Abstract

Background:

The role of dopamine and dopaminergic dysfunction has been established in the pathogenesis of depression. It has been reported that sensitization of D_{2}-like dopamine receptors in the mesolimbic dopamine system may represent a 'final common pathway' in antidepressant action. However, the involvement of specific dopamine receptors in depression is still a matter of debate.

Various experimental and clinical studies have suggested a link between cholesterol and depression. Antidepressant action of various antidepressants is reported to be compromised if patients have hypercholesterolemia. Thus, lowering of cholesterol levels with HMGR inhibitors (HMG CoA reductase inhibitors or statins) may improve the effectiveness of these agents. The precise role link between depression and cholesterol levels has not been established in chronic mild stress condition, a predisposing factor to cause the depression.

The central BDNF gene expression has been reported to be up regulated in major depression. There are independent reports suggesting a possible involvement of exon II transcripts of central BDNF gene in the actions of antidepressant agents. However, no studies have reported the alteration in the expression of BDNF gene in chronic stress models of depression in correlation with changes in circulating lipid levels.

Objectives:

1. To investigate the possible involvement of dopamine or dopamine D_{2} receptor on simvastatin induced beneficial effect in mice treated with acute or chronic mild stress condition, having its potential implication in the treatment of depression.
2. To investigate the influence of dopamine or dopamine D2 receptor on the expression of exon II transcripts of central BDNF gene in mediating the HMGR pathway.

3. To investigate the correlation of circulating lipids with depression by investigating the interaction of antidepressants with statins.

Materials and Methods:

We have studied the effects of in chronic treatment (30 days) of simvastatin and their interaction with subtherapeutic doses of dopaminergic agents such as bromocriptine, haloperidol, bupropion and levodopa on immobility time and compared it time with the immobility in higher doses the same dopaminergic agents using the forced swim test (FST) and tail suspension test (TST) of depression in mice.

Two sets of experiments were performed in FST. In the first set of experiment, mice were given i.p injection of previously reported higher doses of bromocriptine mesylate, haloperidol, bupropion hydrochloride and levodopa as 2 mg/kg, 0.1 mg/kg, 40 mg/kg and 200 mg/kg, respectively. Imipramine hydrochloride was administered in the dose of 10 mg/kg, p.o. Mice were initially subjected to a pretest wherein all animals were subjected to have 15 minutes forced swimming. After 15 minutes pretest, mice were subjected to the treatment of imipramine hydrochloride and the higher doses of bromocriptine mesylate, haloperidol, bupropion hydrochloride and levodopa as mentioned above. All animals were then subjected to the final test of 5 minutes after one hour of last dose.

In second set of experiment, mice were given previously reported sub therapeutic doses of bromocriptine mesylate, haloperidol, bupropion hydrochloride and levodopa as 0.5 mg/kg, 0.06 mg/kg, 10 mg/kg and 12.5 mg/kg respectively. In this set of experiment, 30 days oral treatment of simvastatin in the dose of 10 mg/kg was given. All other animals were given an equal amount of saline. Initially a pretest was
performed on day 29 wherein all animals were subjected to have 15 minutes forced swimming. After 15 minutes pretest, mice of group 8 to 12 were given the treatment of dopaminergic agents as mentioned above. The treatment of these dopaminergic agents was again repeated at 19 hrs and 23 hrs after pre test. On day 30, all animals were then subjected to the final test of 5 minutes after one hour of last dose.

In both sets of experiments of FST, animals were placed in the chamber (35 × 15 × 10) containing water up to a height of 15 cm at 25 +/- 2° C. The animals were not allowed to touch the bottom of the chamber with its hind limbs or tail or climb over the edge of the open chamber. Immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water. A decrease in the duration of immobility is indicative of an antidepressant-like effect. The animals were used only once in this test.

In the tail-suspension test (TST) also two sets of experiments were performed in a similar manner as in the forced swim test. In the first set of experiment, mice were subjected to the treatment of imipramine hydrochloride and the higher doses of bromocriptine mesylate, haloperidol, bupropion hydrochloride and levodopa as mentioned in FST. All animals were then subjected to tail suspension test after one hour of last dose. In second set of experiment of TST, mice of group 8 to 12 were given a daily dose of simvastatin (10 mg/kg) p.o. for 30 days. All other animals were given an equal amount of saline. On day 29, all animals were given i.p. injection of dopaminergic agents at an interval of 1hr, 19 hrs and 23 hrs. On day 30, all animals were then subjected to tail suspension test after one hour of last dose. In both sets of experiments of TST, mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1cm from the tip of the tail. Immobility time was recorded during a 5 min period. The animal was considered to be immobile when it does not show any movement of body and hanged passively.
The interaction of dopamine modulators with or without simvastatin was studied using changes in sucrose intake as the index of anhedonia in depression. Animals were subjected to chronic mild stress for 21 days. Two sets of experiment were done in the CMS. In the first set of experiment, total 48 mice were initially subjected to sucrose intake test at an interval of day 0, day 3, day 6, day 9 and day 12. After day 14, mice of determined groups were given the treatment of dopamine D_2 receptor modulators such as bromocriptine or haloperidol for 7 days. Sucrose intake was estimated consequently on day 15, day 18 and 21. In this the set of experiment, 12 mice were housed under normal condition. They were subdivided into two groups: Group 1 served as normal control and Group 2 received simvastatin (10 mg/kg). Rests of 36 mice were housed under chronic mild stress conditions. After 18 days of adaptation period for sucrose solution, all mice were further divided into subgroup as per the matched base line value of sucrose intake. Mice were initially trained to consume a 2% sucrose solution for 7 days. After 7 days, baseline tests for sucrose solution intake were performed (two tests per 7 days) over a period of 18 days for all mice. These tests involved a 3-h period of food and water deprivation followed by the offering of a sucrose solution for 1 h. Intake was determined by weighing the bottles containing sucrose solution at the beginning and at the end of each test. After measuring the base line value of sucrose intake for the period of 18 days, all mice were further divided into subgroup as per the matched base line value of sucrose intake as mentioned above.

In second set of experiment, another 48 mice were taken and divided into two Groups of normal mice and six groups of stressed mice in similar manner as first set of experiment with respect to their weight. The blood collection was done through retro-orbital plexuses under light ether anaesthesia for the biochemical assay of different lipid parameters such as total cholesterol, triglyceride, HDL, LDL, VLDL, total cholesterol/LDL, HDL/LDL at an interval of day 0, day 7 and day 14. After day 14,
mice were given the treatment of dopamine D$_2$ receptor modulators such as bromocriptine or haloperidol for 7 days. The same lipid parameters as mentioned above were estimated consequently at day 21. The estimation of lipid parameters was done using standard protocol methods. Serum total cholesterol, triglyceride were estimated by the method of CHOD-PAP and HDL by the method of GPO-PAP. LDL and VLDL were calculated by using Friedwald formula and VLDL: TG/5 respectively. Total Cholesterol/ HDL ratio and LDL /HDL ratio were calculated. In both set of experiments of chronic mild stress, the stress scheme, to produce chronic mild stress conditions was followed as per previously reported paradigm. This stress scheme included several stressors. Each as follows: three 5-h periods of food and water deprivation, immediately prior to sucrose tests, one additional 16-h period of water deprivation, two periods of intermittent illumination; two periods (7 and 12h) of 45 degrees cage tilting, one 12h period in a soiled cage (100 ml water in sawdust bedding) and three periods (7, 9 and 12h) of low intensity stroboscopic illumination (150 flashes/min). These stressors were scheduled for each day e.g. Monday to Sunday for total 21 days. In addition, in both set of experiments, mice of Groups 2, 4, 6 and 8 were given a daily dose of simvastatin (10 mg/kg) p.o. for 21 days. After day 14, mice received daily one intraperitoneal injection of bromocriptine mesylate (2 mg/kg) or haloperidol (0.1 mg/kg) for 7 days as mentioned above. All other animals were given equal amount of saline.

In yet another set of experiment, a correlation analysis was done in continuous 14 days drug treatment study, in which mice were exposed to CMS for 14 days during which they were treated daily with simvastatin with or without bromocriptine (2 mg/kg) or haloperidol (0.1 mg/kg) or levodopa. All the animals were subjected to sucrose intake test and this sucrose intake was taken as the index of depression. Blood samples were collected for the estimation of serum lipid levels to establish the indirect evidence for the phosphorylation state of HMG Co A reductase (HMGR). The expression of exon II
transcripts of BDNF gene was studied in mice brain by RT PCR method using specific gene primers and cDNA of respective samples as template. The band intensity of each lane on the exposed films was analysed and the ratio of the corresponding density (BDNF II/ HPRT) was calculated.

**Results:**

The treatment of higher doses of bromocriptine, bupropion and levodopa significantly decreased immobility time and the higher dose of haloperidol significantly increased the immobility time in FST, when compared to normal control and positive control (imipramine). As compared to normal control and positive control (imipramine), the treatment of sub therapeutic doses of bromocriptine, bupropion, levodopa and haloperidol did not significantly affect the immobility time in FST.

Oral administration of simvastatin (10 mg/kg) for 30 days along with the sub therapeutic doses of bromocriptine (0.5 mg/kg), bupropion (10 mg/kg) and levodopa (12.5 mg/kg) decreased immobility time to a greater extent than treatment of simvastatin (10 mg/kg) alone. The same treatment of simvastatin as mentioned above along with the sub therapeutic dose of haloperidol did not affect the immobility time as compared to simvastatin alone.

The treatment of higher doses of bromocriptine, bupropion and levodopa significantly decreased immobility time and the higher dose of haloperidol significantly increased the immobility time in TST, when compared to normal control and positive control (imipramine). As compared to normal control and positive control (imipramine), the treatment of sub therapeutic doses of bromocriptine, bupropion, levodopa and haloperidol did not significantly affect the immobility time in TST.

Oral administration of simvastatin (10 mg/kg) for 30 days along with the sub therapeutic doses of bromocriptine (0.5 mg/kg), bupropion (10 mg/kg) and levodopa (12.5 mg/kg) decreased immobility time to a greater extent than treatment of
simvastatin (10 mg/kg) alone. The same treatment of simvastatin as mentioned above along with the sub therapeutic dose of haloperidol did not affect the immobility time as compared to simvastatin alone.

Treatment of mice with simvastatin for 21 days did not produce any change in sucrose intake in normal mice. The intake of sucrose was decreased in stressed mice as compared to normal mice. The combination of simvastatin and bromocriptine increased the sucrose intake to a greater extent than simvastatin alone. Intra peritoneal injection of haloperidol with oral administration of simvastatin reversed the increased level of the sucrose intake in stressed mice.

Treatment of mice with simvastatin for 21 days did not produce any change in total cholesterol level in normal mice. Level of total cholesterol was increased in stressed mice as compared to normal mice. The combination of bromocriptine and simvastatin decreased the level of total cholesterol to a greater extent than simvastatin alone. The combination of haloperidol and simvastatin did not affect the level of total cholesterol in stressed mice.

Treatment of mice with simvastatin for 21 days did not produce any change in level of total cholesterol, HDL, triglyceride, VLDL, LDL, total cholesterol/HDL, LDL/HDL in normal mice. There was increase in all such parameters with exception of decrease HDL level in stressed mice as compared to normal mice. However, the level of triglyceride and VLDL was initially found to be decreased after 7 days stress treatment followed by abrupt increase in day 14 and day 21. All such alterations in parameters were prevented by the combination of bromocriptine and simvastatin to a greater extent than simvastatin alone. However, the combination of haloperidol and simvastatin did not affect such alteration in lipid levels.

In continuous 14 days drug treatment study, either CMS induced alteration in the sucrose intake and lipid levels or influence of drug treatments in such CMS induced
alterations were in similar manner to that 21 days study. There was a decrease in sucrose levels and increase in serum cholesterol and triglycerides levels with decrease in HDL. All these alterations were prevented by simvastatin. Such action of simvastatin was synergized by bromocriptine and levodopa. Haloperidol prevented significantly the simvastatin induced increase in sucrose intake but not the alterations in lipids. There was upregulation in the expression of BDNF exon IIA and IIB transcripts by CMS but not the exon IIC transcripts. Simvastatin could increase expression of exon IIC transcripts in stressed mice. This was partially synergized by bromocriptine whereas haloperidol significantly prevented simvastatin induced increase in expression of BDNF exon IIC transcripts.

The correlation coefficient analysis showed that, there was a negative correlation of decreased level of sucrose intake in stressed mice as compared to normal mice, with increase in both total cholesterol ($r = -0.6121$) and expression of exon IIB transcripts ($r = 0.8920$). The treatment with simvastatin, prevented this decrease in sucrose intake in stressed mice with positive correlation between simvastatin induced increase in sucrose intake and increase in both expression of exon IIA transcripts ($r = 0.8334$) and exon IIC transcripts ($r = 0.7698$). Such simvastatin induced increase in above mentioned both transcripts (exon IIA transcripts and exon IIC transcripts) showed a negative correlation with simvastatin induced decrease in triglyceride level ($r = -0.9995$ and $r = -0.8713$ respectively) in stressed mice. In the presence of bromocriptine, simvastatin showed more increase in sucrose intake as compared to simvastatin alone. Such enhancement with increase in sucrose intake in presence of bromocriptine, showed positive correlation of increase in sucrose intake with increase in expression of exon IIC transcripts ($r = 0.8229$) with decrease in level of triglyceride ($r = -0.8615$). Although, simvastatin also showed more increase in sucrose intake as compared to simvastatin alone in presence of levodopa, no correlation was found with levodopa and expression.
of exon IIC transcripts or triglyceride level. There was a strong correlation between prevention of simvastatin induced increase in sucrose intake and decrease in expression of exon IIC transcripts \((r = 0.9765)\) in presence of haloperidol and this was irrespective of changes in lipid levels.

**Conclusions:**

The results of the present investigations indicate the possible involvement of dopamine or dopamine D\(_2\) receptor in simvastatin induced beneficial effect in mice treated with acute or chronic mild stress, having its potential implication in the treatment and pathogenesis of depression. Thus, it raised the possibility for the possible interaction between the dopaminergic pathway and mevalonate pathway in depression. The present study also suggests the preferential effects of dopaminergic agents on the function of statins which demonstrated that the signal transduction mechanism in the receptor effector linkage of dopamine D\(_2\) receptor may be mediated through HMGR pathway, which in turn may regulate the expression of exon II transcripts of BDNF gene especially of BDNF exon IIC transcript, having its potential utilization in the future treatment of depression.

**Keywords:** BDNF exon II transcripts, Cholesterol, Depression, Dopamine, D\(_2\) receptor modulators, Mice, Stress, Statins
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