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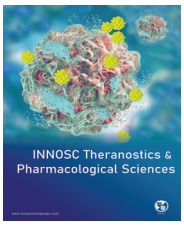

RESEARCH ARTICLE

Long-term Administration of Lovastatin and Rivastigmine: An *In Vivo* Evaluation on Cognitive Functions and Brain Acetylcholinesterase Activity

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Abstract:

Background. There is not much evidence illustrating that statins could be responsible for memory loss or dementia, although increased exposure to statins has been reported to cause cognitive side effects. The present study investigated the effect of lovastatin in combination with rivastigmine on cognitive function as well as brain acetylcholinesterase (AChE) activity in normal mice.

Methods. The mice were categorized into four groups, and they were treated with normal saline, lovastatin, rivastigmine, and the combination of lovastatin and rivastigmine, respectively, by oral administration for 60 days. The treatment effect on cognitive functions was assessed by behavioral tests, namely, the passive avoidance test and spontaneous alternation test, as well as the measurement of brain AChE activity by Ellman's method.

Results. In this study, a significant reduction ($P < 0.01$) of brain AChE activity and positive effects ($P < 0.01$) on cognitive functions was observed in mice treated with the combination of lovastatin and rivastigmine as compared to rivastigmine alone. However, no significant differences ($P < 0.05$) were observed on brain AChE activity as well as cognitive functions in mice treated with lovastatin when compared with those treated with normal saline.

Conclusion. This study suggested that lovastatin did not contribute to any improvements in cognitive functions and brain AChE activity, but it potentiated the effect of rivastigmine.

Keywords: Lovastatin, Rivastigmine, Cognition, Behavioral, Brain acetylcholinesterase activity

1 Introduction

Cognition is an intellectual procedure of gaining information and understanding through attention, skill, and the senses [1]. Cognitive function impairments are associated with loss of interest in learning, declined thinking skills, loss of analysis and interpretation skills, difficulty in detection, dilemma in explaining, and loss of memory [1]. While being affected by aging mostly, cognitive function impairments are the result of declines in the gray and white matter volume in the brain, the decrease in the neurotransmitter levels and changes in white matter [2].

Belonging to the statins family, lovastatin is a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor that is well known for its cholesterol-lowering effect in hypercholesterolemia and widely prescribed for the prevention of coronary heart disease. Apart from that, lovastatin has other health benefits such as improving endothelial functions through the stimulation of nitric oxide release [3] and ameliorating heart diseases through the regulation of antioxidant activities [4,5].

Furthermore, lovastatin exerts anti-inflammatory responses through the downregulation of pro-inflammatory markers such as tumor necrosis factor-α [6-9]. Moreover, statins were also reported to exert immunosuppressant activities [10]. Nonetheless, whether lovastatin could be used for treating cognitive impairments is still debatable.

Lovastatin could alter the cognitive function as it may lead to cognitive impairment and affect memory [11-13]. In addition, lovastatin-treated adults also manifested a small decline in cognitive performance on neuropsychological tests [14]. Reportedly, treatment with lovastatin showed no positive outcome on cognitive functions [14], and it did not improve the flight-related performance on military aircrew [15]. However, an *in vivo* study illustrated that lovastatin improved the spatial learning and attention capabilities in mice [16]. Therefore, the effect of lovastatin in cognitive functions is not yet fully understood and further investigations are needed.

Rivastigmine is a pseudo-irreversible and intermediate-acting acetylcholinesterase (AChE) inhibitor. It normally works by lengthening the neurotransmitter acetylcholine action at synaptic cleft and binding to the active site of acetylcholine, thereby enhancing the cognitive function [17]. Rivastigmine improved memory functions in patients who experienced traumatic brain injury [18]. Furthermore, Alzheimer's disease (AD) patients had enhanced cognitive participation in activities of daily living after the administration of rivastigmine [19]. This line of evidence suggests that rivastigmine is a potential candidate in improving the cognitive function of patients with cognitive impairment. The diminution of brain acetylcholine level is believed to be responsible for the pathogenesis of AD [20]. AChE is a type of enzyme that degrades acetylcholine, and its inhibition by rivastigmine enhances the levels of acetylcholine in the brain, which could be responsible for the improvement in cognitive functions [21].

Herein, this study aims to investigate the combined effects of lovastatin and rivastigmine on cognitive function as well as brain AChE activity *in vivo* through the evaluation of behavioral tests and brain AChE activities. With this, we can uncover whether the treatment of lovastatin and rivastigmine can provide cognitive benefits to patients who are suffering from cognitive impairment.

2 Materials and methods

2.1 Chemicals and reagents

The bovine serum albumin and Folin–Ciocalteu's phenol reagent were purchased from Merck, India. The glucose assay kits were obtained from Span Diagnostics, India. The acetylthiocholine iodide was purchased from Across Organic, USA, and DTNB or 5,5'-dithiobis-(2-nitrobenzoic acid) was procured from HiMedia Laboratories, India.

2.2 Animals

The 12-week-old Swiss albino mice weighing 25 – 35 g were procured from the Central Animal House Facility of Hamdard University, New Delhi. The animals were housed in the air-conditioned room, given a normal diet (Sai Feeds, Bengaluru, India) and water *ad libitum*. The animal experiment was approved by the registered Institutional Animal Ethics Committee, Jamia Hamdard, New Delhi (173/CPCSEA).

The mice were randomly categorized into four treatment groups: (i) Control group which was given 10 mL/kg of normal saline through oral administration, (ii) lovastatin group which was given 5 mg/kg of lovastatin through oral administration, (iii) rivastigmine group which was given 1.5 mg/kg of rivastigmine through oral administration, and (iv) lovastatin + rivastigmine group which was administered with a combination of 5 mg/kg of lovastatin and 1.5 mg/kg of rivastigmine through oral route. Each group consisted of eight animals.

2.3 Behavioral tests

2.3.1 Passive avoidance test

Passive avoidance behavior of rodents is defined as the suppression of the inherent predilection for the shady partition of the experimental machinery (or stepping down from a prominent platform) following disclosure to an inescapable shock. The passive avoidance test is carried out to assess the short-term memory [1], or to evaluate memory retention deficit.

A passive avoidance test was carried out in accordance with the descriptions from the past

literature coupled with some modifications [22-24]. The shuttle box, also known as passive avoidance device, is used for assessing both memory acquisition and retention in mice. The shuttle box which is divided by a middle wall has a light chamber and a dark chamber which is of equal sizes. The middle wall has a trapdoor to allow passage of the mice between the two chambers. The floor of the shuttle box is composed of a metal grid connecting to a shock scrambler.

In the memory acquisition experiment, the drug was administered continuously to the mice and they were kept in the light chamber on day 60. The acquisition transfer latency, defined as the time taken for the mice to enter the dark chamber with all its four legs from the light chamber on the opening of the trapdoor, was recorded. Instantaneously after the mice entered the dark chamber, the trapdoor was shut and an electric current (0.8 mA) was delivered on the grid floor for 3 s. Afterward, the mice were relocated from the dark chamber to their residence cage.

Twenty-four hours after the memory acquisition experiment, the memory retention experiment was performed in the same manner without electric shock. The retention transfer latency, which was defined as the time taken for the mice to enter the dark chamber with all its four legs from the light chamber after the electric shock, was recorded [25]. If the mice did not enter the dark chamber within the cutoff period of 180 s, it was assumed that the mice remembered the previous electric shock experience [22,26].

2.3.2 Spontaneous alternation test

Spontaneous alternation is a quantification of spatial working memory which is commonly assessed using a maze. The spontaneous alternation test is used to quantify cognitive deficits in the mice and evaluate the effects of treatments on cognition. This test investigates the participation of mice in all arms of the maze. Driven by innate curiosity, the mice would remember the previously explored arm and show the tendency to explore the formerly unvisited regions [27].

The experiment was conducted on a plus maze. The apparatus was made of plywood and painted blue. Having four arms (23.5 × 8 × 10 cm), each arm extends from the middle platform (8 × 8 cm) at the height of 38.5 cm above the floor. The arms were labeled A, B, C, and D. The mice were placed in the center and allowed to travel in the maze freely for 6 min. The order of the arms the mice entered was recorded. A spontaneous alternation occurs when a mouse enters a different arm of the maze in each of four consecutive arm entries. An order of arm entries involving B-C-A-C was not recognized as an alternation. Using this method, possible alternation sequences are equal to the number of arm entries minus four [28].

The percent alternation was calculated using the equation as follows:

$$\%\ \text{Alternation} = \frac{\text{Actual number of spontaneous alternations}}{\text{Number of arm entries} - 4} \times 100$$

2.4 Estimation of brain AChE activity

The mice were euthanized by immediate decapitation 2 h after the drug administration on day 61. The entire brain was quickly removed and kept on ice before the estimation of AChE activity. The homogenate of brain tissue was made in 0.32 M sucrose solution. The homogenate was centrifuged at 3000 rpm for 15 min followed by another centrifugation at 10,000 rpm for 10 min at 4°C. Then, 1 mL of the supernatant was mixed with 9 mL of sucrose solution to obtain a 1% post-mitochondrial supernatant (PMS). AChE activity was calculated using 1% PMS by Ellman's method [29].

2.5 Statistical analysis

The data were expressed as mean ± standard error means. Statistical significance of the difference between the treatment group was determined using one-way ANOVA followed by Dunnett's *t*-test. The difference with $P < 0.05$ is considered statistically significant.

3 Results

3.1 Effect of lovastatin, rivastigmine, and their combination on memory acquisition and retention

Based on **Table 1**, the acquisition transfer latency and retention transfer latency of mice treated with rivastigmine as well as a combination of lovastatin and rivastigmine were significantly increased

as compared to the mice in the control group ($P < 0.01$). Nonetheless, there was no significant difference observed in the acquisition and retention transfer latencies of mice treated with lovastatin alone when compared to control. Interestingly, the acquisition and retention transfer latencies of mice treated with a combination of lovastatin and rivastigmine were significantly higher than the mice treated with rivastigmine alone ($P < 0.01$).

3.2 Effect of lovastatin, rivastigmine, and their combination on spontaneous alternation behavior

Based on **Table 2**, the percentage alternation on days 10 and 60 was significantly improved in mice of rivastigmine group and lovastatin + rivastigmine group as compared to those in the control group ($P < 0.01$). However, there was no significant difference in the percentage alternation in mice treated with lovastatin alone when compared to the control. Strikingly, the percentage alternation on days 10 and 60 in mice treated with lovastatin and rivastigmine was significantly increased in comparison to those treated with rivastigmine alone ($P < 0.05$).

Table 1. Effect of lovastatin, rivastigmine, and their combination on memory acquisition and retention in mice

Group	Transfer latency (s)	
	Acquisition	Retention
Control	25.13±8.82	119.15±10.43
Lovastatin	25.50±7.33	121.29±10.12
Rivastigmine	29.25±6.20*	164.43±9.34*
Lovastatin+ Rivastigmine	36.13±7.20*#	175.43±8.41*#

The data are expressed as mean ± SEM. *P<0.01 compared to the control group; #P<0.05 compared to rivastigmine group. SEM: standard error mean

Table 2. Effect of lovastatin, rivastigmine, and their combination of spontaneous alternation behavior in mice

Group	% Alternations	
	Day 10	Day 60
Control	36.13±7.81	61.25±9.32
Lovastatin	37.50±9.31	59.13±10.32
Rivastigmine	46.14±6.23*	70.63±7.90*
Lovastatin+ Rivastigmine	54.40±7.23*#	76.38±6.31*#

The data are expressed as mean ± SEM. *P<0.01 compared to the control group; #P<0.05 compared to rivastigmine group. SEM: standard error mean

3.3 Effect on brain AChE activity in mice

The brain AChE activity was determined using Ellman's method, which colorimetrically measured the yellow color produced by thiocholine when it reacts with dithiobisnitrobenzoate ion. Thus, a higher yellow intensity indicates a higher brain AChE activity [29]. Based on **Figure 1**, the mice of the rivastigmine group and lovastatin + rivastigmine group had significantly lower brain AChE activities as compared to the control group ($P < 0.01$). In contrast, there was no significant difference in the brain AChE activity between the mice treated with lovastatin and normal saline. In addition, mice treated with a combination of lovastatin and rivastigmine exhibited a significant reduction in the brain AChE activity as compared to mice treated with rivastigmine alone ($P < 0.01$).

4 Discussion

Rivastigmine is used to improve cognition, behavioral symptoms, daily living, and global functioning in mild-to-moderate AD [30]. In the present study, rivastigmine treatment contributed to a significant reduction in brain AChE activity and enhanced cognitive functions in mice. As expected,

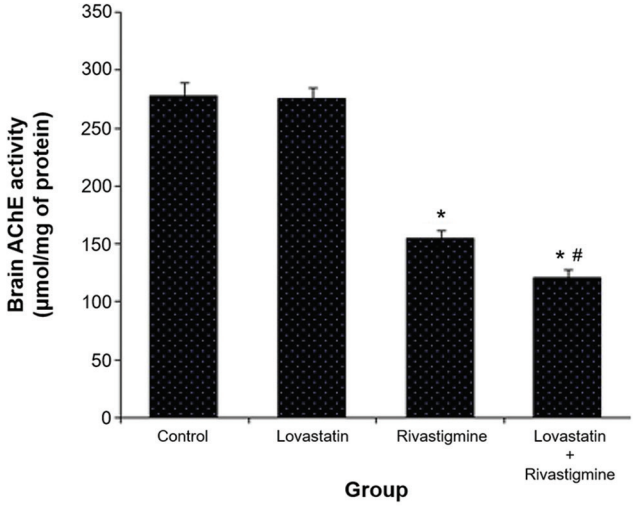

Figure 1. Effect of lovastatin, rivastigmine, and their combination on brain acetylcholinesterase activity in mice. The data are expressed as mean ± standard error mean. *$P < 0.01$ as compared to control group; #$P < 0.05$ compared to rivastigmine group.

rivastigmine treatment rendered a longer time taken for the mice to enter the darkroom. Interestingly, the combination treatment of lovastatin and rivastigmine further lengthened the retention transfer latency as the mice took a longer time to enter the darkroom. This indicates that coupled with the additional drug effect of lovastatin, the rivastigmine-treated mice were better at acquiring the memory of having experienced electric shock when entering the darkroom. In the spontaneous alternation test, the mice which were treated with the combination of both lovastatin and rivastigmine had higher percent alternations as compared with other treatment groups. This shows that the combined treatment with lovastatin and rivastigmine can improve the spatial working memory.

AChE is a type of enzyme that is responsible for the hydrolyzation of acetylcholine into acetate and choline. This reaction terminates the impulse transmission at the cholinergic synapse [31]. An excessive increase in AChE activities which result in a deficit of acetylcholine is implicated in the progression of cognitive impairments which leads to AD [32]. The administration of rivastigmine could decrease brain AChE activities in mice [33]. Accordingly, the inhibition of AChE restores the level of acetylcholine at the synapse [34,35]. In the current study, we found that combined administration of lovastatin and rivastigmine significantly inhibits the AChE activities in mice, suggesting the continuous acetylcholine transmission at the synapse which contributes to the improvement of cognitive functions.

Lovastatin is a statin medication that is used to lower blood cholesterol levels [36]. It is still controversial whether lovastatin could lead to cognitive decline or otherwise improve the condition [37]. Nevertheless, it is important to note that lovastatin did not result in any improvements in cognitive function, as reported in a number of studies [14,15]. In addition, a previous study reported that the lovastatin did not contribute to psychological distress and considerably alter the cognitive function [14]. Another negative impact of lovastatin is a cognitive impairment which particularly affecting memory [11,12]. On another note, lovastatin does not have impacts on cognitive functions [38].

The present study showed that lovastatin alone did not contribute to any significant effects on either the rodents' cognitive functions or brain AChE activity. However, when lovastatin and rivastigmine were used in combination as a treatment, the brain AChE activities were reduced, and behavioral performances of the mice were improved significantly when compared with those treated with rivastigmine alone. Thus, this finding shed some light on the potential potentiation of rivastigmine effect on enhancing cognitive function through the combined use of lovastatin.

Hyperlipidemia, characterized by the excessive lipid accumulation in blood, is one of the risk factors contributing to cognitive impairments, which could lead to AD [39]. AD is associated with increased intra-neuronal β-amyloid deposition and amyloid plaque development [40,41]. Lovastatin has been shown to decrease the formation of β-amyloid which is responsible for neuronal degeneration in AD [42]. It is feasible to preliminarily deduce the mechanism of rivastigmine potentiation with the aid of lovastatin in light of lovastatin's effect on amyloid formation. In addition, free radical scavenging activity, hypolipidemic activity [43,44], and anti-inflammatory activity [6] of lovastatin may also collectively form the underlying mechanism leading to the potentiation of rivastigmine effect. Nevertheless, further study is still required to decipher the exact mechanism.

5 Conclusion

Taken together, the combined administration of lovastatin and rivastigmine further improves the cognitive functions and increases the brain AChE activities *in vivo*, supporting the role of this treatment as a potential therapeutic strategy for the patients with cognitive impairment. The present study demonstrated that lovastatin could potentiate rivastigmine effect on enhancing cognitive function.

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Conflicts of interest

The authors have no conflicts of interest.

Author contributions

J.A. and M.A. (Arif) conceived and designed the experiments. B. performed the experiments. M.I.K. analyzed the data. M.M. contributed reagents/ materials/analysis tools. B. wrote the paper. M.A. (Ahmad) reviewed drafts of the paper. J.A. verified the analytical methods. B. conceived the main ideas and review outline. All authors read and approved the final manuscript.

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