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Effect of Lovastatin on PTZ-Induced Seizure Threshold in Mice

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Abstract: The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) have been unequivocally shown to reduce cardiovascular morbidity and mortality by their lipid-lowering actions. It has been suggested that statins appear to have therapeutic benefits in diseases that are unrelated to elevated serum cholesterol levels. The aim of this study was to evaluation the effect of intraperitoneally injection of different doses of lovastatin on PTZ-induced seizure threshold in mice. Mice were divided into 9 groups randomly: the first group received saline normal (i.p) (control group); the second group received carboxymethylcellulose (CMC) 0.5% (i.p) (vehicle group) and the next groups received respectively different doses of lovastatin (1, 5, 10, 20, 40, 80 and 100 mg/kg b. wt. daily) for 4 days (ip) before determination of seizure threshold induced by PTZ. Results showed that PTZ-induced seizure threshold in control mice was 35.48±1.39 mg/kg b. wt.. Intraperitoneal injection of lovastatin showed significant ($P<0.05$) increase of PTZ-induced seizure threshold in a dose dependently manner. According to our results, lovastatin has anticonvulsant effects on mice. Nevertheless, new studies must be carried out in order to determine the beneficial effects of statins in treatment of epilepsy.

Key words: Lovastatin · Seizure · PTZ · Threshold · Mice

INTRODUCTION

Epilepsy is one of the major neurological diseases in humans and about one percent of the population is involved [1, 2]. The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) have been unequivocally shown to reduce cardiovascular morbidity and mortality [3, 4]. Statins are among the most widely used prescription drugs and exert their lipid-lowering actions by reversible and competitive inhibition of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting step in the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, a precursor of cholesterol [5]. It has been suggested that statins appear to have therapeutic benefits in diseases that are unrelated to elevated serum cholesterol levels, such as rheumatologic diseases and ischemic stroke [6]. Statins are also known for their pleiotropic effects, which are independent of their lipid-lowering properties. Among the effects of statins, the most relevant are anti atherosclerotic and anti-inflammatory actions, improvement of endothelial dysfunction, anti-thrombosis and anti-oxidant actions, prevention of Alzheimer's disease and antineoplasic actions [7]. Statins might exert beneficial effects beyond cholesterol reduction; include improving endothelial function, decreasing vascular inflammation, inhibiting smooth-muscle proliferation and immunomodulation. Most of these effects are mediated through inhibition of isoprenoid synthesis, with subsequent effects on multiple downstream signaling pathways [8]. Also statins might exert beneficial neuroprotective effects in various animal disease models and clinical studies [9-11]. Furthermore, studies have shown that statins reduces the extent of brain damage after ischemic insult [12]. Therefore the aim of this study was to evaluate the effect of intraperitoneal injection of different doses of lovastatin on PTZ-induced seizure threshold in mice.

MATERIALS AND METHODS

Animals: Experiments were performed on 72 weighing 22-25 g adult NMRI male mice in their 8-9 week (n=8 for each group), purchased from Razi Institute (Iran). Animals were housed 8 per cage in the Animal House of Veterinary Faculty of Tabriz Branch, Islamic Azad University in a

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temperature (20-22°C) and humidity (50±10%) controlled environment under a 12-hour light/dark cycle (lights on at 7 am). Food and water were available *ad libitum*. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Research affairs of Tabriz University of Medical Sciences, Tabriz-Iran. All efforts were made to minimize the number of animals which were used and their suffering degree. Animals were divided into 9 groups randomly: the first group received saline normal (ip) (control group); the second group received carboxy methyl cellulose (CMC) 0.5% (ip) (vehicle group) and the next groups received respectively different doses of lovastatin (1, 5, 10, 20, 40, 80 and 100 mg/kg b. wt. /daily) for 4 days (i.p) before PTZ-induced seizure threshold.

Chemicals: PTZ (Pentylenetetrazole) was purchased from Sigma-Aldrich. Lovastatin was provided from Merck Pharmaceuticals. All other reagents were of analytical grade. Lovastatin was prepared by being dissolved in 0.5% carboxy methyl cellulose (CMC) and the vehicle group was given an equal volume of vehicle.

PTZ-Induced Clonic Seizure Threshold: Behavioral experiments were done in a quiet, temperature-controlled (20-22°C) room between 10 am and 4 pm. PTZ-induced clonic seizure threshold was determined by inserting a 30-gauge needle into the tail vein of mice and infusion of 0.5% PTZ solution at a constant rate of 1 ml/min to unrestrained freely moving animals. Minimal dose of PTZ (mg/kg) needed to induce forelimb clonus followed by full clonus of the body was recorded as an index of clonic seizure threshold [13, 14].

Data Analysis: Group data are presented as mean±SEM and analyzed statistically using student test. Time course data were analyzed using one-way ANOVA followed by Tukey's post hoc test. The level for statistical significance was set at a $P<0.05$.

RESULTS

PTZ-induced seizure threshold in control mice was 35.48±1.39 mg/kg. In vehicle group, CMC have not shown significant change on PTZ-induced seizure threshold compared with control group (Fig. 1). Intraperitoneal injection of lovastatin showed significant ($P<0.05$) increase of PTZ-induced seizure threshold in a dose dependently manner compared with control and vehicle groups (Fig. 1).

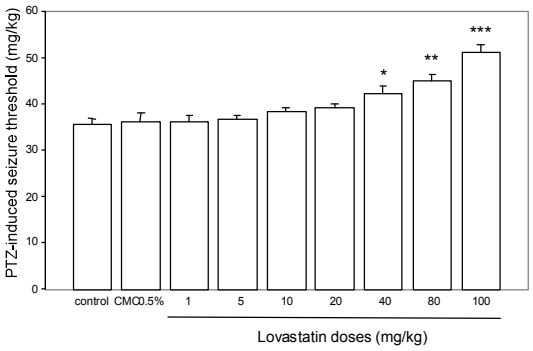

Fig. 1: PTZ-induced seizure threshold in mice (mg/kg). Effect of intraperitoneal injection of different doses of lovastatin on seizure threshold. Each column represents mean±SEM of 8 mice. *P<0.05, **P<0.01 and ***P<0.001 compared with control and vehicle groups.

DISCUSSION

Pentylenetetrazole (PTZ) has been used widely to produce the animal model of chemically induced seizure because this model is highly sensitive for comparing different chemical under standardized conditions [13, 14]. In this study PTZ-induced seizure threshold in control mice was 35.48±1.39 mg/kg and lovastatin increased PTZ-induced seizure threshold in a dose dependently manner. Anti-nociceptive and anti-inflammatory effects of statins have been shown in different experimental animal models of pain and inflammatory and clinical studies [15-19], but so fare effects of statins on epilepsy are very limited [10,11]. Results of this study showed that lovastatin has beneficial neuroprotective effects on PTZ seizure model in mice that in agreement with a recent study that reported an effective neuroprotective action of statins after an acute brain injury and epilepsy [9-11]. Furthermore, recent studies have shown that lovastatin partially suppressed acute experimental autoimmune encephalomyelitis (EAE) in rat spinal cord injury model [20]. Also, studies reported that statin treatment provided anti-inflammatory effects on human multiple sclerosis and X-linked adrenoleukodystrophy [21-28]. Moreover, other research groups have reported the similar neuroprotective role of statins in other neurological disease models, such as traumatic brain injury, brain ischemia and Alzheimer's disease [29-31]. Rangel *et al.* demonstrated that lovastatin treatment was able to prevent hippocampal neuronal loss in CA1 subfield after an epileptic insult [11]. The molecular mechanisms of the anticonvulsant and immunomodulatory properties of statins have not been

fully clarified. Rangel *et al*. suggested that reactive oxygen species (ROS) are a part of normal human metabolism; however, when produced in excess, ROS can cause tissue injury including lipid peroxidation, DNA damage and enzyme inactivation. ROS is a common denominator among acute neurological conditions, including epilepsy. In the pilocarpine model, there is an involvement of excitotoxic neuronal injury and ROS production has been considered to be a part of mechanisms involved with glutamatergic excitotoxicity *in vitro* and *in vivo*. Moreover, it was demonstrated that lovastatin treatment inhibits free radical injury. Thus, this antioxidant effect of statins could explain the neuroprotective properties found in their study [11]. Also, they had been suggested that there is one classical argument supporting a possible role of nitric oxide (NO) in convulsive phenomena: excitatory amino acids, such as N-methyl- D- aspartate (NMDA) and kainate, are known to be potent convulsants and the activation of NMDA receptors is accompanied by the formation of NO. In fact, the role of NO in epileptogenesis has been examined in a number of studies, suggesting to be a proconvulsive endogenous substance. Furthermore, some studies have revealed that statins inhibit the production of NO in brain parenchyma, indicating that statins, secondarily, may play the role of an anticonvulsant substance, does not promoting glutamate-mediated neurotoxicity. Other possibility is that the inhibition of brain endothelial nitric oxide synthase (eNOS) leads to increased blood pressure, which, in turn, may affect the excitability of central nervous system; however, it was demonstrated that statins are able to upregulate eNOS, may be pivotal in enhance cerebral arterial vasodilator responses, decreasing with this, the firing threshold [11]. Also, they had been suggested that anti-inflammatory effects of statins could also contribute to neuroprotection after pilocarpine-induced status epilepticus. Moreover, several studies have implicated a number of cytokines in seizure-related pathology. Therefore lovastatin treatment may provide an important approach to suppression of the inflammatory responses after status epilepticus [11]. In other study, Lee *et al.* reported that statins are inhibitors of HMG-CoA reductase, a rate limiting enzyme for synthesis of cholesterol and isoprenoids, thus, their biological roles are expected to be mediated by their inhibitory roles in cholesterol and isoprenoid synthesis. Indeed, the inhibitory role of statins in cholesterol biosynthesis previously reported to inhibit NMDA mediated excitotoxicity *in vitro* neuron culture study. Also has been reported that statin treatment of cultured neuron cells leads to instability of AMPA receptor by disruption of cholesterol and sphingolipid rich membrane microdomains called "detergent resistant/insoluble membrane domains (DRM/DIM)" or "lipid rafts". Therefore, they had suggested that the possible involvement of cholesterol biosynthesis in statin-mediated anti-excitotoxic activity [10]. At present, whether cholesterol biosynthesis plays a role in statin-mediated anti-seizure and anti-excitotoxicity in the *in vivo* animal model is not known. In the brain, cholesterol has a very long half life (up to several months). Therefore, statins are not expected to change brain cholesterol level readily. Previous studies have reported that systemic statin treatment does not affect cholesterol levels in the brain of guinea pigs. Nevertheless, previously reported reduction in cholesterol levels in lipid raft fractions of synaptosomal plasma membrane suggests that statin treatment may affect intercellular or intracellular distribution of cholesterol which is associated with glutamate receptor function in induction of status epilepticus and excitotoxicity [10]. Along with cholesterol, isoprenoids including farnesyl-pyrophosphate and geranylpyrophosphate are lipid byproducts produced by the mevalonate pathway and implicated in membrane attachment and function of small GTPases. Since the small GTPases (i.e. RhoA and H-Ras) are involved in various neuronal functions and excitotoxic signaling cascades, statins are also expected to exert their anti-seizure and anti-excitotoxic activities through inhibition of isoprenoid synthesis and interfering with small GTPase signaling. Recent *in vivo* reports have demonstrated that inhibition of H-Ras farnesylation by franeyltransferase inhibitor (FTI) treatment inhibits NMDA-mediated excitotoxicity in the rat brain. This report supports that statins may modulate KA-mediated seizure activity and excitotoxicity by down-regulation of H-Ras isoprenylation. However, whether statins exert their anti-seizure and anti-excitotoxic roles through inhibiting synthesis of isoprenoid or cholesterol or both of them is not known at present [10]. Also, Lee *et al.* reported that neuroprotective efficacies of statins are mediated by their anti-inflammatory activity under various neurological disease conditions such as human multiple sclerosis and its animal models, Spinal cord injury, X-linked adrenoleukodystrophy, Alzheimer's disease, traumatic brain injury and stroke. In these studies, statins inhibited inflammatory signal cascades and gene expression through inhibition of isoprenylation of small GTPase (Ras and RhoA). A similar inhibitory role

of statins in inflammatory reactions was observed in KA-treated rats. However, they do not expect that the observed statin-mediated reduction in inflammatory reactions as well as apoptotic cell death in KA-treated rat is mediated by its own anti-inflammatory activity observed in their previous studies. Since post-seizure inflammation and leakage of blood-brain barrier are known to directly correlate with activity and frequency of status epilepticus, the decreased status epilepticus by statin treatment appears to attenuate inflammatory reaction in KA-treated rats [10]. Statins have been used to reduce inflammation, tame immune cell activation, or arrest degenerative processes. Because of their widespread use and long-term safety record, some physicians prescribe statin therapy for non approved indications. A number of case reports describe the dramatic effects of statins added to standard therapies, but beneficial effects need to be confirmed in controlled studies [32]. Nevertheless, new and completely studies must be carried out in order to determine in more detail the beneficial actions of statins regarding the reduction of epilepsy.

CONCLUSION

According to our results, lovastatin has anticonvulsant effects on mice. Nevertheless, new studies must be carried out in order to determine the beneficial effects of statins in treatment of epilepsy.

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