



The Effects of Simvastatin on Experimental Schizophrenia Models in Mice

ORIGINAL
INVESTIGATION

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ABSTRACT

Objective: Statins, or 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, which are potent inhibitors of cholesterol synthesis, are widely used in the treatment of hypercholesterolaemia and prevention of coronary artery diseases. Evidence from clinical trials also show other beneficial actions of statins unrelated to their cholesterol lowering effects. In experimental animal studies, it was reported that simvastatin, a statin agent, may alter dopaminergic and glutamatergic functions. Dopaminergic overactivity and dysfunction of glutamatergic transmission is associated with the pathogenesis of schizophrenia. This study aims to examine the chronic effects of simvastatin on experimental psychosis models in mice.

Materials and Methods: All the experiments were performed with the decision of the Local Ethics Committee for Animal Experimentation of Eskişehir Osmangazi University (26.04.2011-2017). Simvastatin was administered to male Swiss albino mice via oral gavage for 4 weeks at doses of 3, 10, and 30mg/kg. Saline was administered to control group for the same period and route. Apomorphine-induced climbing, MK-801-induced hyperlocomotion, and haloperidol-induced catalepsy were used as experimental psychosis models, and climbing time, the number of total movements, and duration of cataleptic posture were recorded respectively. Results were statistically analyzed with Kruskal–Wallis test.

Results: There was no significant difference between the groups in terms of climbing time, the number of total movements, and duration of cataleptic posture ($p>0.05$).

Conclusion: We suggest that chronic administration of simvastatin does not possess antipsychotic effect at all doses when assessed with the above-mentioned psychosis models.

Keywords: Simvastatin, experimental animal models, schizophrenia

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INTRODUCTION

Statins are a class of drugs usually used as lipid-lowering medications, and it has been shown that they decrease cardiovascular risk factors. Statins lower lipid levels by inhibiting 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase. Evidence from several studies suggests that statins also have considerable pleiotropic effects in addition to their lipid-lowering effects (1). Beyond their indication for lowering lipid levels, statins have other potential application areas in central nervous system (CNS) diseases. Recent studies point out that statins have anti-inflammatory and neuroprotective properties which may be beneficial in the treatment of multiple sclerosis, as well as other central CNS diseases such as traumatic brain injury and Alzheimer's disease (2, 3, 4). Sierra et al. (5) reported that simvastatin, compared to other statins, is more suitable for the prevention of neurodegenerative conditions because of its high capacity to penetrate the blood–brain barrier, strong cholesterol lowering effect on neurons, and protection against neural cell death.

There is an increasing number of data about the role of statins in CNS diseases; however, little is known on their direct effects on central receptors, behavioral effects, and neuropsychiatric disorders. Statins are reported to be advantageous in schizophrenia and mood disorders due to their neuroprotective effects (6). Along with the neuroprotective effects of statins, their effects on central receptors have also been investigated. It was reported that simvastatin altered dopamine (DA) D1, D2, and glutamate N-methyl-D-aspartate (NMDA) receptors, as well as the amount of dopamine in different brain regions (7, 8, 9). The dopamine hypothesis of schizophrenia proposes that overactivity of mesolimbic DA neurons cause the positive symptoms, where underactivity of mesocortical DA neurons cause the negative, cognitive, and affective symptoms of schizophrenia. On the other hand, the glutamatergic hypothesis suggests NMDA receptor hypofunctioning in schizophrenia. It has been proved that NMDA receptors interact with dopamine systems (10) such that dopaminergic systems are modified by NMDA receptors (11).

Furthermore, the data suggest that anti-inflammatory treatment may be beneficial in the treatment of schizophrenia, and statins are candidates as adjunctive therapies to improve the symptoms of schizophrenia in preliminary studies due to their anti-inflammatory properties, and thus are similar to the Cox-2 inhibitors, which have shown some ability as adjuncts to improve the symptoms of schizophrenia in preliminary studies (12).

Taken together, in this study, we aimed to investigate the effects of chronic simvastatin administration on experimental schizophrenia models in mice in order to determine an antipsychotic-like activity of simvastatin. For this purpose, apomorphine-induced climbing, haloperidol-induced catalepsy, and MK-801-induced hyperlocomotion tests were used. Apomorphine is a nonspecific agonist of both dopamine D1 and D2 receptors, and it generates climbing behavior in rodents (13). MK-801 is a non-competitive antagonist of glutamate NMDA receptors, and it induces behavioral hyperactivity in rodents (14). Apomorphine-induced climbing and MK-801-induced hyperactivity tests are considered to reflect the positive symptoms of schizophrenia. Haloperidol-induced catalepsy test is used to screen the potential extrapyramidal side effects of antipsychotic drugs (15).

MATERIALS and METHODS

Animals

Male Swiss albino mice (25gr–30gr) were used for the study. Animals were sheltered in standard laboratory conditions of 12h-light/dark cycle and temperature of $22\pm 2^{\circ}\text{C}$. Experiments were carried out with the permission of the Local Ethical Committee for Animal Experimentation in Eskisehir Osmangazi University (Decision date and number: 26.04.2011-207).

Drugs

Simvastatin (Nobel Pharmaceutical Company; İstanbul, Turkey), (+)-MK-801 hydrogen maleate (Santa Cruz Biotechnology; Heidelberg, Germany), and haloperidol (Sigma; Taufkirchen, Germany) were dissolved in saline. R(-) apomorphine hydrochloride hemihydrate (Sigma Taufkirchen, Germany) was dissolved in saline containing 0.1% ascorbic acid. Simvastatin was administered via oral gavage, (+)-MK-801 hydrogen maleate and haloperidol were administered intraperitoneally (i.p.), and R(-) apomorphine hydrochloride hemihydrate was administered subcutaneously (s.c.).

Experimental Design

A hundred and twenty mice were divided into 5 main groups as follows: vehicle, control, simvastatin 3, 10, and 30mg/kg. Each main group was also divided into 3 subgroups in order to subject the animals to MK-801 induced hyperlocomotion, apomorphine-induced climbing, and haloperidol-induced catalepsy tests separately. There were 8 mice in each subgroup. Vehicle groups received saline only, while control groups received saline with one of the test-inductive agents (MK-801, apomorphine hydrochloride hemihydrate, or haloperidol).

Experimental Protocol

Simvastatin was administered to mice via oral gavage for 4 weeks at doses of 3mg/kg, 10mg/kg, and 30mg/kg. Saline was also administered to mice in vehicle or control groups via oral gavage for 4 weeks. Animals were assessed in experimental schizophre-

nia models on 30th day of chronic drug or saline treatment. An hour after the last administrations of drug or saline administration, MK-801, apomorphine hydrochloride hemihydrate, or haloperidol were injected to mice, and then the animals were subjected to appropriate schizophrenia model. In vehicle groups, 1 hour after the last administrations of saline, the mice received saline again and were then subjected to experimental schizophrenia models without injections of MK-801, apomorphine hydrochloride hemihydrate, or haloperidol.

Experimental Schizophrenia Models

Three different tests were applied as schizophrenia models in mice as described below:

1. MK-801-induced hyperlocomotion test: Locomotion was recorded using activitymeter device (MAY-AMS 02 Animal Activity Monitoring System, Commat Ltd. Ankara/Türkiye). Mice were placed in the apparatus for 60 minutes for habituation. Thereafter, mice were removed from the apparatus and treated with either saline or test compounds and then quickly returned to the chamber. One hour after, saline or 0.3mg/kg MK 801 was injected i.p. Immediately, mice were placed individually on the device, and the number of total movements were recorded automatically for 1 hour (16-18).

2. Apomorphine-induced climbing test: One hour after the last administration of simvastatin or saline to mice, 1.5mg/kg apomorphine was injected s.c. to mice, and mice were immediately placed in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm). The total climbing time on the inside of the cage was recorded for 30 min (19).

3. Haloperidol-induced catalepsy test: Thirty minutes after the last administrations of simvastatin or saline to mice, 0.5mg/kg haloperidol was injected i.p. to mice, and 30 minutes after haloperidol injection mice were observed in bar test. Mice were positioned with both front limbs on a 5cm high bar (20). The time that mice kept this position was recorded for a maximum period of 30 seconds. Data were the mean of three measures separated by 1-minute intervals (21).

Statistical Analysis

Statistical analyses were performed using Statistical Packages for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to examine normal distribution ($X^2=0.14$, $p=0.090$). Numeric variables were compared using Kruskal-Wallis test. $p<0.05$ was considered to be statistically significant.

RESULTS

Results of Apomorphine-Induced Climbing Test

The total climbing time was recorded for 30 min. We did not observe any significant difference between control and simvastatin groups ($p>0.05$). All three doses used in this study did not alter the total climbing time induced by apomorphine as shown in Table 1.

Results of Haloperidol-Induced Catalepsy Test

Three records separated by 1-minute intervals were obtained for haloperidol-induced cataleptic posture. The data were given as the

Table 1. The effect of simvastatin on climbing time in apomorphine-induced climbing test

Groups	Mean±Std Dev	Median (25%-75%)	p
Control	1281.25±460.94	1126.50 (960.00–1615.00)	0,628
Sim 3 mg/kg	1132.67±425.56	1030.50 (888.00–1546.00)	
Sim 10 mg/kg	895.71±696.01	873.00 (230.00–1537.00)	
Sim 30 mg/kg	1015.43±529.51	1019.00 (688.00–1435.75)	

The results are given as both mean±Std Dev and median (25%–75% percentiles) Sim: Simvastatin

Table 2. The effect of simvastatin on catalepsy time in haloperidol-induced catalepsy test

Groups	Mean±Std Dev	Median (25%-75%)	p
Control	23.25±7.38	25.00 (15.50–30.00)	0.800
Sim 3 mg/kg	22.60±8.59	25.00 (16.00–30.00)	
Sim 10 mg/kg	20.67±7.31	17.00 (15.00–30.00)	
Sim 30 mg/kg	24.63±9.96	30.00 (19.50–30.00)	

The results are given as both mean±Std Dev and median (25%–75% percentiles) Sim: Simvastatin

Table 3. The effect of simvastatin on the number of total movements in MK-801-induced hyperlocomotion test

Groups	Mean±Std Dev	Median (25%-75%)	p
Control	2553.17±2100.60	2252.50 (640.00–4704.00)	0,235
Sim 3 mg/kg	4192.50±1257.31	4249.50 (3670.00–4614.00)	
Sim 10 mg/kg	5062.13±1856.49	5163.00 (3154.50–6988.50)	
Sim 30 mg/kg	4265.50±2011.25	4231.00 (2452.00–6207.50)	

The results are given as both mean±Std Dev and median (25%–75% percentiles) Sim: Simvastatin

mean of these three records. The cut-off time was 30 seconds. Simvastatin at all doses used in this study made no significant change in the catalepsy time induced by haloperidol compared to control ($p>0.05$). There was also no significant difference between simvastatin dose groups ($p>0.05$). The results are represented in Table 2.

Results of MK-801-Induced Hyperlocomotion Test

In this test, the total number of movements were recorded for 1 hour. There was no significant difference between groups in terms of total number of movements ($p>0.05$). Simvastatin at all doses did not decrease or increase the number of total movements compared to control. The results are shown in Table 3.

DISCUSSION

In the present study, we examined the potential antipsychotic activity of simvastatin on experimental psychosis models in mice. MK-801-induced hyperlocomotion, apomorphine-induced climbing, and haloperidol-induced catalepsy tests were used as psychosis models. We observed that simvastatin did not decrease the total climbing time in apomorphine-induced climbing test, as well as it did not reduce the total number of movements in MK-801-induced hyperlocomotion test at all doses (3, 10, and 30 mg/kg), representing no antipsychotic activity on these models. We also observed that all doses of simvastatin exhibit no cataleptogenic effect in haloperidol-induced catalepsy test.

Along with the neuroprotective effects of statins, their effects on central receptors have also been investigated. It was reported that simvastatin increased dopamine (DA) D1 and D2 receptors in the prefrontal cortex and changed the amount of dopamine in different brain regions (7, 8). Simvastatin was also shown to upregulate NMDA receptor binding, and to possess hyperlocomotive and anxiolytic-like activities (9).

Recently, the effects of statins other than lipid-lowering are new areas of interest. There are considerable number of studies focusing on anti-inflammatory and neuroprotective effects of statins and their use in CNS diseases. The influence of statins on the receptors and neurotransmitters in CNS has also been investigated. Wang and Zengin (9) reported that simvastatin up-regulated the binding of MK-801 to NMDA receptors. These researchers also observed a hyperlocomotive activity with simvastatin and associated this hyperlocomotive activity with the up-regulation of NMDA receptors by simvastatin. They concluded that simvastatin exhibited NMDA antagonist-like effects. NMDA receptors are the member of ionotropic glutamate receptor family and are involved in many brain functions. The hypofunction of these receptors were associated with schizophrenia (22). Additionally, it was shown that NMDA receptor antagonists induced hyperlocomotor activity (23) and represented symptoms of schizophrenia (24). MK 801 is a non-competitive NMDA receptor antagonist (25). MK-801-induced hyperactivity is considered to predict the positive symptoms of schizophrenia (17), and it serves as a model of glutamate hypo-

function (18). On the contrary, enhancers of NMDA receptor function improved the symptoms of schizophrenia (26). However, other investigators studied the effects of atorvastatin, another member of statins, on open field test and found that atorvastatin made no alteration in locomotor activity in the open field test (27). Although we used MK-801-induced hyperlocomotion to evaluate the locomotor activity, this result is consistent with the results of our study in which we observed that neither dose of simvastatin (3, 10, and 30 mg/kg) significantly altered the number of total movements compared to control.

Apomorphine-induced climbing behavior appears to be associated with the activation of striatal dopaminergic receptors and is a reliable test for screening potential antipsychotic agents (28). In this study, we examined the effect of chronic administration of simvastatin for 4 weeks on apomorphine-induced climbing test in mice and found that simvastatin did not alter the total climbing time. Wang et al. reported that simvastatin (10 and 30 mg/kg) increased dopamine D1 and D2 receptors in the prefrontal cortex and suggested that statins can change dopaminergic functions in the prefrontal cortex (7). In another study, Wang et al. (8) found that simvastatin increased dopamine levels in the striatum but decreased in the prefrontal cortex.

Study Limitations

In this study, we couldn't detect the levels of brain dopamine and glutamate levels due to financial limitations.

CONCLUSION

We suggest that simvastatin do not show antipsychotic effect at all doses when used in chronic administration in experimental psychosis models in rats. The antipsychotic effects of simvastatin should be assessed with different models of schizophrenia, and brain levels of neurotransmitters associated with the schizophrenia pathogenesis should be determined.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Animal Experimentation of Eskişehir Osmangazi University.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: EY., BK. Performed the experiments or case: EY., BK. Analyzed the data: EY., BK. Wrote the paper: EY., BK. All authors have read and approved the final manuscript.

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