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Effect of atorvastatin on behavioral alterations and neuroinflammation during epileptogenesis

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ABSTRACT

Temporal lobe epilepsy (TLE) is the most frequent and medically refractory type of epilepsy in humans. In addition to seizures, patients with TLE suffer from behavioral alterations and cognitive deficits. *Poststatus epilepticus* model of TLE induced by pilocarpine in rodents has enhanced the understanding of the processes leading to epilepsy and thus, of potential targets for antiepileptogenic therapies. Clinical and experimental evidence suggests that inflammatory processes in the brain may critically contribute to epileptogenesis. Statins are inhibitors of cholesterol synthesis, and present pleiotropic effects that include antiinflammatory properties. We aimed the present study to test the hypothesis that atorvastatin prevents behavioral alterations and proinflammatory state in the early period after pilocarpine-induced *status epilepticus*. Male and female C57BL/6 mice were subjected to *status epilepticus* induced by pilocarpine and treated with atorvastatin (10 or 100 mg/kg) for 14 days. Atorvastatin slightly improved the performance of mice in the open-field and object recognition tests. In addition, atorvastatin dose-dependently decreased basal and *status epilepticus*-induced levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ) and increased interleukin-10 (IL-10) levels in the hippocampus and cerebral cortex. The antiinflammatory effects of atorvastatin were qualitatively identical in both sexes. Altogether, these findings extend the range of beneficial actions of atorvastatin and indicate that its antiinflammatory effects may be useful after an epileptogenic insult.

1. Introduction

Epilepsy is a brain disease in which affected individuals have an enduring predisposition to present unprovoked recurrent seizures [1]. With a worldwide prevalence of 0.5–1% in the general population [2] and affecting at least 50 million people worldwide [3], epilepsy is a major worldwide public health problem, being one of the most frequent neurological conditions [4]. In addition to seizures, behavioral comorbidities of epilepsy like anxiety, psychosis, depression, and cognitive deficits exist in many patients with epilepsy, worsening their quality of life [5,6].

Temporal lobe epilepsy (TLE) is a common type of this disease, and it is often elicited by a brain insult [7]. Such event triggers a myriad of cellular and molecular changes that increase the chance of developing epilepsy [7]. The period between the initial insult and the appearance of spontaneous seizures is called epileptogenesis, and may represent the best window of opportunity to modify the disease progression [8]. One of the possible targets of epilepsy-preventing strategies is the inflammatory response that establishes in the brain after the initial epileptogenic injury [9]. In fact, compelling evidence from experimental and clinical studies has suggested that inflammatory mediators in the brain play an etiological role in epileptogenesis, as well as in the accompanying comorbidities and neuropathology of epilepsy [10]. Accordingly, strategies aiming to reduce the levels of inflammatory cytokines and other mediators may constitute a potential antiepileptogenic therapy [9].

Atorvastatin is the leading drug of the class of statins, the first-line medications for the treatment of hypercholesterolemia and prevention of associated cardiovascular burden [11]. Evidence of antiinflammatory and neuroprotective effects of atorvastatin has been obtained in many experimental models of neurological diseases, including epilepsy [12–14]. Moreover, atorvastatin protects from behavioral comorbidities of epilepsy [14,15]. Therefore, considering the need for developing disease-modifying strategies for epileptogenesis, in the present study, we investigated whether atorvastatin improves

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Table 1
Data about animals used in the study.

	Male	Female
Number of animals used to *status epilepticus* induction[a]	83	82
Mice with *status epilepticus*[b]	39	43
Mice resistant to *status epilepticus*[c]	12	10
Mortality during *status epilepticus*[d]	32	29
Total mortality[e]	15	11
Control animals[f]	27	28
Control animals mortality[g]	3	0
Total	110	110

[a] Number of animals submitted to pilocarpine injections.
[b] Number of animals that entered *status epilepticus*.
[c] Number of animals that did not enter in *status epilepticus* after 6 pilocarpine injections.
[d] Number of animals that died within the 60-minute *status epilepticus* period.
[e] Number of animals in *status epilepticus* that died in the 14-day follow-up.
[f] Number of animals submitted to NaCl 0.9% (pilocarpine vehicle) injections.
[g] Number of control animals that died in the 14-day follow-up.

short-term inflammatory response and behavioral alterations after pilocarpine-induced *status epilepticus* (SE). Given the importance of including both sexes in the preclinical research [16], and the need to test new drugs to treat epilepsy in both sexes [17,18], we performed the present study in male and female mice.

2. Materials and methods

2.1. Animals

One hundred ten C57BL/6 mice (25–35 g; 30–60 day-old) of each sex were used. They were kept under appropriated environmental conditions (12 h light–dark cycle, in a room temperature of 22 ± 1 °C). Standard rodent chow (Puro Lab 22 PB, Puro Trato) and filtered tap water were provided ad libitum. All experimental procedures were conducted in accordance with national (Guidelines of the Brazil's National Council for the Control of Animal Experimentation, revised in 2016) and international legislations (Guidelines of the National Institutes of Health of United States of America for the care and use of laboratory animals, revised in 2011), and with the approval of the Ethics Committee for Animal Research of our University (Process #6165230415/2015). We made every possible effort to limit animal's suffering and to keep their number to a minimum.

2.2. Pilocarpine-induced status epilepticus

Epileptogenesis was elicited by a single *status epilepticus* induced by pilocarpine, using a multiple low dose protocol as described previously [19]. Because of the severe peripheral adverse cholinergic associated with pilocarpine, mice received a previous injection of methylscopolamine (1 mg/kg *ip*; Sigma-Aldrich). After 30 min, pilocarpine hydrochloride (Sigma-Aldrich) (100 mg/kg, *ip*) was injected every 20 min until the onset of *status epilepticus*. The maximum number of pilocarpine injections per animal was 6. *Status epilepticus* was stopped after 60 min with diazepam (10 mg/kg, *ip*, Santisa). Age- and weight-matched control animals also received methylscopolamine and diazepam, but NaCl 0.9% instead of pilocarpine. During the 3 days after *status epilepticus* induction, all mice received special attention for welfare purposes. Special care included hand-offering of softened chow, inserting fresh fruits (apples and bananas) into their homecages, and injections of Ringer-lactate solution containing 5% dextrose.

Complete data about animals and *status epilepticus* are shown in Table 1.

2.3. Treatment with atorvastatin

Treatment with atorvastatin started 3 h after diazepam injection and lasted 14 days. Control and *status epilepticus* mice received daily doses of vehicle (0.9% NaCl) or atorvastatin (10 or 100 mg/kg) by intragastric gavage. Atorvastatin solution was freshly prepared by dissolving commercial tablets (Lipitor®; Pfizer, SP, Brazil) to 1 or 10 mg/ml [20,21] in 0.9% NaCl. All solutions were administered at 10 ml/kg. The selection of atorvastatin dosing was based on previous studies [22] and on pilot studies.

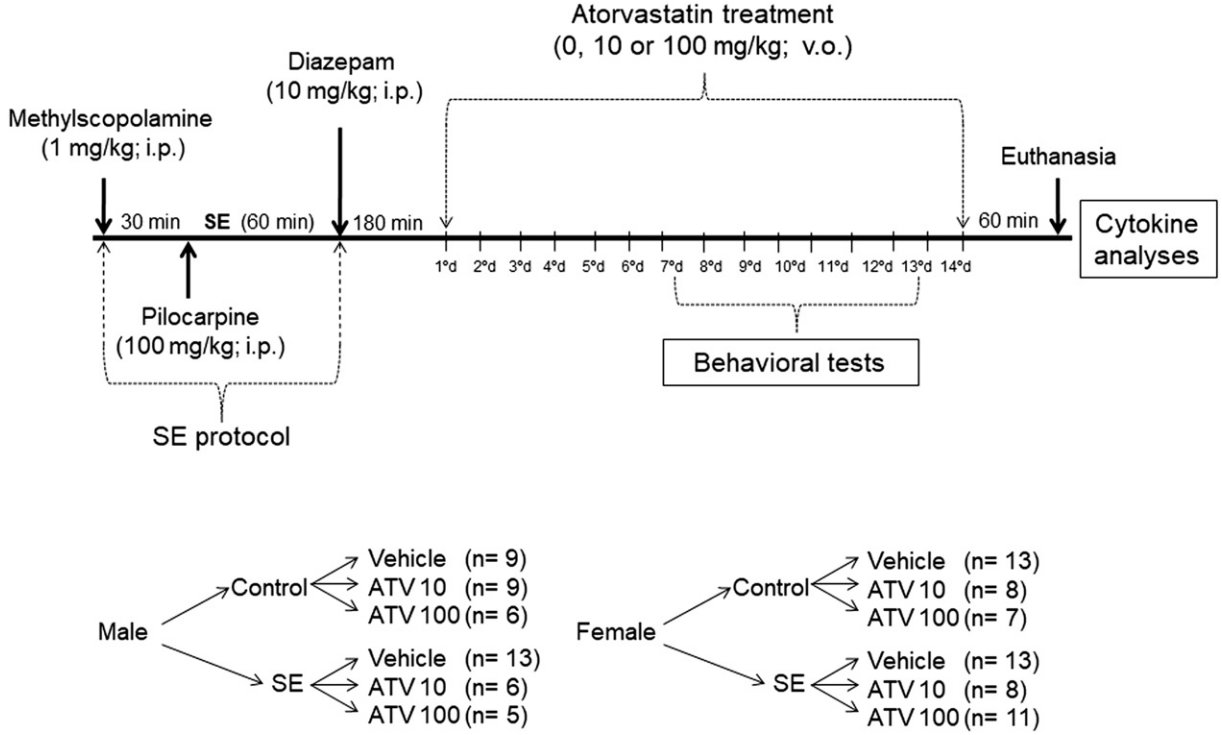

Fig. 1. Schematic illustration of the experimental protocol used in this study. Numbers in parentheses indicate the number of animals in each experimental group.