Original Article

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Anticonvulsant activity of atorvastatin against seizure induced by pentylenetetrazole and maximal electroshock in mice

Mohammad Javad Khoshnoud[1*], Nader Tanideh[2], Samaneh Namdarian[1]

[1]Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Pharmacology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Statins are inhibitors of HMG-CoA reductase and inhibit cellular synthesis of cholesterol and isoprenoids. Studies have previously demonstrated the anti-inflammatory and vasoprotective effects of statins on cultured brain cells (astrocytes and microglia) and endothelial cells. Atorvastatin also prolonged latency (time to appearance of spike potentials) and diminished the amplitude and frequency of spike potentials, which indicate epileptic discharges. In some studies observed that pre-treatment with atorvastatin efficiently reduced seizure activities, hippocampal neuron death, monocyte infiltration and proinflammatory gene expression.In this study the protective effects of atorvastatin on seizures induced by pentylenetetrazole (PTZ) and maximal electroshock stimulation (MES) were investigated. Intraperitoneal pentylenetetrazole was used to induce seizures in mice. It was found that atorvastatin (ED_{50}=5.12±0.98) has antiseizure effects comparing to control group. Atorvastatin treatment significantly increased the seizure threshold (p<0.01) and decreased the incidence of tonic seizure and death which is induced by intraperitoneal pentylenetetrazole. The effect of atorvastatin on seizure induced by MES in mice was evaluated and the results demonstrated that it is not able to produce anticonvulsant activity.

Keyword: Seizure, Atorvastatin, Pentylenetetrazole, Maximal electroshock, Mice.

Introduction

Epilepsy is a neurological disorder characterized by repeated seizures caused by excessive discharge of neurons. Cognitive impairments are frequent in patients with epilepsy, particularly in memory function. Pentylenetrazol (PTZ) has been accepted as an experimental animal model for analyzing epilepsy and estimating the effectiveness of antiepileptic drugs. PTZ is considered an adequate model of human generalized tonic–clonic seizures and this model is suitable for studying seizures and memory impairment associated with epilepsy. PTZ is thought to act as a noncompetitive antagonist of γ-aminobutyric acid type A (GABA-A) receptor (1).

Atorvastatin (inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase) is used in the prevention of coronary artery disease and treatment of hypercholesterolemia and is believed to be safe (2-4). It has been shown that, independent of its cholesterol-lowering effects, atorvastatin has neuroprotective and anti-excitotoxic effects in the settings of cerebral ischemia and trauma, as well as other cerebral pathophysiologies. (5,6)

Epidemiological studies have suggested that individuals older than 50 who were receiving statins had a lower risk of developing dementia (1). In addition, statins reduce stroke incidence and may reduce the risk of Alzheimer's disease (7). On the other hand, one disadvantage of statin therapy is cholesterol inhibition because cholesterol synthesis is essential for the normal functioning of neurons. In consideration of this information, our aim in this study was to elucidate the effects of atorvastatin pretreatment on seizure susceptibility in PTZ and Maximal Electroshok Stimulation (MES).

2. Materials and methods

2.1. Animals

Adult male mice (25-30 g) were purchased from the Animal House of Shiraz University of Medical Sciences Shiraz, Iran. The animal house temperature

Corresponding Author: Mohammad Javad Khoshnoud
Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, 71345-1583 Shiraz, Iran
E-mail: khoshnoudm@sums.ac.ir

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was maintained at 22±2ºC with a 12 h light/dark cycle. All animals were kept for one week prior to experimentation and were given free access to food and tap water. Each animal was tested once. All animal experiments were carried out in accordance with recommendations of the Declaration of Helsinki and internationally accepted principles for the use of experimental animals.

Behavioral tests were performed on groups consisting 10 mice. In order to study on anticonvulsant activity, at least three different concentrations of atorvastatin and diazepam as positive control were prepared freshly.

PTZ was dissolved in normal saline, (NS) and diazepam and atorvastatin were dissolved in 40% dimethyl sulfoxide, (DMSO) and 60 % normal salin (NS). Control groups received NS and DMSO(60:40).

All controls and atorvastatin were administrated intraperitoneally (IP) in volumes of 10 ml/kg body weight.

The time for DZP and other treatments to reach the maximum effect was determined to be 30 min after IP injection.

2.2. Pentylenetetrazole seizure model

Animals were treated with DZP, NS, DMSO or atorvastatin. Thirty min later, seizure was induced by the IP administration of 90 mg/kg of PTZ. The following parameters were recorded during the first 30 min after PTZ administration and 1 hour after atorvastatin administration:

1. Latency to the onset time of myoclonic, Straub tail and tonic-clonic seizures.
2. Protection from Hindlimb Tonic Extention (HLTE) and death.

2.3. Maximal electroshock seizure model

Electro-convulsive shock usually induces HLTE in 99.9% of the animals. The electrical stimulus (120 V, 50 Hz, 1.5 s duration) was applied through ear-clip electrodes using a stimulator apparatus. Animals were treated with DZP, NS, or atorvastatin. Thirty minutes later and 1 hour after atorvastatin administration, seizure was induced by electroshock and protections from HLTE were recorded (8).

Table 1. ED50 of diazepam and atorvastatin on HLTE induced by MES and death by PTZ models in mice.

Compound	ED50(mg/kg)	
	MES	PTZ
Diazepam	1.98±0.4	1.4±0.3
Atorvastatin	_	5.12±0.98

2.4. Statistical analysis

The dose of the compound required for inducing anticonvulsant effect in 50% of animals and its associated 95% confidence limit were calculated by SPSS software and probit regression. Data obtained from delay convulsion behavior were expressed as Mean±SEM and were analyzed by One-way ANOVA along with Dennett's post test. $P<0.05$ was considered significant.

3. Results and discussion

The results demonstrated that atorvastatin has anticonvulsant activity in PTZ, but it is not able to produce anticonvulsant activity in MES seizure model. Table 1 shows the ED_{50} with confidence limits of diazepam and atorvastatin in HLTE induced by MES and death by PTZ models. It was found that atorvastatin in PTZ model has antiseizure effects comparing to control group.

Among the tests used for evaluation of anticonvulsant activity, the MES and PTZ tests are of predictive relevance regarding the clinical spectrum of activity of experimental compounds, since the MES and PTZ tests are assumed to identify anticonvulsant drugs effective against human generalized tonic-clonic and absence seizures, respectively (8,9). MES-induced seizure can be prevented either by drugs that inhibit voltage-dependent Na^+channels such as phenytoin, Sodium valproate, felbamate and lamotrigine; or by drugs that block glutamatergic receptor such as felbamate. On

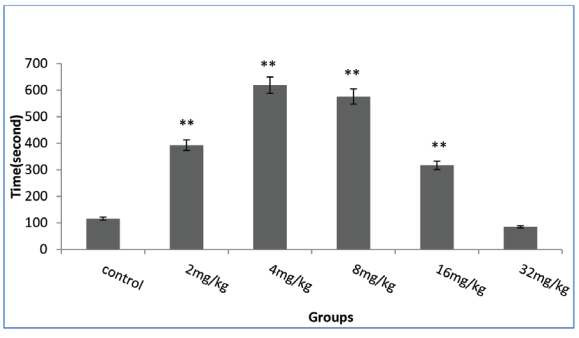

Figure 1. Effect of intraperitoneal injection of different doses of atorvastatin on myoclonic seizure onset time (Mean±SEM) induced by pentylenetetrazole 90 mg/kg. (n=10). **$p<0.01$.

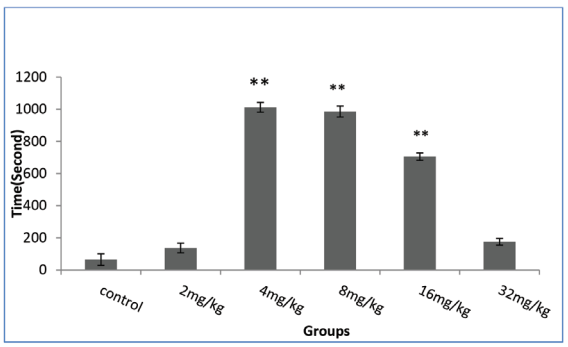

Figure 2. Effect of intraperitoneal injection of different doses of atorvastatin on tonic-clonic seizure onset time ((Mean±SEM)) induced by pentylenetetrazole 90 mg/kg. (n=10). **$p<0.01$.

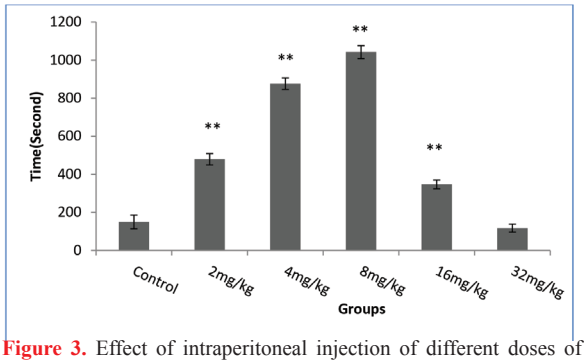

Figure 3. Effect of intraperitoneal injection of different doses of atorvastatin of straub tail onset time ((Mean±SEM)) induced by pentylenetetrazole (90 mg/kg). (n=10) **(*p*<0.01).

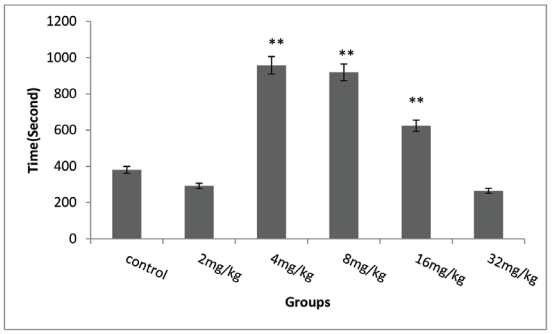

Figure 4. Effect of intraperitoneal injection of different doses of atorvastatin HLTE seizure onset time ((Mean±SEM)) induced by pentylenetetrazole (90 mg/kg). (n=10) **(*P*<0.01).

the other hand, drugs that reduce T-type Ca^{++} currents, such as ethosuximide can prevent seizures induced by PTZ. Drugs that enhance gamma amino butyric acid type A (GABAA) receptor mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital and perhaps valproate and felbamate can prevent this type of seizure. Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 75% of the patients (10). Furthermore, undesirable side effects from the drugs used clinically often render treatment difficulty; so, there are demands for new types of anticonvulsants drugs.

In the present study, the effect of atorvastatin on seizure induced by MES and PTZ in mice was evaluated and the results evidently demonstrated that atorvastatin is able to produce potent anticonvulsant activity in PTZ seizures (Figs. 1 to 5). Atorvastatin increased latency to the onset of myoclonic in a dose-dependent manner compared with the control group (*P*<0.01, Fig. 1). Atorvastatin also increased the time to the onset of tonic-clonic seizure compared with the control group *(P*<0.01, Fig. 2). As shown in Fig 3 atorvastatin at the studied doses, i.e. 2, 4, 8, and 16 mg/kg, increased the time to the onset of straub tail compared with the control group (*P*<0.01, Fig. 3). Moreover, atorvastatin caused a delay in HLTE seizure onset time (*P*<0.01, Fig.4) and the onset time of PTZ-induced death (*P*<0.01, Fig. 4).

In the present study, oral administration of atorvastatin was shown to possess anticonvulsant effects on PTZ-induced clonic seizures in a dose-independent manner at a wide range of different doses. Statins' beneficial effects have traditionally been recognized as cholesterol-lowering agents through inhibition of HMG-CoA reductase. However, accumulating evidences suggest that the overall benefits observed with statins may not be mediated solely by their lipid-lowering properties, but possibly through cholesterol-independent mechanisms (pleiotropic effects) (11,12). At least, one of the following mechanisms is involve in pleiotropic effects of atorvastatin; inhibition of inflammatory responses, reduction in oxidative stress, as well as reduction in inducible nitric oxide synthase and restoration of nitric oxide (NO) bioavailability by upregulation of endothelial NO synthase, modulation of some cytokines, and antiapoptotic effects (13,14). Moreover, in the central nervous system, statins have been to possess neuroprotective effects in animal models of Alzheimer's disease and ischemic brain injury through NO-dependent pathways, which indicates the predominant role of NO as a second messenger system of statins (15,16). In an in vitro study, atorvastatin exerted antiexcitotoxic effects through reducing intracellular calcium levels (17). Also, atorvastatin prevented kindling development and the long-term memory deficit caused by epileptic seizures (1). Our results are in line with these studies indicating that atorvastatin could be considered as an anticonvulsive agent in prevention of epileptic seizures. The relationship between epileptic seizures and statins has been investigated in very few studies. In one study, pretreatment with atorvastatin reduced seizure activity and hippocampal neuron death in PTZ–induced seizure model (18). While recent reports have speculated that atorvastatin may exert anticonvulsant effects on seizure threshold, its mechanism of action remains unknown. Among the most prominent mechanisms responsible for statins' actions are their effects through the NO pathways. NO primarily affects

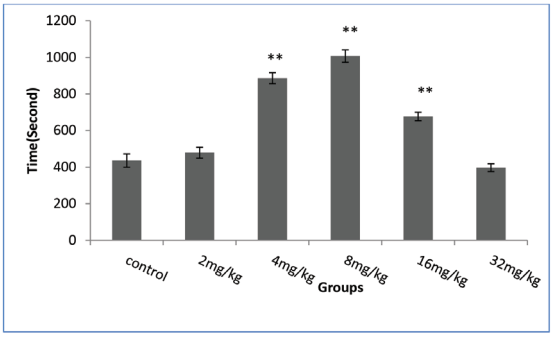

Figure 5. Effect of intraperitoneal injection of different doses of atorvastatin on death onset time (Mean±SEM) induced by pentylenetetrazole (90 mg/kg). (n=10) **(*p*<0.01).

the vasculature as an endogenous vasodilator and antithrombotic molecule, inhibits platelet adherence, and regulates endothelial permeability to lipoproteins. Moreover, NO has been shown to act as a neuronal messenger or neurotransmitter in the central nervous system and has been suggested as a modulator of seizure susceptibility with diverse anticonvulsant and/or proconvulsant effects in different seizure models (19).

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