Research Article

EFFECT OF CILOSTAZOL AGAINST THE COGNITIVE DEFICIT OBSERVED IN POST-TRAUMATIC STRESS DISORDER

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ABSTRACT
The Large conductance calcium activated potassium channel activators has a pivotal role in reducing neuronal damage due to traumatic, ischemic events or due to neurodegenerative processes which is a realistic perspective. The present investigation was designed to carry out the effect of Cilostazol, a non-selective BK channel opener against cognitive impairments in mice exposed to the stressor underwater trauma (UWT). Mice were randomly allocated into five groups comprising of a control, stressed, two test groups with Cilostazol (20 mg/kg and 40 mg/kg) and a standard group with fluoxetine (20 mg/kg). Prior to the exposure to the Under Water trauma paradigm, in which the mice were exposed to a constraint under water for 30 seconds, both the test groups and the standard group were treated with the respective drugs orally for nine days. Animals were then tested in the Morris water maze (MWM). After UWT exposure, the traumatized rats performed poorly in the spatial memory task in the Morris water maze which resulted in behavior aversive effects. However, the performance of Cilostazol (40 mg/kg) ($p < 0.001$) treated group was more pronounced than that of Cilostazol (20 mg/kg) treated group and displayed significant memory improvement when compared to the traumatized group. From the present study, the results shows the protective efficacy of Cilostazol against cognitive deficit of post traumatic stress disorder and paves a path for further exploration of the therapeutic role of BK channels in various other neurological disorders.
Keywords: Cilostazol (CZ), Post traumatic stress disorder (PTSD), Under water trauma (UWT), Large conductance calcium activated potassium channel activator (BK).

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a serious condition which is characterized with a stress response due to a life threatening experience and often ruined with chronic mental illness, causing occupational disability, psychiatric and medical morbidity and severe psychosocial distress[1]. Stress can initiate neuronal responses which may result in the development of behavioral disorders that are characterized with cognitive impairment, memory dysfunction and anxiety[2]. BK channels are one type of calcium activated potassium channel which are activated by depolarizing membrane potentials as well as by an increase in the internal calcium concentration[3]. They are widely expressed in neurons of the brain including those in the lateral amygdala (LA), which is closely involved in developing stress disorders[2]. In nervous system, the BK channels activation is critical for shaping action potentials and in regulating the neuronal excitability[4]. It has been documented that stress can cause hyper excitability of neurons of amygdala and the effects of stress on the excitability can be reversed by the activation of the BK channels[2]. Cilostazol is a phosphodiesterase III and adenosine uptake inhibitor who's antithrombotic and vasodilator properties have been approved in the United States for reduction of intermittent claudication[5]. Cilostazol also has the advantage of therapeutic plasma concentration ranging from 1 to 5μM, which can also add the BK channel opening property to the drug[6]. Hence, we propose the present study to evaluate Cilostazol, a non-selective BK channel opener drug for its effect against cognitive impairments observed in UWT paradigm in post traumatic stress disorder.

MATERIALS AND METHODS

Animals

Adult albino Swiss mice of either sex weighing 25-35 g were housed in groups of six per cage. They were maintained in well-ventilated room temperature with relative humidity of 45-55 % and natural 12 h: 12 h day-night cycle in propylene cages. All the experiments were carried out between 10:00 am to 2:00 pm. The animals were housed for one week, prior to the experiments to adapt laboratory temperature. Food was withdrawn 3 h before the experiment and the animals were fed with water during experiment. The study protocol was approved by the Institutional Animal Ethics Committee IAEC Ref. No. 290/CPCSEA/2009-PH-PCOL-01.

Drugs/Chemicals

The drugs used were Cilostazol (Cilodoc, Lupin Laboratories, India), Fluoxetine (Prozac, Cygnus Healthcare, India). All chemicals and reagents used were of analytical grade. Cilostazol was made into suspension in 10 % aqueous Tween 80 for oral administration. Tween 80 was obtained from Sisco research laboratories Private (Ltd), Mumbai, India. Cilostazol (Tab), Fluoxetine (Tab), were purchased from Supermed pharmacy, Thiruninravur, Chennai, India. All chemicals and reagents used were of highest quality analytical grade.

CTZ treatment

Cilostazol was made into suspension in 10 % aqueous tween 80 for oral administration. Animal dose was calculated according to the body mass surface ratio[7].

Methods
Under water trauma
The division of animals into groups and their treatment are mentioned in the Table 1-Experimental Design. Learning and spatial memory performance was measured using MWM. The water maze consisted of a pool of water (diameter: 1.7 m; 50 cm high). For a spatial learning task a (12, 3, 12-cm) escape platform was hidden at one of four positions in the pool with the top surface 2-3 cm below the water level. In this way the platform was not visible to the performing mouse. The actual swimming path of the mice was recorded manually. Mice were given two blocks of three trails per day for 8 days followed by, 1-min quadrant analysis test (the time spent by the mice in the quadrant where the platform was placed during training period was noted), with no escape platform in the maze, at the end of the eighth day. On the ninth day, mice in the entire group were given 1 minute to swim, and then held under water for 30 s using a special metal net. The location of the trauma was restricted to the part of the water maze opposite to quadrant 1, to exclude possible aversive conditioning to avoid that quadrant. Rats were then put back in a resting cage until commencing the post trauma tests. Control rats were given 1 minute to swim and then are put in a resting cage[8].

Table 1: Experimental Design

Group No	Name	Treatment for 9 days
Group 1	Control (non-traumatized)	10 % aq Tween 80 (10 ml/kg, p.o)
Group 2	Stressed (traumatized using UWT)	10 % aq Tween 80 (10 ml/kg, p.o)
Group 3	CTZ (traumatized using UWT)	(20 mg/kg, p.o)
Group 4	CTZ (traumatized using UWT)	(40 mg/kg, p.o)
Group 5	FXE (traumatized using UWT)	(20 mg/kg, p.o)

Post Trauma Tests
After 20 minutes in the resting cage, the mice were given a second quadrant analysis test. The pre-trauma and post-trauma performances in all the quadrants were recorded for all the group of animals and the results were compared[8].
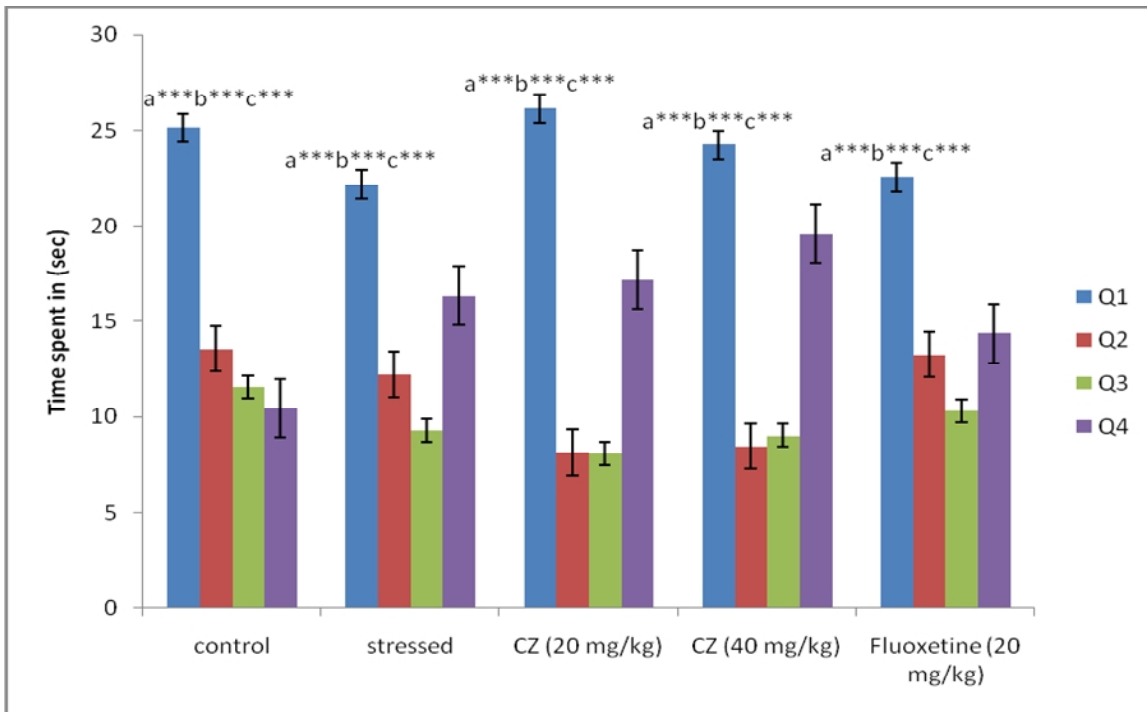
Statistical analysis
The pre-trauma and post trauma performance of mice for spatial memory task were analyzed using one way analysis of variance across the five groups. Dunnet's 't' test was further used as the post-hoc to detect the differences between the four quadrants (Q_1, Q_2, Q_3, Q_4) of all the groups. All data were expressed as means ± S.E.M. All tests were two-sided and statistical significance was defined as $p < 0.001$.
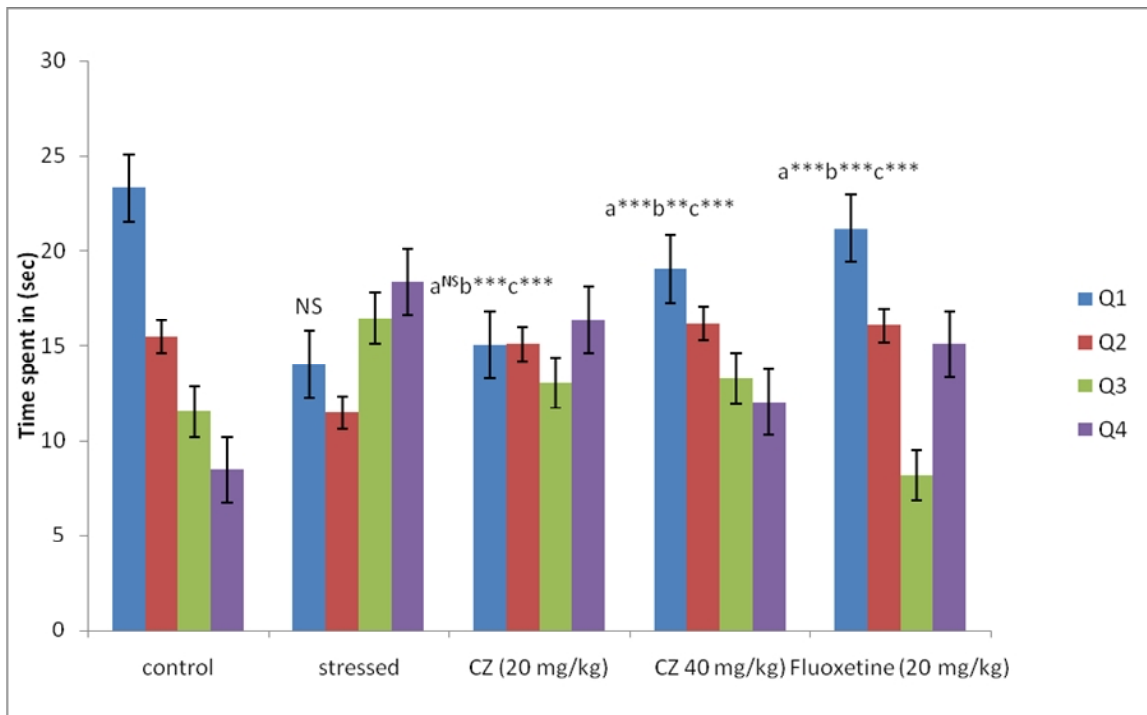
RESULTS AND DISCUSSION
Under Water Trauma
In the pre-trauma stage, all the groups of mice showed a significant bias towards swimming in the quadrant where platform was previously placed. In the second quadrant analysis test following the trauma, mice in the control group showed a significant bias towards the quadrant in which the platform had previously been located, indicating that all the mice acquired the task. In contrast, the stressed group showed no such bias. Mice in group treated with CZ (40 mg/kg) showed significant bias when compared to the stressed group. While the mice in group treated with CZ (20 mg/kg) did not spent much time in the quadrant in which the platform was previously placed. Fluoxetine was also found to have significant effect compared to that of the stressed group with more time spent in the quadrant in which the platform was previously placed. Results are expressed in the Figure 1 and Figure 2. PTSD undergoes neurological and psychological changes stemming from altered brain activity. A decreased size of hippocampus may affect the processing and integration while abnormal activation of amygdala may be tied to fear response[9]. Hence we have studied the cognitive performance in traumatized and drug treated animals. The underwater trauma model provides an important relationship between stress, cognition and learning[10]. The underwater trauma is a life-threatening situation, and as a brief experience may model a brief trauma and it may be a more "natural" setting then other type of stressors, such as electrical tail shocks and restraint[11]. Furthermore, in the water maze effect of such on performance of a memory task can be later evaluated in context of the trauma[10]. A 30-s underwater trauma resulted in behavior aversive effects. Twenty minutes after the trauma, the traumatized mice performed poorly in the spatial memory task in the water maze[12]. The difference between performance of the traumatized and CZ (40 mg/kg) treated groups showed significant memory improvement, since the drug may be effective against cognitive deficit of post traumatic stress disorder.

Figure 1: Pre-trauma performance for spatial memory task in morris water maze

Comparison was made between Q1 and Q2, Q3, Q4 of all groups
a - comparison between Q1 and Q2; b –comparison between Q1 and Q3;
c - comparison between Q1 and Q4 of all groups. ***p < 0.001

Figure 2: Post-trauma performance for spatial memory task in Morris water maze

Comparison was made between Q1 and Q2, Q3, Q4 of all groups
a - comparison between Q1 and Q2; b - comparison between Q1 and Q3;
c - comparison between Q1 and Q4 of all groups. ***p < 0.001

CONCLUSION

Cilostazol, a phosphodiesterase III and adenosine uptake inhibitor can activate the BK channels apart from its primary action has been found to be a promising drug against post-traumatic stress disorder. In the present study, emphasis was laid on the preliminary study of Cilostazol against post traumatic stress disorder. Hence the detailed exploration of its neuroprotective effect using different experiment animal models, different dose level, duration and detailed mechanisms remain to be studied and this work also provides an arena for future development.

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