



Effect of memantine in experimental models of depression in swiss albino mice

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ABSTRACT

The present study is undertaken to evaluate the antidepressant activity of Memantine in swiss albino mice. They were divided into four groups containing six mice in each group. First group mice were given normal saline (control) 10ml/kg, Amitriptyline 10mg/kg as standard for second group and for third group Memantine 3mg/kg (test drug) and Memantine plus Amitriptyline (3mg/kg + 10mg/kg) for fourth group intraperitoneally daily for 7 consecutive days. Duration of immobility was observed for 4 minutes in forced swimming test. Duration of locomotor activity was observed in Photoactometer. Results were analyzed by ANOVA followed by Post hoc Tukey's test. 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 Amitriptyline. NMDA (N-methyl-D-aspartate) antagonist, Memantine has showed significant antidepressant activity in experimental models of depression in mice.

Key words: Memantine, NMDA-antagonist, Forced swim test, Locomotor activity, Anti-depressant.

INTRODUCTION

Depression is one of the major mental disorders. It is common in women, who have lifetime prevalence for 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. 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 (AEs) related to 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 NMDA receptor[4]. This is a departure from previous thinking, which had focused on serotonin and norepinephrine. It is evident that neurotransmission via NMDA receptors is deregulated in depression.

Indeed, there is a wealth of evidence indicating the involvement of the NMDA receptor complex in the modulation of depression. Both pre-clinical and clinical studies indicate that compounds that reduce transmission at NMDA receptors show antidepressant-like actions[5]. Other antagonists of glutamate N-methyl-D-aspartate (NMDA) receptors like Ketamine[6], Topiramate[7] have showed antidepressant effects. It was reported that the statin dose independently improves depression and anxiety via NMDA receptors[8]. Memantine is a non-competitive NMDA antagonist which is used in Alzheimer's disease. There are no studies in literature showing effect of Memantine in depression models of swiss albino mice. Thus, Memantine is evaluated for its antidepressant activity in this study.

EXPERIMENTAL SECTION

2.1. Aims and objectives- To study the effect of Memantine on behavioral parameters of depression in mice.

2.2. Animals- Swiss albino mice of either sex weighing between 25 and 30 Gms were obtained from the Central Animal House of MVJ Medical College & Research Hospital. The animals were housed in cages and kept under controlled environmental condition (temperature 22 ± 2 °C, humidity 50–55 %, natural light/day cycle). All the experiments were performed in daytime between 09:30 and 15:30 hours. 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.

2.3. Drugs and Chemicals- Memantine (SunPharma drugs Pvt.Ltd. India), Amitriptyline (IntasPharmaceuticals Ltd.India) diluted in Normal saline were used.

2.4. Experimental Groups- In the experiment mice are divided into following groups (n= 6).

Group 1- control (normal saline, 10 ml/kg i.p.)

Group 2- Memantine (3mg/kg, i.p.)

Group 3- Amitriptyline (10 mg/kg, i.p.)

Group 4- Memantine (3mg/kg, i.p.) + Amitriptyline (10 mg/kg, i.p.)

Memantine, Amitriptyline, normal saline were administered intraperitoneally daily for 7 days of experimental period to see their effects on '0' and day '07'. The mice are administered respective drugs/normal saline intraperitoneally as scheduled, and behavioral assessment was conducted 30 minutes after drug administration.

2.5. Assessment of behavioral tests-

2.5.1 Forced swim test- The forced swimming model to test for antidepressant activity was developed by Porsolt et al [9]. The mice are forced to swim in a plastic cylinder measuring 30 X 30 cm containing water at room temperature to a depth of 20 cm. After an initial 2 minute 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 minutes of total 6 minute test.

2.5.2 Locomotor activity [10] - 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.

2.6. Statistical Analysis- Results are presented as Mean \pm SEM. One way ANOVA is used for comparison between the groups, followed by post hoc Tukey's test. For all the tests 'P' value of 0.05 or less is considered statistically significant.

RESULTS AND DISCUSSION

Table1: Effect of single dose observation in Forced swim test on day '0'

| Groups, (dose) | Duration of Immobility (sec) |
|-----------------------------------|--------------------------------|
| 1. Normal saline (10 ml/kg, i.p.) | 150.4 \pm 4.52 |
| 2. Amitriptyline (10mg/kg, i.p.) | 78.2 \pm 2.85 [*] |
| 3. Memantine (3mg/kg, i.p.) | 138.6 \pm 3.47 ^{††} |
| 4. Memantine + Amitriptyline | 60.3 \pm 2.39 [‡] |

(n=6), values expressed as mean \pm SEM. (^{*}p < 0.001 vs. normal saline-control), ([†]p < 0.05, ^{††}p < 0.01 vs. Amitriptyline), ([‡]p < 0.001 vs. Memantine)

Table2: Effect of multiple dose observation in Forced swim test on day '7'

| Groups, (dose) | Duration of Immobility (sec) |
|-----------------------------------|-------------------------------|
| 1. Normal saline (10 ml/kg, i.p.) | 156.2 \pm 3.14 |
| 2. Amitriptyline (10 mg/kg, i.p.) | 70.6 \pm 1.97 [*] |
| 3. Memantine (3mg/kg, i.p.) | 81.3 \pm 1.73 ^{††} |
| 4. Memantine + Amitriptyline | 65.4 \pm 2.18 [‡] |

(n=6), values expressed as mean \pm SEM. (^{*}p < 0.001 vs. normal saline-control), ([†]p < 0.05, ^{††}p < 0.001 vs. Amitriptyline), ([‡]p < 0.001 vs. Memantine)

Mice treated with Amitriptyline as a standard, showed significant decrease (p < 0.001) in immobility period on day '0' and day '07' as compared to control group (as shown in table1 and table2).

When Memantine treated group compared to control group, mice showed slight decrease in immobility period on day '0', but not statistically significant (table1).Whereas, on day '07' there was significant decrease ($p < 0.001$) in immobility period (table2).

Mice treated with Memantine as a test drug when compared to Amitriptyline treated group, mice showed significant decrease ($p < 0.001$) in immobility period on day '0' and significant decrease ($p < 0.05$) on day '07'(as shown in table 1 and table 2).

On day '0' & day '07', (Memantine + Amitriptyline) treated group showed significant decrease ($p < 0.001$) in immobility period when compared to Memantine alone(as shown in Table 1 and table 2).

Table3:Effect of single dose observation on Locomotor activity on day '0'

| Groups, (dose) | No. of Counts/5 min |
|-----------------------------------|--------------------------|
| 1. Normal saline (10 ml/kg, i.p.) | 196.1± 4.01 |
| 2. Amitriptyline (10mg/kg, i.p.) | 205.3±5.23 [*] |
| 3. Memantine (3mg/kg, i.p.) | 198.5±2.64 ^{††} |
| 4. Memantine + Amitriptyline | 209.2±3.54 [‡] |

($n=6$), values expressed as mean±SEM. (^{*} $p < 0.001$ vs. normal saline-control), ([†] $p < 0.05$, ^{††} $p < 0.01$ vs. Amitriptyline), ([‡] $p < 0.001$ vs. Memantine)

Table4:Effect of multiple dose observation on Locomotor activity on day '7'

| Groups, (dose) | No. of Counts/5 min |
|-----------------------------------|---------------------------|
| 1. Normal saline (10 ml/kg, i.p.) | 199.5±3.06 |
| 2. Amitriptyline (10 mg/kg, i.p.) | 208.2±2.81 [*] |
| 3. Memantine (3mg/kg, i.p.) | 197.1±2.42 ^{*††} |
| 4. Memantine + Amitriptyline | 213.6±6.85 [‡] |

($n=6$), values expressed as mean±SEM. (^{*} $p < 0.001$ vs. normal saline-control), ([†] $p < 0.05$, ^{††} $p < 0.01$ vs. Amitriptyline), ([‡] $p < 0.001$ vs. Memantine)

Group treated with Amitriptyline on '0' and '07' days, showed slight increase in locomotor activity but not statistically significant when compared to control group (as shown in table3 and table4).

Group treated with Memantine as a test drug on '0' and '07' days, showed slight increase in locomotor activity but not statistically significant when compared to control group and Amitriptyline treated group (as shown in table3 and table4).

Group treated with (Memantine + Amitriptyline) on '0' and '07' days, showed slight decrease in locomotor activity but not statistically significant when compared to Memantine alone (as shown in table3 and table4).



Fig.1 Showing forced swimming activity of mouse keeping its head above water

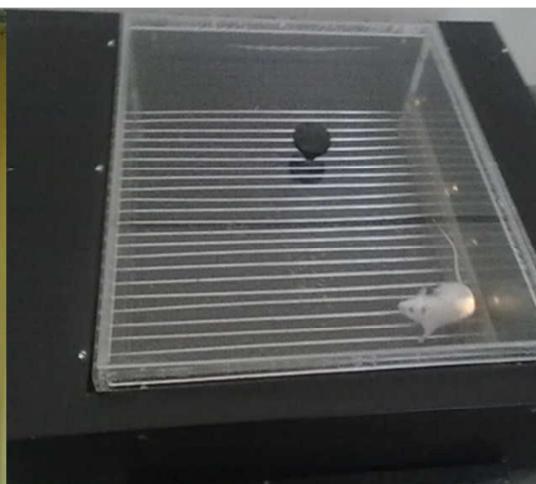


Fig2. Showing the interception of photo beams by mouse in Photoactometer

Antidepressant activity of selective monoamine reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and monoamine oxidase (MAO) inhibitors is by potentiating monoaminergic neurotransmission.

Selection of best possible antidepressant for an individual should be based on its proven efficacy, safety and tolerability of the drug. Among 160 patients who took antidepressants, 26.87% reported adverse drug reactions, with

at least one possible causality[11]. Side effects and medication non-adherence to therapies are two reasons for inadequate responses to antidepressant agents. Approximately 20% of patients will discontinue their antidepressant medications[12]. In spite of the fact that several meta-analyses found no association between increased suicidal risks with use of antidepressants, yet few studies revealed that people on antidepressants have the tendency to commit suicide after 10–14 days of commencement of antidepressant[13].

(FST) Forced swim test[9] and Photoactometer[10] are widely used tests to screen the compounds for their antidepressant activity[14]. The immobility observed during FST corresponds to human depression[14]. The locomotor activity of animals was measured to differentiate between sedative and central nervous system stimulant activity of drugs. Mice tried to explore the area and during their movement they intercepted the photo beams.

In the present study Memantine treated group when compared to control group, revealed significant reduction in the duration of immobility in FST. Memantine significantly reduced the immobility in FST, on day '7' when compared to day '0'. But, no significant effect on locomotor activity in Photoactometer test was observed.

There also exists statistically significant difference in the FST immobility period in the groups treated with both Memantine (3 mg/kg) and Amitriptyline (10 mg/kg) compared to Memantine alone treated group on '0' day, but not significant on '7' day. This demonstrated synergistic interaction between Memantine and Amitriptyline in their antidepressant activity.

Memantine is a non-competitive NMDA receptor antagonist, but, differs with other most potent NMDA receptor blockers, like Ketamine, Phencyclidine and Dizocilpine (MK-801), has a low affinity for the receptor and its action is voltage/use dependent [15-17]. Moreover it has been recently found that Memantine selectively blocks the extra synaptic (excitotoxic) receptor but preserves the normal synaptic function[18]. These peculiar pharmacological properties explain the lack of psychotomimetic/psychedelic effect and of interference with the normal physiological functions memory and learning, synaptic plasticity, etc.[19].

The immobility time in FST, is also reduced by CNS stimulants, but they tend to increase the locomotor activity[20] in the animals as opposed to the anti-depressants, which does not bring much change in locomotion[21]. In the present study, Memantine administered for '07' days, did not show any significant change in locomotor activity of mice, as compared to the control group, which helps to confirm the anti-depressant like activity (Table 3 and table 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 was significantly decreased by activation of glutamate or glycine binding site at NMDA receptor when measured in the forced swim test in mice[22-24]. The above findings of our behavioral tests are similar with other previous study by Karve, et al[25].

The increase in extracellular 5-HT represents a critical step in the mechanism of action of antidepressants, and the combined treatment with clinically tolerated NMDA antagonists such as Amantadine could reduce the delay in therapeutic onset of antidepressants as well as possibly enhance their efficacy[26]. The infusion of NMDA into either the raphe nuclei or frontal cortex has revealed to alter local 5-HT release and also serotonergic transmission[27].

The neurotropic hypothesis of depression and anti-depressant action was originally based on findings in rodents that acute or chronic stress decreases expression of BDNF in the hippocampus and that diverse classes of antidepressant treatment produce the opposite effect and prevent the actions of stress.

It has been reported that activation of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) glutamate receptors increases BDNF expression, and stimulates neurogenesis and neuronal sprouting, in the hippocampus[28]. Recent studies also highlighted the role of hypothalamus pituitary adrenal (HPA) axis over activity in both depression disorders. The amygdala and the hippocampus control the activity of the HPA axis in a counter-balancing way, and through many neuropeptides such as corticotrophin-releasing factor (CRF), substance P, galanin, vasopressin and neuropeptide Y (NPY)[29].

The previous study by Wieronska JM et al [30] 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[31]. Activity of Memantine on NPY activity, 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 3mg/kg, i.p. has demonstrated antidepressant activity which was comparable to Amitriptyline. 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 Amitriptyline. 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 clinical scenario.

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