and antidepressant treatment.[8] Furthermore, several clinical studies have shown that BDNF serum levels are decreased in depressed patients.[9]

Therefore, it appears that NMDA receptor antagonists may be key to developing a new generation of improved treatments for major depression. Antidepressant-like effects have been demonstrated by several types of NMDA receptor antagonists in different animal models.[10,11] These antagonists include competitive and non-competitive, antagonists and partial agonists at strychnine-insensitive glycine receptors, and also antagonists acting at polyamine binding sites. MK-801 (a use-dependent channel blocker or uncompetitive antagonist) and CGP 37849 (a competitive antagonist) have shown antidepressant properties in preclinical studies, either alone or combined with traditional antidepressants.[12,13]

Other antagonists of glutamate NMDA receptors like ketamine[14] topiramate[15] have showed antidepressant effects. It was reported that the statin dose independently improves depression and anxiety through NMDA receptors.[16] Similar to ketamine, other NMDA receptor antagonists have been shown to have antidepressant-like effects and to potentiate the effects of classical antidepressants in rodents.[17] Because ketamine and other NMDA antagonists have severe side effects and the potential for abuse, other glutamate-based approaches, including the potentiation of AMPA (alfa-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid) receptors, have increasingly become the focus of preclinical studies. The medical need for newer, better-tolerated and more efficacious treatments remains high. Hence, newer potent antidepressant with minimal side effects should be investigated. Memantine is a non-competitive NMDA receptor antagonist, with peculiar pharmacological properties, which explain its excellent safety and tolerability profile, at variance with the most potent NMDA receptor blockers such as ketamine, phencyclidine, and MK-801.[18]

Memantine is a non-competitive NMDA antagonist which is used in Alzheimer's disease. Still, there are no conclusive findings showing the antidepressant effects of Memantine. Some studies have shown antidepressant effects of Ketamine (NMDA receptor antagonist) but, it is also known psychedelic and dissociative drug. On the other side, Memantine (NMDA receptor antagonist) has excellent safety and tolerability profile. In C57BL/6 strain mice, Memantine antidepressant activity was observed by Kos and Popik.[19]

There are hardly any studies in literature showing the effect of Memantine in depression models of Swiss albino strain mice. Thus, in the present study, we evaluated Memantine for its antidepressant activity in BALB/c strain Swiss albino mice.

MATERIALS AND METHODS

Swiss albino mice of either sex weighing between 25 and 30 g were used in our study. The animals were housed in cages and kept under controlled environmental condition (temperature 22 ± 2°C, humidity 50–55%, and natural light/day cycle). All the experiments were performed in the daytime between 09:30 and 15:30 h. Care of animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was duly approved by the Institutional Animal Ethics Committee. Drugs and Chemicals - Memantine (Sun Pharma drugs Pvt. Ltd. India), Fluoxetine (Zydus Cadila Ltd. India) diluted in Normal saline were used. In the experiment, mice are divided into following groups ($n = 6$).

1. Group 1 - control (normal saline, 10 mL/kg i.p.)
2. Group 2 - memantine (3 mg/kg, i.p.)
3. Group 3 - fluoxetine (20 mg/kg, i.p.)
4. Group 4 - memantine (3 mg/kg, i.p.) + fluoxetine (20 mg/kg, i.p.)

Memantine, Fluoxetine, and normal saline were administered intraperitoneally daily for 15 days of the experimental period to see their effects on the 8th day and 15th day. The mice are administered respective drugs/normal saline intraperitoneally as scheduled, and behavioral assessment was conducted 30 min after drug administration.

Assessment of Behavioral Tests

1. Forced swim test (FST) - the forced swimming model to test for antidepressant activity was developed by Porsolt *et al*.[20] The mice are forced to swim in a plastic cylinder measuring 30 cm × 30 cm containing water at room temperature to a depth of 20 cm. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility is recorded during next 4 min of total 6-min test.

2. Locomotor activity[21] - animal is kept in photoactometer for the first 3 min, and then locomotor activity is recorded using photoactometer for a period of 5 min. The apparatus is placed in darkened, light-sound attenuated and ventilated testing room. Each mouse is observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive photocells using digital photoactometer and values expressed as counts per 5 min.

Statistical Analysis

Results are presented as mean ± SEM. One-way ANOVA is used for comparison between the groups, followed by *post hoc* Tukey's test. For all the tests $P = 0.05$ or less is considered statistically significant.

RESULTS

Mice treated with Fluoxetine as a standard showed a significant decrease ($P < 0.001$) in immobility period on the 8th day and 15th day as compared to control group as shown in Tables 1 and 2.

When Memantine treated group compared to control group, mice showed a slight decrease in immobility period on the 8th day, but not statistically significant [Table 1] whereas, on 15th day there was significant decrease ($P < 0.001$) in immobility period [Table 2]. Mice treated with Memantine as a test drug when compared to Fluoxetine-treated group, mice showed a significant decrease ($P < 0.001$) in immobility period on the 8th day and significant decrease ($P < 0.05$) on the 15th day as shown in Tables 1 and 2.

On 8th day and 15th day, Memantine + Fluoxetine-treated group showed a significant decrease ($P < 0.001$) in immobility period when compared to Memantine alone as shown in Tables 1 and 2. Group treated with Fluoxetine on the 8th day and 15th day, showed slight increase in locomotor activity but not statistically significant when compared to control group as shown in Tables 3 and 4.

Group treated with Memantine as a test drug on the 8th day and 15th day, showed a slight increase in locomotor activity but not statistically significant when compared to control group and Fluoxetine treated group as shown in Tables 3 and 4. Group treated with Memantine + Fluoxetine on the 8th day and 15th day showed slight decrease in locomotor activity but not statistically significant when compared to Memantine alone as shown in Tables 3 and 4.

DISCUSSION

In the present study Memantine treated group when compared to control group, revealed a significant reduction in the duration of immobility in FST. Memantine significantly reduced the immobility in FST, on the 15th day when compared to the 8th day. However, no significant effect on locomotor activity in photoactometer test was observed. There also exists a statistically significant difference in the FST immobility period in the groups treated with both Memantine (3 mg/kg) and Fluoxetine (20 mg/kg) compared to Memantine alone treated group on the 8th day, but not significant on the 15th day. This demonstrated synergistic interaction between

Table 1: Effect of single dose observation in FST on the 8th day

Groups (dose)	Duration of immobility (s)
Normal saline (10 mL/kg, i.p.)	148.4±3.52
Fluoxetine (20 mg/kg, i.p.)	76.2±3.85*
Memantine (3 mg/kg, i.p.)	136.6±2.47††
Memantine+Fluoxetine	58.3±1.39‡

n=6, values expressed as mean±SEM. *P<0.001 versus normal saline - control, †P<0.05, ††P<0.01 vs. Fluoxetine, ‡P<0.001 versus Memantine, FST: Forced swim test

Table 2: Effect of multiple dose observation in FST on the 15th day

Groups (dose)	Duration of immobility (s
Normal saline (10 mL/kg, i.p.)	158.2±2.14
Fluoxetine (20 mg/kg, i.p.)	72.6±2.97*
Memantine (3 mg/kg, i.p.)	83.3±2.73*,†
Memantine+fluoxetine	67.4±1.18‡

n=6, values expressed as mean±SEM. *P<0.001 versus normal saline - control, †P<0.05, ††P<0.001 versus Fluoxetine, ‡‡P<0.001 versus Memantine, FST: Forced swim test

Table 3: Effect of single dose observation on locomotor activity on the 8th day

Groups (dose)	Number of counts/5 min
Normal saline (10 mL/kg, i.p.)	191.1±4.01
Fluoxetine (20 mg/kg, i.p.)	204.3±5.23*
Memantine (3 mg/kg, i.p.)	194.5±2.64††
Memantine+fluoxetine	203.2±3.54‡

n=6, values expressed as mean±SEM. *P<0.001 versus normal saline - control, †P<0.05, ††P<0.01 versus Fluoxetine, ‡P<0.001 versus Memantine

Table 4: Effect of multiple dose observation on locomotor activity on 15th day

Groups (dose)	Number of counts/5 min
Normal saline (10 mL/kg, i.p.)	197.5±3.06
Fluoxetine (20 mg/kg, i.p.)	206.2±2.81*
Memantine (3 mg/kg, i.p.)	195.1±2.42*,††
Memantine+fluoxetine	211.6±6.85‡

n=6, values expressed as mean±SEM. *P<0.001 versus normal saline - control, †P<0.05, ††P<0.01 versus Fluoxetine, ‡P<0.001 versus Memantine

Memantine and Fluoxetine in their antidepressant activity. The immobility time in FST is also reduced by central nervous system stimulants, but they tend to increase the locomotor activity in the animals as opposed to the antidepressants, which does not bring much change in locomotion.[22] In the present study, Memantine administered for 15 days, did not show any significant change in locomotor activity of mice, as compared to the control group, which helps to confirm the antidepressant-like activity [Tables 3 and 4], which is not a false positive.

The outcomes of the present studies are in agreement with previous experiments which indicated that antidepressant-like activity of CGP 37849 and L-701,324 by a significant reduction in the duration of immobility when measured in the FST in mice.[23,24] The above findings of our behavioral tests are similar with another previous study by Karve *et al*.[25] Similar antidepressant activity study of Memantine was done by us wherein we used amitriptyline as a standard control which belongs to (tricyclic antidepressant class),[26,27] but, in the present study, we used Fluoxetine as a standard drug having less side effects clinically, which belongs to selective serotonin reuptake inhibitors class for comparison to avoid the bias.

Our study limitations were that we could not correlate our findings with the biochemical estimation of BDNS, neuropeptide Y (NPY), AMPA, and hypothalamus pituitary adrenal (HPA) levels due to lack of laboratory facilities. The previous study by Wierosnka *et al*.[28] indicates that in the amygdala, the NMDA receptors mediated glutamatergic transmission may regulate NPY neurons. There is also evidence showing topiramate (NMDA receptor modulator) alter the NPY activity in Flinders Sensitive Line "Depressed" rats.[29] Future studies are required to elucidate the activity of Memantine on these biochemical parameters, which might contribute to the antidepressant activity, which cannot be ruled out giving new way for its further exploration.

CONCLUSION

Memantine at a dose of 3 mg/kg, i.p. has demonstrated antidepressant activity which was comparable to Fluoxetine. Memantine could be producing its antidepressant activity by blocking NMDA receptor. However, its modulating effect on NPY which might contribute to antidepressant activity cannot be ruled out. There was synergism in antidepressant activity of Memantine and Fluoxetine. Further research is required to gain closer insights into the exact mechanism of action of Memantine and which might be of benefit to depressed patients in a clinical scenario.

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How to cite this article: Bagewadi HG, Rajeshwari, Patil BV. Behavioral assessment of antidepressant activity of Memantine - a NMDA receptor antagonist in animal models: An experimental study. Natl J Physiol Pharm Pharmacol 2018;:964-968.

Source of Support: Nil, **Conflict of Interest:** None declared.