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ANTIDEPRESSANT ACTIVITY OF AMANTIDINE IN ALBINO MICE

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ABSTRACT: ***Background:*** The glutamate system has been implicated in depression recently. This is a departure from previous thinking, which had focused on serotonin and norepinephrine. The glutamate system may represent a new avenue for treatment and research. NMDA and AMPA are receptors for the neurotransmitter glutamate. Blocking NMDA increases the activity of another receptor, AMPA, and this boost in AMPA activity is crucial for rapid antidepressant actions. Amantidine being a non-competitive antagonist at NMDA receptor is evaluated for its antidepressant activity in this study.

Objectives*:* To evaluate the antidepressant activity of amantidine and compare it with Imipramine in albino mice.

Methodology*:* Total of 18 swiss albino male mice were used. They were divided into three treatment groups and with normal saline (control) 10mg/kg, Imipramine (standard) 10mg/kg and amantidine 26 mg/kg (test drug) given orally. Each group contained 6 animals. Duration of immobility was observed for 6 minutes in tail suspension test and for 4 minutes in forced swimming test on separate set of animals.

Results: Results were analyzed by ANOVA followed by Post hoc Tukey's test. Amantidine at the dose of 26 mg/kg significantly reduced the immobility time in both the tests compared to control ($p < 0.05$).

Conclusion*:* Non-competative antagonist, amantidine has significant antidepressant activity in acute models of depression.

Key words: NMDA antagonists, amantidine, imipramine

INTRODUCTION

Major depressive disorder (MDD) is a mental disorder characterized by an all-encompassing low mood accompanied by low self-esteem, and by loss of interest or pleasure in normally enjoyable activities. Major depressive disorder is a disabling condition which adversely affects a person's family, work or school life, sleeping and eating habits, and general health. In the United States, around 3.4% of people with major depression commit suicide, and up to 60% of people who committed suicide had depression or another mood disorder.[1] Depression is a major cause of morbidity worldwide.[2] Lifetime prevalence varies widely, from 3% in Japan to 17% in the US. In most countries the number of people who would suffer from depression during their lives falls within an 8–12% range.[3,4] In North America the probability of having a major depressive episode within a year-long period is 3–5% for males and 8–10% for females.[5,6] Population studies have consistently shown major depression to be about twice as common in women as in men, although it is unclear why this is so, and whether factors unaccounted for are contributing to this.[7] The relative increase in occurrence is related to pubertal development rather than chronological age, reaches adult ratios between the ages of 15 and 18, and appears associated with psychosocial more than hormonal factors.[7]

Researchers have discovered associations between clinical depression and the function of three major neurochemicals. These substances are serotonin, norepinephrine, and dopamine. Antidepressants influence the overall balance of these three neurotransmitters within structures of the brain which regulate emotion, reactions to stress, and the physical drives of sleep, appetite, and sexuality.[8]

Most antidepressant medications increase the levels of one or more of the monoamines the neurotransmitters serotonin, norepinephrine and dopamine in the synaptic cleft between neurons in the brain. Some medications affect the monoamine receptors directly.

Approximately two-thirds of the depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing.[5] More over these drugs have unusual side effects. The medical need for newer, better-tolerated and more efficacious treatments remains high. Hence newer potent antidepressant with minimal side effects should be investigated. The glutamate system has been implicated in depression recently. This is a departure from previous thinking, which had focused on serotonin and norepinephrine. The glutamate system may represent a new avenue for treatment and research. [9]

NMDA and AMPA are receptors for the neurotransmitter glutamate. A new study in mice by Zarate et al. shows that blocking the NMDA receptor is an intermediate step. According to this study, blocking NMDA increases the activity of another receptor, AMPA, and this boost in AMPA activity is crucial for rapid antidepressant actions. Amantidine being a non-competitive antagonist at NMDA receptor is evaluated for its antidepressant activity in this study.

MATERIALS AND METHODS

Animals: Ethical clearance was taken from Institutional Ethics Committee of J.J.M.Medical College, Davangere, Karnataka, India, before conducting the present study. Male swiss albino mice weighing 25-35 gm, were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, J.J.M Medical College, Davangere, Karnataka, India under suitable conditions of housing, temperature, ventilation and nutrition. The study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs and chemicals

The standard antidepressant drug imipramine was obtained from our institutional pharmacy. The test drug Cap. Amantidine (100 mg) was dissolved in distilled water and used for the present study.

Experimental design

Total 18 (n=18) albino mice were used for this study. Animals were divided randomly into three groups of six mice in each group. Group 1 received the vehicle, normal saline (10ml/kg) and served as the control group, group 2 received imipramine (10mg/kg) served as standard, group 3 received the test drug amantidine (26 mg/kg) per orally. Drugs/vehicle was administered to the animals 60 minutes prior to the study. The antidepressant activity of the test drug was evaluated using the experimental models of depression tail suspension test (TST) and forced swim test (FST).

Tail suspension test (TST): The method was similar to that described by Steru et al.[10] Animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Initially the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility was noted during 6 minute period. Each animal was used only once.

Forced Swim Test (FST): The forced swimming model to test for antidepressant activity was developed by Porsolt et al.[11] The model used in the present study was similar to the original method described. The animals were 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 was recorded during next 4 minutes of total 6 minute test. The changes in immobility duration were studied after administering drugs in separate group of animals. Each animal was used only once.

Statistical analysis: Results are presented as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by Tukey's post hoc test for comparison between groups. For all the tests a 'P' value of 0.05 or less was considered for statistical significance.

ANOVA (Analysis of variance): In statistics, analysis of variance is a collection of statistical models and their associated procedures, in which the observed variance is partitioned into components due to different explanatory variables. In its simplest form ANOVA gives a statistical test of whether the means of several groups are all equal and therefore generalizes Student's two sample t-test to more than two groups.

Post-hoc test: Post-hoc tests (or post-hoc comparison tests) are used at the second stage of the analysis of variance (ANOVA) if the null hypothesis is rejected. The question of interest at this stage is which groups significantly differ from others in respect to the mean. In the present study Tukey's test was used for post-hoc comparison.

RESULTS

Table I and II shows immobility period of amantidine in tail suspension test and forced swim test respectively. A significant (P<0.01) decrease in the duration of immobility is seen with the standard drug imipramine and amantidine as compared to the control group in both the tests.

Table I Effect of Amantidine on immobility period in Tail Suspension Test

Group No.	Drug treatment	Number of animals	Dose (kg-1)	Immobility Time in (secs) (Mean ± SEM)
1.	Control (Normal Saline)	6	10ml	193.3± 8.160
2.	Imipramine	6	10mg	86 ± 5.877*
3.	Amantidine	6	26mg	77.33± 15.05*

Statistical analysis of data was carried out by one-way ANOVA followed by Tukey's test.
*p < 0.05 as compared to control.

Table II Effect of Amantidine on immobility period in Forced swimTest

Group No.	Drug treatment	Number of animals	Dose (kg-1)	Immobility Time in (secs) (Mean ± SEM)
1.	Control (Normal Saline)	6	10ml	199.5± 6.587
2.	Imipramine	6	10mg	104.2 ± 4.792*
3.	Amantidine	6	26mg	95.17± 20.07*

Statistical analysis of data was carried out by one-way ANOVA followed by Tukey's test.
*p < 0.05 as compared to control.

The table III and IV shows difference between three groups (tukey's multiple comparision test) in immobility period. There is significant difference between group 1 & 2 (control and imipramine group) and group 1 &3 (control and amantidine group). This shows amantidine has significant antidepressant activity compared to control.

Table III. Tukey's multiple comparison test showing difference between groups in Tail suspension test

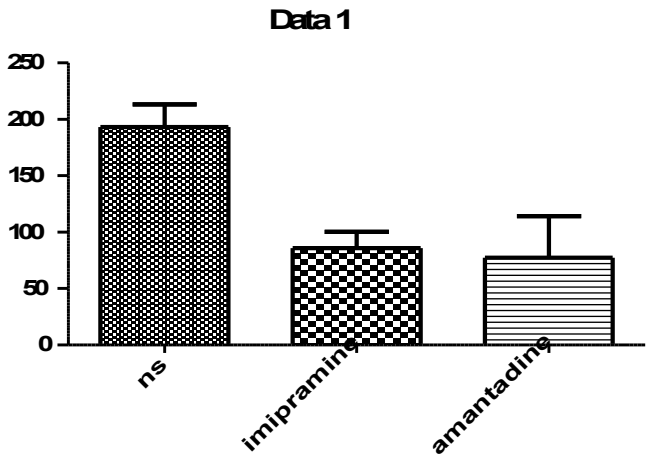
DIFFERENCE BETWEEN GROUPS		
GROUPS COMPARED	MEAN DIFFERENCE	(P <0.05) significant/not significant
Group 1 & 2	107.3	S
Group 1 & 3	116.00	S
Group 2 & 3	8.667	NS

S – Significant, NS – Not significant

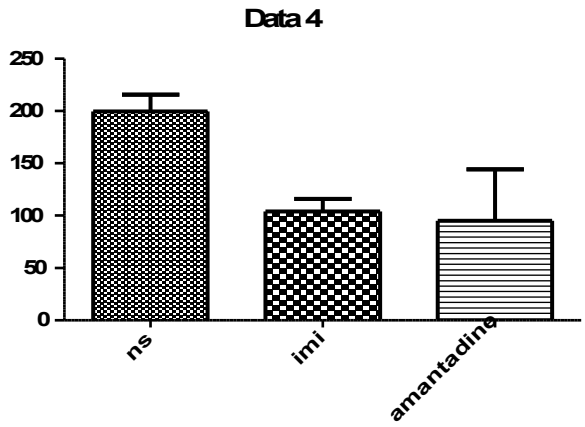
Table IV. Tukey's multiple comparison test showing difference between groups in Forced Swim test

DIFFERENCE BETWEEN GROUPS		
GROUPS COMPARED	MEAN DIFFERENCE	(P <0.05) significant/not significant
Group 1 & 2	95.33	S
Group 1 & 3	104.3	S
Group 2 & 3	9.00	NS

S – Significant, NS – Not significant



Bar diagram showing duration of immobility in Tail Suspension Test (TST)



Bar diagram showing duration of immobility in Forced swim Test (TST)

DISCUSSION

For several decades, the monoamine theory of depression has been predominant with regard to the aetiology of the illness itself as well as the rationale behind the bulk of treatments available in the clinic. Currently, the most widely prescribed antidepressant drugs have high degrees of selectivity for the 5-hydroxytryptamine (5-HT) transporter, the selective serotonin reuptake inhibitors (SSRIs) and, to a lesser extent, those with a high degree of selectivity for the noradrenaline transporter, the selective noradrenaline reuptake inhibitors (SNRIs). Despite the potency of drugs in these classes, they offer little therapeutic improvement on earlier generations of antidepressants. Although generally having a markedly superior side-effect profile, they are similarly not clinically effective in a significant proportion of patients.[12] Furthermore, SSRIs and SNRIs require a period of several weeks for full therapeutic effect to occur.[13] This time lag is clearly emotionally undesirable for the patient and can be a serious consideration in those patients at high risk of suicide.[12] Although the exact mechanism responsible for this delay in therapeutic onset is still under debate, there is a general agreement that this must involve neuroadaptive changes at the cellular and/or receptor level, leading to net alterations in neurotransmission. [13]

Over the past decade, interest has turned to a potential role of the glutamatergic system in depression, particularly with regard to the NMDA receptor.[14] It has been found that a variety of NMDA receptor antagonists demonstrate antidepressant activity comparable to conventional antidepressants in animal models of the illness. These include both competitive[15] and noncompetitive [16] NMDA receptor antagonists. Moreover, conversely, a significant number of antidepressants have been demonstrated to alter the NMDA receptor in a manner that would be consistent with a resulting decrease in functional activity at this site [17-20]. Unfortunately, in the case of NMDA receptor antagonists, many of these compounds have very limited value in patients, partly as a result of extremely poor CNS penetration or unacceptable side effects; although quite recently Berman et al demonstrated a long-lasting antidepressant effect of ketamine following intravenous infusion of the drug into patients.[21]

Amantadine appears to act through several pharmacological mechanisms, none of which has been identified as the one chief mode of action. It is a dopaminergic, noradrenergic and serotonergic substance, blocks monoaminoxidase A (MAO-A) and NMDA receptors, and seems to raise beta-endorphin/beta-lipotropin levels.[22] However, it is still uncertain which of these actions are relevant in therapeutic doses. It is suggested that amantadine might work as an antidepressant not through one, but through several mechanisms thought to be related to antidepressant activity.

Recently, amantadine and memantine, both weak NMDA receptor antagonists, have been shown to have a synergistic effect with conventional antidepressants in a model of depression. [23, 24] These authors observed that fluoxetine, venlafaxine and imipramine all synergized with amantadine or memantine to give improved performance by rats in the forced swim test. In other study shown increase in the cortical extracellular 5-HT level following the administration of two weak NMDA antagonists with SSRIs, resulting in the potentiation of antidepressant activity.[25]

Although the underlying neurochemical mechanisms by which weak NMDA antagonists are able to potentiate cortical 5-HT levels by antidepressants are therefore unclear, they could involve direct alterations in serotonergic transmission or effects on synthesis of the transmitter.

Alternatively, amantadine has both been shown to alter monoamine metabolism. These studies have largely focused on the effects of these drugs on the dopaminergic system due to their clinical role in Parkinson's disease. Both have been shown to potentiate the activity of L-DOPA decarboxylase as well as inhibiting monoamine oxidase B and to increase extracellular dopamine.[26,27] Fisher & Starr have also reported regional effects of these drugs on the activity 5-hydroxy-tryptophan decarboxylase in the substantia nigra and striatum of rats. Additionally, amantadine has been reported to increase cerebral 5-HT turnover (Altagracia et al.1993).[28]

Recently possible role of immunological dysregulation in the pathogenesis of depression has been noted. The combination of amantadine and fluoxetine enhanced the production of the negative immunoregulator interleukin-10. The antidepressive efficacy of a combination of fluoxetine and amantadine given in suboptimal doses may be related to the negative immunoendocrine effects of these drugs.[29]

CONCLUSION

This study shows, amantidine has significant antidepressant activity. This drug can be an alternative to conventional antidepressant drugs. Hence clinical trials are required for further study on these NMDA antagonists to reveal both efficacy and safety profile.

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