

Acute antidepressant and anxiolytic effects of simvastatin and its mechanisms in rats

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ABSTRACT

الأهداف: التحقق من تأثير عقار السيمفاستاتين المضاد للاكتئاب والقلق لدى الجرذان.

الطريقة: أُجريت هذه الدراسة في معمل اختبار الحيوانات، قسم الصيدلة، جامعة إيسكيسيهير أوزمانغازي للعلوم الطبية، إيسكيسيهير، تركيا وذلك خلال الفترة من مارس إلى مايو 2010م. شملت الدراسة 64 جردي ذكر بالغ من النوع سبراغو دولي (تبلغ من العمر: 8-9 أسابيع، ووزنها: 200-250 غرام). لقد قمنا باستخدام اختبار الإرغام على السباحة، وارتفاع اختبار المتاهة من أجل تحليل مدى تأثير عقار السيمفاستاتين المضاد للاكتئاب والقلق. وقد قُسمت الحيوانات إلى 8 مجموعات كالتالي: مجموعة الشاهد (محلول ملحي)، مجموعة 10 ملغ/كغ من السيمفاستاتين، و30 ملغ/كغ من السيمفاستاتين، و50 ملغ/كغ من السيمفاستاتين، ومجموعة 10 ملغ/كغ من أميرتريبتيلين، ومجموعة 5 ملغ/كغ من السيترالين، ومجموعة تركيب 30 ملغ/كغ من السيمفاستاتين مع 10 ملغ/كغ من أميرتريبتيلين، وأخيرا مجموعة تركيب 30 ملغ/كغ من السيمفاستاتين مع 5 ملغ/كغ من السيترالين.

النتائج: لقد قامت كافة جرعات عقار السيمفاستاتين بإنقاص وقت عدم الحركة بشكل واضح، كما قامت جرعات العقار التي بلغت 10 و30 ملغ/كغ بزيادة الوقت المستغرق في الذراع المفتوحة ولم تؤثر جرعة 50 ملغ/كغ على هذا الوقت. وأظهرت الجرعة الواحدة من أميرتريبتيلين (10 ملغ/كغ) تأثيرا واضحا في إزالة القلق والاكتئاب، فيما أظهرت جرعة السيترالين (5 ملغ/كغ) تأثيرا واضحا في إزالة الاكتئاب ولم يكن لها أي تأثير على القلق. ولم يكن لعقار السيمفاستاتين أي تأثير على إزالة القلق والاكتئاب وذلك عند تركيبه مع عقار أميرتريبتيلين، ولقد تمت ملاحظة التكمين وتضاؤل تأثير السيمفاستاتين على إزالة القلق وذلك عند تركيبه مع السيترالين.

خاتمة: أثبتت الدراسة بأن تأثير عقار السيمفاستاتين في إزالة الاكتئاب والقلق مشابه لمثبطات استرداد السيروتونين الانتقائية، كما أن التفاعل بين تأثير السيمفاستاتين على بعض الإنزيمات ووظائف الدوبامين قد يؤدي إلى تسهيل تأثير هذا العقار وإزالته للاكتئاب والقلق.

Objective: The aim of this study is to investigate the antidepressant and anxiolytic effects of simvastatin in rats.

Methods: Sixty-four male adult (8-9 weeks old) Sprague-Dawley rats (200-250 g) were used. The forced swimming test and the elevated plus maze test were used for testing the antidepressant and anxiolytic effects. Eight groups were formed: control (saline), simvastatin 10, 30, and 50 mg/kg, amitriptyline 10 mg/kg, sertraline 5 mg/kg, simvastatin 30 mg/kg+amitriptyline 10 mg/kg, and simvastatin 30 mg/kg+sertraline 5mg/kg combinations. The study was conducted at the Animal Experiment Laboratories, Department of Pharmacology, Eskisehir Osmangazi University Medical School, Eskisehir, Turkey from March to May 2010.

Results: The immobility periods were significantly reduced by all doses of simvastatin. Simvastatin 10 and 30 mg/kg but not 50 mg/kg increased time spent in the open arm. A single dose of amitriptyline 10 mg/kg showed significant antidepressant and anxiolytic effects. Sertraline 5 mg/kg showed significant antidepressant, but not anxiolytic effects. There was no change in the antidepressant and anxiolytic effects of simvastatin when combined with amitriptyline. A potentialization in antidepressant effect, and a decrease in anxiolytic effect of simvastatin were observed in combinations of simvastatin and sertraline.

Conclusion: Simvastatin presents significant antidepressant and anxiolytic effects in rats similar to selective serotonin reuptake inhibitors, and interactions between the effects of simvastatin on indoleamine 2,3-dioxygenase enzymes, N-methyl-D-aspartic acid receptor blockade, and dopaminergic functions possibly mediate its antidepressant and anxiolytic effects.

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The antioxidant and antidepressant properties of statins have been investigated in clinical studies.^{1,2} However, in some studies statins are reported to be associated with depression,³ while other studies found antidepressant features.⁴ The beneficial effects of statins in cardiovascular diseases are related to their cholesterol lowering effect. At the same time, the results of clinical studies exhibit useful and direct (pleiotropic) actions of statins in improving endothelial dysfunction, reducing atherogenesis, in dementia and Alzheimer's disease, and anti-inflammatory and antithrombotic effects that are not related to their cholesterol lowering effects.^{5,6} These potential advantages of statins have led to an increasing preference in clinical use.⁷ Recently, it was thought that low cholesterol levels were associated with depression, behavioral disturbances, and an increased tendency to violence and suicide.^{1,2} There is sufficient data on the effects of statins on multiorgan functions. Currently, there is an increasing interest in their psychotropic effects. The results of clinical and preclinical studies introduce a relation between cholesterol and depression. The entity of 'vascular depression' presents indirect evidence that hypercholesterolemia is a risk factor in the pathophysiology of depression.¹⁰ It was also found that hypercholesterolemia as well as other cardiovascular risk factors are associated with ineffective treatment and the severity of depression.^{11,12} The aim of this study is to examine the antidepressant and anxiolytic effects of simvastatin, a drug classified in statins, in connection with its mechanism of action in rats.

Methods. This study was conducted at the Department of Pharmacology Animal Experiment Laboratories, Eskisehir Osmangazi University Medical School, Eskisehir, Turkey from March to May 2010 with the permission of the Eskisehir Osmangazi University Medical School local ethical committee for animal experimentation (19-08-2009/No: 129).

Drugs. Simvastatin (Nobel, Istanbul, Turkey), amitriptyline (Deva, Istanbul, Turkey) and sertraline (Pfizer, Istanbul, Turkey) were dissolved in saline. Rats in the control group were injected with saline. All the drugs and saline were injected intraperitoneally.

Animals. Sixty-four male Sprague-Dawley rats weighing 200-250 g (8-9 weeks old) were used for the study. Rats were sheltered in standard laboratory conditions with a 12-hour light/dark cycle at 25±1°C, and were allowed to access standard pellet food and

tap water freely. All the experiments were performed in the Pharmacology Department laboratory, Eskisehir Osmangazi University Medical School, Eskisehir, Turkey. Animals were divided into 8 experimental groups each containing 8 rats as follows: group one (control group): saline; group 2: 10 mg/kg simvastatin; group 3: 30 mg/kg simvastatin; group 4: 50 mg/kg simvastatin; group 5: 10 mg/kg amitriptyline; group 6: 10 mg/kg amitriptyline+30 mg/kg simvastatin; group 7: 5 mg/kg sertraline; and group 8: 5 mg/kg sertraline+30 mg/kg simvastatin.

Experimental procedure. Drugs and/or saline were injected into the rats i.p. one hour before the experimentation. The forced swimming test and the plus maze test were used to assess the antidepressant and anxiolytic effects of simvastatin as described below.

Plus maze test. The test was performed as described by Pellow et al.¹³ The plus maze set up is 50 cm high from the floor and has 2 open (50 cm x 10 cm), and 2 closed arms (50 cm x 10 cm x 50 cm). Rats were put on the center (10 cm x 10 cm) of the set up, which connects the open and closed arms, and the times spent in the open and closed arms were observed for 5 minutes. Immediately after the plus maze test session, each rat was assessed with the forced swimming test.

Forced swimming test. The test was performed as described by Porsolt et al.¹⁴ Rats 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 rats were observed for 5 minutes and immobility and struggling times were recorded. A posture in which the rats stopped struggling and floated motionless with small movements to keep on top of the water was accepted as immobile. Rats were forced to swim for 15 minutes as a training session one day before the experimentation session without any recording.

Statistical analysis. Results were analyzed with Mann-Whitney U test. Statistical analyses were performed using the Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). A $p < 0.05$ was considered for statistical significance, and 95% confidence intervals for mean (lower-upper bound) are shown in Table 1.

Results. Results of the forced swimming test. Immobility time was significantly reduced in all doses of the simvastatin alone groups compared with the controls. Also, immobility time was significantly decreased in the amitriptyline 10 mg/kg alone, and sertraline 5 mg/kg alone groups compared with the controls. In addition, the combined use of simvastatin 30 mg/kg with amitriptyline 10 mg/kg and sertraline 5 mg/kg reduced the immobility time compared with

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Table 1 - The 95% confidence intervals of all studied groups of rats (lower-upper bound).

Groups	Immobility time	Struggle time	Open arm time
	(Lower-upper bound)		
Control	95.68-165.56	134.42-204.32	17.76-57.74
Sim 10 mg/kg	35.67-82.03	208.27-274.23	46.91-71.09
Sim 30 mg/kg	16.82-50.12	249.88-283.12	50.69-80.31
Sim 50 mg/kg	13.05-51.45	248.50-286.90	9.39-35.61
Amit 10 mg/kg	-1.93-28.67	271.32-301.92	61.21-87.03
Amit 10 mg/kg + Sim 30 mg/kg	4.62-50.88	249.12-295.38	60.35-87.39
Sert 5 mg/kg	57.00-104.50	175.90-262.90	11.95-36.79
Sert 5 mg/kg + Sim 30 mg/kg	3.09-49.15	248.18-297.06	3.87-18.87

Sim - simvastatin, Amit - amitriptyline, Sert - sertraline

the controls. The struggling time was significantly increased in all groups except in the sertraline 5 mg/kg alone group compared with the controls. The results are shown in Figures 1 & 2.

Results of the plus maze test. Simvastatin showed an anxiolytic effect on the time spent on the open arms. Both 10 and 30 mg/kg simvastatin significantly increased the time spent on the open arms compared with the

controls, while 50 mg/kg simvastatin had no significant effect compared with the controls. It was also observed that 10 mg/kg amitriptyline alone, and in combination with 30 mg/kg simvastatin enhanced the time spent on the open arms compared with the controls. However, 5 mg/kg sertraline alone, and in combination with 30 mg/kg simvastatin had no significant effect on the time spent on the open arms compared with the controls, even 5 mg/kg sertraline decreased the time spent on the open arms observed with 30 mg/kg simvastatin alone. The results are shown in Figure 3.

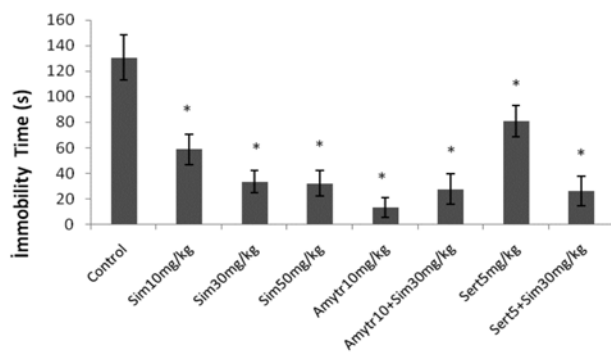


Figure 1 - Immobility time observed in the forced swimming test in rats. Sim - simvastatin, Amit - amitriptyline, Sert - sertraline, * $p < 0.05$ compared with controls, s - seconds.

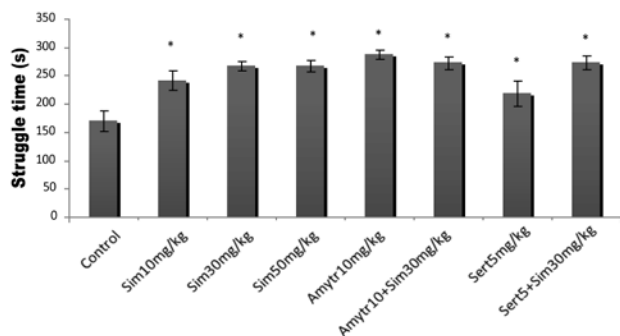


Figure 2 - Struggle time observed in the forced swimming test in rats. Sim - simvastatin, Amit - amitriptyline, Sert - sertraline, * $p < 0.05$ compared with controls, s - seconds.

Discussion. *Evaluation of simvastatin and antidepressant effects.* In this study, we observed that all doses of simvastatin, amitriptyline alone, sertraline alone, and the combined use of simvastatin with amitriptyline and sertraline decreased immobility time, while increasing the struggling time, except for sertraline alone, indicating an acute antidepressant effect. However, the antidepressant effect observed with sertraline alone was lower than the other groups, while combined use of sertraline and simvastatin had an antidepressant effect closer to simvastatin 10 and 30 mg/kg alone. At

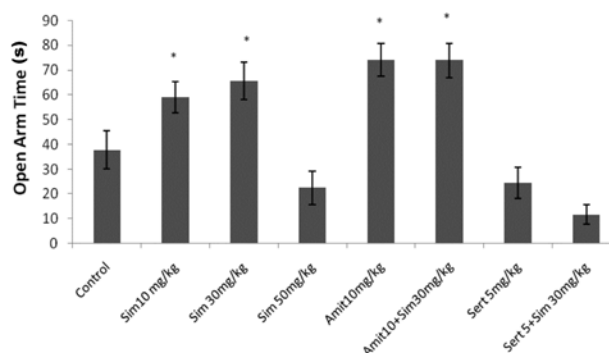


Figure 3 - Time spent on open arms of the elevated plus maze by rats. Sim - simvastatin, Amit - amitriptyline, Sert - sertraline, * $p < 0.05$ compared with controls, s - seconds.

first sight, these effects observed with simvastatin seem to be associated with its cholesterol lowering effect. However, it was also found that statins suppressed secretion of Th1 type cytokines IFN γ , IL 2, and IL 12 while enhanced production of Th2 type cytokines IL 4, IL 5, and IL 10.¹⁵ In addition, it was shown that the atorvastatin dose dependently attenuated the activity of indoleamine 2,3-dioxygenase (IDO).¹⁶ Indoleamine 2,3-dioxygenase is an IFN γ -inducible enzyme that degrades tryptophan in the kynurenine pathway. It is commonly observed even in chronic heart diseases that tryptophan degradation accelerates due to activation of IDO enzymes related to endogenous formation of IFN γ , which is a Th1 type cytokine. Tryptophan is a precursor of the neurotransmitter serotonin. Low tryptophan levels were associated with depression.¹⁷ Additionally, it was also reported that there is a relationship between low serum tryptophan levels due to immune activation and poor life quality in patients with colorectal cancer.¹⁸ We suggest that simvastatin shows its antidepressant effects by increasing tryptophan levels by blocking the IDO enzyme. It may be proposed that statins reduce the risk of depression.

According to the results of our study, we consider that simvastatin may be preferred due to its antidepressant features in hypercholesterolemic patients with depression eliminating the need for an antidepressant drug.

Evaluation of simvastatin and anxiolytic effects.

Simvastatin, amitriptyline alone, and in combination with simvastatin enhanced the time spent on the open arms indicating an acute anxiolytic effect. Simvastatin showed an acute anxiolytic effect with 10 and 30 mg/kg doses used in this study. High doses of simvastatin had no anxiolytic effect. However, sertraline alone, and in combination with simvastatin had no anxiolytic effect, even sertraline decreased the anxiolytic effect observed with simvastatin alone. The present study was designed to investigate the effect of acute simvastatin treatment on anxiety and its association with N-methyl-D-aspartic acid (NMDA) receptors in the brain of rats.¹⁹ In one study,²⁰ it became a current issue that the effects of statins in the CNS are not associated with their hypocholesterolemic effects. The contradictory findings may result from different cholesterol metabolisms in human beings and rodents.²¹ It was reported that the statin dose independently alleviates depression and anxiety via NMDA receptors.³ In our study, we also observed that 10 and 30 mg/kg doses of simvastatin had an anxiolytic effect; however, 50 mg/kg simvastatin exposed an anxious effect. However, in several human studies contradictory results on the association between statins and anxiety and depression were acquired. The reasons for the conflicting outcomes may result from their different methodology.²² It is highly probable

that inhibition on NMDA receptor upregulation in the hippocampus mediates the anxiolytic effects of simvastatin.²³ In another study, it was also observed that a similar effect was obtained with inhibition of NMDA receptors with MK-801 in the basolateral complex of the amygdala.^{24,25} However, these effects were observed with doses of 10 and 30 mg/kg, but not 50 mg/kg simvastatin. Probably, although simvastatin has antioxidant properties, high dose (50mg/kg) simvastatin presented an oxidant manner in line with a common property of antioxidants. In rats, 10 and 30 mg/kg doses of simvastatin increased the time of staying in the open arms. This reflects an ability of simvastatin to produce a profoundly anxiolytic effect when administered at doses of 10 and 30 mg/kg in rats. These results indicate a close association between the upregulation of NMDA receptors and the anxiolytic effect due to treatment with doses of 10 and 30 mg/kg simvastatin. It was observed that simvastatin affected dopamine levels and dopamine metabolism *in vivo*.²⁶ It has been well documented that there is a close interaction between the regulation of NMDA receptors and the dopaminergic system.²⁷ It was reported that NMDA antagonists like amantadine increased striatal dopamine.²⁸

We may suggest that the action of simvastatin both on dopaminergic systems and on IDO enzymes mediates its antidepressant effect, while it has an anxiolytic effect not dependent on its effects on IDO enzymes. Ten and 30 mg/kg/day doses of simvastatin increased D1 and D2 receptor expressions in the prefrontal cortex. These results suggest that lipophilic statins can alter dopaminergic functions in the prefrontal cortex possibly via a central mechanism.²⁶

Combinations. Simvastatin showed analogous to amitriptyline but more potent antidepressant effects than sertraline. In the combined use, simvastatin potentiated the antidepressant effects of sertraline, but did not change amitriptyline's effect. We suggest that an IDO enzyme related mechanism plays a role in this potentiating effect of simvastatin. It is not surprising that the sertraline and simvastatin combination have a more potent effect in line with the selective serotonin reuptake inhibitors (SSRI) property of sertraline. It may be suggested that sertraline increases tryptophan levels. We observed that sertraline enhanced anxiety and this effect was not changed when combined with simvastatin. We also found that 10 and 30 mg/kg simvastatin doses had an anxiolytic effect similar to amitriptyline, and there was no influence on this effect with the combination of amitriptyline and simvastatin.

In conclusion, amitriptyline, a tricyclic antidepressant, made no change to the anxiolytic and antidepressant effect of simvastatin. In light of our results, and those of other studies, we suggest that simvastatin presents

its anxiolytic and antidepressant effect in a similar way to SSRIs. We suggest that the interactions between the effects of simvastatin on IDO enzymes, NMDA receptor blockade, and dopaminergic functions mediate the anxiolytic and antidepressant effects of simvastatin. In this study we did not examine IDO enzymes, NMDA receptors, and dopamine levels. These are limitations of our study, however, we are planning future studies on these issues. The results of our current study provide a contribution to the treatment of patients with depression and anxiety who also use simvastatin.

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