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Effects of statins on liver cell function and inflammation in septic rats

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ABSTRACT

Background: Several studies suggest that the presence of statins may be beneficial during sepsis, but this idea is controversial. The aim of this study was to investigate the effects of long-term statin treatment in the livers of septic animals, focusing on its antioxidant, antiinflammatory, and metabolic properties.

Materials and methods: Male Wistar rats were treated orally with simvastatin, atorvastatin, or vehicle once a d. After 30 d, sepsis was induced by cecal ligation and puncture (CLP) in Control, Simvastatin-treated, and Atorvastatin-treated groups, while the Sham group underwent only laparotomy. The Basal Simvastatin and Basal Atorvastatin groups received only their respective drugs without surgery. Twenty-four h after CLP or laparotomy, samples were collected from anesthetized rats for evaluation of hepatic oxidative stress, liver histology, hepatic mitochondria enzyme activity, leukocyte counts in blood and peritoneal cavity, gene expression of hepatic superoxide dismutase and *TNF-2*, and plasma biochemistry.

Results: Most parameters that we tested exhibited expected changes upon sepsis induction. However, statin treatment only improved liver mitochondrial enzymatic activity. In other parameters, simvastatin and atorvastatin failed to protect the liver against injuries incurred upon the CLP-induced polymicrobial sepsis model.

Conclusions: Pretreatment with simvastatin or atorvastatin alone before sepsis induction improved mitochondrial activity in the liver; however, this result was not reproduced in other biomarkers of liver function and leukocyte migration during sepsis. Future studies should be performed to evaluate whether statins can be combined with other drugs to increase the efficacy of sepsis therapy.

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1. Introduction

Sepsis is a systemic inflammatory condition caused by infectious agents that invade host organisms and establish serious endotoxemia. It is characterized by generalized, systemic inflammation with cytokine overproduction and circulatory system disruption [1]. Moreover, oxidative stress is known to play an important role in the pathogenesis of sepsis [2]. During sepsis, the liver is the second organ affected, after the lungs [3]. Several changes in hepatic metabolism occur, and they may have an important role in disease outcome since the liver plays a role in host defense mechanisms; for example, liver-resident Kupffer cells inactivate and clear bacteria and bacterial products, and are also responsible for production as well as clearance of inflammatory mediators during sepsis. In contrast, inflammatory cytokines released during sepsis may affect metabolic pathways in hepatocytes [4].

Statins, such as simvastatin and atorvastatin, are hypocholesterolemic drugs that possess pleiotropic effects, including antioxidant [5] and antiinflammatory properties, that are either dependent or independent of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibition. In particular, the antiinflammatory properties correlate with reduced sepsis-induced morbidity and mortality [6,7]. However, there is no consensus in the literature for this protective effect, as some studies called attention to the lack of randomized controlled trials in these earlier reports [8]. There are also studies demonstrating that statins have an adverse effect on sepsis [9]. Additional investigation evaluating the long-term effect of statin use for sepsis is needed, since most of the existing studies focused on the effects of acute or single-dose administration [10,11]. Evaluating the safety of statins in sepsis during long-term treatment is also important. Although statins are considered safe drugs, hepatotoxicity has been reported [12]. In the present study, we directly tested the effect of statins on sepsis, and compared the effect of two different statins: simvastatin, chosen for its widespread use, and atorvastatin, chosen as a new-generation statin that is likely more effective.

Since sepsis remains the primary cause of mortality and morbidity in noncoronary intensive care units [13], and the positive effects of statins on sepsis are not yet clear, studies testing the therapeutic effects of different statins and other agents on sepsis are necessary. Therefore, the major objective of this study is to investigate the effects of long-term statin treatment on hepatic function in experimental sepsis, focusing on the antioxidant, antiinflammatory, and metabolic properties of statins.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by the Ethical Committee for Animal Use of the Biological Sciences Section of Federal University of Paraná (certificate #475). Male Wistar rats (*Rattus norvegicus*) weighing 200–250 g were housed at 22 ± 2°C and maintained in a 12-h dark-light cycle. The animals had free access to water and standard laboratory chow during treatment.

2.2. Oral treatment

Rats were treated via oral gavage once a d for 30 consecutive d. The animals were separated into eight different groups. The drug delivery vehicle was composed of polysorbate 80 and distilled water (30 μL polysorbate/mL water). The Sham group ($n = 6$) and Control group ($n = 6$) received vehicle only. The low-dose Simvastatin 1 (Galena, Campinas, SP, Brazil) ($n = 6$) and Atorvastatin 1 (Pharma Nostra, Anápolis, GO, Brazil) ($n = 6$) statin groups received 1.17 mg/kg and 0.59 mg/kg of each drug, respectively. These doses were obtained by allometric extrapolation [14] of the indicated doses for the hypercholesterolemia treatment in humans. The high-dose Simvastatin 2 ($n = 6$) and Atorvastatin 2 ($n = 6$) statin groups received 5.85 mg/kg and 2.95 mg/kg, respectively, which is a 5-fold higher dose than the other statin-treated groups. Basal Simvastatin ($n = 5$) and Basal Atorvastatin ($n = 5$) groups also received 5.85 mg/kg and 2.95 mg/kg, respectively, but did not receive any surgical procedure to induce sepsis. We chose to give statin treatment before sepsis induction, as there is evidence to support that individuals who use statins on a regular basis, previous to being septic, have lower mortality rates during sepsis [8].

2.3. Cecal ligation and puncture–induced sepsis

Briefly, the animals were anesthetized intraperitoneally with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). Sepsis was induced in the Control, Simvastatin 1 and 2, and Atorvastatin 1 and 2 groups. Three punctures were made with a 16-gauge needle, and the cecum was squeezed to extrude the fecal material from the wounds. A 50% survival rate index was observed within 7 d using this protocol, allowing the collection of viable material to evaluate sepsis biomarkers. We chose this protocol because this was the most effective number of punctures among the ones we tested in our preliminary studies (data not shown). Several hours post–cecal ligation and puncture (CLP), the animals developed hypothermia, as evidenced by abdominal contraction, prostration, pyloerection, and lethargy. The Sham group experienced only the laparotomy and the animals did not present clinical signs of infection or pain. Both the Basal Simvastatin and Atorvastatin groups were not subjected to any surgical procedures. Animals undergoing surgical procedures received sterile saline subcutaneously (10 mL/kg) immediately after skin suture completion for fluid resuscitation. After 12 h of CLP or laparotomy, the rats were fasted for an additional 12 h to avoid variations in plasma biochemistry.

2.4. Sample collection

Twenty-four h after the surgical procedure, animals were anesthetized as described earlier for CLP, and blood was drawn from the abdominal vena cava with heparinized syringes. Plasma was separated by centrifugation and stored at −70°C for further analysis. The liver was rapidly harvested,