EFFECT OF ATORVASTATIN AND ROSUVASTATIN ON LEARNING AND MEMORY IN RATS WITH DIAZEPAM-INDUCED AMNESIA

Maria T. Georgieva-Kotetarova*, Ivanka I. Kostadinova
Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University, Plovdiv, Bulgaria

ABSTRACT
During the past decade, evidence has emerged that statins have neuroprotective effects. The aim of this study was to investigate the effects of atorvastatin and rosuvastatin on learning and memory in rats with diazepam-induced amnesia.

Material and methods: Experiments were carried out on 48 white male Wistar rats, divided into 6 groups, each of 8 rats. The experimental animals were treated per os for 14 days with atorvastatin and rosuvastatin in doses of 10 mg/kg and 20 mg/kg body weight, respectively. To induce amnesia diazepam was administered intraperitoneally in a dose of 2.5 mg/kg bw. Cognitive skills of the animals were examined after the induction of amnesia with active avoidance test using autonomic reflex conditioner (shuttle box) and passive avoidance tests (step-through and step down) (Ugo Basile, Italy). The following parameters were assessed: number of conditioned responses (avoidances), number of unconditioned responses (escapes) and number of intertrial crossings in the active avoidance test; latency of reactions was measured in the passive avoidance tests.

Results: We found a significant increase of conditioned responses in atorvastatin treated animals (in a dose of 10 mg/kg bw) in active avoidance training. In the animals treated with rosuvastatin in both doses there was a statistically significant increase of unconditioned responses. In the step-through passive avoidance test there was significant improvement of short-term and long-term memory following administration of atorvastatin (10 mg/kg bw). Rosuvastatin (10 mg/kg bw) preserves long-term memory. In the step-down passive avoidance test, atorvastatin (10 mg/kg bw) and rosuvastatin (10 mg/kg and 20 mg/kg bw) preserve long-term memory.

Conclusions: Atorvastatin (10 mg/kg bw) and rosuvastatin (10 mg/kg and 20 mg/kg bw) improve cognitive functions in rats with diazepam-induced amnesia and preserve long-term memory.

Key words: statins, neuroprotective effect, diazepam-induced amnesia, active avoidance, passive avoidance

INTRODUCTION
Frequent and prolonged use of statins as lipid-lowering agents raises the issue of their pleiotropic effects and the need to extend their indications for clinical use. Considerable evidence has emerged in the past decade supporting the neuroprotective effect of statins. Clinical studies have demonstrated that cognitive decline is slowed in Alzheimer’s patients that have been receiving statins.\(^1\)\(^,\)\(^2\) Statins have been reported to be effective in Parkinson’s disease.\(^3\) The neuroprotective effect of statins has been studied in multiple sclerosis\(^4\) and in traumatic brain injury.\(^5\) The effect of the most common statins such as simvastatin and atorvastatin has been studied experimentally in different models of memory impairment. The results of these studies are rather contradictory, which can be attributed to the different degrees of cognitive deficit. Learning ability and memory have been found to improve after treatment with statins in a number of studies conducted after traumatic brain injury, and in experimental models of vascular dementia and amnesia.\(^1\)\(^,\)\(^6\) Statins have been reported to reduce the ischemic area after occlusion of the middle cerebral artery in mice and rats.\(^7\) In a model of olfactory bulbectomized mice with marked cognitive deficits no improvement of learning and memory has been reported after treatment with statins.\(^8\) There is little data in available

*Correspondence and reprint request to: M. Georgieva-Kotetarova, Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University, Plovdiv; E-mail: mariatgeorgieva@yahoo.com; Mob.: +359 896 104 816
15A Vassil Aprilov St., 4002 Plovdiv, Bulgaria

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literature on the effect of atorvastatin and rosuvastatin on learning and memory in an experimental model of diazepam-induced amnesia.

AIM
The aim of this study was to investigate how learning and memory are affected by two-week administration of atorvastatin and rosuvastatin in rats with diazepam-induced amnesia.

MATERIAL AND METHODS
White male Wistar rats (average weight of 180-200 g) were used in the study; they were maintained under standard laboratory conditions (temperature 26 ± 1.0°C, 45% humidity, 12:12 hour light-dark cycle), providing them with food and water ad libitum. The experimental protocol was approved by the Ethics Committee of Plovdiv Medical University. The animals were treated daily for two weeks. They were allocated into 6 groups of 8 white rats each:

Group I - a control group treated with physiological saline solution.

Group II – a control group of animals treated intraperitoneally with diazepam (2.5 mg/kg bw) for 14 days.

Group III - animals treated orally with atorvastatin (10 mg/kg bw) and intraperitoneally with diazepam (2.5 mg/kg bw) for 14 days.

Group IV - animals treated orally with atorvastatin (20 mg/kg bw) and intraperitoneally with diazepam (2.5 mg/kg bw) for 14 days.

Group V - animals treated orally with rosuvastatin (10 mg/kg bw) and intraperitoneally with diazepam (2.5 mg/kg bw) for 14 days.

Group VI - animals treated orally with rosuvastatin (20 mg/kg bw) and intraperitoneally with diazepam (2.5 mg/kg bw) for 14 days.

After the two-week period the animals were evaluated to test their ability to remember and store memory traces.

Diazepam in a dose of 2.5 mg/kg was injected intraperitoneally half an hour after oral administration of atorvastatin and rosuvastatin at doses of 10 mg/kg and 20 mg/kg bw, respectively. The cognitive and memory functions of the rats were assessed half an hour after the administration of diazepam. Tests were used to evaluate active and passive learning.

A standard two-way active avoidance box (autonomic reflex conditioner, shuttle box, Ugo Basile, Italy) was used in studying active learning in rats. Training lasted 5 days with 30 training sessions daily, each consisting of a 6-second light and sound stimulus (670 Hz and 70 dB), and electrostimulation (0.4 mA) in the last 3 seconds delivered through the cage grid floor. Each session was followed by 12 seconds of rest. Seven days after this training session (day 12) retesting was done for one day to track the storage of memory traces. The following parameters were assessed: number of conditioned responses (avoidances), number of unconditioned responses (escapes) and number of intertrial crossings.

The passive avoidance test was performed using passive avoidance controller (step-through) (Ugo Basile, Italy), which represents a cage with two compartments, one of them darkened. Learning ability and short-term memory were studied in three consecutive days; each session lasted 3 minutes; 3 trials were performed daily with the following parameters: opening the door between the compartments after 7 seconds, electrostimulation on the floor of the cage for 9 seconds with intensity of 0.4 mA. On day 9 a long-term memory retention test was performed. The parameter assessed was latency of reactions in seconds (how long the animals remained in the illuminated part of the cell).

A passive avoidance test was also carried out using passive avoidance controller (step-down) (Ugo Basile, Italy) with penalty reinforcement. Training was performed for 2 days. Each day included 2 training sessions with a 60-minute interval. The animal was placed on a plastic platform which vibrates vertically after switching on the apparatus. When the rat stepped down off it on 3 or 4 paws, electrostimulation (0.4 mA) was delivered by the cage floor outside the platform. Animals were tested for short-term memory on day 3 of experiment, and for long-term memory - on day 7. The latency of reactions was measured in seconds (the time during which the animal remained on the platform).

STATISTICAL ANALYSIS
Statistical analysis was performed using SPSS 17.0. For each of the parameters an arithmetic mean was determined and the standard error of the mean with level of significance p < 0.05. ANOVA was used to compare the groups’ parameters followed by Bonferroni multiple comparisons test.

RESULTS
ACTIVE AVOIDANCE TEST
Assessing the parameter “number of conditioned responses” we found a significant increase of the
number of avoidances in the control group treated with saline solution on days 3, 4, 5, and 12 compared with the number on the first day of experiment. We also found a significant reduction of the number of conditioned responses in the control group with diazepam-induced amnesia compared with the control group with saline solution during entire training period and in the study of long-term memory. On days 2, 3, 4 and 12 we found a statistically significant increase of the number of conditioned responses in animals treated with atorvastatin in a dose of 10 mg/kg bw in comparison with the control group with diazepam treated controls. In the group treated with rosuvastatin in a dose of 10 mg/kg bw a significant increase in the number of avoidances on days 2 and 3 was found in comparison with diazepam treated controls, and for the animals treated with the higher dose of rosuvastatin - on day 2 in comparison with the same control group (Fig. 1).

Analyzing the parameter “number of unconditioned responses” we found a statistically significant increase of the number of escapes in the saline solution controls on days 4, 5, and 12 in comparison with day 1 of the study. There was a significant reduction of unconditioned responses in the diazepam treated controls compared to the number in the saline treated controls on days 1 and 2 of training session. No statistically significant difference was found between the animals treated with atorvastatin in both doses and the controls with diazepam-induced amnesia. We found a significant increase of unconditioned responses on days 1, 2 and 5 of training session in the animals treated with rosuvastatin in a dose of 10 mg/kg bw and in the memory retention test on day 12 compared with the controls with diazepam-induced amnesia. Similar results were obtained in the trial with a higher dose of rosuvastatin (Fig. 2).

Analyzing the number of intertrial crossings for atorvastatin and rosuvastatin treated animals there was no statistically significant increase in

\[ p < 0.05 \] compared with day 1, physiological solution controls; \[ * p < 0.05 \] compared with the physiological solution controls for the respective day; \[ ** p < 0.05 \] compared with the diazepam controls for the respective day.

**Figure 1.** Number of conditioned responses by days in active avoidance test (shuttle box) in rats treated with atorvastatin and rosuvastatin.
Effect of Atorvastatin and Rosuvastatin on Learning and Memory in Rats with Diazepam-Induced Amnesia

the locomotor activity, which renders the data for parameters “number of conditioned responses” and “number of unconditioned responses” significant (Table 1).

Table 1. Number of intertrial crossing by days in active avoidance test (shuttle box)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of study</th>
<th>Physiological solution controls (n = 8) mean ± SEM</th>
<th>Diazepam controls (2.5 mg/kg bw) (n = 8) mean ± SEM</th>
<th>Atorvastatin (10 mg/kg bw) diazepam (2.5 mg/kg bw) (n = 8) mean ± SEM</th>
<th>Atorvastatin (20 mg/kg bw) diazepam (2.5 mg/kg bw) (n = 8) mean ± SEM</th>
<th>Rosuvastatin (10 mg/kg bw) diazepam (2.5 mg/kg bw) (n = 8) mean ± SEM</th>
<th>Rosuvastatin (20 mg/kg bw) diazepam (2.5 mg/kg bw) (n = 8) mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>14.37 ± 1.43</td>
<td>17 ± 4.39</td>
<td>15.25 ± 5</td>
<td>23.25 ± 10</td>
<td>15 ± 1.34</td>
<td>16.75 ± 1.88</td>
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<td></td>
<td>2</td>
<td>16.75 ± 2.22</td>
<td>17 ± 4.94</td>
<td>16.12 ± 3.77</td>
<td>11.87 ± 3.06</td>
<td>13 ± 2.62</td>
<td>16 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>16 ± 1.87</td>
<td>19 ± 3.35</td>
<td>19.25 ± 2.35</td>
<td>14.25 ± 3.26</td>
<td>14 ± 0.98</td>
<td>14.13 ± 1.39</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15.12 ± 1.89</td>
<td>16.75 ± 3.56</td>
<td>18.5 ± 1.8</td>
<td>13.62 ± 2.27</td>
<td>16.37 ± 2.01</td>
<td>19.25 ± 1.19</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>15.125 ± 1.37</td>
<td>13.87 ± 2.23</td>
<td>19.37 ± 2.01</td>
<td>19.37 ± 2.91</td>
<td>14.7 ± 2.21</td>
<td>20.25 ± 2.38</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.
solution group on days 2, 3 and 9 compared with the latency time measured on day 1. The short- and long-term memory tests found statistically significant shorter latency period in diazepam-induced amnesia controls compared with the saline control group. The parameter we assessed in the animals treated with atorvastatin (10 mg/kg bw) was greater than that in the control animals with diazepam induced amnesia on days 3 and 9 of trial. The latency of reactions for the atorvastatin treated animals (20 mg/kg bw) was significantly increased compared with that for the controls with diazepam induced amnesia on day 2 of training session. The group receiving rosvastatin (10 mg/kg bw) scored significantly longer latency time in comparison with the controls with diazepam-induced amnesia on days 1 and 9 of training session. Statistically significant increase of the latency time was found for the animals treated with the higher dose of rosvastatin compared with the controls with diazepam-induced amnesia on days 1 and 2 of training session (Fig. 3).

In the step-down passive avoidance test the saline treated control group showed longer latency time on days 3 and 7 than the latency scored at baseline. The difference between the saline treated controls and the controls with diazepam induced amnesia could not reach statistical significance. In the long-term memory retention test, the latency period measured in the 10 mg/kg atorvastatin treated group was significantly longer than that of the diazepam treated controls. These results were confirmed for the animals treated with rosvastatin in doses of 10 mg/kg and 20 mg/kg bw. There was no significant difference between the group receiving 20 mg/kg b.w of atorvastatin and the diazepam treated control group (Table 2).

**DISCUSSION**

The diazepam in the present study was administered intraperitoneally in a dose of 2.5 mg/kg to induce anterograde amnesia. The animals receiving statins were found to improve their learning abilities and preserve their long-term memory compared with the controls with diazepam-induced amnesia. The animals treated with atorvastatin in a dose of 10
mg/kg bw were found to increase the number of conditioned responses, while the animals treated with rosuvastatin in both doses scored a greater number of unconditioned responses. The different degrees of effect on memory capacity is probably associated with differences in the pharmacokinetics of the preparations, although no clinical differences in the neuroprotective properties of lipophilic and lipophobic statins have been reported. Atorvastatin is highly lipophilic while rosuvastatin is water soluble, yet shows good affinity toward transport carriers. With the passive avoidance step-through test we found that the short-term and long-term memory are ameliorated after administration of atorvastatin in a dose of 10 mg/kg but not after atorvastatin in the higher dose. Rosuvastatin in a dose of 10 mg/kg b.w preserves long-term memory. These results were confirmed in the step-down passive avoidance test - atorvastatin (10 mg/kg bw) and rosuvastatin (10 mg/kg bw and 20 mg/kg bw) preserve long-term memory.

Research on the neuroprotective effect of statins is carried out in different experimental models of brain injury. Investigating statins in experimental models of amnesia induced by alpazolam, Parle et al. found that atorvastatin and simvastatin were efficient in reversing the memory deficits. There have been reports that benzodiazepines mediate some of their actions by modulating the signalling pathway NO/cGMP. In memory formation nitric oxide acts as a retrograde messenger that modulates synaptic function and affects short- and long-term memory. It has been found that statins increase the expression of eNOS and inhibit iNOS thereby increasing the level of nitric oxide. Our results with atorvastatin in a dose of 10 mg/kg are similar to those reported by Parle et al. Similar results in a model of neurotoxicity induced by quinolone acid were reported by Kalonia et al. for atorvastatin, simvastatin and fluvastatin.

The neuroprotective effect of statins has also been studied in an experimental model of neurodegenerative disorder caused by neonatal iron loading which induces cognitive impairment in adult rats. Rech et al. found that three-week administration of rosuvastatin improved short-term memory in a novel object recognition test in animals that received iron in the neonatal period and improved long-term memory in rats with age-related cognitive deficits.

Our results, reported for the model of diazepam-induced amnesia and with rosuvastatin in a dose of 10 mg/kg are consistent with those of Rech et al. about the effect of this drug on long-term memory which can be explained by the mechanisms of inducing brain injury. According to Rech et al. one of the possible mechanisms by which rosuvastatin improves cognitive disorders is reduction of the oxidative damage of the cortex and the hippocampus in adult rats.

Neuroprotective effect of statins has also been studied in transgenic mice overexpressing the mutant amyloid β precursor protein where there is a significant accumulation of amyloid β in the brain causing brain disorder and pathology typical of Alzheimer’s disease. It has been found that pitavastatin and atorvastatin improve behavioral memory in animals aged 5 to 20 months and reduce deposits of amyloid β. In active and passive avoidance tests rosuvastatin and atorvastatin in a dose of 10 mg/kg bw in our study ameliorated long-term memory.

The neuroprotective effect of statins is not confirmed by all studies. Douma et al. studied the influence of simvastatin on cognitive functions in...
mice with olfactory bullectomy, resulting in severe cognitive deficit in learning and memory. Administration of simvastatin in this study did not have any behavioral effect as tested in open field, passive avoidance step-through and object-recognition tests, which differs from the results we have obtained. The cognition of the animals was improved in intact rats after treatment with simvastatin.8 This can be most likely accounted for by modulation of the signalling pathways involved in synaptic plasticity and formation of spatial memory.15 The level of NMDA receptors has been demonstrated to increase after chronic treatment with statins.16 These receptors play an important role in learning and memory17, which may be part of the complex mechanism of improving learning and memory by administration of statins.

In studying the effect of atorvastatin in cognitive abilities in mice with scopolamine-induced brain disorder, an improvement of spatial learning has been found as well as retention of short-term memory in passive avoidance step-through, which has been confirmed by our results in a model of diazepam-induced amnesia.18

CONCLUSIONS

Atorvastatin in a dose of 10 mg/kg bw improves learning in active avoidance test and retention of long-term memory in passive and active avoidance tests in rats with diazepam-induced amnesia.

Rosuvastatin in doses of 10 and 20 mg/kg bw leads to amelioration of memory deficit in rats with diazepam-induced amnesia and preservation of long-term memory.

REFERENCES

ВЛИЯНИЕ ATORVASTATIN-A И ROSUVASTATIN-A НА ПРОЦЕССЫ ОБУЧЕНИЯ И ПАМЯТИ У КРЫС С DIAZEPAM-ИНДУЦИРОВАННОЙ АМНЕЗИЕЙ

М. Георгиева-Котетарова, И. Костадинова

РЕЗЮМЕ

ВВЕДЕНИЕ: В последние десятилетия появились данные о нейропротективном действии статинов.

ЦЕЛЬ: Изучить влияние Atorvastatin-a и Rosuvastatin-a на процессы обучения и памяти у крыс с Diazepam-индуцированной амнезией.

МАТЕРИАЛ И МЕТОДЫ: Опыты поставлены на 46 белых мужских крысах породы Wistar, разделенных на 6 групп по 8 крысам. Подопытные животные в течение 14 дней перорально получали atorvastatin и rosuvastatin в дозах 10 mg/kg b.w и 20 mg/kg b.w. С целью причинить амнезию диазепам вводят интратеритонеально (i.p.) в дозе 2,5 mg/kg b.w. Исследованы когнитивные способности животных после индуцирования амнезии с помощью теста активного обучения посредством autonomic reflex conditioner (shuttle box) и пассивного обучения посредством passive avoidance controllers (step-through и step down) (Ugo Basile, Italy). Установлены следующие показатели: число условных ответов (avoidance), число безусловных ответов (escapes) и межтренировочные переходы при тесте активного обучения; латентный период при тестах пассивного обучения.

РЕЗУЛЬТАТЫ: При активном обучении наблюдается значимое увеличение условных ответов у животных, получавших atorvastatin 10 mg/kg b.w. У животных, получавших rosuvastatin и при обеих дозах статистически значимо увеличиваются безусловные ответы. При тесте пассивного обучения step-tough step-tough регистрируется значимое улучшение кратковременной и долговременной памяти, после приема atorvastatin 10 mg/kg b.w. Rosuvastatin 10 mg/kg b.w сохраняется долговременная память. При тесте пассивного обучения step-down atorvastatin 10 mg/kg b.w и rosuvastatin 10 mg/kg b.w и 20 mg/kg b.w сохраняется долговременная память.

ВЫВОДЫ: Применение atorvastatin-a 10 mg/kg b.w и rosuvastatin-a 10 и 20 mg/kg b.w улучшает когнитивные функции у крыс с Diazepam-индуцированной амнезией и сохраняет долговременную память.