Original Article

Evaluation of Antidepressant and Anxiolytic Activity of Phosphodiesterase 3 Inhibitor - Cilostazol

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ABSTRACT

Background: Cyclic nucleotide Phosphodiesterases (PDEs) are ubiquitously distributed in mammalian tissues and play a major role in cell signaling by hydrolyzing cyclic Adenosine Monophosphate (cAMP) and cyclic Guanosine Monophosphate (cGMP). Impairments in signal transduction have been implicated as possible mechanism of reduced plasticity and neuronal survival in major depressive disorders. PDE inhibitors possess a potentially powerful means to manipulate secondary messengers involved in learning, memory and mood. Cilostazol is an antiplatelet agent indicated for the treatment of intermittent claudication with peripheral artery occlusion and for the prevention of ischemic stroke worldwide. Various animal studies have reported neuroprotective, anti apoptotic, cognition and cerebral blood flow improvement properties of cilostazol. **Materials and Methods:** In this study, the antidepressant and anxiolytic effects of cilostazol were evaluated in mice using behavioral tests sensitive to clinically effective antidepressant compound. **Results:** Cilostazol, administered intraperitoneally (20 mg/kg), decreased immobility time of mice when subjected to forced swim test and tail suspension test as compared to standard fluoxetine (20 mg/kg). Cilostazol also produced significant decrease in the number of marbles buried as compared to fluoxetine in marble burying model. **Conclusion:** The present study suggests that cilostazol possesses potential antidepressant and anxiolytic activity, which could be of therapeutic interest for use in patients with depressive disorders.

Key words: *Cilostazol, forced swim test, marble burying behavior model, PDE3 inhibitor, tail suspension test*

INTRODUCTION

According to WHO, depression is the leading cause of disability leading to suicide. Patients with cardiovascular disease are at increased risk of developing depression and, when depression develops, cardiovascular risk is exacerbated further.[1,2] Depression is associated with an approximately two-fold increase in cardiac morbidity and mortality for various coronary heart disease (CHD) populations, including patients with recent acute myocardial infarction (AMI), patients awaiting coronary artery bypass graft (CABG) surgery, and patients post revascularization.[3] Cilostazol has been used as an antiplatelet agent and in intermittent claudication in patients with peripheral vascular disease.[4,5] Moreover, it has antithrombotic, vasodilatory, lipid lowering, and anti proliferative effects.[6] PDE3 are found in hippocampus, striatum, and other sites of brain and may affect of calcium ions and electroshock may modify their activity.[7] Furthermore, studies on CNS by Cilostazol have tried to explore about amelioration of neuronal damage, neuroprotection, improvement of cognitive function, prevention of cerebral ischemia through various mechanisms in preclinical studies.[8-10] Phosphodiesterase (PDE)

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inhibitors present a potentially powerful means to manipulate second messengers involved in learning, memory, and mood.[11-14] PDE inhibitors are currently being investigated as possible memory enhancers, antidementia drugs, antidepressants, and antipsychotic agents due to location of PDEs in brain at discrete sites.[15-19] Cilostazol served as an alternative to milnacipran for the treatment of patients with post stroke depression as it leads to decrease in Hamilton rating scale for depression (HAM-D) after switching from milnacipran to cilostazol (100mg/day).[20] Thus, cilostazol is being evaluated for antidepressant and anxiolytic activity in mice.

MATERIALS AND METHODS

Animals and environment

Experiments were performed on Swiss albino mice (20-25 gm.) of either sex obtained from Torrent Research Centre, Ahmedabad, Gujarat, India. The animals were maintained in a well ventilated room with 12:12 hour light/dark cycle in polypropylene cages under well controlled temperature (25±2ºC) and humidity (55%±5%). Commercially available standard pellet diet (Pranav Agro, Vadodara, India) and drinking water was provided ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, were followed and prior permission was granted by the Institutional Animal Ethics Committee of Shri Sarvajanik Pharmacy College, Mehsana with approval number 06/2011 for conducting the study.

Materials

Fluoxetine and Cilostazol were gifted samples from Cadila Pharmaceuticals, Ahmedabad, Gujarat, India.

Grouping of animals and treatment

Animals were divided into three groups of six animals each. Each group was given treatment as shown in [Table 1].

Cilostazol was dissolved in 0.5% Na CMC and administered at a daily dose of 20mg.kg-1 to group 3 for 15 days. Fluoxetine was dissolved in distilled water and administered at a dose of 20mg.kg-1 for 7 days.[21] Control animals were administered 0.5% Na CMC solution for 15 days. All treatments were given intraperitoneally. Dosing of animals was carried out every day between 10 am-11:30 am IST. After the dosing period of 7 days (fluoxetine) and 15 days (sodium carboxy methyl cellulose and cilostazol) all animals were moved from their housing colony to laboratory conditions for 1 hr and then exposed to marble burying test, tail suspension test and forced swim test.

Table 1: Grouping of animals

Group	Treatments
Control (*n*=6)	Treated with vehicle (0.5% Na.CMC i.p.)
Standard (*n*=6)	Treated with fluoxetine (20 mg/kg i.p., 1 week).
Test (*n*=6)	Treated with cilostazol (20 mg/kg i.p., 2 weeks).

Parameters assessed

Antidepressant activity

Tail suspension test

Tail suspension test (TST).[22] was employed for screening antidepressant activity in mice. Sodium carboxy methyl cellulose (Na CMC) and Cilostazol were administered for 15 days. Fluoxetine was administered for 7 days. Treatment drugs were administered 1 hr prior to testing on last day of dosing. Each animal was individually suspended on the edge of a shelf 50cm above a table top with the help of adhesive tape. The total period of immobility was recorded through a video recorder for 6 min and then subsequently analyzed. Mice were considered to be immobile in absence of body movement, hung passively, and were completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test.[21,23]

Forced swim test

Forced swim test (FST)[24] was used for screening of antidepressant activity in mice. Na CMC and Cilostazol were administered for 15 days. Fluoxetine was administered for 7 days. Treatment drugs were administered 1 hr prior to testing on last day of dosing. Mice were individually forced to swim in an open circular glass chamber of diameter 15 cm and height 25 cm containing fresh water to a height of 15 cm and maintained at 25±1ºC. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind paws or tail. Water in the chamber was changed after subjecting each animal to FST since used water alters the behavior. Animal shows vigorous movements during initial 2 min swimming session. The duration of immobility was recorded for a period of next 4 min of the total 6 min swimming session with the help of video recorder and subsequently analyzed.[24]

Mice were considered immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried, and returned to their housing condition.[23]

Anxiolytic activity

Marble-burying behavior model

In this method, animals were individually placed in a transparent poly carbonate cages (22 x 32 x13.5 cm), containing 5 cm layer of saw dust and twenty four glass marbles (1.5 cm in diameter) that were evenly distributed on the saw dust in the cages. Sodium CMC and Cilostazol were administered for 15 days. Fluoxetine was administered for 7 days. Treatment drugs were administered 1 hr prior to testing on last day of dosing. Animals were kept in the cages for a period of 30 minutes and the number of marbles at least two-third buried in the saw dust was recorded.[25,26]

Statistical analysis

All the data represent Mean±S.E.M values for immobility time and number of marbles buried as shown in [Table 2]. The data were analyzed by using Graph Pad in Stat by one way analysis of variance (ANOVA). The test was followed by Tukey-kramer multi comparison test, *P* values less than 0.05 were considered as statistically significant. Analysis was performed using Graph Pad in Stat software (version 3.01, GraphPad Software Inc.).

RESULTS

Tail suspension test

In this test [Figure 1], animals treated with cilostazol (20 mg/kg) showed decrease in immobility time, which was significant (102±5.66; $P<0.01$) when compared with control (143.33±9.6). Animals treated with Fluoxetine (20 mg/kg), also showed a significant decrease in the immobility time (63±5.22; $P<0.001$) as compared to control (143.33±9.6). Similarly animals treated with Cilostazol showed significant decrease in immobility time (102±5.66; $P<0.01$) and thus effective antidepressant activity when compared to Fluoxetine (63±5.22).

Forced swim test

In this test [Figure 2], after intraperitoneal administration of cilostazol (20 mg/kg) there was extremely significant decrease in immobility time (70.66±2.27; $P<0.001$) as compared to control (123.33±9.44). Animals treated with fluoxetine (20 mg/kg), showed significant decrease in the immobility time (32±3.12; $P<0.001$) when compared to control (123.33±9.44). Cilostazol also showed significant decrease in immobility time (63±5.22; $P<0.001$) and thus marked antidepressant activity when compared to Fluoxetine (32±3.12).

Marble burying model

In this test [Figure 3], a significant decrease in the number of marbles buried (8.33±0.42; $P<0.001$) was observed with intraperitoneal administration of cilostazol (20 mg/kg) as compared to control (16.33±1.33). Similarly, animals treated with fluoxetine (20 mg/kg) showed significant decrease in the number of marbles buried (2.83±0.47; $P<0.001$) when compared to control (16.33±1.33). Cilostazol also showed

Table 2: Effects of fluoxetine and cilostazol on tail suspension test, forced swim test and marble burying behavior

Group	Treatment	Tail suspension test forced swim test		Number of marbles buried
		Immobility time in seconds		
I	Control (0.5% Na.CMC)	143.33±9.6	123.33±9.44	16.33±1.33
II	Fluoxetine (20mg/kg)	63±5.22**	32±3.12**	2.83±0.47**
III	Cilostazol (20mg/kg)	102±5.66*,†	70.66±2.27**,††	8.33±0.42**,††

Note: Values represented in (Mean±S.E.M.) (n=6). *$P<0.01$, **$P<0.001$, P compared vs group I. †$P<0.01$, ††$P<0.001$, P compared to fluoxetine-treated group

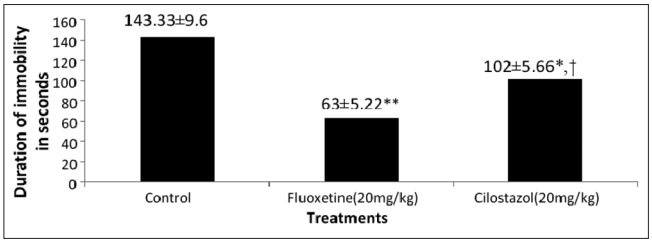

Figure 1: Effects of cilostazol and fluoxetine on duration of immobility in the TST. Note: Results are expressed as Mean±S.E.M (n=6). *$P<0.01$ and **$P<0.001$ as compared to control group. †$P<0.01$ as compared to fluoxetine

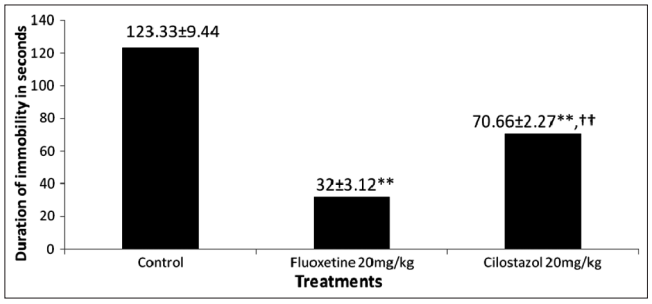

Figure 2: Effects of Cilostazol and fluoxetine on duration of immobility in the FST. Note: Results are expressed as Mean±S.E.M (n = 6). **$P<0.001$ as compared to control group. ††$P<0.001$ as compared to fluoxetine

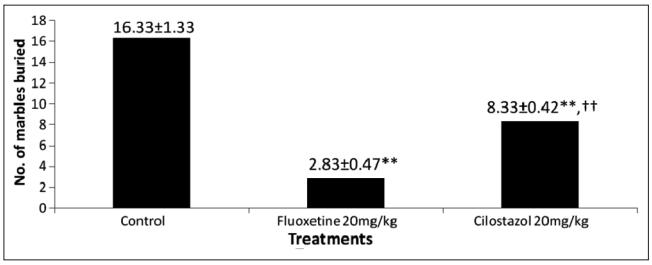

Figure 3: Effects of cilostazol and fluoxetine on number of marbles buried in the marble burying model. Note: Results are expressed as Mean±S.E.M (n=6). **$P<0.001$ as compared to control group. ††$P<0.001$ as compared to fluoxetine

significant decrease in marble burying (8.33±0.42; $P<0.001$) and thus marked anxiolytic activity when compared to fluoxetine (2.83±0.47).

DISCUSSION AND CONCLUSION

This study was initiated with an objective to evaluate the preclinical antidepressant activity of cilostazol at a dose equivalent to clinically therapeutic antiplatelet dose (50 mg BD) of cilostazol and the duration of treatment was also same as that for clinical antiplatelet response.[27] In the present study, the tail suspension test and forced swim test were carried out to assess antidepressant activity of cilostazol and the marble burying behavior model was done to assess the anxiolytic activity of cilostazol.

In forced swim test and tail suspension test, a normal animal submitted to a non-soluble aversive situation alternate between agitation and immobility. The reason of agitation is searching, it is highly energy consuming, while the purpose of immobility is energy conservation. Animals after antidepressant treatment struggle more even in desperate situation, and they spend less time with immobility.[28]

Fluoxetine and cilostazol treated animals showed significant reduction in immobility time as compared to control group in forced swim test and tail suspension test, which is indicative of antidepressant activity of cilostazol. Moreover, clinical study has shown that cilostazol possesses antidepressant effect as evidenced through decrease in Montgomery-Asberg Depression rating Scale scoring compared to baseline ratings. Cilostazol when used in cardiovascular patients with depression who underwent angioplasty and on adjuvant dual antiplatelet therapy has shown prominent results for treatment of mild to moderate depression.[29]

Anxiety is a feeling of apprehension, worry, or uneasiness that may or may not be based on reality.[30] The co-occurrence of anxiety and depression represents more severe and chronic illness and anxiety has been shown to increase the risk of suicidality in older patients with depression. Marble burying behavior is thought to be an expression of defensiveness, some forms of anxiety, obsessiveness, or compulsiveness.[31] In the present study, cilostazol was also evaluated for its anxiolytic activity through marble burying behavior model. Cilostazol showed a significant decrease in number of marbles buried as compared to control suggesting the anxiolytic activity of cilostazol.

It can be concluded that cilostazol possesses antidepressant and anxiolytic like activity by increasing cAMP in hippocampus through PDE3 inhibition, which could be of therapeutic interest for use in patients with depressive disorders. Moreover, cilostazol could serve as dual antiplatelet and antidepressant in patient with cardiovascular disease and depression. Although it has been established that cilostazol improves cognitive function in mice by increasing the production of insulin-like growth factor-I in the hippocampus and that the peripheral and central production of insulin-like growth factor-I is responsible for antidepressant like behavior.[10,32] Further studies are required to confirm the exact mechanism for antidepressant and anxiolytic activity of cilostazol.

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