

Evaluation of Antidepressant Activity of Simvastatin in Albino Mice

*Ayesha Rubina, Swetha E S, Umakanth N Patil

Department of Pharmacology, S. S. Institute of Medical Sciences and Research Centre, NH-4 Road, Davangere-577004, Karnataka, India.

ABSTRACT

OBJECTIVE: Evaluation of acute antidepressant property of Simvastatin in albino mice. **BACKGROUND:** Cholesterol is an important determinant of the structure and function of cell membranes, and plays an integral role in many neural functions that contribute to mood state. Cholesterol may have a role in the pathophysiology of depression. Lowering cholesterol levels with statins reduces risks for cardiovascular events, and statins exert neuroprotective properties by increasing BDNF level. **METHOD:** Total of 84 mice was taken, 42 were taken for Tail suspension test (TST) and 42 for Forced swimming test (FST). Each model had 7 groups of 6 mice in each group. Group 1 is taken as a control, group 2 standard Fluoxetine [SSRI](5mg/kg), group 3 standard Amitriptyline [TCA](10mg/kg), group 4 and 5 test drug (simvastatin) in doses of 5mg/kg and 10mg/kg, group 6 combination of simvastatin(5mg/kg) and sub effective dose of fluoxetine(2.5mg/kg), group 7 combination of simvastatin(5mg/kg) and sub effective dose of amitriptyline(5mg/kg). **RESULTS:** Simvastatin presents significant antidepressant effect at all doses, indicated by reduction in immobility time in both FST and TST. There was significant reduction in immobility period when simvastatin was combined with sub effective dose of fluoxetine[SSRI] and sub effective dose of amitriptylin[TCA]. **CONCLUSION:** Simvastatin shows significant antidepressant effect in mice. It potentiates the action of sub effective dose of Fluoxetine and Amitriptyline when given in combination, raising the possibility that simvastatin can be used to facilitate the action of other antidepressants or can themselves can be used as antidepressants in humans.

Keywords: Amitriptyline, fluoxetine, simvastatin, forced swimming test and tail suspension test

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*Address for correspondence:

Dr. Ayesha Rubina,

Department of Pharmacology, S. S. Institute of Medical Sciences and Research Centre, NH-4 Road, Davangere-577004, Karnataka, India.

E-mail:ayesharubina@yahoo.com

INTRODUCTION

Simvastatin is widely used to reduce serum low density lipoprotein cholesterol by inhibiting the rate-limiting enzyme hydroxymethylglutaryl-coenzyme.

Simvastatin reduces the risk of ischemic heart disease events and cerebrovascular stroke and has potential applications in multiple sclerosis, traumatic brain injury, Alzheimer's disease, anxiety and anti-inflammatory and antithrombotic effects that are not related to their cholesterol lowering effects [1,2]. These potential advantages of statins have led to an increasing preference in clinical use. In 1990's, it was thought that low cholesterol levels were associated with depression, behavioral disturbances, and an increased tendency to violence and suicide [1,2]. But

the entity of 'vascular depression presents indirect evidence that hypercholesterolemia is a risk factor in the pathophysiology of depression [3]. It was also found that hypercholesterolemia as well as other cardiovascular risk factors are associated with ineffective treatment and the severity of depression [4]. However, recent large-scale controlled and population-based studies have a positive role of statins or low cholesterol levels on psychological well-being [5]. It was also shown that rats maintained on a lovastatin-enriched diet for 30 days were more sensitive to the antidepressant-like effects of a low (sub effective) dose of fluoxetine [6].

NMDA receptors are involved in the pathophysiology of depression [7].

Preclinical data have demonstrated that blocking the NMDA receptor complex produced anxiolytic and antidepressant activity in animal tests [8-10]. Amitriptyline (TCA) and Fluoxetine (SSRI) which are monoamine uptake blocker type antidepressants acts mainly by having an inhibitory effects over NMDA receptor [11,12]. Statins weakens the binding between NMDA receptors and statins, probably due to the decrease of the association between NMDA receptors and lipidic rafts [13], thereby can act as antidepressant. With this background we are evaluating the antidepressant effect of simvastatin in forced swimming test and tail suspension test, animal models of antidepressant drugs

OBJECTIVES:

1. To evaluate the antidepressant activity of simvastatin in albino mice
2. To study the effect of combination of sub effective dose of simvastatin and standard drug fluoxetine and amitriptyline to check for synergistic effect.

MATERIALS AND METHODS:

ANIMALS: Albino mice of either sex of weighing between 20-25g which are inbred in central animal house of S.S. Institute of medical Sciences and Research Centre, Davangere are used. Animals are randomly housed in cages at an ambient temperature and humidity, with a 12 hour light and 12 hour dark cycle. The animals have free access to food and water *ad libitum*. The experiment was approved by the Institutional Animal Ethics Committee (IAEC). The animals were used according to the CPCSEA guidelines for the use and care of experimental animals.

EXPERIMENTAL DESIGN

Total of 84 animals were taken, 42 mice were taken for FST and 42 mice were taken for TST each model had 7 groups. Mice were divided randomly into control and experimental groups with 6 animals in each group (n=6), Group 1 received the vehicle, normal saline (10ml/kg) and served as the control group, group 2 received the standard drug fluoxetine (5mg/kg), group 3 standard drug amitriptyline (10mg/kg), groups 4 and 5 the test drug [Simvastatin] in the doses of 5mg/kg and 10mg/kg

intraperitoneally respectively. Group 6 contains combination of simvastatin (5mg/kg) with sub effective dose of fluoxetine (2.5mg/kg), and group 7 contains combination of simvastatin (5mg/kg) with sub effective dose of amitriptyline (5mg/kg) these groups are taken for TST, same set of groups are taken for FST.

Drugs/vehicle was administered intraperitoneally to the animals 30 minutes prior to the evaluation. Forced swimming test and Tail suspension test was used to assess the antidepressant effect in albino mice

FORCED SWIMMING TEST (FST)

The test was performed as described by Porsolt et al [14]. Mice were forced to swim in a Plexiglas cylinder with a diameter of 18 cm. The water level in the cylinder was 15 cm, and the water temperature was 25±0.5°C. The mice were observed for 6 minutes, immobility and struggling time was recorded. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Each mouse was tested separately, and the time of immobility was recorded during the last 4 min of the 6-min testing period, that is after 2 min of habituation. Results are expressed as the immobility time during the 240-s test period for the 6 mice tested in each group. water was changed after each test. Rats were forced to swim for 15 minutes as a training session one day before the experimentation session without any recording.

TAIL SUSPENSION METHOD (TST):

The "Tail suspension Test" has been conducted as described by Steru et al [15]. For the test the Mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was observed for a period of 6 minutes, last 4 minutes of the observation were taken for calculation. Mice were considered immobile only when they hung passively and were completely motionless.

STATISTICAL ANALYSIS

Data is described in terms of mean and standard error of mean (SEM) and further analysed by using one way ANOVA followed by student t-test. P<0.05, is considered as probability for statistical significance.

RESULTS

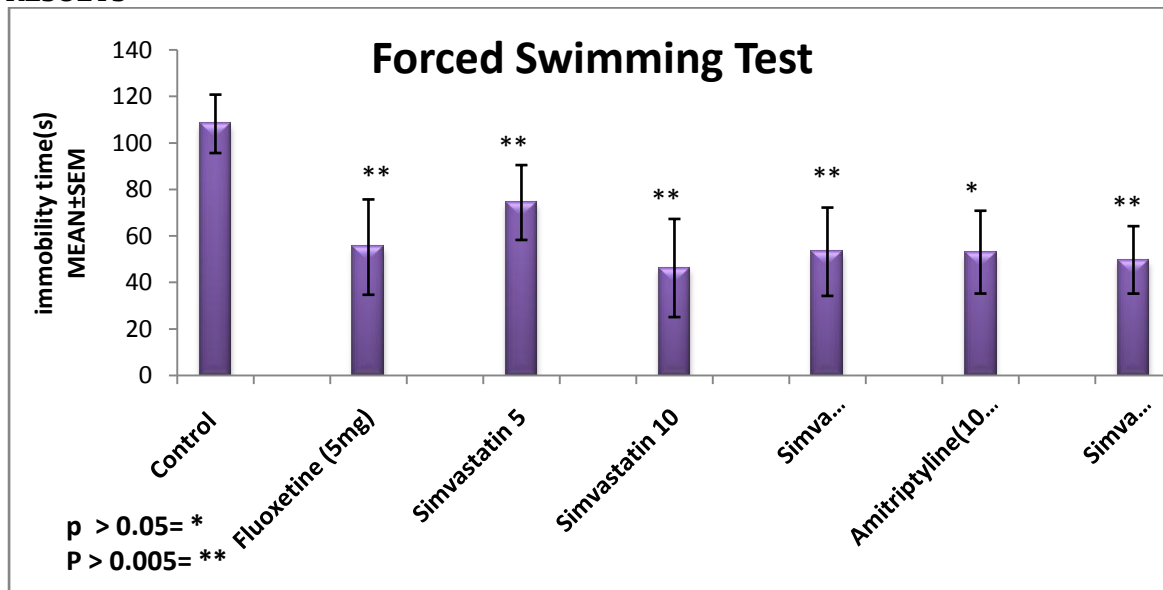


Figure 1: Forced Swimming Test

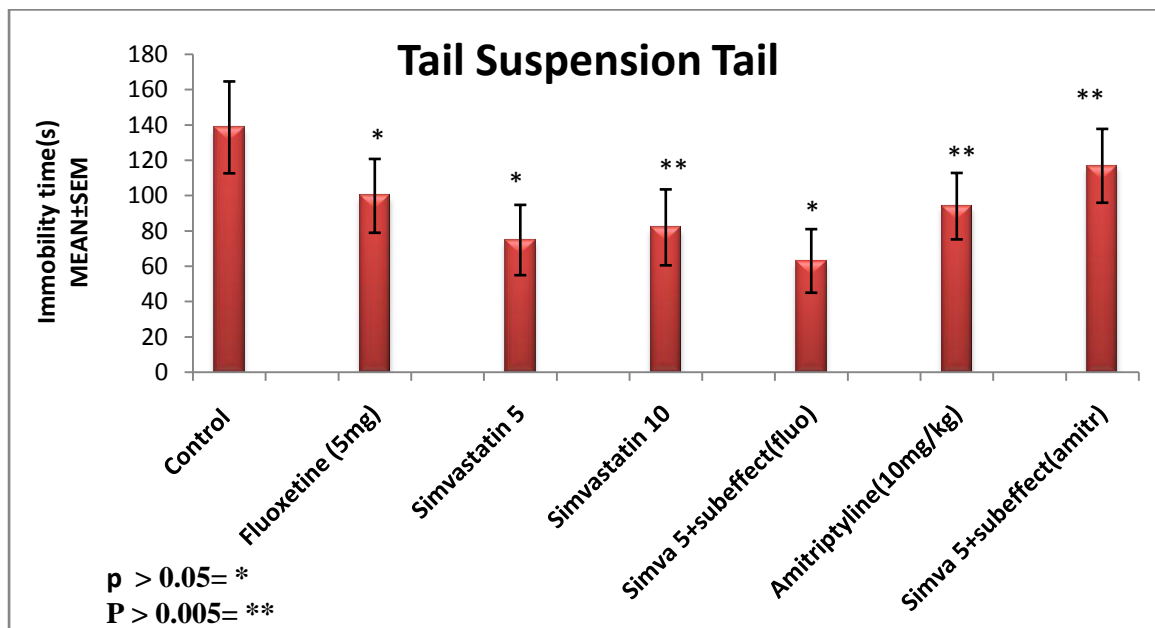


Figure 2: Tail suspension test

Immobility time was significantly reduced in all groups of the simvastatin compared with the controls in both tail Suspension test and forced swimming test. There was reduction in immobility time in both the standards fluoxetine and amitriptyline compared to control. In FST, there was highly significant reduction ($P < 0.005$) in time noticed in all groups as well as combination with sub effective doses of fluoxetine and amitriptyline whereas in TST, there was significant ($P < 0.05$) reduction in time noticed with simvastatin

5mg/kg and in combination with sub effective dose of fluoxetine but simvastatin 10mg/kg and in combination with sub effective dose of amitriptyline greater reduction in time was seen ($P < 0.005$) compared to controls.

DISCUSSION

Results show greater reduction in immobility in all groups of simvastatin and also when used in combination with sub effective dose of fluoxetine and amitriptyline in both FST and TST models of depression.

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, which are potent inhibitors of cholesterol biosynthesis. There is clinical and pre-clinical evidence that the statins may have additional pleiotropic properties that are potentially neuroprotective, independent of their effect on serum cholesterol [16].

Brain derived neurotrophic factor (BDNF) and neurotrophic growth factor (NGF) are two important neurotrophic factors that induce the survival, development, and function of neurons [17]. The statin-induced increase of these growth factors may enhance neuronal and synaptic plasticity, which improves functional recovery by increasing BDNF and NGF level via PI3K/Akt pathway [18].

Neuronal cell death is caused by excitotoxicity which is mediated by glutamate mediated ion channel [19]. The NMDAR subtype of glutamate receptors probably plays the major role because of its high calcium permeability. NMDAR-mediated calcium influx activates nitric oxide synthase (nNOS). nNOS-mediated production of nitric oxide and subsequent formation of reactive oxygen radicals is a cause of excitotoxic neuronal death, and inhibitors of nNOS protect cultured cortical neurons from excitotoxicity [20]. nNOS are found in sterol-rich plasma-membrane microdomains called lipid rafts and depletion of membrane [21]. Reduction in cholesterol associates with protection from excitotoxicity, cholesterol alters localization, composition, and function of protein complexes associated with rafts [22]. Statins may alter the sterol content of lipid rafts, thereby uncoupling nNOS activation from NMDAR-mediated calcium flux. Statins weakens the binding between NMDA receptors and statins, due to the decrease of the association between NMDA receptors and lipidic rafts [23].

Current immune-mediated concepts on the etiology of depression include increased proinflammatory cytokines, and final activation of tryptophan and serotonin degrading enzyme indoleamine 2, 3-deoxygenase, which may cause a reduction of serotonergic neurotransmission in MDD [24]. Proinflammatory cytokines such as

interleukin-2, interferon- γ , or tumor necrosis factor- α activate the tryptophan and serotonin-degrading enzyme indoleamine 2,3-dioxygenase (IDO). Simvastatin significantly decrease levels of circulating IL-6 and TNF- α , in addition to IL-1, in patients with hypercholesterolemia [25].

Considering the anti-inflammatory and immunomodulatory properties of statins [16], adjunctive use of statins with SSRIs may block or reverse a cascade of immune-mediated serotonin depletion in depression. Accordingly, in the brains of suicide victims and patients with depression, a reduced glycine binding site of the NMDA receptor was found [26,27]. NMDA receptor antagonists increase the serotonin levels in the brain [28][29]. It has been suggested on the basis of in vitro studies that tricyclic antidepressants interact with the N-methyl-D-aspartic acid (NMDA)-receptor complex to block the action of NMDA [30].

In the present study greater reduction in time for both FST and TST was noticed when sub effective dose of simvastatin was used with sub effective concentration of amitriptyline than with sub effective concentration of fluoxetine, implying that may be simvastatin has an antidepressant effect by acting on NMDA receptors.

CONCLUSION

Simvastatin shows significant antidepressant property in albino mice. Further clinical evaluation is needed to be tried in human subjects, as it will be very helpful for the patients having hyperlipidemia and depression.

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