Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

ANTIDEPRESSANT LIKE EFFECT OF NEWLY SYNTHESIZED COMPOUND 2[(N-BENZYLACETAMIDO) MERCAPTO] BENZIMIDAZOLE (VS 25) AND ITS POSSIBLE MECHANISM BY INHIBITION OF MONOAMINE OXIDASE ENZYME IN MICE

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Received: 21 Nov 2014 Revised and Accepted: 15 Dec 2014

ABSTRACT

Objective: The objective of the study was to investigate the antidepressant activity of a newly synthesized compound 2 [(N-benzylacetamido) mercapto] benzimidazole (VS 25) by forced swimming test in mouse and to explore the mechanism of action by its effect on monoamine oxidase enzyme in mouse brain.

Methods: Swiss albino mice were randomly divided into 4 groups, each containing 6 animals. Group one was controlled and received 1 % cmc, p. o., Group II received moclobemide (50 mg/kg) p. o., group three and four received VS25 (30mg/kg and 60 mg/kg) once a day for 14 days.

Results: In the forced swim test the decrease in immobility was 38.10% in moclobemide treated mice compared to 34.66% in VS25 (30 mg/kg) and 28.77% in VS 25 (60 mg/kg) treated mice. The percentage inhibition of the monoamine oxidase enzyme in mouse brain mitochondria was in moclobemide treated animals was 75.19% compared to 66.94% in VS 25 (30mg/kg) and 55.52% in VS 25 (60 mg/kg) treated animals. Moclobemide did not inhibit MAO B enzyme while VS25 (30 mg/kg) showed 6.48% and VS 25 (60 mg/kg) showed 4.75% inhibition.

Conclusion: It is concluded that both moclobemide (50 mg/kg) and VS25 (30 mg/kg) showed antidepressant activity in mice. Moclobemide selectively inhibited MAO A enzyme in mouse brain. VS 25 (30 mg/kg) was equipotent as MAO A inhibitor but differed from moclobemide with respect to weak inhibition of MAO B activity also.

Keywords: Forced swim test, Monoamine oxidase enzyme Inhibition, Moclobemide, VS25.

INTRODUCTION

Depression is an extremely common psychiatric condition. It is the most common mood affective disorder which refer to a pathological change in mood state, it may range from very mild to severe psychotic depression and is accompanied by hallucinations and delusions. (Rajput, et al 2011)Depression is characterized by low or sad mood and loss of interest in activities previously enjoyed. According to world health organization depression is currently the fourth major cause of disease burden worldwide. An estimated 340 million people suffer from depression. It is predicted that depression will be the second leading cause of disease burden worldwide by the year 2020 [1, 2].

Stress is an important environmental risk factor associated with the pathogenesis of mental disorders like depression and anxiety [3, 4]. Forced swim test used to induce stress leads to alteration in behavioral, neurochemical, and endocrine changes. It is frequently used animal model for studying antidepressant activity [5].

Antidepressants are clinically prescribed, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenergic reuptake inhibitors (SNRIs), and other atypical antidepressant drugs and monoamine oxidase inhibitors (MAOIs) but emerging data have questioned the safety and efficacy of antidepressants [6].

Historically monoamine oxidase inhibitors, were used as first line antidepressants, continue to have a nook in the treatment of psychiatric and neurological disorders. Significant progress in antidepressant therapy has been substantially made to manage the depression over the past half century following numerous reports of their neuroprotective actions *in vivo* and *in vitro* [7]. Recently the new generations of antidepressants with high degrees of selectivity for MAO inhibitors have become the most widely prescribed drugs in clinical application.

In the present study, we have selected compound VS 25 which was synthesized in the chemistry laboratory of our college. Dr. Suhas Shelke has synthesized a series of compounds through the pharmacophore study of 82 molecules. The pharmacophore was developed using PHASE software. This pharmacophore was matched using ZINC database and out of 1, 40, 000 molecule 11 hits were obtained [8]. From this, 1 hit was selected and modified. The designed molecule was subjected to docking study by using 'Glide'. Total 25 compounds were synthesized and characterized and the pilot studies were carried out on a small number of animals.

The objective of the present investigation was to evaluate the antidepressant effect of selected synthesized compound 2 [(N-Benzylacetamido)] Benzimidazole (VS 25) and moclobemide in mice subjected to forced swim test and also to study the effect of selected compounds on mouse brain monoamine oxidase.

MATERIALS AND METHODS

Selection of doses

The selection of doses of test compound VS 25 (30 mg/kg and 60 mg/kg) was based on the equivalent dose of moclobemide 50 mg/kg.

Drugs and chemicals

5 Hydroxytryptamine (Sigma-Aldrich's. Louis, U. S. A), Moclobemide® (Trima 150) Intas pharmaceuticals Ltd., Mumbai., India. Acetic acid, chloroform, sucrose, sodium hydroxide (S. D. Fine Chemicals, Mumbai, India), sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, Tris, EDTA (Hi Media Laboratories, Mumbai, India) and sodium carboxy methyl cellulose(CMC) (Research Lab, India) were purchased from respective companies. The test compound VS-25 was synthesized by Dr. Suhas Shelke, department of pharmaceutical chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, India and supplied to us for evaluation of pharmacological activity.

Preparation of drug solution

VS 25 and moclobemide were suspended in 1% Sodium carboxy methyl cellulose (CMC) solution. The route of administration was oral.

Experimental animals

Male Swiss albino mice (20-25 gm) were procured from the National Toxicology Centre, Pune, India and housed in animal house in groups of six animals in polypropylene cages. The animals were housed at 25±2°C, relative humidity of 45% to 55% and under standard environmental conditions of 12 h light 12 h dark cycle. All the animals were acclimatized for 10 days to the animal house conditions prior to the start of an experimental protocol. The animals had free access to food (Amrut laboratory animal feed, Sangali, MS, India) and water *ad libitum.*

The research protocol was approved by the Institutional Animal Ethical Committee (IAEC) constituted as per the directions of the committee for the purpose of control and supervision of an experiment on animals (No: CPCSEA/ 31/14). All experiments were carried out between 12:00-16:00 hours

Acute toxicity study

An acute toxicity study was carried out as per OECD guideline- 425. Five female Swiss albino mice were used for studies which were fasted overnight, but had free access to water. The test compound was administered orally at one dose level of 2000 mg/kg body weight and animals were observed continuously for the first 4 h and then periods up to 24 h for toxicity and mortality.

Forced swimming test (FST)

Forced swimming test first proposed by Porsolt et al (1978, 1979) is a most frequently used model for screening antidepressant like activity in rodents [9, 10]. The method is used to induce the depressive behaviour. The mice were divided into 4 groups of six animals each.

The group I represented as a vehicle control group received (1% CMC). Group II received moclobemide (50 mg/kg p. o.) Group III and IV were tested compound treated groups and received two different doses of VS25 30 mg/kg and 60 mg/kg respectively. Following the 14 day drug treatment, mice were subjected to forced swim test as described by Porsolt et al (1979) with slight modification. A mouse was individually forced to swim inside vertical Plexiglas cylinder (Height: 38 cm; Width 75 cm) containing water maintained at 26^0 $\pm1^0$c. Two swimming sessions were conducted as pre-test session (15 min habituation) and 24 h later the test session (6 min). After 15 min swim in water the mouse was removed and allowed to dry for 15 min in a heated enclosure (32^0c) and then returned to their home cages. Water in the cylinder was changed after subjecting each animal to FST because used water has been shown to alter the behaviour [11].

Animals were placed in the cylinder, 24 h later, and each animal showed vigorous movement during an initial 2 min period of the test. The duration of immobility was recorded by the stop watch during the next 4 min of the total 6 min testing period. The mice were judged to be immobile whenever they ceased struggling and remained floating passively in the water in a slightly hunched but upright position, its head just above the surface.

Measurement of MAO-A and MAO-B

At the end of the experiment, mice were sacrificed and the brain samples were collected and mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne, (1976). The MAO activity was assessed spectrophotometrically [12]. Briefly, the buffer, washed brain sample was homogenized in 9 volumes of cold 0.25 M sucrose, 0.1 M Tris, 0.02 M EDTA buffer (pH 7.4) and centrifuged twice at 800 g for 10 min at 4°C in cooling centrifuge (Remi instruments, Mumbai, India). The pellet was discarded. The supernatant was then centrifuged at 12000 g for 20 min at 4°C in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-Tris-EDTA buffers and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mixed well at 4°C for 20 min. The mixture was then centrifuged at 15000 g for 30 min at 0°C and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method using bovine serum albumin as the standard [13].

For estimating MAO A activity, 2.75 ml sodium phosphate buffers (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxytryatpamine were mixed in a quartz cuvette. This was followed by the addition of 150 µl solutions of the mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded by a double beam spectrophotometer (JASCO, Japan) at a wavelength of 280 nm against the blank containing sodium phosphate buffer (100 mM) and 5 hydroxytryptamine (4 mM). For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 0.1 M benzylamine were mixed in a quartz cuvette at a wavelength of 249.5 nM against the blank containing sodium phosphate buffer and benzylamine. This was followed by the addition of 150 µl solutions of the mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded.

Statistical analysis

All the results were expressed as mean ± SEM. The data was analyzed by one way ANOVA followed by Dunnett's test. The statistical analyses were performed using Graph Pad Prism 5 software (San Diego, CA). Data was considered statistically significant at $P<0.05$.

RESULTS

Effect of moclobemide and VS 25 on immobility period of mice in forced swimming test

Mice pretreated with the test compound VS 25 and moclobemide for 14 successive days showed significant ($p< 0.001$) decrease in the duration of the immobility period in the forced swim test. Vehicle treated mice remained immobile for 123.96 ± 5.57 Sec. Mice pretreated for 14 days with moclobemide (50 mg/kg) showed immobility for 76.72 ± 3.65 Sec. I. e. a reduction of 47.24 Sec or 38.10 %. In case of VS 25 (30 mg/kg and 60 mg/kg) treated animals the immobility periods were 80.99± 5.13Sec and 88.05±3.31 sec. The reduction of immobility period of 42.97 Sec or 34.66% and 35.67 Sec or 28.77%. The reduction of immobility by moclobemide and VS 25 was statistically significant ($p< 0.001$). (table-1)

Table 1: Effect of moclobemide and VS 25 on immobility period of mice in forced swimming test

Groups	Treatment	Oral dose(mg/kg) × 14 days	Immobility period (Sec)	The percentage decrease in immobility
1	Vehicle Control	cmc (35mg/kg)	123.96± 5.57	
2	Moclobemide	50mg/kg	76.72±3.65***	38.10
3	VS 25	30 mg/kg	80.99±5.13***	34.66
4	VS 25	60mg/kg	88.05±3.31***	28.77

Values were expressed as mean ± SEM; n=6, One Way ANOVA followed by Dunnett's test, *** $p<0.001$.

Effect of moclobemide and VS 25 on brain MAO A activity of mice

In vehicle treated mice both MAOA and MAO B activities in the brain were not inhibited. On the other hand, the percent inhibition of MAO A in moclobemide (50 mg/kg) treated animals was 75.19 %, which was slightly higher than 66.94% shown by VS25 (30mg/kg) and was significant at ($p< 0.01$). On the other hand, higher dose (60 mg/kg) of VS 25 showed significantly less inhibition ($p< 0.05$) of MAO A activity (55.52%). The paradoxical finding may be due to saturation kinetics of the enzyme. Similar findings were observed in the forced swim test wherein the percentage decrease in immobility was less i. e. 28.77% with VS25 (60 mg/kg) compared to the percentage decrease of immobility of 34.66 with lower doses of VS 25 (30 mg/kg). In case of MAO B activity moclobemide (50mg/kg) did not inhibit the

enzyme (-1.47%) whereas VS 25 showed inhibition of 6.48 % after 30 mg/kg and 4.75% after 60 mg/kg pretreatment. The results thus indicated that moclobemide is a selective inhibitor of MAO A enzyme. VS 25 (30 mg/kg) was equipotent in inhibition of MAO A activity compared to moclobemide, but also showed MAO B inhibitory activity although of small magnitude (Table-2).

Table 2: Effect of moclobemide and VS 25 on inhibition of MAO A and MAO B activities in mouse brain

Groups	Treatment	Oral dose (mg/kg) ×14 days	% Inhibition of MAO A	% Inhibition of MAO B
1	Vehicle Control	cmc (35mg/kg)		
2	Moclobemide	50mg/kg	75.19	-1.47
3	VS 25	30 mg/kg	66.94	6.48
4	VS 25	60mg/kg	55.52	4.75

DISCUSSION

Acute stress is used in animal models to induce behavioural, physiological and neural changes relative to human depression [14].

Porsolt et al (1978) described an animal model for assessing the effect of antidepressant drugs [9]. The animal model was based on behavioural despair, i. e. the rat after placing in water become immobile and float with stretched limbs which is an indication of depression. Drugs which reduced the period of immobility belonged to the group of antidepressants. The Porsolt test is an extensively used, validated model and included in the battery of test for screening drugs having antidepressant like activity [15].

In the present study vehicle control mice remained immobile for about 2 min (123.96± 5.7 Sec) and in both moclobemide and VS 25 treated mice showed a reduction in the period of immobility significantly indicated antidepressant activity. Moclobemide has clinically used antidepressant drug. Reduction in immobility time by VS 25 (30 mg/kg) was identical with that exhibited by moclobemide (50 mg/kg). However a dose dependent effect could not be established in the case of VS 25 as the immobility period declined at higher dose of 60 mg/kg.

According to the monoaminergic theory of depression the majority of the antidepressant molecules that have been developed in the past were aimed at increasing extracellular levels of biogenic amines within the brain., the focus has been on the monoaminergic neurotransmitter systems, thought to support the behavioural and visceral manifestations of mood disorders. One of the first neurochemical theories of depression was the monoamine hypothesis. According to this hypothesis, major depression results from a deficiency or imbalance of available monoamines (serotonin and catecholamine's- norepinephrine and dopamine) or subnormal monoamine receptors functioning in certain regions of the brain [16]. The subsequent development of monoamine oxidase inhibitors was based on a similar approach, namely indirect elevation of extracellular concentration of the biogenic amines.

Monoamine oxidase is a mitochondrial enzyme (MAO) which catalyzes the oxidative deamination of a variety of monuments, such as serotonin, dopamine, and norepinephrine. The pathophysiology of depression involves the abnormal activity of the enzyme which leads to dysfunction in monoaminergic neurotransmission in central nervous system [17]. MAO inhibitor is one of the important classes of antidepressants which act by inhibiting monoamine oxidase and leads to increase the neuronal monoamine level produces antidepressant activity.

Researchers reported different methods for the estimation of monoamine oxidase inhibitory activity like Manometric [18, 19], Microfluorimetric [20], Fluorimetric [21] and radioactive tracer techniques [22]. In the polarographic assay of monoamine oxidase, during oxidative deamination of substrates by the enzyme the oxygen consumption may be determined polarographically, using an oxygen electrode [23]. A simple and sens1itive spectrophotometric determination of monoamine oxidase activity was used by many researchers in studying the MAO inhibitory activity [24].

MAO enzyme is present in two isoform MAOA and MAOB, which have been distinguished based on relative substrate specificity. Monoamine oxidase A is more specific for epinephrine, norepinephrine and 5-hydroxytryptamine, whereas monoamine oxidase B is more specific for phenylethanolamine and benzylamine. Dopamine and tyramine are handled equally well by both isoenzymes [25]. Some MAO A inhibitors are effective for treating depression [26]. Many targets are reported for the treatment of depression like inhibition of serotonin, norepinephrine uptake and one of the important targets is a monoamine oxidase inhibitor, inhibition of which produces antidepressant activity. It may result from inhibition of selective MAO A which leads to increase in brain serotonin, norepinephrine, and dopamine level in brain [27]. Moclobemide Inhibited monoamine oxidase A, but did not inhibit MAO B enzyme. Test compound VS 25 at both the doses showed inhibitory effects on MAO A enzyme and partial inhibition of the MAO B enzyme. Thus, this study confirmed the non selective MAO inhibitory activity of VS 25.

CONCLUSION

It is concluded that VS 25 (30 mg/kg) showed antidepressant activity similar to that of moclobemide (50 mg/kg) in the forced swim test in mice. The mechanism of action of antidepressant activity appears to be primarily due to non selective inhibition of brain monoamine oxidase enzyme activity.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

ACKNOWLEDGMENT

The authors would like Acknowledge Dr. K. R. Mahadik, Principal, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, India and Dr Suhas Shelke, Dr. Mugdha Suryawanshi for providing test compound to carry out the study.

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