Original Article

The effect of Simvastatin on Picrotoxin-induced seizure in mice

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Abstract

Objective: To study the effect of simvastatin on picrotoxin-induce seizures in mice in order to understand the impact of gabaergic system on neuronal cell death.

Methods: The study was held between July and September 2011, at the Karadeniz Technical University in Trabzon, Turkey. Balb/c mice weighting 20-40g were randomly selected and divided into five groups of six each. The first group was designated as control group; and the second as the picrotoxin (10mg/kg; intraperitoneal) alone group. The rest of the groups were administered simvastatin in dozes of 10, 20 and 40mg/kg respectively. Onset, number and duration of seizures, and death time were measured in mice for one hour. At the end of the study, the brain was removed from mice and normal and degenerative pyramidal neurons were counted in hippocampal CA1, CA2, CA3 region by light microscope. Using SPSS 17, Mann=Whitney U and Chi square and student-T tests were performed for statistical analysis.

Results: Simvastatin (10mg/kg) significantly decreased the number and duration of picrotoxin-induced seizures in mice. In addition, Simvastatin (10, 20, and 40mg/kg) significantly reduced the total number of abnormal pyramidal cells in CA1 and CA3 hippocampal regions compared to the picrotoxin-alone group.

Conclusion: The effect of simvastatin on picrotoxin-induced seizures may be the result of increase in gabaergic activity and decrease in glutamatergic activity. More studies are needed to validate these results.

Keywords: Seizures, Simavastain, Picrotoxin. (JPMA 62: 1187; 2012)

Introduction

Epilepsy is a neurological disorder characterised by seizures that start as paroxysmal, short-lived and sometimes leading to loss of consciousness. These seizures can occur via electroshock and chemical convulsant or without provocation. Epileptic seizures are characterised by increasing excitability in brain structures (such as within the cortex and subcortical area).

The regulation of excitability is the role of the glutamatergic excitatory system and gabaergic inhibitory system. Increased excitability is seen to occur with increasing excitatory activity (glutamatergic activity) or decreasing inhibitor activity (gabaergic activity). The neuronal ionic mechanism of excitability is regulated by Ca2+ inflow in brain neurons (such as hippocampal pyramidal neurons). Also, neuronal depolarisation can be inhibited via Potassium Chloride (KCl) entry in neurons.[1-4]

Neurological diseases are believed to be associated with neuronal cell death (excitotoxicity) component in their pathogenesis. It is a well-established fact that neuronal cell death is a Calcium-dependent process, with the calcium signalling affecting its various underlying pathways. It is also reported that the inhibition of glutamate-gated ion channels prevent neuronal cell death under various neurological diseases. Studies have shown that activating gamma-aminobutyric acid (GABA) receptor leads to a direct neuroprotective effect.[4-8] In contrast, there are studies in literature reporting that GABA receptor activation is not protective in neuronal cell death.[9,10]

Statins are hypolipidaemic drugs and are thought to have neuroprotective effects independent of their cholesterol-lowering action in experimental studies.[11-16] It has been reported that neuroprotective effect of statins depend on the prevention of glutamate-induced neuronal toxicity in brain neurons (e.g hippocampal neuron). The results of a cohort study done in 2010 suggested that the administration of statins decreases the risk of hospitalisation for epilepsy by preventing epileptic seizures.[17]

Picrotoxin, a specific GABAA-chloride channel blocker, is used in chemical convulsant models in animal studies. The effect of simvastatin as a statin on neuronal cell death and epileptic seizures via gabaergic system is not clear in literature. We aimed to study the effect of simvastatin on picrotoxin-induced seizures in mice.

Material and Methods

The study was held between July and September, 2011, at the Behavioural Pharmacology Laboratory of

Department of the Pharmacology at Karadeniz Technical University, in Trabzon. For this randomised controlled experimental animal study, Balb/c mice of either gender weighing 20-40g were randomly selected. The animals were housed in cages with free access to food and water. The cages were placed in a quiet and temperature-humidity controlled room (22±2°C and 60±5% respectively) in which a 12:12-h light-dark cycle was maintained. Experiments were conducted between 9am and 5pm to minimise the diurnal variation. The experimental protocol was approved by the Local Ethics Committee of the University's Faculty of Medicine.

The 30 animals were divided into five groups, the first group was the control group; the second group was the picrotoxin-alone (10mg/kg) group. Simvastatin at doses 10, 20, 40 mg/kg were administered via oral gavage to mice in the third, fourth and fifth groups respectively. Picrotoxin (10mg/kg) was administered after 60 minutes of simvastatin application in the last three groups. Simvastatin (Merck Sharp & Dohme, Turkey) was dissolved in phosphate buffered saline (PBS). It was administrated by oral gavage, while Picrotoxin (PTX) was administrated intraperitoneally. Time to onset of seizures, the number of seizures, the duration of seizures as well as the death time were measured in mice for a period of one hour. At the end of experimental study, the brains of mice were removed, and normal and degenerative pyramidal neurons were counted in the hippocampal CA1, CA2, CA3 regions by light microscopy.

The region of the brain containing the hippocampal formation was removed. For light microscopic investigation, whole hemispheres of brains were fixed in 10% neutral buffered formalin solution for 48h, dehydrated in increasing concentrations of ethanol, and embedded in paraffin. Brain tissues were then sectioned at 5 µm using a microtome (Leica RM 2255, Tokyo, Japan). Hippocampal sections, including those from the CA1, CA2 and CA3 regions, were cut coronally. Approximately 5 µm thick sections were stained with cresyl violet for general morphology. All brain tissue slides were examined with a light microscope (Olympus BX-51; Olympus Optical Co., Ltd., Tokyo, Japan) at high magnification by a histologist blinded to the animal groups. Photographs were taken using a light microscope with a camera attachment (Olympus DP 71, Japan). Digital images from hippocampal substructures were projected onto a monitor screen, before CA1, CA2 and CA3 regions were analysed. Normal and degenerative pyramidal neurons were counted. For cell-counting, only cells with nuclei were included. The criteria for abnormal cells were the presence of one or more of the following characteristics; dark-stained cytoplasm, condensed cell nucleus and shrunken or swollen in shape. The numbers of abnormal cells in the defined anatomical regions were counted with an Analysis 5 Research programme (Olympus Soft Imaging Solutions, Germany) in a blind fashion.

Statistical analyses were performed using SPSS software version 17. Comparison of the changes in measured data were carried out using Mann-Whitney U test and comparison of the data obtained by counting were carried out using square test (χ^2) and student-T test. The level of significance was set at $p < 0,05$.

Results

Simvastatin 10mg/kg significantly decreased the number and duration of seizures on picrotoxin-induced seizures in mice (respectively; $p<0,03$, $p<0,01$). However, at doses of 20 or 40mg/kg it did not show any changes in the onset, number and duration of seizures and the death time (Table-1).

Table-1: The effect of time to onset of seizures, number of seizures, duration of seizures and death time of simvastatin (10, 20, 40 mg/kg) on picrotoxin-induced seizures.

	Time to onset of seizures (minute)	Number of seizures	Duration of seizures (second)	Death time (minute)
PTX group	7.82 ± 1.43	25.17 ± 10.39	229.83 ± 5.29	17.63 ± 3.83
S10 + PTX group	8.25± 0.85	13.33 ± 3.84 **	156.33 ± 8.54 *	19.72 ± 3.38
S20 + PTX group	8.26 ± 1.41	21.50 ± 7.08	185.17 ± 6.17	18.65 ± 4.91
S40 + PTX group	8.67 ± 1.15	22.17 ± 7.12	207.67 ± 5.86	19.07 ± 3.34

*: Compared to picrotoxin ($p<0,01$)
**: Compared to picrotoxin ($p<0,03$).
PTX: Picrotoxin. S: Simvastatin.

Table-2: The effect of simvastatin on number of degenerative neuron on picrotoxin-induced seizures in hippocampal CA1, CA2 and CA3 pyramidal cell.

	CA1	CA2	CA3
Control	3.67 ± 0.74*	5.33 ± 1.10	9.00 ± 0.81*
PTX group	57.67 ± 5.91	8.50 ± 1.70	68.17 ± 7.22
S10 + PTX group	8.67 ± 1.48*	6.50 ± 1.38	12.83 ± 2.78*
S20 + PTX group	13.00 ± 2.37*	9.67 ± 3.89	16.00 ± 1.15*
S40 + PTX group	9.50 ± 0.50*	8.17 ± 0.68	13.50 ± 1.80*

*: Compared to picrotoxin ($p <0,0001$).
PTX: Picrotoxin. S: Simvastatin.

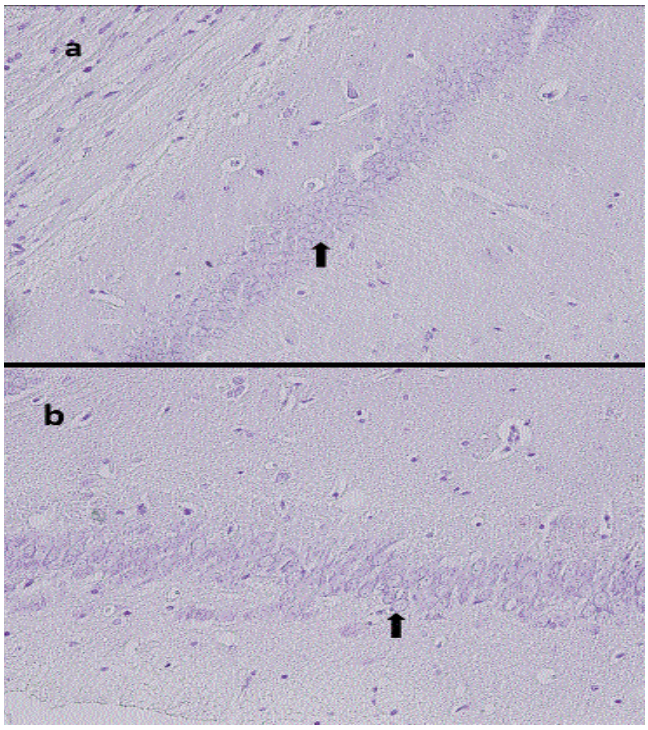

Figure-1: Hippocampal sections showed normal (↑) and increased number of abnormal pyramidal cells (▲) with dark-stained cytoplasm, shrunken shape at the CA1 (a) and CA3 regions (b) in the control group (Cresyl violet X 100).

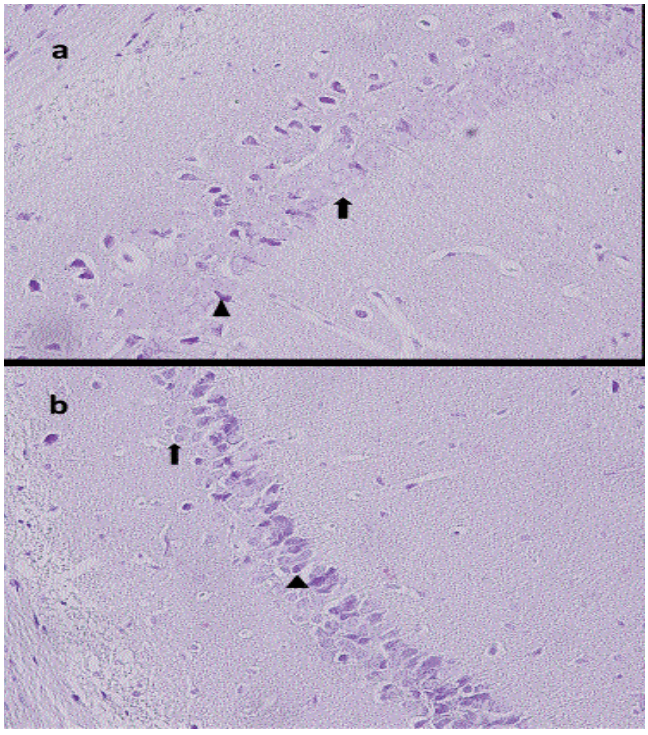

Figure-2: Hippocampal sections showed normal (↑) and increased number of abnormal pyramidal cells (▲) with dark-stained cytoplasm, shrunken shape at the CA1 (a) and CA3 regions (b) in picrotoxin group (Cresyl violet X 100).

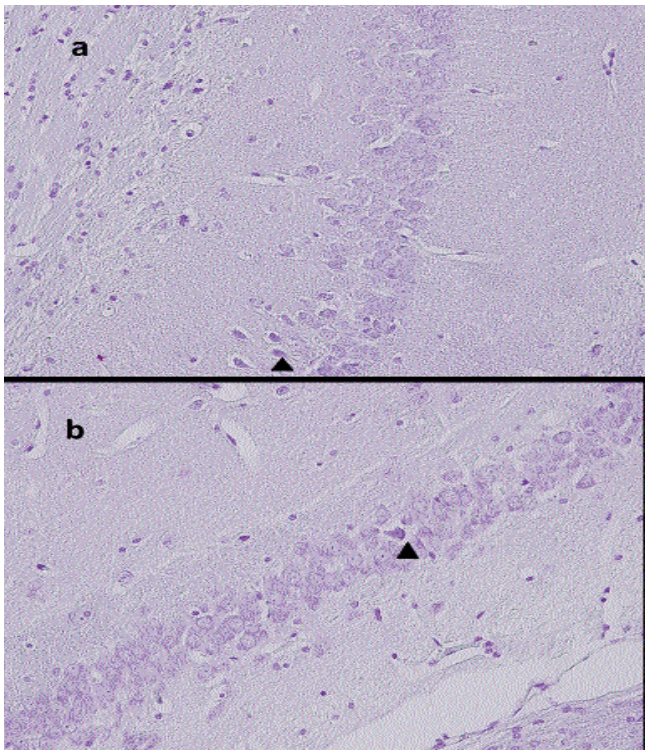

Figure-3: Hippocampal sections showed normal (↑) and increased number of abnormal pyramidal cells (▲) with dark-stained cytoplasm, shrunken shape at the CA1 (a) and CA3 regions (b) in simvastatin + picrotoxin group (Cresyl violet X 100).

The morphology of pyramidal cells of CA1, CA2 (not shown), CA3 regions hippocampus sections in the control group were normal (Figure-1). In the picrotoxin group, significantly increased number of degenerative cells with an abnormal, dark-stained cytoplasm and shrunken morphology compared to the control group were seen, especially in the CA1 and CA3 regions ($p < 0,0001$) (Table-2, Figure-2). Simvastatin significantly reduced the total number of abnormal pyramidal cells compared to picrotoxin group in CA1 and CA3 hippocampal regions at all doses used in the study ($p < 0,0001$) (Table-2, Figure-3).

Discussion

Simvastatin is mainly prescribed for the treatment of hypercholesterolemia. Apart from the lipid-lowering effects of statins, the pleotropic effects of these drugs have been investigated in various animal models for second beneficial effects. Statins have been shown to be neuroprotective in a group of animal disease models and clinical studies.[11,17] Statins are shown to prevent hippocampal neuron death in kainic acid (KA), pilocarpine, pentylentetrasole and quinolinic acid (QA)-induced epileptic seizures.[11,13]

In our study, simvastatin (10 mg/kg) significantly decreased the number and duration of picrotoxin-induced

seizures in mice. Simvastatin (10, 20, 40mg/kg) also reduced the total number of abnormal pyramidal cells in CA1 and CA3 hippocampal regions as well, by a significant manner, when compared to the picrotoxin-alone group.

A study has shown that atorvastatin (10 mg/kg) prevented hippocampal (CA1 and CA3 region) cell death due to quinolinic acid-induced seizures in mice.[12] Similar results have been demonstrated via atorvastatin on hippocampal (CA1 and CA3 region) neuronal cell death due to kainic acid-induced seizures in rats.[13] It has been shown that statins (simvastatin, atorvastatin, pravastatin, rosuvastatin, mevastatin) prevented N-Mythyl-D-Aspartic (NMDA)-induced excitotoxic cell death in statin-treated cultured neurons; simvastatin and rosuvastatin demonstrated stronger neuroprotective effect potency compared to the other statins in mice.[14] In another study, lovastatin (20mg/kg) reduced neuronal cell death in hippocampal CA1 region on pilocarpine-induced status epilepticus in rats.[15] These results in experimental studies are partially supportive of the results of our study.

Epileptic seizures have been related to both increase in glutamatergic activation and decrease in Gabaergic inhibition. It has been shown that gabaergic and glutamatergic synaptic activity are very important on epileptic seizures in hippocampal pyramidal CA3 cells, and epileptic seizures are caused by the decrease in the gabaergic activity of a rat.[3] Picrotoxin, a specific GABAA-chloride channel blocker, blocked the GABAA receptor activation. We have demonstrated picrotoxin-induced neuronal cell death in hippocampal CA1, CA2, CA3 pyramidal cells in our study. This result may be related to the decrease in gabaergic activity and increase in glutamatergic activity in the hippocampal area. However, a lot of studies have reported the importance of increased glutamatergic activity on neuronal cell death.[13,18,19] We have demonstrated the decrease in neuronal cell death by simvastatin in hippocampal pyramidal CA1 and CA3 cells, and decrease in the number of epilectic seizures and duration of seizures. This result of simvastatin may be related to the neuroprotective effect in hippocampal pyramidal neuron.

There are studies showing the role of ionic mechanisms on neuronal cell death. It has been shown that increase in intracellular Ca2+ caused neuronal cell death.[8,20] A study has demonstrated that atorvastatin decreased the glutamate-induced increase of intracellular Calcium in primary cortical neurons.[16] We have shown that simvastatin decreased the neuronal cell death on hippocampal pyramidal cells in picrotoxin-induced seizures in mice. This result of simvastatin in our study may be related to the prevention of calcium-induced neuronal death cell as a result of increase in gabaergic activity and decrease in glutamatergic activity.

Conclusion

Simvastatin may prevent neuronal damage by causing a decrease in glutamatergic activation and increase in gabaergic inhibition in hippocampal slices on picrotoxin-induced seizures. Further studies are needed to validate the results.

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21. Ref # 8 repeating as Ref # 21.