EFFECT OF BUPIVACAINE AND POSACONAZOLE ON ANIMAL MODELS FOR DEPRESSION IN ALBINO RATS

Rajesh M* and Divya R

Assistant Professor, Department of Pharmacology, Sri Muthukumaran Medical College & Research Institute, Chennai, Tamilnadu, India.

ABSTRACT

OBJECTIVE: Depression is a disturbance in mood, thought, and body characterized by varying degrees of sadness, disappointment, loneliness, hopelessness, self-doubt, and guilt. Management of depression with Tricyclic Antidepressants, Selective serotonin reuptake Inhibitors and Selective Noradrenaline reuptake inhibitors are troublesome due their side effects, Latency of onset, overdose toxicity and Non-compliance. Anti-depressants like amitriptyline and anti-convulsants like carbamazepine, gabapentin, pregabalin, lamotrigine have been used, but with uncertain efficacy. Hence this study was designed evaluate the antidepressant effect of Bupivacaine and Posaconazole on Forced Swim test and Locomotor activity by Actophotometer in Wister Albino Rats.

MATERIALS & METHODS: For this study 30 healthy male albino rats were selected and divided into 6 equal groups. Digital Forced Swim test was conducted to rats of groups 1 to 5, 30 mins after oral administration of following drugs, distilled water, Amitriptyline(2mg/kg), Bupivacaine(5mg/kg) Amitriptyline+Bupivacaine (2mg/kg+5mg/kg) and Posaconazole (40mg/kg) respectively. After two weeks of washout period the same animals were used for testing Locomotor activity using Actophotometer. The results were analysed statistically. RESULTS: Amitriptyline plus Bupivacaine combination treated group showed significant (P < 0.05) antidepressant activity compared to amitriptyline alone treated rats. There was no significant change in the locomotor activity in the amitriptyline, posaconazole, and bupivacaine treated animals. CONCLUSION: Amitriptyline plus Bupivacaine combination has significant antidepressant activity compared to placebo. Posaconazole failed to show significant antidepressant activity.
KEYWORDS: Posaconazole, Bupivacaine, Digital forced swim apparatus, Actophotometer.

INTRODUCTION
Depression is a common disabling mental illness which affects millions of people each year and impairs all aspects of everyday life.\cite{1} According to the World Health Organization (WHO), some 121 million people are currently suffering from depression, with an annual prevalence of 5.8% for men and 9.5% for women.\cite{2} By the year 2020, depression is projected to reach second place in the ranking of Disability Adjusted Life Years (DALYs) calculated for all ages and both sexes.\cite{3} Tricyclic antidepressants (TCA), SSRIs, SNRIs, etc were introduced based on monoaminergic neurotransmission for the management of depression. But these drugs have their own limitations comprising of troublesome side effects, latency of onset, overdose toxicity & non-compliance.\cite{4} There is still considerable need for safer, faster-acting and more effective agents that go beyond the “solely monoaminergic” perspective.\cite{5} Thorough literature search have revealed that Bupivacaine inhibit the binding of radiolabeled substance P\cite{6}. Studies have shown that Substance P antagonist Capsaicin elevates the mood in the subjects. Posaconazole, a broad spectrum antifungal, causes cortical excitation which may be due to imbalance between glutamate and GABA.\cite{7,8}

Hence this study was designed to evaluate the effect of Bupivacaine and Posaconazole on Forced Swim test using Digital forced swim apparatus and Locomotor activity using Actophotometer in Wister Albino Rats.

MATERIALS AND METHODS
For this study 30 healthy male Wister albino rats weighing 150-250mg, were procured from the Central animal house of the Institute (Sri Muthukumaran Medical College & RI) and were kept in the Pharmacology experimental laboratory for about 10 days. They were maintained at room temperature (25±2°C) under standard 12:12 hr L:D cycle, fed on germinating grams and water ad libitum. All the drugs were administered via intragastric route except Bupivacaine which was administered via i.p. route. Digital Forced swim test was conducted to rats of group 1 to 5. After two weeks of washout period the same animals were used for testing Locomotor activity using Actophotometer (group 6 to 10). The study protocol was approved by the Institutional animal ethics committee.
Plan of study

<table>
<thead>
<tr>
<th>Groups - Digital Forced swim test</th>
<th>Drug</th>
<th>Dose</th>
<th>Groups- Locomotor activity in Actophotometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water</td>
<td>0.5ml</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Amitriptyline</td>
<td>2mg/kg</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>Bupivacaine</td>
<td>5mg/kg</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Amitriptyline + Bupivacaine</td>
<td>2mg/kg + 5mg/kg</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Posaconazole</td>
<td>40mg/kg</td>
<td>10</td>
</tr>
</tbody>
</table>

Digital Forced Swim test[^9]

The test was conducted 30 mins. after administration of test and control drug. Rats were individually allowed to swim inside the digital forced swim apparatus. The apparatus consists of a wheel with rods so that the animal moves on rods. The wheel is surrounded by a container in which water required for animal to swim is poured. There is a sensor detector which detects when every time the animal makes one full rotation and the readings are noted digitally. The animal was allowed to swim for 6 min. Total duration of mobility was calculated. Post treatment score for all the groups was recorded on the same day except for the animals treated with drug Posaconazole. Since, posaconazole reaches study state plasma concentration on 14th day[^10], the post treatment score for the same was recorded after two weeks following daily intragastric administration posaconazole.

Locomotor activity by Actophotometer[^9]

The test was conducted 30 mins after administration of test and control drugs. Rats were placed individually in the activity cage for 10min. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. The locomotor activity score for each animal was observed for 10 mins.

Statistical Analysis

The results were analysed statistically by Paired T test and One-way ANOVA followed by LSD post hoc test using SPSS16.0 software. The data were expressed as mean ±standard error mean (SEM) and P < 0.05 was considered significant.

OBSERVATIONS AND RESULTS

Digital Forced swim test

The mean digital forced swim test score in the Amitriptyline and Amitriptyline plus Bupivacaine combination treated groups were significantly (P<0.05) greater than the control
group. The animals treated with Posaconazole and Bupivacaine alone failed to show significant antidepressant activity (Figure 1). The antidepressant activity of Amitriptyline plus Bupivacaine combination treated was significantly ($P < 0.05$) greater than amitriptyline treated group (Table 1).

Table 1. Effect of Drugs on Digital forced swim test in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Digital Forced Swim test score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>1</td>
<td>Distilled water</td>
<td>0.5ml</td>
<td>12.00±0.82</td>
</tr>
<tr>
<td>2</td>
<td>Amitriptyline</td>
<td>2mg/kg</td>
<td>11.17±0.87</td>
</tr>
<tr>
<td>3</td>
<td>Bupivacaine</td>
<td>5mg/kg</td>
<td>11.00±0.73</td>
</tr>
<tr>
<td>4</td>
<td>Amitriptyline + Bupivacaine</td>
<td>2mg/kg + 5mg/kg</td>
<td>10.83±0.79</td>
</tr>
<tr>
<td>5</td>
<td>Posaconazole</td>
<td>40mg/kg</td>
<td>10.67±0.67</td>
</tr>
</tbody>
</table>

Between group - One-way ANOVA followed by LSD post hoc test * $P < 0.05$  Within group – Paired t test # $P < 0.05$.

Figure 1: Effect of Drugs on Digital forced swim test in albino rats

Locomotor activity

There was no significant change in the locomotor activity in the amitriptyline, posaconazole, and bupivacaine alone treated groups in comparison to control group. Amitriptyline plus bupivacaine combination treated group also failed to produce significant change in the locomotor activity when compared to control animals (Table 2).
DISCUSSION

In the last 50 years the monoamine hypothesis has been the major pharmacological target for the treatment of depression. Even the atypical, heterocyclic or “second generation” antidepressants (maprotiline, nomifensine, trazodone, mianserin, etc.) introduced in the 1970s, and the third generation of antidepressants, the selective serotonin reuptake inhibitors, SSRIs (fluoxetine, sertraline, citalopram and paroxetine), introduced in the late 1980s, and the more recent noradrenaline reuptake inhibitor, NARI (reboxetine), or dual-acting serotonin norepinephrine reuptake inhibitors, SNRI (venlafaxine, duloxetine) or the presynaptic receptor antagonist, mirtazapine, continue to employ the same action mechanism as the classic drugs, that is, the modulation of monoaminergic neurotransmission at a synaptic level.[13]

Thus all the above drugs initiate their effect after more than 3-4 weeks, produce classical side-effects, as well influence their lack of effectiveness in approximately 30% of patients with major depressive disorder (MDD). Hence, there is still considerable need for safer, faster-acting and more effective agents that go beyond the “solely monoaminergic” perspective.[13]

Substance P, a neurokinin that acts on NK-1 receptors, has aroused enormous interest in psychiatry due to its identification in the circuits related to fear and anxiety, circumstances involving the release of neurokinin, together with its location alongside serotonin and noradrenaline. Antagonists of these receptors, MK-869, appear to exhibit experimental anxiolytic and antidepressant properties. Bupivacaine was found to inhibit tachykinin mediated neurotransmission by inhibiting the NK1 receptor. A study by Dalia A, et al proved that, Posaconazole-Vincristine Coadministration Triggers Seizure in a Young Female Adult, which may be due to imbalance between glutamate and GABA.[7],[8]

### Table 2. Effect of drugs on Locomotor activity using Actophotometer in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Locomotor activity score for 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Distilled water</td>
<td>0.5ml</td>
<td>608.17 ± 18.49</td>
</tr>
<tr>
<td>7</td>
<td>Amitriptyline</td>
<td>2mg/kg</td>
<td>582.50 ± 32.20</td>
</tr>
<tr>
<td>8</td>
<td>Bupivacaine</td>
<td>5mg/kg</td>
<td>629.33 ± 28.14</td>
</tr>
<tr>
<td>9</td>
<td>Amitriptyline + Bupivacaine</td>
<td>2mg/kg + 5mg/kg</td>
<td>592.00 ± 29.14</td>
</tr>
<tr>
<td>10</td>
<td>Posaconazole</td>
<td>40mg/kg</td>
<td>600.83 ± 36.88</td>
</tr>
</tbody>
</table>

One-way ANOVA followed by LSD post hoc test
Hence, the present study was undertaken to evaluate the potential antidepressant activity of Bupivacaine, a NK 1 antagonist and Posaconazole, found to cause cortical excitation, in animal model of depression. Forced swim test is a behavioral despair was proposed by Porsolt et al (1978), is a standard model predictive of antidepressant activity.

The results showed that Amitriptyline plus Bupivacaine combination has significant antidepressant activity compared to placebo. That was greater than that seen with amitriptyline alone (Table 1). This significantly greater antidepressant activity among combination treated group compared to amitriptyline alone could be due to Bupivacaine (Fig.1).

A study by Li YM et al proved that Bupivacaine inhibits radiolabeled binding of substance P to its receptor (NK1). Neurokinin antagonists (NK) of these receptors appear to exhibit experimental anxiolytic and antidepressant properties. In another study by Berton O et al showed that, the initial excitement aroused by MK-869, an NK-1 antagonist, with antidepressant efficacy in humans similar to that of paroxetine, fell off in the wake of later studies in phases II and III, in which there was observed a high response to placebo; in turn, interest in developing these antagonists waned. The antidepressant like activity produced by bupivacaine could be due to raised substance P (NK antagonism) level in the brain. The profile of neurokinin antagonists could be useful as complements to antidepressants, be it by increasing their effects, decreasing the delay in their action onset or reducing their adverse side effects.

Locomotors activity is considered as an index of alertness and a decrease in that indicates a sedative effect. In our study, there is no significant change in the motor activity is seen with standard and the test drug, thus indicating non-sedative effect of the test drug. In our study, Posaconazole another test drug failed to show significant antidepressant activity. Further studies with these and other similar drugs can prove to develop novel drugs for the management of depression.

**CONCLUSIONS**

Bupivacaine potentiates the antidepressant activity of amitriptyline when given in combination. The study results of test drug indirectly support the non-monoaminergic hypothesis of depression. However posaconazole did not show significant effect on animal models for depression in albino rats.
SUMMARY

Bupivacaine, a local anaesthetic, also was found to block the NK1 receptors and literature reports revealed that Capsaicin, a Substance P antagonist (NK1 receptor blocker) was found to elevate mood of subjects. Posaconazole, an antifungal drug, was found to cause cortical excitation in some studies. Taking these two reports into consideration, this study was designed to evaluate these two drugs for their potential antidepressant activity, acting through novel mechanisms, in animal models of depression.

In the Forced Swim test, Bupivacaine (in the therapeutic dose) did not reveal any antidepressant activity but was found to significantly potentiate the antidepressant activity of Amitriptyline. Posaconazole, in the therapeutic dose, did not reveal any antidepressant activity in the Forced swim test. All the drugs used in this study (the test and the control) did not show any change in the locomotor activity in Actophotometer signifying that sedation was not a reason for decrease in swimming by animals. Thus, Bupivacaine can be used to potentiate the antidepressant action of amitriptyline and this drug can be taken up for further studies to elucidate its exact mechanism of action.

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