ANTIDEPRESSANT ACTIVITY OF KETAMINE IN ALBINO MICE

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Summary

The present study is shown the antidepressant activity of ketamine in albino mice. And has no potentiating effect with imipramine when combined together.Total of 24 (n=24) swiss albino male mice were used. They were divided into four groups containing six mice in each group. First group animals were given normal saline (control) 10ml/kg, similarly imipramine (standard) 15mg/kg for second group and for third group ketamine 2mg/kg (test drug) and ketamine plus imipramine (2mg/kg + 15mg/kg) for fourth group intraperitoneally. 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 were analyzed by ANOVA followed by Post hoc Tukey's test. Ketamine at the dose of 2mg/kg significantly reduced the immobility time in forced swim test compared to control ($p < 0.05$). NMDA (N-methyl-D-aspartate) antagonist, ketamine has significant antidepressant activity in acute models of depression.

Key words*:* N-methyl-D-aspartate antagonists, forced swim test, tail suspension test, ketamine

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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.

Depression is a major cause of morbidity worldwide.[1] Lifetime prevalence varies widely, from 3% in Japan to 17% in the US. And in India prevalence of depression is estimated to be 15%. In most countries the number of people who would suffer from depression during their lives falls within an 8–12% range.[2,3] 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.[4,5] Population studies have consistently shown major depression to be about twice as common in women as in men.[6]

Researchers have discovered associations between clinical depression and the function of three major neurotransmitters-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.

Most antidepressant medications increase the levels of one or more of these neurotransmitters 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.[4] 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. [7] NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) are receptors for the neurotransmitter glutamate. A new study in mice by Zarate et al shows that blocking the NMDA receptor increases the activity of another receptor, AMPA and this boost in AMPA activity is crucial for rapid antidepressant actions.

Ketamine being an 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 inj. ketamine (50mg/ml) was purchased from pharmacy shop and used for the present study.

Experimental design: Total 24 (n=24) albino mice were used for this study. Animals were divided randomly into four groups of six mice in each group. Group 1 received the vehicle, normal saline (10ml/kg) and served as the control, Group 2 received imipramine (15mg/kg) served as standard, Group 3 received the test drug ketamine (2mg/kg) and Group 4 received ketamine plus imipramine (2mg/kg + 15mg/kg) intraperitoneally (i.p). Drugs/vehicle was administered to the animals 30 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.[8] 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.[9] 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 ketamine in Tail Suspension Test (TST) and Forced Swim Test (FST) respectively. A significant ($P<0.05$) decrease in the duration of immobility is seen with the ketamine as compared to the control group in forced swim test but not in tail suspension test.

Table I Effect of ketamine on immobility period in Forced swim Test Test:

Group No.	Drug treatment	Number of animals	Dose (kg-1)	Immobility Time in (secs) (Mean ± SEM)
1.	Control (Normal Saline)	6	10ml	199.5±6.5
2.	Imipramine	6	15mg	104.2±11.7 *
3.	Ketamine	6	2mg	117.2±21.8*
4.	Ketamine +	6	2mg+15mg	192.7±27.3

	imipramine			

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 ketamine 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 (NS)	6	10ml	193.3±8.16
2.	Imipramine	6	10mg	86.00±5.87*
3.	Ketamine	6	2mg	160.00±26.79
4.	Ketamine + imipramine	6	2mg+15mg	158.00±38.47

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 ketamine group). This shows ketamine has significant antidepressant activity compared to control. Also there is significant difference between 3 & 4 (ketamine and ketamine + imipramine) in forced swim test, this shows ketamine alone has antidepressant activity in comparison with combination of ketamine and imipramine. There is no potentiation of antidepressant activity by combining ketamine and imipramine.

Table III. 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	82.33	S
Group 2 & 3	-13.00	NS
Group 1 & 4	6.833	NS
Group 2 & 4	-88.50	S
Group 3 & 4	-75.50	S

S – Significant, NS – Not significant

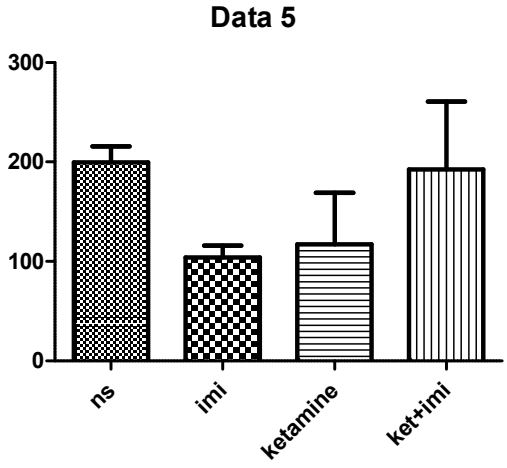
Table IV. Tukey's multiple comparison test showing difference between groups in Tail suspension test:

DIFFERENCE BETWEEN GROUPS		
GROUPS COMPARED	**MEAN DIFFERENCE**	**(P <0.05) significant/not significant**
Group 1 & 2	107.3	S
Group 1 & 3	33.33	NS
Group 2 & 3	-74.00	NS
Group 1 & 4	35.33	NS
Group 2 & 4	-72.00	NS
Group 3 & 4	2.00	NS

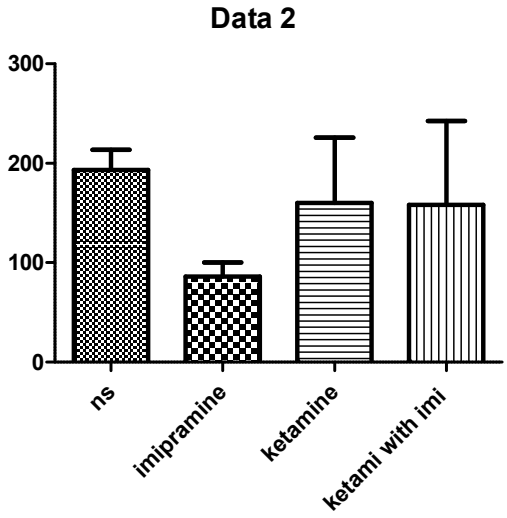
S – Significant, NS – Not significant

Bar diagram showing duration of immobility in Forced Swim Test and Tail Suspension Test

Forced SwimTest:



Tail Suspension Test:



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 are the selective serotonin reuptake inhibitors (SSRIs) and, to a lesser extent, 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.[10] Furthermore, SSRIs and SNRIs require a period of several weeks for full therapeutic effect to occur.[11] This time lag is clearly emotionally undesirable for the patient and can be a serious consideration in those patients at high risk of suicide.[10] 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. [11]

Over the past decade, interest has turned to a potential role of the glutaminergic system in depression, particularly with regard to the NMDA receptor.[12] 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 and noncompetitive NMDA receptor antagonists.[13,14] 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. [15-18] 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.[19] Leda et al demonstrated anti depressant activity of NMDA antagonists like ketmine in rodents by forced swim tests. [20]

Although ketamine is a high-affinity NMDA receptor antagonist, it has less, but potentially relevant, affinity for the μ opiate receptors and weak antagonist activity for the dopamine transporter. [21] Additionally, NMDA receptor agents may potentially affect mood via known secondary effects on monoamine and opiate systems.[22-24] Profound and transient cognitive deficits and euphoria, as evidenced by increases in BPRS(Brief Psychiatric Rating Scales)scores, were also induced by ketamine infusion, as also observed in other subject populations.[25,26]

All available antidepressants commonly take weeks to begin achieving results, but ketamine alleviates depressed mood almost immediately and exhibit residual antidepressant effect when they were retested after two weeks. Although our findings suggest the potential benefit of further exploration of NMDA antagonists as potential antidepressant agents, clinical applicability of this strategy may be limited by the psychotomimetic effects and the potential for abuse of many of these agents. Conversely, NMDA receptor antagonists without psychotomimetic properties in humans (e.g., memantine, eliprodil, and 1-aminocyclopropanecarboxylic acid) merit testing for antidepressant activity.

Conclusion

This study shows ketamine has significant antidepressant activity. There is no potentiation of antidepressant activity when imipramine and ketamine are given together. Hence ketamine alone 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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