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Research Article

Antidepressant activity of Simvastatin in behavioral models of depression in rats

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ABSTRACT

Background: There is evidence, that statins can augment the antidepressant effects of fluoxetine in rats. Hence the present experimental study was designed to evaluate the effect of Simvastatin on duration of immobility in acute forced swim test (Acute FST) and Chronic forced swim test (Chronic FST), as models of behavioral despair in rats.
Methods: In acute FST and Chronic FST models, effects of simvastatin (Smv) and fluoxetine (Flx) per se and in combination, on immobility of rats were compared. Open field test was performed to discriminate between the general behavioral stimulation and antidepressant effect of study drugs.
Results: In Acute FST, duration of immobility decreased (171.33 ± 6.15 sec) non-significantly in simvastatin group, & decreased significantly in the groups of rats which received fluoxetine alone (161.33 ± 8.68, $P < 0.01$) or in combination with simvastatin (167.66 ± 7.71 sec, $P < 0.001$). The 3 treatment groups did not differ from each other. In chronic FST duration of immobility lowered significantly in both, the fluoxetine treated group (147.66 ± 8.73) and the combination treated group (130.5 ± 5.68 sec) with significant fall in the combination group ($P < 0.001$) compared to the individual therapy groups.
Conclusions: Lowering cholesterol levels with statins not only reduces risks for cardiovascular events, but also affect serotonergic neurotransmission, leading to clinical efficacy of standard antidepressants. Simvastatin can augment the antidepressant effects of fluoxetine in rats, raising the possibility that statins could be used to facilitate the effects of antidepressants in humans.

Keywords: Simvastatin, Antidepressant, Fluoxetine, Forced swim test

INTRODUCTION

In India, Coronary Artery Disease (CAD) rates have increased during the last 30 years, whereas declining trends have been noticed in developed Western countries.[1] Various independent epidemiological studies[2] conducted in north India suggest that the prevalence of CAD has increased from 1% in 1960 to 10.5% in the urban population and a two fold increase in the rural population.[3] A higher prevalence of 7.4% was observed in some parts of rural South India.

Among patients with CAD, hyperlipidemia is present in 2/3rd of patients.[5] Statins are first line drugs for the treatment of hyperlipidemia. HMG – CoA reductase catalyzes the reaction which is the rate limiting step in cholesterol biosynthesis. Statins inhibit this enzyme and decreases cholesterol synthesis. This leads to increased hepatic LDL receptor activity and accelerate clearance of circulating LDL.

In today's scenario depression and CAD are in close association. Prospective studies have shown that depression

increases the risk for death or nonfatal cardiac events approximately 2.5 fold in patients with CAD.[6] Depression is characterized by depressed mood and/or the loss of interest or pleasure in nearly all activities for a substantial period of time, causing significant distress. All over the world, depression represents a major public health issue. According to WHO (World Health Organization), it is the fifth disease in the world in years of illness, and by 2020, it would become the second in the whole world population.[7] Currently three major groups of antidepressants are recognized: 1) Selective Serotonin Reuptake Inhibitors (e.g. citalopram, sertraline, fluoxetine) 2) Monoamine oxidase Inhibitors (e.g. phenelzine, moclobemide) and 3) Other newer heterocyclic antidepressants. The Selective Serotonin Reuptake Inhibitors (SSRIs) are regarded as first-line pharmacotherapy for major depressive disorder. (MDD). Indians are among the worlds most depressed. According to WHO sponsored study, while around 9% of people in India had an extended period of depression in their lifetime, nearly 36% suffered from Major Depressive Disorder (MDD). It is characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy and poor concentration, besides feeling depressed.

Recently, statins have been proven to have additional properties other than hypolipidemic actions. These are anti-inflammatory, antioxidant and antiplatelet actions, referred as pleiotropic effects.[8] There is evidence from animal studies that Lovastatin can augment the antidepressant effects of a low dose of fluoxetine in rats, raising the possibility that statins could be used to facilitate the effects of antidepressants in humans.[9] However, the literature search revealed lack of sufficient data either from animal studies or clinical trials related to the antidepressant activity of statins. Hence present experimental study has been designed to evaluate antidepressant activity of simvastatin from the group of available statins.

This study was conducted to analyze the antidepressant activity, if any, of Simvastatin (a lipid lowering drug), alone and in combination with Fluoxetine (standard antidepressant), by measuring the time duration (seconds) of immobility, in Acute Forced Swim Test (Acute FST) and Chronic Forced Swim Test (Chronic FST), as the two selected models of behavioral despair in rats.

METHODS

Animal Ethics Committee Permission

Permission of the Institutional Animal Ethics Committee of Seth G.S. Medical College and K.E.M. Hospital was taken before the commencement of the study.

Experimental Animals

Twenty four Wistar rats of either sex, weighing 150 to 250 gms bred in the Institutional Centre for Animal Studies were used for the study. They were housed in the air conditioned rooms with filtered fresh air changes per hour in polypropylene cages with stainless steel top grill having facilities for food and drinking water in glass bottles with stainless steel sipper tube. The temperature was maintained at 22 ± 3ºC and relative humidity approx. 30 -70 %.

Study drugs and doses

The following solutions of drugs were prepared to be administered orally

1) Carboxy methyl cellosolve (CMC) used as solvent.
2) Simvastatin dissolved in CMC (10 mg/Kg body weight)
3) Fluoxetine dissolved in CMC (10 mg/Kg body weight)
4) Simvastatin (10mg/Kg) + Fluoxetine (10 mg/Kg).

Simvastatin (Lipid lowering drug) served as the experimental test drug Fluoxetine (SSRI antidepressant) served as positive control. CMC served as vehicle control

Study Methodology

The study was divided into following:

Part I : Acute Forced Swim Test

Part II : Chronic Forced Swim Test

In each part, the rats were randomly allocated to the four study groups as shown below. The drugs and the dosage regimen used in Part I and Part II are presented in Table 1 and Table 2 respectively. The details of methodology and efficacy variables for individual parts are described in the following paragraphs.

Table 1: Experimental groups for Acute Forced Swim Test (Part I).

Groups	Vehicle/Drugs	Oral dose and schedule 24 hr, 5 hr, 1 hr before test
1	Carboxymethylcellosolve (CMC)	1 ml
2	Fluoxetine	10 mg/Kg
3	Simvastatin	10 mg/Kg
4	Simvastatin + Fluoxetine	10 mg/Kg each

6 Wistar rats per Gp. CMC was used to prepare suspension for active drugs.

Part I: Acute Forced Swim Test

Experiments were carried out according to the method of Porsolt and co-workers.[10] For this a glass cylindrical water tank of height 40 cm and diameter 18 cm was used. The tank was marked from the bottom at an interval of 5 cm up to the top. This test was conducted in *two sessions*:

Table 2: Experimental groups for Chronic Forced Swim Test (Part II).

Groups	Vehicle/Drugs	Oral dose and schedule Once daily for 14 days
1	Carboxy methyl cellosolve (CMC)	1 ml
2	Fluoxetine	10 mg/Kg
3	Simvastatin	10 mg/Kg
4	Simvastatin + Fluoxetine	10 mg/Kg each

6 Wistar rats per Gp. CMC was used to prepare suspension for active drugs.

Pretest Session: This test was performed 24 hours prior to the actual test. The glass cylindrical water tank was filled with lukewarm water (25 ºC) to a height of 15 cm. All the rats were placed individually in the tank and allowed (forced) to swim for 15 minutes. After the pretest, rats were removed, wiped and dried in a separate cage before returning to their home cages. The animals were then randomly divided into four groups as shown in Table 1. Rats from Group 1 served as control group and received CMC while those from Groups 2, 3 & 4 were administered test drugs using gavage tube 24 hr., 5 hr. and 1 hr. before the test session.

Test Session: The rats were exposed again to the conditions outlined above and total time of immobility (in seconds), over a period of 5 minutes was recorded, using a stopwatch. They initially struggled to escape from water, but later adopted a posture of immobility in which they only made the movements necessary to keep their head above water. A rat was judged to be immobile whenever it remained floating in the water, in an upright position, making only small movements to keep its head above water. The rats are forced to swim because of its inability to escape from water, leading to a condition of helplessness and despair.

Efficacy variable

The time period (in seconds) during which the rats were immobile was taken as a measure of depression. So the decrease in period of immobility was considered as the endpoint for antidepressant activity.

Part II: Chronic Forced Swim Test

Animals used in Part 1 were used in Part 2, after a washout period of 14 days. They were treated with either vehicle or drugs (Table 2) for 14 days. Thirty minutes after the administration of last dose, they were subjected to forced swim test for 6 min. Immobility period was recorded for each animal during this period.

Efficacy variable

Decrease in period of immobility was considered as the endpoint for antidepressant activity.

***Open Field Test*[11]**

All the animals underwent the Open Field Test (OFT) 5 minutes before subjecting them to acute or chronic FST to assess the locomotor activity. In order to see whether a change in immobility is associated with changes in motor activity, animals treated with the study drugs were tested for activity in an open field. The apparatus for open field test comprised of a wooden square box, 60 cm X 60 cm with 30 cm high walls. Its floor was divided into nine smaller squares of equal dimensions (20 cm X 20 cm). Hand operated counters were employed to score locomotion (number of line crossings within 5 minutes) and rearing frequencies (number of times an animal stood on its hind legs). Each rat was placed in the centre of the arena and its behavioral parameters were recorded for 5 minutes. The apparatus was washed with a detergent solution before keeping the next animal to eliminate possible odour clues left by the earlier animal.

Efficacy variables

1. Mean number of times line is crossed and
2. Mean number of times standing on hind limbs

Statistical Analysis

The observations recorded as time (in seconds), were expressed as Mean ± SD. Intergroup comparison was done using ANOVA followed by post hoc Turkey's Test. A 'P' value < 0.001 was considered to be significant.

RESULTS

Part I: Acute Forced Swim Test (Table 3)

CMC alone showed an immobility time of 183.66 ± 9.52 sec. Simvastatin administration decreased the duration of immobility (171.33 ± 6.15 sec) but the difference was not significant. The duration of immobility decreased significantly in the groups of rats which received fluoxetine alone (161.33 ± 8.68, P < 0.01) or in combination with simvastatin (167.66 ± 7.71 sec, P < 0.001). However, the 3 treatment groups did not differ from each other.

Table 3: Duration of immobility in rats subjected to Acute Forced Swim Test.

Group No	Drug administered	Duration of Immobility
1	Carboxymethylcellosolve (CMC)	183.66 ± 9.522
2	Fluoxetine	161.33 ± 8.687 **
3	Simvastatin	171.33 ± 6.15 $^{NS}{}_{1}{}^{NS}{}_{2}$
4	Simvastatin + Fluoxetine	167.66 ± 7.71* $^{NS}{}_{2}{}^{NS}{}_{3}$

All fig represent Mean ± S. D.

* P< 0.001, ** P < 0.01, NS1: Nonsignificant (P> 0.05) v/s CMC

NS_2: Nonsignificant (P> 0.05) v/s Fluoxetine

NS_3: Nonsignificant (P> 0.05) v/s Simvastatin

Part II: Chronic Forced Swim Test (Table 4)

In Chronic FST, the duration of immobility in the CMC treated group (182.33 ± 4.32 sec) and simvastatin treated group (175.33 ± 5.71 sec) did not differ statistically. But the duration was found to be significantly lower in both, the fluoxetine treated group (147.66 ± 8.73) and the combination treated group (130.5 ± 5.68 sec) as were compared to the CMC treated rats. Intergroup comparison revealed that there is a significant fall in the combination group (P < 0.001) compared to the individual therapy groups.

Table 4: Duration of immobility in Chronic Forced Swim Test in Rats.

Group No	Drug administered	Duration of Immobility in Seconds (Mean ± S. D.)
1	Carboxymethylcellosolve (CMC)	182.33 ± 4.32
2	Fluoxetine	147.66 ± 8.73*
3	Simvastatin	175.33 ± 5.71$^{NS\#}$
4	Simvastatin + Fluoxetine	130.5 ± 5.68$^{*\ \#\ \$}$

NS: Nonsignificant v/s CMC; * P < 0.001 v/s CMC, # P < 0.001 v/s Fluoxetine

$^{\$}$P < 0.001 v/s Simvastatin

Part III: Open Field Test (Table 5 and 6)

The OFT study showed no significant difference in the frequency of line crossings and rearings when drug treated groups were compared with rats treated with CMC (P > 0.05), ruling out any nonspecific stimulant/ depressant activity.

Table 5: Total number of line crossings, rearings and defecation in Open Field Test (OFT) for Acute FST.

Drug	Crossing	Rearing	Defecation
Control(CMC)	63.83 ± 9.152	40.56 ± 5.167	1.00 ± 0.632
Fluoxetine (Flx)	62.83 ± 6.882^{NS}	36.33 ± 5.715^{NS}	1.00 ± 0.894 ^{NS}
Simvastatin(Smv)	70.33 ± 6.25 ^{NS}	40.33 ± 5.78 ^{NS}	1.00 ± 0.63 ^{NS}
Flx + Smv	68.50 ± 5.00 ^{NS}	33.16 ± 5.67 ^{NS}	1.50 ± 0.83 ^{NS}

NS: Non significant, P > 0.05 v/s Control Group

Table 6: Total number of line crossings, rearings and defecation in Open Field Test (OFT) for Chronic FST.

Drug	Crossing	Rearing	Defecation
Control(CMC)	61.66 ± 10.28	28.33 ± 3.03	1.00 ± 0.632
Fluoxetine (Flx)	55.16 ± 10.02^{NS}	32.33 ± 5.64 ^{NS}	1.33 ± 0.81 ^{NS}
Simvastatin(Smv)	61.33 ± 5.75 ^{NS}	36.00 ± 4.98 ^{NS}	1.16 ± 1.16 ^{NS}
Flx + Smv	53.55 ± 5.08 ^{NS}	35.66 ± 5.98 ^{NS}	1.66 ± 1.21 ^{NS}

NS: Non significant, P > 0.05 v/s Control Group

DISCUSSION

In the present study the antidepressant action of simvastatin was evaluated using acute FST and chronic FST as models of behavioral despair in rats. These FST models were chosen as they are widely used to screen antidepressant drugs.[12] In FST rats are forced to swim in a restricted space from which they cannot escape. The rats are induced to a characteristic behavior of immobility and this behavior reflects a state of despair that can be reduced by several agents which are therapeutically effective in human depression. The FST model is sensitive to mono-aminergic manipulations. However, positive response in FST is also shown by antistress drugs, adaptogens, anti-anxiety drugs and drugs which increase exercise tolerance. FST also provides a useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses.[13]

Simvastatin was selected amongst all statins as it is the most lipid soluble and readily crosses brain blood barrier (BBB). However, simvastatin did not find to reduce duration of immobility in acute FST significantly when given individually whereas fluoxetine showed a significant decrease. Such response to fluoxetine has been reported in literature,[14] and our findings prove its worth as positive control. Lack of effect on immobility by simvastatin in this model indicates that it does not have any antidepressant activity.

High partial or non-response rates constitute other major challenges in the treatment of MDD (major depressive disorder). Approximately 30 to 50% of patients treated with antidepressants do not achieve remission.[15] Hence it was of interest to test the combination of simvastatin and fluoxetine. When tested, the combination of both, simvastatin and fluoxetine significantly reduced duration of immobility. However, the observed effect was comparable to that of fluoxetine. This means that combination effects can be attributed to the antidepressant action of Fluoxetine alone and presence of simvastatin in this combination does not augment effects of Fluoxetine.

In the model of chronic FST effects of Simvastatin and Fluoxetine given individually were similar to those observed in acute FST. Simvastatin, as a single dose, did not significantly reduce duration of immobility while Fluoxetine, as expected, showed a significant reduction. However, the point to note was simvastatin in combination with fluoxetine exhibited greater reduction in the duration of immobility than either of the individual agents. Thus in chronic FST model, simvastatin significantly potentiated antidepressant activity of fluoxetine. These results are consistent with one previous study wherein lovastatin potentiated antidepressant effects of fluoxetine only in chronic FST.[16]

Open field test is usually performed to discriminate between the general behavioral stimulation (false positives) and antidepressant effect of study drugs. The OFT study showed no significant difference in the frequency of line crossings, rearings and defecation among all the groups. This ruled out any nonspecific CNS stimulant/depressant activity by either agents, and thereby confirmed the assumption that the observed effects in FST models are specific to the antidepressant activity.

Our study did not prove antidepressant potential of simvastatin when used alone. Conventionally it is known that therapeutic effects of antidepressant agents take 2 to 3 weeks or more to become evident. However, in our study duration of treatment period with simvastatin was shorter in the model of acute FST. This could be one of the reasons for failure of simvastatin as antidepressant. In chronic FST the duration of therapy was 2 weeks but still no effect was observed when simvastatin was used alone. Here the duration may be adequate but statin alone may not be potent enough to exhibit the effect.

As statins potentiated action of fluoxetine on chronic administration, there is possibility that statins can have synergistic effect with fluoxetine and can reduce dose of fluoxetine in patients of depression. This may help to minimize adverse effects of fluoxetine like gastrointestinal disturbances (the most frequently reported side effects). [17] Also as hypercholesterolemia has been shown to be associated with non-response or resistance to antidepressants, statins by reducing cholesterol levels can also improve outcome in patients of resistant depression.

Thus it appears that simvastatin can serve as adjunct to SSRIs like fluoxetine. Simvastatin may facilitate serotonergic function and thereby improve treatment outcomes. It may help to reduce the incidence of depression in IHD patients and will reduce the increased morbidity and mortality due to depression in IHD patients. There is a possibility that it may help to reduce the dose of fluoxetine thereby reducing adverse effects of fluoxetine. However randomized, controlled clinical trials are needed in future to estimate the impact of adding simvastatin to fluoxetine in patients of depression.

Considering the high rate of partial or non-response to antidepressants such as serotonin selective reuptake inhibitors (SSRI),[15] which are frequently used as first-line agents for the treatment of depression, cholesterol-lowering drugs like simvastatin as adjuncts to SSRIs may improve treatment outcomes.

Thus to conclude, this study showed that although simvastatin did not exhibit independent antidepressant activity, it can have synergistic effect with SSRI like fluoxetine. This raises the possibility that it can improve clinical efficacy of antidepressants

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