

Research Article

Evaluation of Antianxiety Activity of Angiotensin Receptor Blockers in Albino Mice

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Abstract: Anxiety is one of the most common conditions present globally adding to economic burden along with health concern. It is most common existing condition along with other psychiatric illness as well as co morbid conditions like hypertension. Recently the role of angiotensin is gaining importance in the field of research as it modulates various brain physiologies. Hence the present study was planned to evaluate the antianxiety activity of Losartan 5mg/kg, Losartan 10mg/kg, Olmesartan 5mg/kg, Olmesartan 10mg/kg, Telmisartan 5mg/kg and Telmisartan 10mg/kg in comparison with control and Diazepam 0.5mg/kg. Anti-anxiety activity was evaluated using Elevated plus maze and Social interaction test in mice. Administration of Losartan 10mg/kg ($p < 0.01$), Olmesartan 10mg/kg ($p < 0.01$) and Telmisartan at both the doses ($p < 0.001$) increased the time spent in open arms when compared to control in Elevated plus maze test and in social interaction, Losartan, Olmesartan and Telmisartan, at both 5mg/kg and 10mg/kg significantly increased the time spent in social interaction ($p < 0.00$). Finally, it can conclude that the Angiotensin receptor blockers like Losartan, Olmesartan and Telmisartan have got significant antianxiety property in both lower and higher doses.

Keywords: anxiety, angiotensin system, elevated plus maze, social interaction.

INTRODUCTION

Anxiety disorders are the most prevalent mental health condition affecting about 7-30% of the world's population and is one of the most commonly encountered problems in the psychiatry outpatient department [1]. Anxiety is nothing but a state of fear characterised by motor tension as well as sympathetic over activity which causes substantial distress to the person. This has worry as the key component, with associated symptoms of restlessness, fatigue, impaired concentration, irritability, muscle tension, and sleep disturbance [2]. There are several types of anxiety disorders amongst which generalised anxiety disorder (GAD) is the most common. Studies and hypothesis have suggested the role of various neurotransmitters like serotonin and GABA in the pathophysiology of anxiety. According to recent guidelines except in the acute anxiety states, SSRIs are preferred as first line of drugs. In acute anxiety state diazepam is preferred [3]. Considerable evidences suggest the role of brain Renin Angiotensin System in anxiety states [4-6]. Angiotensin contributes for the regulation of neurotransmitters like serotonin, norepinephrine and GABA where it modulates the release [7]. There is upregulation of angiotensin II and its receptors during stress [8]. Angiotensin receptor blockers are presently used for cardiovascular conditions like hypertension and

congestive cardiac failure. There is increased prevalence of anxiety in these patients which contributes to morbidity and mortality [9]. Hence this study is planned to evaluate the anti-anxiety effects of the three angiotensin receptor blockers, losartan, olmesartan and telmisartan.

MATERIALS AND METHODS

Ethical approval was taken from the institutional ethics committee of SSIMSRC, Davangere. The mice inbred in the central animal house of S.S. Institute of Medical Sciences and Research Centre are used. A total of 96 ($n=96$) animals will be divided into 16 groups of 6 each. The previous day of the experiment, the animals were weighed and were randomly housed in cages as 6 animals per cage. The temperature was maintained at $21 \pm 3^{\circ}\text{C}$, with 12-hour light-dark cycle. The animals had free access to food and water, *ad libitum*. Swiss albino mice of either sex weighing 20-25 g were used and divided into 8 groups of 6 each. Group I received 1 ml distilled water, Group II received Diazepam 0.5mg/kg, Groups III & IV received Losartan 5 and 10mg/kg, Groups V & VI received olmesartan 5 and 10mg/kg, and groups VII & VIII received telmisartan 5 and 10mg/kg respectively. Two models were used. Social interaction test and elevated plus maze test. Same animals were used for

both the models after a washout period of 2weeks.

Social interaction test[10]: Pre-treated mice were isolated for 1 hour before the test. In the test arena, an open topped box (22 × 15 × 12 cm), the mice were observed for cumulative time spent in social interaction for a period of 5mins. The social interaction included genital investigation, sniffing a partner, following, grooming, kicking, biting, wrestling, climbing over and under, neck licking and boxing. **Elevated plus maze [10]:** The plus maze apparatus consisted of two open arms, measuring 16 × 5 cm, and two closed arms, measuring 16 × 5 × 12 cm, connected to a central platform (5 × 5 cm). The maze is elevated to a height of 25 cm above the floor. Pre-treated animals were placed individually for 5mins in the maze with its head facing the open arm. The number of entries into the open and closed arm, time spent in each arm and the percentage preference to the open arms was noted.

The percent time spent on the open arms was determined as follows:

$\% = 100 \times \text{Number of seconds spent on open arms} / 300$
total seconds (5 min observation time).

Statistical analysis:

All the results were expressed as Mean ± SEM. Data were analyzed by ANOVA followed by Tukey Kramer's multiple comparison test in Graph Pad Instat (GPIS) package, version 3.05. $P < 0.05$ was considered as significant.

RESULTS:

Social interaction test:

Diazepam (0.5 mg/kg) significantly ($P < 0.001$) increased the time spent in social interaction among mice as compared to its effect in the distilled water-treated group [Table 1]. The test groups losartan 10mg/kg ($P < 0.01$), olmesartan 10mg/kg ($p < 0.001$), telmisartan 5 and 10mg/kg ($P < 0.001$ and $P < 0.01$) significantly increased the time spent in social interaction as compared to distilled water-treated group [Table 1]. The results were comparable to standard diazepam treated group. No significant effects were produced with losartan 5mg/kg and olmesartan 5mg/kg treated groups when compared to control.

Elevated plus maze test:

Administration of diazepam (0.5 mg/kg) significantly increased the amount of time spent in the open arms ($P < 0.001$) compared to distilled water-treated group [Table 2]. The test groups losartan 5 and 10mg/kg ($P < 0.001$), olmesartan 5 and 10mg/kg ($p < 0.01$) and telmisartan 5mg/kg ($p < 0.05$), 10mg/kg ($p < 0.001$) significantly increased the time spent in the open arms when compared to control. However, the percentage preference to open arms and the number of open arm entries did not significantly increase with standard as well as test groups when compared to distilled water treated group.

Table-1: Effect of different drugs in social interaction test observed during 5 minutes duration

GROUPS	Time spent in social interaction in seconds.(Mean±SEM)	Significance (P<0.05)
Distilled water(C)	38.33±4.65	P<0.001
Diazepam(S)	155.5±3.92	P>0.05(ns)
Losartan 5mg	66.33±11.7	P<0.01
Losartan 10mg	89.0±13.4	P>0.05(ns)
Olmesartan 5mg	74.67±4.48	P<0.001
Olmesartan 10mg	110.33±9.35	P<0.001
Telmisartan 5mg	102.67±8.88	P<0.01
Telmisartan 10mg	90.83±12.21	P<0.001

c-control, s-standard, ns- not significant

Table-2: Effect of different treatment groups on the time spent by mice in Elevated plus maze test

Groups	Open arm entry	Open arm duration in seconds(Mean±SEM)	Closed arm entry	Closed arm duration in seconds(Mean±SEM)
Distilled water(c)	14.3±1.6	133.5±13.0	11.8±1.1	146.8±15.4
Diazepam(s)	8.8±1.1	189.5±4.0*	6.5±1.3	110.5±4.0
Losartan 5mg	16.3±2.0	189.5±4.0*	8.8±1.8	84.5±18.2
Losartan 10mg	15.7±2.1	213±8.7*	9.3±1.4	87.0±8.7
Olmesartan 5mg	12.8±2.3	179.3±9.6#	10.8±1.8	120.7±9.6
Olmesartan 10mg	13.2±0.8	185.5±10.8#	10.0±1.4	115.8±10.8
Telmisartan 5mg	10.5±1.7	173.7±5.1^	12.0±1.0	126.3±5.1
Telmisartan 10mg	9.3±2.0	204.3±1.8*	8.0±0.8	95.7±1.8

*- very highly significant, #- highly significant, ^- significant, C-control, S-standard.

DISCUSSION

According to recent reports anxiety burdens every 1 in 13 individuals globally and therefore offers an important challenge in the treatment as well opens doors in the fields of drug development research[11]. The role of serotonin is well established in anxiety by various preclinical as well as clinical studies. It is also involved in the regulation of mood, impulse control, sleep, vigilance, eating, libido and cognitive functions which includes learning and memory. In addition, serotonin is important in the modulation of behaviours such as fear and impulsiveness in suicidal and other violent acts [12-13]. Recently brain RAS is gaining importance for its influence in various physiologies like thirst, sympathetic activity and cognition[14-15]. Various studies highlighted the role of RAS in the brain.

Brazsko et al showed the anxiogenic potential of ARBs is mainly due to the blockade of $AT_1 > AT_2$ receptors in the brain[16].

Pavel et al studied the effects of peripherally administered angiotensin receptor blockers on brain and concluded that the ARBs can behave as anti-stress compounds[17].

In our study, ARBs losartan, olmesartan and telmisartan showed significant anti-anxiety effect in elevated plus maze test as well as social interaction test consistently almost comparable to diazepam. Losartan and olmesartan doesn't cross the Blood brain barrier (BBB) yet exhibited anti-anxiety effect whereas telmisartan crosses the BBB. The exact mechanism of anti-anxiety effect is not known. Anxiety state triggers the up regulation of angiotensin level in the brain as well its receptors especially in the circumventricular organs which remain outside the blood brain barrier. Stimulation of periventricular AT_1 receptors leads to release of noradrenalin. Angiotensin II also influences the release and synthesis of serotonin[18-4]. These factors may be responsible for the anti-anxiety effect of ARBs. Angiotensin receptors have been localised in various regions of human brain. AT_{1A} receptor subtypes are found in the areas involved in the regulation of blood pressure and fluid homeostasis, whereas AT_{1B} is found in the glandular tissue such as anterior pituitary, pineal gland and adrenal gland. In depression, there is dysregulation of HPA axis and the stimulation of AT_{1B} in the pituitary gland and adrenal gland may be one of the reasons. Therefore blockade of this receptor is compatible with enhanced activity of HPA axis and also decreased sympathetic activity. Certain genetic and pharmacological models further substantiates the direct correlation between enhanced activity of brain AngII and increased anxiety, HPA axis stimulation along with central and peripheral sympathetic Activity. AT_2 are densely expressed in the lateral septum, thalamic nuclei, locus ceruleus and inferior olive[19]. Stimulation of AT_2 stimulates axonal regeneration and linking

neurogenesis to improvement in anxiety symptomatology would justify seeking new treatments that increase neurogenesis, plasticity and cell survival[20]. There have been lots of evidences to show that there is counteracting effects of existing between AT_1 and AT_2 stimulation. A balanced negative crosstalk occurring between these receptors is hypothesized to oppose signal transduction mechanisms in order to ensure the homeostasis. AT_2 also has a role to play in drinking mechanisms and vasopressin release. Whether it is related to its behavioural effects is not known. Angiotensin II acts on AT_4 receptor to bring about cognition enhancement[19]. This shows that the RAS system has a significant role to play in behavioural disorders. In our study, losartan and olmesartan at lower doses (5mg/kg) did not show consistent anti-anxiety effect in the anxiety paradigms used. This may be attributed to receptor occupancy as well as the crossing across the BBB. Both the drugs at higher dose and telmisartan at both the doses exhibited significant anti-anxiety effect which may be due to the blockade of AT_1 receptors in the circumventricular organs and telmisartan may show better effect when compared to the other drugs because it may block cerebral AT receptors. In addition telmisartan also has PPAR gamma mediated as well as NADPH oxidase mediated activity which may be responsible for its role in oxidative stress, thus may have additional benefit[21]. Few studies have shown the biphasic effects of losartan and telmisartan on anxiety and depression paradigms i.e., at higher doses (50-100mg/kg), ARBs had depressant like effect which may be because of the blockade of AT_2 receptors which show effects opposite to that of AT_1 receptors[19, 22]. Therefore thorough research has to be done in this field to prove the efficacy of angiotensin receptor blockers on behaviour disorders.

CONCLUSION

From this study we can conclude that the ARBs losartan and olmesartan at higher doses, telmisartan at both lower and higher doses possess significant anti-anxiety property which needs to be confirmed by various other animal models as well as human trials. The anti-anxiety property of ARBs would enhance its therapeutic utility as an add-on drug or an alternative to the available anti-anxiety drugs, especially when the patient is hypertensive.

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