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ACUTE EFFECT OF ADMINISTRATION OF PROPRANOLOL ON ANTIDEPRESSANT EFFECT OF ALPRAZOLAM IN ALBINO MICE

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ABSTRACT

Depression is an affective disorder characterized by change of mood and often co-exists with anxiety in depressed patients. Alprazolam, a benzodiazepine anxiolytic, also has antidepressant activity and can be used in Mixed Anxiety and Depressive Disorder. Propranolol, a non-selective beta adrenergic blocker, may be used as adjuvant to benzodiazepines in treatment resistant cases of anxiety. We wanted to know the effect of administration of propranolol on the antidepressant effect of alprazolam in animal models of depression using 'Behavioural despair.' Thirty six adult albino mice of either sex were divided into six groups of six animals each and subjected to Tail suspension test (TST) and Forced swim test (FST) and evaluated for cumulative duration of immobility. Then, groups A through F received distilled water (1ml/kg), imipramine (10mg/kg), propranolol (1mg/kg), alprazolam (5mg/kg), propranolol (1mg/kg) + alprazolam (5mg/kg) and propranolol (1mg/kg) + imipramine (10mg/kg) intraperitoneally. TST and FST were repeated. Statistical analysis was done using one way ANOVA, followed by Dunnett's multiple comparison test, with $P< 0.05$ as level of significance at 95% confidence intervals. Results were expressed as Mean ± SEM. Administration of drugs decreased the baseline cumulative duration of immobility in some groups in both TST and FST, but there was significant difference between baseline and post-drug administration values only in FST. Day 1 FST results revealed that only imipramine and alprazolam groups showed significant antidepressant activity compared to control. Combination of alprazolam and propranolol and imipramine and propranolol groups showed no significant antidepressant activity compared to control. Hence, acute administration of propranolol with alprazolam and with imipramine probably reversed the significant antidepressant activity which was seen after administration of alprazolam and imipramine alone.

KEY WORDS: **Antidepressant, Alprazolam, Propranolol, TST, FST**

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INTRODUCTION

Depression is an affective disorder characterized primarily by change of mood with common clinical features like sad mood, loss of interest and pleasure, low energy, worthlessness, guilt, psychomotor retardation or agitation, change in appetite and sleep, melancholia, suicidal thoughts etc. It is associated with significant morbidity and mortality. The prevalence of major depression in the general population is estimated at 5 %. Among patients, the prevalence ranges from 9% in ambulatory setting to as high as 30% in hospitalized patients.[1]According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioural disorder. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020.[2] Depression can interfere with normal functioning, and frequently causes problems with work, social and family adjustment. Anxiety is an unpleasant emotional state associated with uneasiness, worry, tension and concern for the future. Depression and anxiety often co-exist, as in Mixed Anxiety and Depressive Disorder (ICD 10).[3] Alprazolam, a benzodiazepine sedative/anxiolytic also has antidepressant activity and has been shown to be efficacious in the treatment of unipolar depression in humans.[4] Although its general acceptance as an antidepressant awaits further study, it may be useful in depression with co-morbid anxiety. Propranolol, a non-selective beta adrenergic blocker, is used to mitigate the symptoms of anxiety which are due to sympathetic over-activity and thus is of proven value in the treatment of anxiety. It may be used as adjuvant to benzodiazepines in treatment resistant cases of anxiety. Beneficial therapeutic effect of co-administration of alprazolam and propranolol in anxiety is well known. But, research on concurrent administration of the same drugs in animal models of depression is scarce. Since depressive patients often suffer from co-morbid anxiety, we wanted to know the effect of administration of propranolol on the antidepressant effect of alprazolam in animal models of depression using 'Behavioural despair.' Our objective was to evaluate the effect of acute administration of propranolol on the antidepressant activity of alprazolam in albino mice.

MATERIALS AND METHODS

We performed the following experiments after obtaining clearance from the Institutional Animal Ethics Committee.

Experimental animals:

Thirty six healthy adult albino mice of either sex (20-40g) were obtained from the Central Animal House and kept for one week in the departmental animal house in separate cages, maintaining 12 hours light:dark cycle and free access to laboratory diet and water, as per the recommendations of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA).[5]

Drugs and doses:

Drugs to be used in the study were alprazolam, propranolol and imipramine. Doses of drugs have been taken from previous studies.[6,7]All drugs were suspended in Distilled water (D/W) (1ml/kg). Single doses of Alprazolam 5mg/kg, Propranolol 1mg/kg and Imipramine 10mg/kg were suspended in distilled water and administered intraperitoneally.

Grouping and treatment scheduling:

Healthy albino mice of either sex (20-40g) were grouped into six arms containing six mice each for testing antidepressant activity (n=36). The treatment schedule was as follows:

Group A: D/W (1ml/kg)
Group B: Imipramine (10mg/kg)
Group C: Propranolol (1mg/kg)
Group D: Alprazolam (5mg/kg)
Group E: Propranolol (1mg/kg) + Alprazolam (5 mg/kg) administered separately
Group F: Propranolol (1mg/kg) + Imipramine (10mg/kg) administered separately

Experimental Design:

Acute testing for antidepressant effect was done by Tail suspension test (TST) and Forced Swim Test (FST). After taking baseline values of TST and FST on Day 0, the vehicle and the drugs were administered orally one hour before subjecting them to TST and FST on Day 1.

TST

TST is a simple, rapid and reliable method to screen antidepressants and other class of psychotropics, which was first proposed by Steru, et al,[8] wherein a mouse suspended by the tail develops 'behavioural despair' and shows alternate agitation and immobility which is indicative of a state of depression. The mice were suspended by the tail from a horizontal bar 50cm above the ground using adhesive tape. The duration of immobility was recorded for a period of five minutes. Mice were considered immobile when they hung passively and completely motionless for at least one minute. Groups of animals were treated with the test compounds or the control/vehicle one hour prior to testing.

Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail.

FST

The FST is also a behavioural paradigm predictive of antidepressant activity in rodents. The immobility exhibited by rodents when they are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior.[9] There was a pre-test session which consisted of allowing the animals to swim for 15 minutes. Naive mice were individually forced to swim inside a vertical plastic cylinder (height: 30 cm; diameter: 15 cm, containing 15 cm of water at room temperature). Mice placed in the cylinders for the first time were initially highly active, but after two to three minutes, activity decreased and became interspersed with phases of immobility or floating of increasing duration. An animal was regarded as immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose just above the surface. After five to six minutes, immobility reached a plateau where the mice remained immobile most of the time. After 15 minutes in the water, they were removed and dried and wrapped with soft towels. They were again placed in the cylinder 24 hours later and the total duration of immobility was measured during a five minute test. Floating behaviour was observed during this five minute period. The duration of immobility during the five minute observation period was recorded for all mice in each group. Drugs/control were administered one hour prior to testing.

Antidepressant drugs reduce duration of immobility.

Statistical analysis:

Statistical analysis was done using SPSS statistical software Version 16.0. For demonstration of antidepressant activity, we used one way ANOVA, followed by Dunnett's multiple comparison test for analysing the difference between groups (if any), with $P < 0.05$ as level of significance with 95% confidence interval.

RESULTS

The results of TST and FST expressed as Mean ± SEM are shown in Table 1 and results of one way ANOVA are shown in Table 2. Inter-group comparisons on day of experiment using Dunnett's multiple comparison test are shown in Table 3.

Table 1
Cumulative duration of immobility (seconds) in Tail suspension test (TST) and Forced swim test (FST), expressed as Mean ± SEM

GROUP	TST (D0)	FST (D0)	TST (D1)	FST (D1)
A	177.67±16.03	193.67±10.25	150.00±27.69	189.33±7.26
B	147.33±7.72	159.33±6.02	94.83±34.37	112.83±8.44
C	180.00±32.19	183.33±4.24	168.67±18.62	198.17±6.86
D	152.67±39.78	207.00±13.70	166.83±21.54	107.00±25.69
E	142.83±21.55	174.33±21.76	171.50±17.93	163.17±27.56
F	179.67±21.44	187.02±31.44	171.48±26.55	168.47±91.43

D0 = Day 0 (Baseline) D1 = Day 1 (Day of experiment after drug/control administration)

Table 2
Results of one way ANOVA between observations on D0 (baseline) and D1 (post-drug/control)

Test performed on D0 and D1	*P* value (*P*<0.05)
TST D0	0.827 (Not significant)
FST D0	0.123 (Not significant)
TST D1	0.186 (Not significant)
FST D1	0.002 (Highly significant)

Table 3
Results of Dunnett's Multiple comparison test between groups on day of experiment (Day 1) for FST

	Group	A	B	C	D	E	F
A	t-test						
	P-value						
B	t-test	6.873					
	P-value	0.000**					
C	t-test	0.884	7.847				
	P-value	0.397	0.000**				
D	t-test	3.084	0.216	3.429			
	P-value	0.012*	0.384	0.006**			
E	t-test	0.918	1.747	1.233	1.491		
	P-value	0.380	0.111	0.246	0.167		
F	t-test	1.669	1.149	0.239	0.453	0.126	
	P-value	0.126	0.277	0.816	0.661	0.902	

indicates significant and ** indicates highly significant difference between groups

DISCUSSION

Our objective was to evaluate the acute effect of administration of propranolol on the antidepressant activity of alprazolam in albino mice. In TST, there was a mean decrease in duration of immobility from baseline values following drug administration in control, imipramine and propranolol groups, but not in

alprazolam, alprazolam/propranolol and imipramine/propranolol combination groups. In FST, there was a mean decrease in duration of immobility from baseline values following drug administration in all the groups except propranolol group. Thus, administration of drugs in these groups decreased the baseline parameter, that is, cumulative duration of immobility (in seconds) in TST and FST, which is indicative of antidepressant activity.

Results of one way ANOVA revealed that there was no significant difference between the performances of the groups in TST and FST on Day 0. Thus, the groups were comparable at baseline before drug administration. There was no significant difference between groups on Day 1 (post drug/control) compared to baseline when TST performances are observed. But, we observed a highly significant difference between the performances of the animals in FST on Day 1 compared to Day 0. On comparing the performance of the drug treated groups in FST on Day 1, only imipramine and alprazolam groups showed highly significant and significant antidepressant activity respectively compared to control. However, there was no significant difference between imipramine and alprazolam groups. Propranolol group showed no significant antidepressant activity compared to control and alprazolam group showed highly significant antidepressant activity compared to propranolol. In earlier studies, propranolol was found to cause depression and reverse antidepressant activity of alprazolam and imipramine.[6] Combination of alprazolam and propranolol and imipramine and propranol groups showed no significant antidepressant activity compared to control and also to alprazolam and imipramine groups respectively. That is, acute administration of propranolol with alprazolam and with imipramine probably reversed the significant antidepressant activity which was seen after administration of alprazolam and imipramine alone.

The monoamine hypothesis of depression suggests that depression is related to a deficiency in the amount or function of cortical and limbic serotonin, noradrenaline and dopamine. In addition to the monoamine hypothesis, there is evidence that neurotrophic and endocrine factors play a major role (The Neurotrophic hypothesis).[10] As per the monoamine hypothesis, antidepressants revert the system to a state of equilibrium[11] by increasing neurotransmitter availability in the synaptic cleft by blocking reuptake in the presynaptic neuron. In general, antidepressants enhance serotonergic or noradrenergic transmission although the nature of this effect may change with chronic treatment.[12] Depression may also be due to a change in receptor function, so antidepressants act by modulating receptor function as well. For example, imipramine inhibits pre-synaptic reuptake of the biogenic amines, serotonin and noradrenaline to produce antidepressant action.[13-15] Duncan, *et al* reported that imipramine also induces down-regulation of beta adrenergic receptors.[16] Stimulation of the central beta 2 adrenergic receptor, particularly those located in the hippocampus, produces antidepressant-like effects on behavior.[17] It was speculated that the antidepressant effect of imipramine is related to the stimulation of central beta 2 adrenergic receptors.[18] Blocking beta 1 receptors also produces antidepressant action as evidenced by studies on modulation of immobility in FST in mice on administration of agonists and antagonists at the beta adrenergic receptors.[19] Imipramine may cause down regulation or blockade of beta 1 receptors, disturbing the balance between beta 1 and beta 2 receptors. This leads to the predominance of beta 2 receptor activity which produces an antidepressant effect. Thus, blocking beta 1 or stimulation of beta 2 receptors may mediate the mechanism of imipramine antidepressant action. Imipramine may produce its antidepressant action through GABA-ergic mechanisms, causing the release of catecholamine.[20]

The antidepressant effect of alprazolam may be mediated by a GABA-ergic mechanism that is independent of the benzodiazepine receptor.[21] But, non- GABA-ergic systems are also involved in the

antidepressant effects of alprazolam.[22] Animal studies have suggested that alprazolam causes a net decrease in noradrenergic activity in an anxiety state but produces a net increase in noradrenergic activity in both prefrontal cortex and hippocampus in depression.[23] Studies have also shown that alprazolam treatment resulted in significantly decreased plasma and urinary cortisol level, serotonin platelet-bound, and urinary 3-methoxy 4-hydroxyphenyglycol concentrations, which reinforce its efficacy in depression and anxiety. Thus, central monoaminergic systems are probably partly involved in the antidepressant effect of alprazolam.[24] Alprazolam may enhance the release of serotonin (5-HT) in the hippocampus, and this may at least partly explain its antidepressant activity.[25] Several observations indicate that alprazolam and standard antidepressants have some similar actions, such as the down-regulation of the beta-adrenergic receptor and their anti-anxiety effect.[26] Propranolol, a non-selective beta adrenergic receptor antagonist, alone produced no significant improvement in immobilisation periods of mice in FST, probably due to the blockade of both beta 2 and beta 1 receptors. Activation of central beta 1 receptors leads to enhanced behavioural despair while beta 2 receptor activation reverses this effect,[19] but the net effect of such activity is depression, as proved in earlier studies.[6] Propranolol combined with alprazolam abolished antidepressant effects of alprazolam Alprazolam induced release of mono-amine transmitters, like noradrenaline through GABA-ergic system[20] produces stimulation of the beta 2 adrenoceptors leading to antidepressant action. Administration of propranolol with imipramine produced significant antagonism of imipramine antidepressant effect, probably due to blockade of beta 2 adrenoceptors. Our observation of the depressant effect of propranolol agrees with previous studies which associate beta blockers with induction of symptoms of depression as mentioned above. Propranolol is also known to cause depression as an adverse effect. However, the association between use of beta blockers and the incidence of depression is not clear.[27]

CONCLUSION

We wanted to evaluate the acute effect of administration of propranolol on antidepressant activity of alprazolam in albino mice using behavioural despair models. Propranolol probably decreased the antidepressant activity of alprazolam as well as imipramine, while demonstrating no antidepressant activity of its own.

Thus, we see that if alprazolam is used in treatment of Mixed Anxiety Depression, addition of propranolol as adjunctive treatment for anxiety will reduce the antidepressant efficacy of alprazolam, even though its anxiolytic effect would be enhanced. However, further studies are needed to evaluate the effect of chronic administration of propranolol on antidepressant activity of alprazolam in both animals and humans.

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