Epilepsy & Behavior 75 (2017) 56–59

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Intranasal pitavastatin attenuates seizures in different experimental models of epilepsy in mice

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ARTICLE INFO

Article history:
Received 6 March 2017
Revised 1 July 2017
Accepted 3 July 2017
Available online 18 August 2017

Keywords:
Pitavastatin
Status epilepticus
Intranasal
Epilepsy
Cognitive impairment

ABSTRACT

This study was carried out to evaluate the effect of intranasal pitavastatin (PVS) on pentylenetetrazole (PTZ)-induced seizures, increasing current electroshock (ICES) seizures, and status epilepticus in mice. Intranasal PVS, 0.5 and 1.0 mg/kg, showed significant increase in latency to PTZ-induced seizures and ICES seizure threshold compared to control; however, the effects were dose-dependent and were more significant at higher dose. Further, intranasal PVS (1.0 mg/kg) but not intravenous PVS (50.0 mg/kg) showed effective protection against PTZ-induced status epilepticus. No impairment in cognitive functions was observed following intranasal PVS (1.0 mg/kg), thus making it a prospective therapeutic approach for acute seizures and status epilepticus.

1. Introduction

Epilepsy is a serious CNS disorder affecting more than 70 million people worldwide, making it one of the most common neurological diseases globally [1]. Epilepsy is characterized by recurrent, unprovoked seizures as a result of electrical instabilities in the brain that can range from brief lapses of attention or muscle jerks to severe and prolonged convulsions [1]. The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) are potent cholesterol-lowering drugs which also possess beneficial antiinflammatory, antioxidant, immunomodulatory, and antiexcitotoxic effects [2–4]. Statins have proven neuroprotective effects in several neurological diseases such as Alzheimer's and Parkinson's disease, cerebral ischemia, multiple sclerosis, and traumatic brain injury. Several in vitro and in vivo studies have demonstrated the potential antiseizure properties of statins, particularly atorvastatin [5], simvastatin [6], fluvastatin [7], lovastatin [8], and pitavastatin [9] in epilepsy induced by pentylenetetrazole (PTZ), pilocarpine, or increasing current electroshock (ICES). Most of the published reports demonstrated the antiseizure activity of statins following oral or intravenous doses at high concentration. Probably, statin has to cross highly lipophilic blood–brain barrier, following oral or intravenous dosing, in order to produce the effect resulting in use of their high doses [4]. Further, dose-dependent side effects of statins, in particular myopathy, rhabdomyolysis, and cognitive impairment are of concern [10,11].

Recent development in drug delivery highlights the use of intranasal administration as a novel route for rapid administration of drugs to brain [12–15]. Intranasal drug administration is painless, does not require skilled personnel, and is immediately available for all patients. The nasal cavity permits topically administered drugs to rapidly achieve effective blood levels avoiding gastrointestinal destruction and hepatic first-pass metabolism, improving bioavailability compared with oral administration [13]. Additionally, the intranasal delivery exploits nose–brain pathway to bypass the blood–brain barrier and deliver the drug directly to the brain [14,15]. The present study explores the neuroprotective effect of intranasal pitavastatin (PVS), a new statin that exhibits potent antihyperlipidemic activity, against seizures induced by PTZ [16], ICES [17], and in emergency condition—status epilepticus [18]. Further, the effect of intranasal PVS on cognitive impairment was assessed to evaluate the safety of the treatment.

2. Materials and methods

2.1. Materials

Pitavastatin was received as gift sample from Mylan Laboratories Limited (Hyderabad, India). Pentylenetetrazole (PTZ) and phenytoin

Abbreviations: AEDs, antiepileptic drugs; ICES, increasing current electroshock; PTZ, pentylenetetrazole; PVS, pitavastatin.

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http://dx.doi.org/10.1016/j.yebeh.2017.07.004
1525-5050/

were purchased from Sigma Aldrich, Inc. (St. Louis, MO, USA). Pitavastatin solution was prepared by dissolving its weighed quantity in aqueous solution of PEG 400. Pentylenetetrazole and phenytoin solutions were prepared in normal saline.

2.2. Animals

Healthy Swiss albino male mice weighing between 20 g and 35 g were used for the studies. The animals were housed in a temperature-controlled room (18–24 °C) and provided with free access to food and water. All care and handling of animals were in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with the approval (Approval No. 114) of Institutional Animal Ethics Committee, Jamia Hamdard, New Delhi.

2.3. Drug administration protocol

To assess the effects of intranasal PVS on seizures, status epilepticus, and cognition, the animals were divided into different groups with each group containing 6 mice (n = 6). Five groups were used for PTZ- and ICES-induced seizure response study. Two groups received different doses of intranasal PVS, i.e., 0.5 and 1.0 mg/kg. Untreated animals and vehicle-treated (intranasal PEG aqueous solution) animals were used as controls, whereas group receiving intravenous PVS (50.0 mg/kg) was used for comparison. To study the acute effects of intranasal PVS on seizures, the experiments were performed on the seventh day of dosing, 30 min after the administration of PVS [5]. For status epilepticus studies, three groups (n = 6) were used. One group received intranasal PVS (1.0 mg/kg), untreated animals served as the control, and intravenous PVS (50.0 mg/kg) was used for comparison. To study the effects of intranasal PVS in status epilepticus, where immediate action is required, a single-dose protocol was followed, and the experiment was performed 30 min after a single dose of PVS [5]. For cognitive assessment, two groups (n = 6) were used, and intranasal PVS (1.0 mg/kg) was evaluated against untreated animals as control. The experiments were performed on the seventh day of dosing, 30 min after the administration of PVS.

3. Experimental design

3.1. PTZ-induced seizures

PTZ seizures were induced by intraperitoneal injection of PTZ solution at a dose of 60 mg/kg. This dose of PTZ produced myoclonic jerks and generalized seizures in all the animals without mortality. The latency to myoclonic jerks and generalized seizures was observed immediately after PTZ injection for a period of 30 min. In the absence of seizures, 30 min (1800 s) was taken as the latency time [16].

3.2. ICES-induced seizures

The ICES test was used to evaluate the anticonvulsant effect of PVS. Electroshock, starting with a current of 2 mA, was applied via ear electrode (forceps style) using electro-convulsometer (Microteknik, India). The electroshock was given as a single train of pulse for 0.2 s with linearly increasing intensity of 2 mA/2 s. The current at which tonic hind limb extension (HLE) occurred was recorded as the seizure threshold current. If no tonic HLE was observed by a current of 30 mA, electroshock was terminated, and this cutoff current was used in the analysis [17].

3.3. Status epilepticus

Status epilepticus was induced in the mice by subcutaneously injecting PTZ at a dose of 80 mg/kg in the loose skin behind the neck [18]. The volume of the injection was 0.1 mL/10 g body weight. Two hours prior to PTZ administration, phenytoin sodium (40 mg/kg) dissolved in alkalinized saline was administered intraperitoneally (volume: 0.1 mL/10 g body weight) to prevent the terminal tonic HLE produced by PTZ. The time required for the development of unequivocal sustained clonic seizure activity involving the limbs (isolated myoclonic jerks were not counted) was carefully noted. Seizure-free state for a period of 30 min was taken as protection. Observations were made 30 min after administration of PVS.

3.4. Cognitive assessment

3.4.1. Pole climbing test

Cook's pole climbing apparatus consists of a grid floor composed of stainless steel rods capable of delivering electric shock. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber. The animal under investigation was gently placed on the wooden platform set in the center of the grid floor. This platform served as a shock-free zone. When the mouse stepped down and placed all its paws on the grid floor, electric shock (20 V, 50 Hz, 1 mA, 1 s) was delivered for 15 s and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Mice showing SDL in the range (2–15 s) during the first test were used for the second session and the retention test. The second session was carried out 90 min after the first test. When mice stepped down before 60 s, electric shock was delivered for 15 s. During the second test, animals were removed from shock-free zone if they did not step down for a period of 60 s. Memory retention was tested after 24 h in a similar manner, except that electric shocks were not applied to the grid floor. Each subject was again placed on the platform, and the SDL was recorded, with an upper cutoff time of 600 s.

3.4.2. Forced swim test

Mice was dropped one at a time in a plexiglass cylinder (height 25 cm and diameter 10 cm) containing water up to a height of 9 cm at room temperature and left for 15 min. After a brief spell of vigorous activity, they showed a posture of immobility which is characterized by floating motionless in the water, making only those movements necessary to keep the head above water. This immobility reflects a state of depression. After allowing 1 min for acclimatization, each animal was observed for 15 min for immobility. Thus, immobility time (i.e., total duration of immobility in a period of 5 min) was recorded for each animal.

3.4.3. Elevated plus maze test

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (50 cm × 10 cm) and two covered arms (50 cm × 40 cm × 10 cm). The arms extended from a central platform (10 cm × 10 cm) and the maze was elevated to a height of 50 cm from the floor. The animals were placed individually at the either end of the open arms and allowed to enter the closed arms. If the animal fails to enter the closed arm within 180 s during first screening, it was not included in the experiment. During training, if the animal did not enter the closed arm within 180 s, it was gently pushed in the closed arm. To become acquainted with the maze, the animals were allowed to explore the maze for 20 s after reaching the closed arm and then returned to their home cage. The learning was tested 30 min later on the same day, and the animals were re-tested 24 h after the first day training to test the retention of memory. The time taken by the animal to move from the open arm to the closed arm is noted as transfer latency (TL). A time of 180 s was taken as cutoff, and animals not entering the closed arm in this period were assigned the TL of 180 s. A long latency period to reach enclosed arm indicates poor retention compared to significantly shorter latencies.