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Research Article

EFFECT OF DIFFERENT STATINS IN ANIMAL MODEL OF ANXIETY IN WISTAR RATS

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ABSTRACT

Objective: Pleiotropic mechanisms of statins have been explored in treating neurological disorders such as generalized anxiety disorder and depression. Hence, the aim of the present study is to evaluate the effect of different statins in animal model of anxiety in Wistar rats.

Methods: Sixty rats were divided into five groups of 6 rats each for each model. Two models were used, elevated plus maze and open-field model. Grouping is as follows: Group 1 - normal control (0.9% saline), Group 2 - alprazolam 0.5 mg/kg, Group 3 - atorvastatin 10 mg/kg, Group 4 - rosuvastatin 10 mg/kg, and Group 5 - pitavastatin 10 mg/kg. All drugs were given orally for 30 days.

Results: In open-field model and in elevated plus maze, alprazolam, atorvastatin, rosuvastatin, and pitavastatin in comparison with control showed significant antianxiety effect ($p<0.01$ and $p<0.001$), respectively.

Conclusion: Hence, further clinical trials can be done to see the effect of various statins on generalized anxiety disorder in comparison with standard antianxiety drugs.

Keywords: Alprazolam, Anxiety, Latency period, Line crossing, Pitavastatin, Rosuvastatin.

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INTRODUCTION

Anxiety is defined as a psychological and physiological state which is characterized by cognitive, somatic, emotional, and behavioral components [1]. These components create an unpleasant feeling and are typically associated with feelings of uneasiness, apprehension, fear, or worry. At times, it occurs without identifiable triggering stimuli. Anxiety treatment includes antidepressant medication and cognitive and interpersonal psychotherapies as per the National Treatment Guidelines for major depression [2,3].

Statins and hydroxymethylglutaryl-CoA reductase inhibitors are prescribed to lower blood cholesterol levels and are mainly used in the treatment of hyperlipidemia [4]. Statins reduce the risk of ischemic heart disease and cerebrovascular stroke. In addition, statins play an important role in many central nervous system (CNS) disorders as well and have major applications in multiple sclerosis, traumatic brain injury, and Alzheimer's disease (AD) [5]. In generalized anxiety disorders, there is an increased level of serum cholesterol when compared to others without anxiety. Statins modulate serotonin transporter (SERT) functions by altering serum cholesterol levels. Statins also upregulate the N-methyl-D-aspartate receptor (NMDA) and improve the mood behavior [6]. With the background of the potential role of statins in CNS disorders, the present study was aimed to exaluate the anxiolytic activity of statins and to compare the effects of different statins in anxiety in Wistar albino rats.

METHODS

The study was commenced after approval by the Institutional Animal Ethics Committee (IAEC/KMC/13/2016).

Animals

Male/female albino rats of Wistar stain weighing 180–250 g and 10–12 weeks were used for the study. The animals were housed under standard condition, 12:12 light-dark cycle, 50% humidity, and 28°C temperature and provided with standard food granules and water *ad libitum*.

Chemicals/drugs

Atorvastatin, rosuvastatin, pitavastatin, and alprazolam were used.

Experimental design

Sixty rats were divided into five groups of 6 rats each for each model. Two models were used, elevated plus maze and open-field model. Grouping is as follows:

1. Group 1 - Control rats received normal saline for 30 days
2. Group 2 - Alprazolam 0.5 mg/kg for 30 days
3. Group 3 - Atorvastatin 10 mg/kg for 30 days
4. Group 4 - Rosuvastatin 10 mg/kg for 30 days
5. Group 5 - Pitavastatin 10 mg/kg for 30 days.

Each drug solution was prepared freshly just before the administration. All drugs were administered orally. Dosage strength of all drugs was based on literature evidence [7-10]. Drugs were administered once daily for 30 days, and the last dose was given on the 30th day, before experiment.

Laboratory investigation

Thirty animals were tested initially in plus maze. After the last dose on the 30th day of drug or vehicle administration, each animal was placed in the center square of the plus maze, facing one of the closed arms. The number of entries into closed and open arms, the time spent in open and closed arms, and latency in each arm in a 5-min period were noted. Similarly, 30 animals were tested in open-field apparatus. The number of entries into center, line crossings, time in center, and latency was observed in a session of 2 min. Each time, a new experiment was started, and the entire area was cleaned with spirit to mask the odor left by the animal in previous experiment.

Statistical analysis

The data were analyzed using one-way analysis of variance followed by Tukey's *post hoc* test. $p<0.05$ was taken as statistically significant.

RESULTS

Open field

Alprazolam, atorvastatin, rosuvastatin, and pitavastatin in comparison with control showed a significant increase ($p<0.01$) with respect to latency, center entry, line crossings, and time in center. Rosuvastatin-treated group showed a significant increase in latency when comapred to standard and atorvastatin ($p<0.001$). There was no significant difference ($p>0.05$) between pitavastatin and Groups 3 and 4 with respect to latency. Atorvastatin- and rosuvastatin-treated groups showed a significant increase in numebr of center entries as compared to standard ($p<0.05$). However, there was no significant difference ($p>0.05$) between the three test groups atorvastatin, rosuvastatin, and pitavastatin with respect to center entry, line crossings, and time spent in center (Table 1).

Elevated plus maze

Alprazolam, atorvastatin, rosuvastatin, and pitavastatin in comparison to control group showed a significant increase ($p<0.001$) in latency, time spent in open arm, and entries to open arm. Rosuvastatin showed a significant increase in entries to open arm when compared to group atorvastatin ($p=0.01$). There was a significant increase ($p<0.001$) in time spent in open arms in Groups 3 and 4 as compared to Groups 2 and 5 (Table 2).

Alprazolam, atorvastatin, rosuvastatin, and pitavastatin in comparison to control group showed a significant decrease ($p<0.001$) in entry and time spent in closed arm. Group 2 showed a significant increase ($p<0.001$) in time spent in closed arm when compared with Groups 3–5. Group 5 pitavastatin showed a significant increase ($p<0.001$) in time spent in closed arm as compared to atorvastatin and rosuvastatin where the time spent was less (Table 2).

DISCUSSION

Neuroprotective effects of statins have been reported in several clinical and animal studies. In neuropathologic conditions like stroke, traumatic brain Injury, brain ischemia, excitotoxicity, AD, and seizure-induced neuronal death in rat's statins have exhibited a protective effect [11]. The elevated plus maze is a widely used behavioral assay for anxiety behavior of rodents. It is easy to use, and it is fully automated. The results can be obtained in a short, 5-min testing period. It is said to have face validity, construct validity as well as prediction validity. The patterns of results obtained using this task are replicable across other species, anxiety/affective behavior measures, studies, and laboratories [12].

In our study, in elevated plus maze model, alprazolam, atorvastatin, rosuvastatin, and pitavastatin in comparison to control group showed a significant increase in latency, time spent in open arm, and entries to open arm. Rosuvastatin showed better results when compared with atorvastatin with respect to open arm entry, and both rosuvastatin and atorvastatin showed an increase in time spent in open arm as compared to pitavastatin and standard drug alprazolam.

Open-field model is used comprehensively to assess both locomotor and behavioral activity in rats. It is an easy to perform, non-invasive, and requires no animal handling during the test procedure without special training. One more advantage is multiple animals that can be tested at one time. It is shown to give clinical relevant outcome measures when used as model for anxiety [13].

In our current study, in open field, all drugs showed a significant increase of all parameters when compared with control, and both rosuvastatin and atorvastatin showed an increased number of entries to center in comparison with standard with rosuvastatin showing a significant increase in latency period when compared with atorvastatin. These results strongly indicate that statin treatment results in improved coping with aversive situations, thus leading to a reduced anxiety level. Standard anxiolytic drug alprazolam and statins in both the experimental models showed decreased fear, an increase in exploratory behavior, and the behavioral disinhibitory effect.

The benzodiazepines (BZDs) are relatively safe and are widely used anxiolytic agents. These agents are known to act through the BZD-gamma amino butyric acid (GABA) receptors; the role of GABA in anxiety is well established [14]. The behavioral changes by test drugs, atorvastatin, rosuvastatin, and pitavastatin were better to that produced by alprazolam and are suggestive of anxiolytic effect of the statins. As per literature evidence, serum levels of cholesterol were increased in generalized anxiety disorders [15]. Reports have linked statin modulate SERT functions by altering serum cholesterol levels. Selective serotonin reuptake inhibitors act by inhibiting SERT and increasing the concentration of neurotransmitter serotonin at intraneuronal space in the brain. Statins are also found to act through the same mechanism. It was also shown that statins upregulate the NMDA receptors and improve mood behavior in the brain [16]. Our results obtained were similar to a study which proved the anxiolytic activity of atorvastatin and simvastatin to be comparable to alprazolam [6]. However, several human studies have shown contrary results demonstrating that there was no association between statins and anxiety and depression [16,17].

Table 1: Results of open-field test

Groups	Latency (s) (mean±SEM)	Number of center entries (Mean±SEM)	Number of line crossings (Mean±SEM)	Time in center (s) (Mean±SEM)
I Control	1.33±0.210	1.0±0.365	24.6±0.881	1.5±0.619
II Alprazolam	3.5±0.428*	2.66±0.33*	37.66±1.54*	10.33±0.421*
III Atorvastatin	4.16±0.307*	4.16±0.307*#	36.33±0.881*	9.5±0.428*
IV Rosuvastatin	5.833±0.401*$^	4.0±0.365*#	37.16±1.66*	10.5±0.428*
V Pitavastatin	4.5±0.428*	3.6±0.21*	35.16±1.01*	9.5±0.619*

*p<0.01 as compared to control, $p=0.001 as compared to standard, ^p<0.05 as compared to atorvastatin, #p<0.05 as compared to standard. SEM: Standard error of the mean

Table 2: Results for elevated plus maze test

Groups	Latency (s)	Number of entries to open arm	Number of entries to closed arm	Time in open arm (s)	Time in closed arm (s)
1 Control	1.5±0.223	1.33±0.49	10.83±1.536	9.16±3.27	265±5.7
II Alprazolam	2.5±0.547*	4.66±0.33*	5.6±0.49**	83.16±6.76*	194±6.11**@
III Atorvastatin	1.83±0.30*	4.0±0.516*	5.66±0.49**	177.1±6.4*#	80.5±4.2**
IV Rosuvastatin	2.66±0.33*	6.0±0.258*$	3.33±0.42**	164.3±10.1*#	88.3±8.6**
V Pitavastatin	2.33±0.210*	5.0±0.258*	3.83±0.307**	109±8.73*	138.3±10.54**&

*p<0.001 as compared to control, $p=0.01 as compared to atorvastatin, #p<0.001 as compared to standard and pitavastatin, **p<0.001 as compared to control, @p<0.001 as compared to Groups 3-5, &p<0.001 as compared to Groups 3 and 4

The contrary outcomes may mainly result from their different doses, duration, and follow-up. In chronic statin treatment, antianxiety effects were seen in open-field model in the elevated plus maze model in earlier studies as well [18]. Authors have reported that coadministration of simvastatin with fluoxetine induced a more pronounced anxiolytic activity than treatment with drugs alone in elevated plus-maze and open-field tests pointing toward the anxiolytic effect of simvastatin [19]. In Fast swim test, simvastatin exerts an antidepressant-like effect on diabetic rats and this effect appears to be mediated, by changes in the blood levels of corticosterone and serotonin concentration in the hippocampus [20]. Atorvastatin administration reduced immobilization time, both in the tail suspension test and in forced swim test [21]. Atorvastatin, simvastatin, and pravastatin also reduced anxiety in the open-field test on long-term treatment. These changes were not related to plasma cholesterol levels, which remained unchanged during drug treatment [8]. Some of the clinical studies also point toward the fact that statins can produce antianxiety and antidepressant effect when administered to patients with cardiovascular risk factor including hyperlipidemia. In clinical studies, long-term use of statins in individuals who had a cardiac event was associated with a significant reduction in the risk of depression and anxiety [22]. A recent study in patients with head-and-neck cancer with hyperlipidemia showed that statin use could decrease the risk of anxiety/depression, more so in patients older than 65 years and female patients [23]. Statins, by their pleiotropic effect, shows a promising effect in anxiety. 4 weeks of statin therapy can produce antianxiety effects comparable to alprazolam.

CONCLUSION

The present study suggests that statins have anxiolytic-like effect like that of alprazolam and have a potential to be used as add-on drugs in patients with anxiety. Furthermore, among the different statins, rosuvastatin showed a superior antianxiety effect in different models. Further clinical studies are required to confirm the role of statins as adjuvant antianxiety drugs.

AUTHORS' CONTRIBUTIONS

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CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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