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# Lovastatin decreases the synthesis of inflammatory mediators during epileptogenesis in the hippocampus of rats submitted to pilocarpine-induced epilepsy

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ABSTRACT

Statins may act on inflammatory responses, decreasing oxidative stress and also reducing brain inflammation in several brain disorders. Epileptogenesis is a process in which a healthy brain becomes abnormal and predisposed to generating spontaneous seizures. We previously reported that lovastatin could prevent neuroinflammation in pilocarpine-induced *status epilepticus* (SE). In this context, this study investigated the long-lasting effects of lovastatin on mRNA expression of proinflammatory cytokines (interleukin-1β, tumor necrosis factor α, interleukin-6) and the antiinflammatory cytokine IL-10 in the hippocampus during epileptogenesis by immunohistochemistry and real time polymerase chain reaction (RT-PCR) during the latent and chronic phases in the epilepsy model induced by pilocarpine in rats. For these purposes, four groups of rats were employed: saline (CONTROL), lovastatin (LOVA), pilocarpine (PILO), and pilocarpine plus lovastatin (PILO + LOVA). After pilocarpine injection (350 mg/kg, i.p.), the rats were treated with 20 mg/kg of lovastatin via an esophagic probe 2 h after *SE* onset. All surviving rats were continuously treated during 15 days, twice/day. The pilocarpine plus lovastatin group showed a significant decrease in the levels of IL-1β, TNF-α, and IL-6 during the latent phase and a decreased expression of IL-1β and TNF-α in the chronic phase when compared with the PILO group. Moreover, lovastatin treatment also induced an increased expression of the antiinflammatory cytokine, IL-10, in the PILO + LOVA group when compared with the PILO group in the chronic phase. Thus, our data suggest that lovastin may reduce excitotoxicity during epileptogenesis induced by pilocarpine by increasing the synthesis of IL-10 and decreasing proinflammatory cytokines in the hippocampus.



## 1. Introduction

Epilepsy is a brain disorder characterized by recurrent, spontaneous seizures. A particularly common form of epilepsy is temporal lobe epilepsy (TLE). The presence of spontaneous seizures may trigger inflammatory cascade in specific brain regions, raising the possibility that inflammatory mediators could modify the processes by which a normal neuronal network becomes a seizure-generating focus. This abnormal network generation may occur after a brain insult, and this process is called epileptogensis [1], which results in spontaneous seizure onset [2–4]. According to these authors, this epileptogenic process occurs during a silent period, with the absence of abnormal clinical and electroencephalographic (EEG) signals, being followed by the appearance of spontaneous and recurrent seizures.

Previous reports from our group have described an important role of inflammatory processes in epilepsy, showing an increased expression of several inflammatory markers such as kinin receptors, cytokines, and nitric oxide in the hippocampus of humans and rodents submitted to experimental models of epilepsy [5–10]. Thus, cytokines have been linked to the pathogenesis of epilepsy, since their levels are increased after limbic seizures [3].

Molecules such as IL-1β, TNF-α, and IL-6 are expressed at very low levels in a normal brain. In contrast, their mRNA and protein levels are quickly increased (≤30 min) after the induction of seizures, declining to basal levels within 48–72 h after seizure onset [11]. Thus,

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antiinflammatory strategies have been tested, and new evidence has have emerged showing that antiinflammatory molecules may protect the brain during the epileptogenesis. Consequently, the mechanism of action of several drugs has been studied in brain, after a severe insult.

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG–CoA) reductase inhibitor and are orally administered as cholesterol-lowering drugs. The enzyme HMG–CoA reductase catalyzes the conversion of HMG–CoA to L-mevalonate. Statins prevent biological activities downstream of L-mevalonate because of their inhibitory effect [12]. Furthermore, several lines of evidence have associated lipid metabolism with inflammatory processes linked to the progressive nature of neurodegenerative diseases [13].

Statin intake has been associated with a decrease in the incidence of Alzheimer's disease (AD) [14], cerebral ischemia [15], and multiple sclerosis [16].

In epilepsy, previous data from our group have shown that lovastatin is able to decrease the neuronal death rate induced by long-lasting *status epilepticus* (SE) [17]. We also showed that statin controls hyperthermia, reducing the levels of IL-1β, TNFα, IL-6, kinin B1, and B2 receptor mRNA production in the hippocampus of rats during SE [7].

Thus, this work was delineated to understand how lovastatin can exert its long-lasting action in the hippocampus of rats during the epileptogenic process triggered by SE. In this context, the present work analyzed the effect of lovastatin in the modulation of IL-1β, TNF-α, IL-6, and IL-10 production during the silent (latent) and chronic periods of pilocarpine-induced temporal lobe epilepsy.

## 2. Materials and methods

### 2.1. Animal treatment

The animal experiments were performed under UNIFESP Ethical Committee Institutional and approved (Hospital São Paulo/Universidade Federal de São Paulo, process n. 0653/10), and all efforts were made to minimize animal suffering. Wistar adult male rats, weighing 250 g, were housed in groups of three to four per cage and maintained in controlled room temperature, humidity, and light–dark cycle (12:12 h) with chow pellets and tap water available ad libitum. The rats received a single dose of pilocarpine (350 mg/kg, intraperitoneal [i.p.]). To prevent peripheral cholinergic effects, scopolamine methylnitrate was injected subcutaneously at a dose of 1 mg/kg, 30 min before pilocarpine administration. Only rats that displayed SE were included in this study. The SE was monitored and terminated with an injection of diazepam (10 mg/kg ip; Roche, Brazil) 3 h after *SE* onset.

### 2.2. Drug administration

We analyzed the latent period (15 days after pilocarpine injection) and chronic period (30 days after first spontaneous seizure), and each group was composed of the following: saline (CONTROL, n = 5), lovastatin (LOVA, n = 5), pilocarpine (PILO, n = 8), and pilocarpine plus lovastatin (PILO + LOVA, n = 8). After pilocarpine injection (350 mg/kg, i.p.), the rats were treated with 20 mg/kg of lovastatin via an esophagic probe after 2 h of *SE* onset. All rats were continuously treated during 15 days, twice daily.

### 2.3. Quantitative real-time PCR

Rats from the above groups (n = 5 for each group) were sacrificed, and their brains were isolated and dissected, and the hippocampi were frozen in liquid nitrogen and stored at −80 °C. Thawed tissue was homogenized in 1-mL TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and total RNA was isolated according to the manufacturer's instructions. Ribonucleic acid was subjected to DNase I digestion followed by reverse transcription to cDNA, and PCR was performed in a 7000 Sequence Detection System (ABI Prism, Applied Biosystems, Foster City, CA) using the SYBR Green core reaction kit (Applied Biosystems). Primers used for cytokine mRNA quantification were as follows:

| | |
|---|---|
| IL-1β | F: 5′-CACCTCTCAAGCAGAGCACAG-3′ |
| | R: 5′-GGGTTCCATGGTGAAGTCAAC-3 |
| TNF-α | F: 5′-AAATGGGCTCCCTCTATCAGTTC-3′ |
| | R: 5′-TCTGCTTGGTGGTTTGCTACGAC-3′ |
| IL-6 | F: 5′-TCCTACCCCAACTTCCAATGCTC-3′ |
| | R: 5′-TTGGATGGTCTTGGTCCTTAGCC-3′ |
| IL-10 | F: 5′-AAAGCAAGGCAGTGGAGCAG-3′ |
| | R: 5′-TCAAACTCATTCATGGCCTTGT-3′ |
| GAPDH | F: 5′-GGGCAGCCCAGAACATCAT-3′ |
| | R: 5′-CCGTTCAGCTCTGGGATGAC-3′ |

Quantitative values for cytokine (IL-1β, TNF-α, IL-6, and IL-10) mRNA transcription were obtained from the threshold cycle number, where the increase in the signal associated with an exponential growth of PCR products begins to be detected. Melting curves were generated at the end of every run to ensure product uniformity. The relative target gene expression level was normalized on the basis of GAPDH expression as endogenous RNA control. $\Delta C_t$ values of the samples were determined by subtracting the average $C_t$ value of cytokine mRNA from the average $C_t$ value of the internal control GAPDH. To express the relative expression data, we used $2\text{-}(\Delta)C_t$ parameter.

### 2.4. Statistical analysis

Expression levels of cytokine mRNA were presented as an n-fold difference relative to the levels expressed during the inflammatory process, and the different treatments were compared using the one-way ANOVA followed by the Student–Newman–Keuls *t*-test. The data were presented as means ± S.D., and $p < 0.05$ was considered statistically significant. Only data that showed a normal distribution were considered.

### 2.5. Immunohistochemistry

The rats were anesthetized with 0.1 mL of chloral hydrate (4%) and subjected to transcardiac perfusion with a solution of paraformaldehyde 1% (pH 7.4, 15 mL/rat, infusion rate: 15 mL/min) followed by a solution of paraformaldehyde (4%) (pH 7.4, 150 mL/rat, infusion rate: 15 mL/min). After perfusion, the brain was carefully removed from the skull, postfixed in paraformaldehyde (4%) for 48 h, and immersed in a solution of sucrose (30%) for cryoprotection for 48 h. Forty-micrometer-thick coronal slices were obtained using a cryostat (HM 505E Micromeria, Zeiss) and stored in 0.1-M phosphate buffer (pH 7.4). The slices were collected throughout the hippocampus and stored in 0.1-M phosphate buffer. The slices were mounted on gelatin-coated slides for immunohistochemistry with the antibodies anti-IL-1β (IBL, 1:100), IL-6 (Abcam, 1:50), IL-10 (R&D, 1:100), and TNF-α (IBL, 1:100).

The immunohistochemistry was performed in the same way for all primary antibodies in all groups. Briefly, free-floating slices were treated with hydrogen peroxide (1%) for 10 min, washed with phosphate-buffered saline (PBS, pH 7.4) several times, and then treated with Triton X-100 (0.4%) for 30 min. Slices were washed with PBS, preincubated with albumin (10%) for 2 h, and incubated with primary antibody overnight at 4 °C. The next day, the slices were washed and then incubated at room temperature with appropriate secondary antibodies (1:200, biotinylated immunoglobulin G, Calbiochem) for 2 h. Sections were well washed and incubated in avidin–biotin–peroxidase complex (ABC Kit, Vector) for 90 min, washed again with Tris–HCl (pH 7.6), and finally developed with diaminobenzidine (DAB, 1 tablet/15 mL of Tris–HCl). Slices were finally washed in PBS and mounted on histological slides.

## 3. Results

### 3.1. Animal behavior

Pilocarpine administration induced the following behavioral changes: akinesia, facial automatisms, and limbic seizures consisting of forelimb clonus with rearing, salivation, and masticatory jaw movements and falling. This type of behavior built up progressively into motor limbic seizures that recurred repeatedly, evolving to long-lasting *SE* [19].

### 3.2. Real-time PCR analysis for cytokines

#### 3.2.1. Latent period

Cytokine mRNA levels were analyzed using a quantitative RNA assay, and IL-1β, TNF-α, and IL-6 showed increased expression in the hippocampus of the PILO group when compared with the CONTROL group. In contrast, the PILO + LOVA group showed an important decrease in IL-1β ($p < 0.05$), TNF-α ($p < 0.001$), and IL-6 ($p < 0.01$) when compared with the PILO group. By contrast, IL-10 mRNA presented similar values in all groups (Fig. 1).

#### 3.2.2. Chronic period

Interleukin-1 beta and TNF-α mRNAs showed an increased expression in the PILO group when compared with the CONTROL group. However, the PILO + LOVA group showed an important decrease in IL-1β ($p < 0.05$) and TNF-α ($p < 0.001$) levels in the hippocampus when compared with the PILO group. Interleukin 6 mRNA expression showed no difference between studied groups. Interestingly, IL-10 mRNA expression levels were increased in the PILO + LOVA group when compared with the PILO group ($p < 0.05$) (Fig. 2).

### 3.3. Immunohistochemistry

Interleukin-1 beta was localized in all hippocampal formations, mainly in experimental animals. In the PILO group, during silent and chronic periods (Figs. 3A III and 4A III), this staining was increased when compared with the CONTROL (Figs. 3A I and 4A I) and LOVA groups (Figs. 3A II and 4A II), mainly in regions such as CA1, CA3, and *stratum lacunosum–moleculare*. However, in the PILO + LOVA group, this immunoreactivity was decreased in the same regions of hippocampal formation during the latent (Fig. 3A IV) and chronic periods (Fig. 4A IV).

Tumor necrosis factor-alpha was visualized in the hippocampal formation as staining of neurons from the dentate gyrus and hilus. However, no difference between the PILO and PILO + LOVA groups was found in either period.

Regarding IL-6 distribution in the hippocampus of PILO group, in both the silent and chronic periods (Figs. 3C III and 4C III), we found cells with an the shape of astrocytes stained with more intensity, when compared with CONTROL (Figs. 3C I and 4C I) and LOVA groups (Figs. 3C II and 4C II). In contrast, the PILO + LOVA group (Figs. 3C IV and 4C IV) presented the same staining observed in the CONTROL and LOVA groups in both periods, showing a disappearance of cells with the shape of astrocytes.

Interleukin-10 was also localized throughout all hippocampal formations of all groups. This immunoreactivity was visualized in major intensity in the PILO group during latent (Fig. 3D III) and chronic (Fig. 4D III) periods when compared with the CONTROL (Figs. 3D I and 4D I) and LOVA groups (Figs. 3D II and 4D II). However, the PILO +LOVA group (Figs. 3D IV and 4D IV) presented more cells with the shape of microglia stained by IL-10 antibody when compared with the CONTROL (Figs. 3D I and 4D I) and LOVA groups (Figs. 3D II and 4D II) during the chronic phase.

## 4. Discussion

Pilocarpine-induced SE ([18,19]) simulates a brain injury and is associated with the development of temporal lobe epilepsy in rodents and is an appropriate model to study epileptogenesis. Thus, events occurring during this period are critically important and may reveal targets for pharmacological intervention. Successfully modifying these targets during epileptogenesis will likely reduce neuronal death and inflammation.

The present study demonstrated that continuous treatment with lovastatin, after induced SE, reduced several important hippocampal cytokines related to brain inflammation during epileptogenesis and during the period of spontaneous seizures. In addition, an increased expression of IL-10, an antiinflammatory cytokine, was increased during the chronic phase, in those rats treated with lovastatin.

Studies with statins have shown that these drugs activate several neuroprotective signaling pathways. Zacco et al. [20] reported that several statins render mouse primary cortical neurons more resistant to excitotoxicity in a cholesterol-dependent manner. This action is related to the efficacy of statins in blocking HMG–CoA reductase [21]. This idea was reinforced by the reversal of this effect that was observed by the addition of mevalonate or cholesterol into cell culture [20].

However, in vivo experiments have demonstrated that statin treatment increases neurogenesis and synaptogenesis without altering serum cholesterol levels after brain damage [22–25].

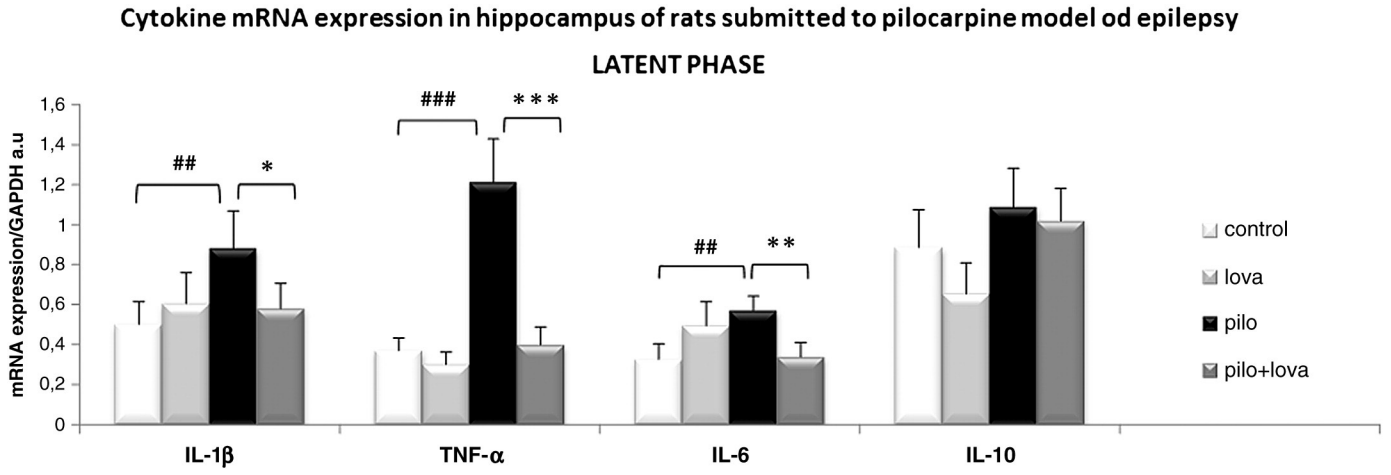


**Fig. 1.** Quantification of mRNA expression by RT-PCR assay. Expression levels of mRNA of IL-1β, TNF-β, IL-6, and IL-10. All data have been normalized for levels of GAPDH expression within the same sample and are expressed relative to levels detected in each experimental group in the latent period. (#) Comparison between the PILO group with the CONTROL group ($^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.001$). (*) Comparison between the PILO + LOVA group with the PILO group ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$). The difference between groups was evaluated using one-way ANOVA followed by the Student Newman Keuls *t*-test.

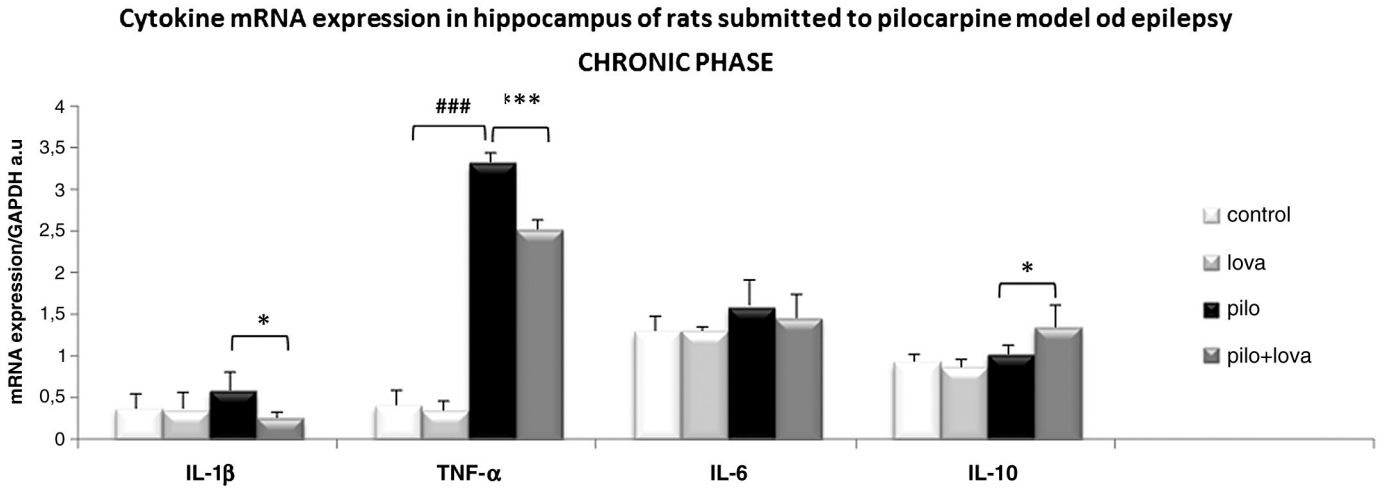


**Fig. 2.** Quantification of mRNA expression by RT-PCR assay. Expression levels of mRNA of IL-1β, TNF-β, IL-6, and IL-10. All data have been normalized for levels of GAPDH expression within the same sample and are expressed relative to levels detected in each experimental group in the chronic period. (#) Comparison between the PILO group with the CONTROL group ($^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.001$). (*) Comparison between the PILO + LOVA group with the PILO group ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$). The difference between groups was evaluated using one-way ANOVA followed by the Student Newman Keuls *t*-test.

Hering et al. [26] also observed that treatment with statins would lead to instability of the AMPA receptor in cultured neurons. This effect occurs due to modification of cholesterol and sphingolipid-rich microdomains known as insoluble membrane domains or "lipid rafts". However, in vivo studies suggested the possible involvement of cholesterol biosynthesis in antiexcitotoxic activity mediated by statins. So far, it is not known how the cholesterol biosynthesis could mediate this activity in vivo. However, the reduction of cholesterol levels in fractions of these "lipid rafts" on the plasma membrane of synaptosomes [27] suggested that statin treatment could affect intracellular and extracellular distribution of cholesterol, which is associated with glutamate receptor function during induced excitotoxicity.

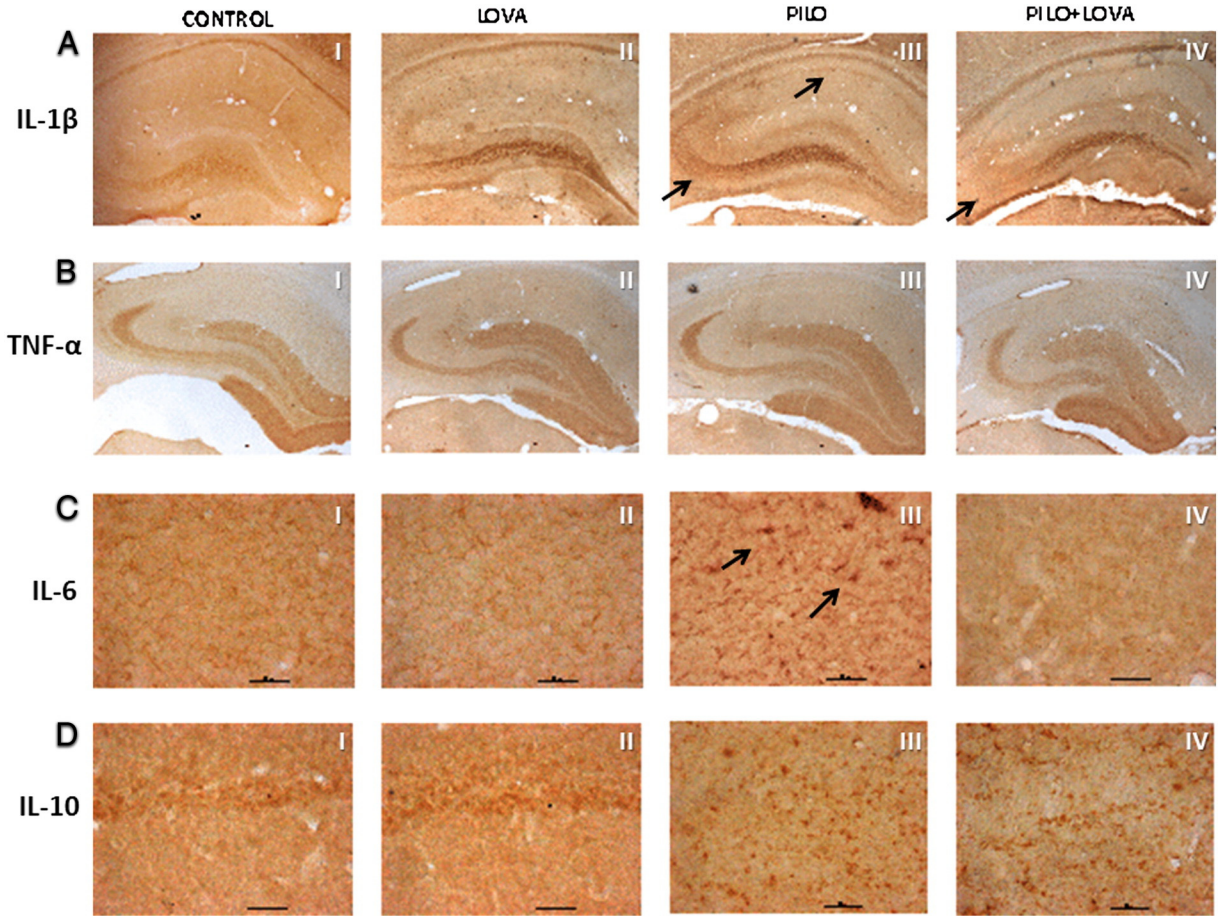


**Fig. 3.** Immunohistochemical analysis of cytokines in the hippocampus in the CONTROL, LOVA, PILO, and PILO + LOVA groups in the latent phase. A: Interleukin-1β (IL-1β). B: Tumor necrosis factor-α (TNF-α). C: Interleukin-6 (IL-6), and D: Interleukin-10 (IL-10). Scale bar = 100 μm.

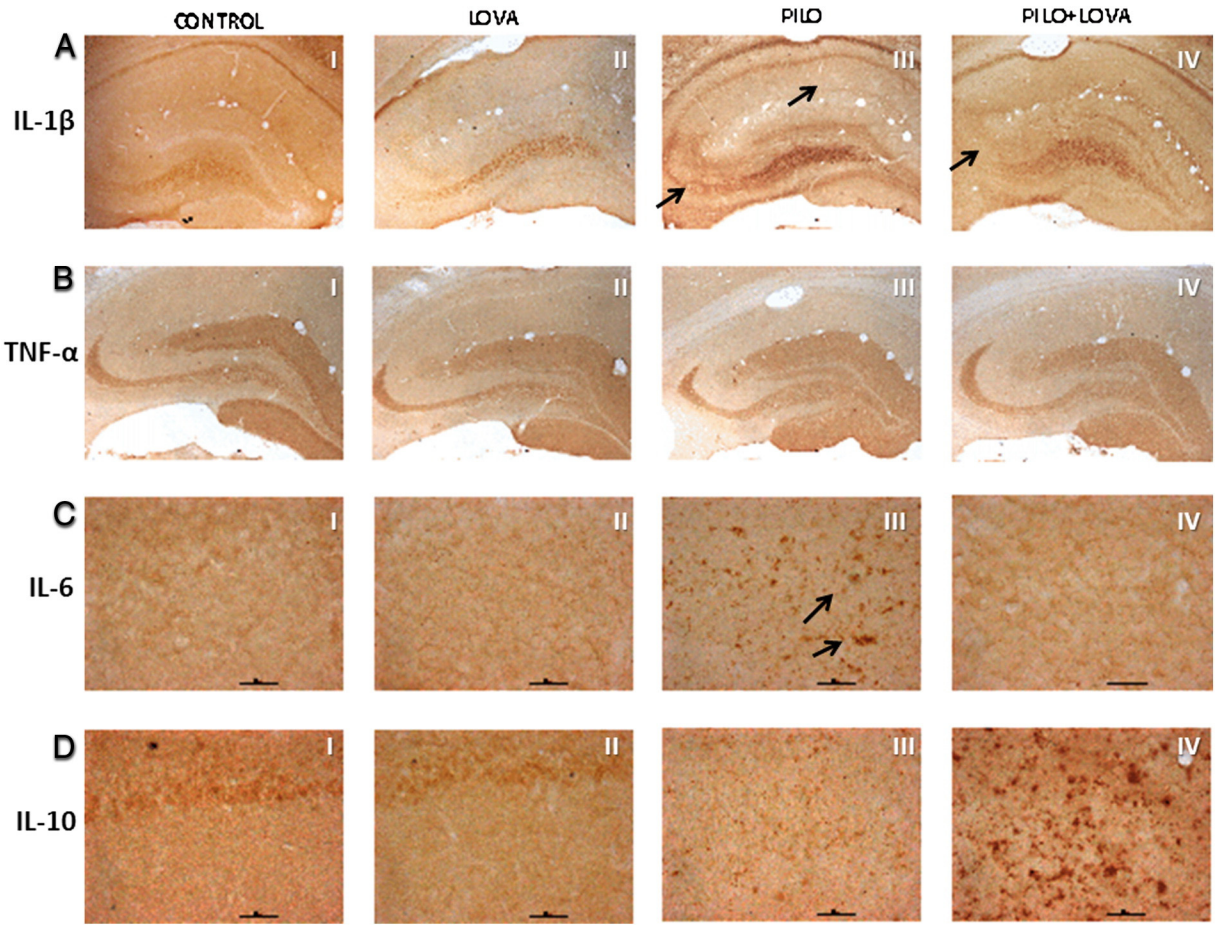


**Fig. 4.** Immunohistochemical analysis of cytokines in the hippocampus in the CONTROL, LOVA, PILO, and PILO + LOVA groups in the chronic phase. A: Interleukin-1β (IL-1β). B: Tumor necrosis factor-α (TNF-α). C: Interleukin-6 (IL-6), and D: Interleukin-10 (IL-10). Scale bar = 100 μm.

In the present study, we observed a significant increase in the IL-1β transcript as well as in its hippocampal distribution in all phases of the model. Intense staining was visualized in CA1 and CA3 in *stratum lacunosum–moleculare* and in the dentate gyrus of the PILO group, which was reduced in animals from the PILO + LOVA group, suggesting that lovastatin promoted an antiinflammatory action, interfering in the inflammatory cascade triggered by induced SE.

Interleukin-1 beta may involve the induced synthesis of other cytokines as well as IL-6 and TNF-α by astrocytes and microglia [41,42], and many actions of IL-1β in the CNS could be mediated by IL-6 and TNF-α [28].

The highest concentration of the TNF-α transcript was verified in the PILO group, especially in the acute phase of the model, decreasing in lovastatin-treated group [7].

Dolga et al. [29] observed that lovastatin increased the selective expression of TNFR2 induced by excitotoxicity in culture of cortical neurons. Levels of TNFR2 were increased in ischemic tissues, and this TNFα receptor has been related to neuroprotective signaling cascade, which includes PKB/Akt and NF-κB [30,31]. However, the mechanism by which the TNFαR2/NF-κB promotes neuroprotection is not yet fully understood.

Additionally, IL-10 has been associated with neuroprotection since this cytokine presents an antiinflammatory action. This mechanism seems mediated by astrocytes and microglia [32]. Indeed, IL-10 mRNA quantification showed a significant increase in the chronic phase in the PILO +LOVA group. This result was confirmed in hippocampal tissue since increased expression of IL-10 was visualized in cells with the shape of microglia.

Cell survival promoted by IL-10 is associated with its antiinflammatory action, protecting the central nervous system against a variety of proapoptotic factors induced during the immune response activation. Interleukin-10 blocks initiator and effector molecules involved in cell death [33]. In addition, IL-10 prevents cell death induced by TNF-α in astrocytes [34]. This cytokine also reduces the death of cerebellar neurons through the inhibition of excitotoxicity induced by NMDA receptor [35,38]. Experiments with IL-10-deficient mice showed the presence of high levels of TNF-α and IL-6 in the brain [39,40], and it is supposed that IL-10 may reduce the expression of proinflammatory cytokines [34]. Interleukin-10 also inhibits mitochondrial-induced cell death in microglia [36], blocking the apoptosis cascade [37]. Thus, our data suggest that lovastin may reduce excitotoxicity during epileptogenesis induced by pilocarpine by increasing the synthesis of IL-10 in the hippocampus.

## 5. Conclusion

These data have relevance to the development of innovative strategies to block the activation of cytokine-mediated signaling in associated with the development and maintenance of epilepsy, thus highlighting potential new targets for therapeutic intervention. Based on this, our data showed that lovastatin decreases the inflammatory process in the hippocampus induced by long-lasting SE. This action was initially identified during SE [7] and confirmed now during the silent and chronic periods in this epilepsy model.

## Conflict of interest

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the

criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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## References

[1] Pitkanen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol 2002;1(3):173–81.
[2] Choi H, Sell RL, Lenert L, Muennig P, Goodman RR, Gilliam FG, et al. Epilepsy surgery for pharmacoresistant temporal lobe epilepsy: a decision analysis. JAMA 2008;300(21):2497–505.
[3] Ravizza T, Vezzani A. Status epilepticus induces time-dependent neuronal and astrocytic expression of interleukin-1 receptor type I in the rat limbic system. Neuroscience 2006;137(1):301–8.
[4] Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia 2005;46(11):1724–43.
[5] Arganaraz G, Regina Perosa S, Cristina Lencioni E, Bader M, Abrao Cavalheiro E, Da Graca Naffah-Mazzacoratti M, et al. Role of kinin B1 and B2 receptors in the development of pilocarpine model of epilepsy. Brain Res 2004;1013(1):30–9.
[6] Arganaraz GA, Silva Jr JA, Perosa SR, Pessoa LG, Carvalho FF, Bascands JL, et al. The synthesis and distribution of the kinin B1 and B2 receptors are modified in the hippocampus of rats submitted to pilocarpine model of epilepsy. Brain Res 2004;1006(1):114–25.
[7] Gouveia TL, Scorza FA, Silva MJ, Bandeira Tde A, Perosa SR, Arganaraz GA, et al. Lovastatin decreases the synthesis of inflammatory mediators in the hippocampus and blocks the hyperthermia of rats submitted to long-lasting status epilepticus. Epilepsy Behav 2011;20(1):1–5.
[8] Perosa SR, Arganaraz GA, Goto EM, Costa LG, Konno AC, Varella PP, et al. Kinin B1 and B2 receptors are overexpressed in the hippocampus of humans with temporal lobe epilepsy. Hippocampus 2007;17(1):26–33.
[9] Silva Jr JA, Goto EM, Perosa SR, Argañaraz GA, Cavalheiro EA, Naffah-Mazzacoratti MG, et al. Kinin B1 receptors facilitate the development of temporal lobe epilepsy in mice. Int Immunopharmacol 2008;8(2):197–9.
[10] Varella PP, Santiago JF, Carrete Jr H, Higa EM, Yacubian EM, Centeno RS, et al. Relationship between fluid-attenuated inversion-recovery (FLAIR) signal intensity and inflammatory mediator's levels in the hippocampus of patients with temporal lobe epilepsy and mesial temporal sclerosis. Arq Neuropsiquiatr 2011;69(1):91–9.
[11] De Simoni MG, Perego C, Ravizza T, Moneta D, Conti M, Marchesi F, et al. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. Eur J Neurosci 2000;12(7):2623–33.
[12] Ginsberg HN. Effects of statins on triglyceride metabolism. Am J Cardiol 1998;81(4A):32B–5B.
[13] Griffin JL, Nicholls AW. Metabolomics as a functional genomic tool for understanding lipid dysfunction in diabetes, obesity and related disorders. Pharmacogenomics 2006;7(7):1095–107.
[14] Li G, Larson EB, Sonnen JA, Shofer JB, Petrie EC, Schantz A, et al. Statin therapy is associated with reduced neuropathologic changes of Alzheimer disease. Neurology 2007;69(9):878–85.
[15] Cimino M, Gelosa P, Gianella A, Nobili E, Tremoli E, Sironi L. Statins: multiple mechanisms of action in the ischemic brain. Neuroscientist 2007;13(3):208–13.
[16] Neuhaus O, Stuve O, Zamvil SS, Hartung HP. Are statins a treatment option for multiple sclerosis? Lancet Neurol 2004;3(6):369–71.
[17] Rangel P, Cysneiros RM, Arida RM, De Albuquerque M, Colugnati DB, Scorza CA, et al. Lovastatin reduces neuronal cell death in hippocampal CA1 subfield after pilocarpine-induced status epilepticus: preliminary results. Arq Neuropsiquiatr 2005;63(4):972–6.
[18] Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalographic and neuropathological study. Behav Brain Res 1983;9(3):315–35.
[19] Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. Epilepsia 1991;32:778–82.
[20] Zacco A, Togo J, Spence K, Ellis A, Lloyd D, Furlong S, et al. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors protect cortical neurons from excitotoxicity. J Neurosci 2003;23(35):11104–11.
[21] Mctaggart F, Buckett L, Davidson R, Holdgate G, Mccormick A, Schneck D, et al. Preclinical and clinical pharmacology of rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. Am J Cardiol 2001;87(5A):28B–32B.
[22] Chen C, Mireles RJ, Campbell SD, Lin J, Mills JB, Xu JJ, et al. Differential interaction of 3-hydroxy-3-methylglutaryl-coa reductase inhibitors with ABCB1, ABCC2, and OATP1B1. Drug Metab Dispos 2005;33(4):537–46.
[23] Chen J, Zhang ZG, Li Y, Wang Y, Wang L, Jiang H, et al. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Ann Neurol 2003;53(6):743–51.
[24] Lu D, Mahmood A, Goussev A, Schallert T, Qu C, Zhang ZG, et al. Atorvastatin reduction of intravascular thrombosis, increase in cerebral microvascular patency and integrity, and enhancement of spatial learning in rats subjected to traumatic brain injury. J Neurosurg 2004;101(5):813–21.
[25] Lu D, Qu C, Goussev A, Jiang H, Lu C, Schallert T, et al. Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. J Neurotrauma 2007;24(7):1132–46.
[26] Hering H, Lin CC, Sheng M. Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J Neurosci 2003;23(8):3262–71.
[27] Kirsch C, Eckert GP, Mueller WE. Statin effects on cholesterol micro-domains in brain plasma membranes. Biochem Pharmacol 2003;65(5):843–56.
[28] Wang Y, Qin Z-H. Molecular and cellular mechanisms of excitotoxic neuronal death. Apoptosis 2010;15(11):1382–402.
[29] Dolga AM, Nijholt IM, Ostroveanu A, Ten Bosch Q, Luiten PG, Eisel UL. Lovastatin induces neuroprotection through tumor necrosis factor receptor 2 signaling pathways. J Alzheimers Dis 2008;13(2):111–22.
[30] Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U. Neurodegenerative and neuroprotective effects of tumor necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. J Neurosci 2002;22(7):RC216.
[31] Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL. Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. J Biol Chem 2004;279(31):32869–81.
[32] Zhou Z, Peng X, Insolera R, Fink DJ, Mata M. Interleukin-10 provides direct trophic support to neurons. J Neurochem 2009;110(5):1617–27.
[33] Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998;281(5381):1305–8.
[34] Pahan K, Khan M, Singh I. Interleukin-10 and interleukin-13 inhibit proinflammatory cytokine-induced ceramide production through the activation of phosphatidylinositol 3-kinase. J Neurochem 2000;75(2):576–82.
[35] Bachis A, Colangelo AM, Vicini S, Doe PP, De Bernardi MA, Brooker G, et al. Interleukin-10 prevents glutamate-mediated cerebellar granule cell death by blocking caspase-3-like activity. J Neurosci 2001;21(9):3104–12.
[36] Strle K, Zhou JH, Broussard SR, Venters HD, Johnson RW, Freund GG, et al. IL-10 promotes survival of microglia without activating Akt. J Neuroimmunol 2002;122(1-2):9–19.
[37] Brodie C. Differential effects of Th1 and Th2 derived cytokines on NGF synthesis by mouse astrocytes. FEBS Lett 1996;394(2):117–20.
[38] Grilli M, Barbieri I, Basudev H, Brusa R, Casati C, Lozza G, et al. Interleukin-10 modulates neuronal threshold of vulnerability to ischaemic damage. Eur J Neurosci 2000;12:2265–72.
[39] Agnello D, Villa P, Ghezzi P. Increased tumor necrosis factor and interleukin-6 production in the central nervous system of interleukin-10-deficient mice. Brain Res 2000;869:241–3.
[40] Sewnath ME, Olszyna DP, Birjmohun R, ten Kate FJ, Gouma DJ, van Der Poll T. IL-10-deficient mice demonstrate multiple organ failure and increased mortality during Escherichia coli peritonitis despite an accelerated bacterial clearance. J Immunol 2001;166:6323–31.
[41] Schobitz B, De Kloet ER, Holsboer F. Gene expression and function of interleukin 1, interleukin 6 and tumor necrosis factor in the brain. Prog Neurobiol 1994;44:397–432.
[42] Benveniste EN. Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. Am J Physiol 1992;263:C1–16.