Simvastatin Effects on Skeletal Muscle Using the Biodistribution of 99mTc Sestamibi

Efeitos da sinvastatina no músculo esquelético usando a biodistribuição do sestamibi-Tc99m

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Study performed at Department of Surgery, Federal University of Rio Grande do Norte (UFRN), Brazil.
Financial support: none.
Conflict of interest: None.
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Submitted: April 10, 2015. Accepted, after review: May 12, 2015.

ABSTRACT

Objective: Examining the effects of simvastatin on skeletal muscles in an experimental model of abdominal sepsis in rats through the biodistribution of 99mTc-sestamibi. **Methods:** Wistar rats were randomly assigned to 2 groups: with abdominal sepsis (n = 12) and without sepsis (n = 12). Six animals with sepsis and 6 controls were injected with a daily dose of 5 mg/kg/day of simvastatin by gavage for 3 days before induction of peritonitis and 4 hours before surgical procedure. The others received 1ml of 0.9% saline solution orally. The animals were anesthetized with 20mg/kg of xylazine and 50mg/kg of ketamine i.p. Cecal ligation and puncture were performed by laparotomy. The rats were under observation for 24 hours and their survival time was recorded. The animals were re-anesthetized and 0.1 ml of 99mTc-sestamibi was administered by i.v. 30 minutes later,the thigh muscle was biopsied for percentage of radioactivity per gram of tissue (ATI%/g) determination, measured by the automatic gamma counter Wizard Counter, PerkinElmer,Finland. **Results:** ATI%/g of Tc99m-sestamibi was higher in muscle samples of groups treated with simvastatin sepsis ((1.82±0.21), compared with saline-treated (1.07±0.19) and control groups (1.18±0.31;1.26±0.24); this is a statistically significant difference ($p<0.001$). **Conclusion:** The data resulting from this study allows for concluding that the pre-treatment with simvastatin contributed to a biodistribution increase of 99mTc-sestamibi into the skeletal muscle in the abdominal sepsis model in rats.

Keywords: Simvastatin. Sepsis. 99mTc-sestamibi. Skeletal muscle. Biological availability.

RESUMO

Objetivo: Estudo com o objetivo de examinar os efeitos da sinvastatina no músculo esquelético em modelo experimental de sepse abdominal em ratos, através da biodistribuição de Tc99m-sestamibi. **Métodos:** Ratos Wistar foram separados aleatoriamente em 2 grupos: com sepse abdominal (n=12) e sem sepse (n=12). Em 6 animais com sepse e 6 controles foi injetada dose única diária de sinvastatina 5 mg/kg/dia, por gavagem, durante 3 dias antes da indução da peritonite e 4 horas antes do procedimento cirúrgico. Os demais receberam 1 ml de solução salina 0,9 % via oral. Os animais foram anestesiados com xilazina 20 mg/kg e cetamina 50 mg/kg i.p. Por laparotomia foi feita ligadura e punção do ceco que em seguida foi recolocado na cavidade abdominal. Sob vigilância durante 24 horas, foi registrado o tempo de sobrevida dos ratos. Em seguida, os animais foram re-anestesiados e 0,1 ml de Tc99m-sestamibi foi administrado i.v. 30 minutos após, o músculo da coxa foi biopsiado para a determinação da atividade radioativa por grama de tecido (%ATI/g), aferida com o contador gama automático Wizard Counter, PerkinElmer, Finland. **Resultados:** O %ATI/g do Tc99m-sestamibi mostrou-se maior nas amostras de músculo do grupo com sepse tratado com sinvastatina (1,82±0,21), quando comparado com o tratado com solução salina (1,07±0,19) e com os grupos controles (1,18±0,31; 1,26±0,24). Diferença estatisticamente significante (p<0,001). **Conclusão:** Os dados resultantes desse estudo permitem concluir que o pré-tratamento com sinvastatina contribuiu para o aumento da biodistribuição do Tc99m-sestamibi em músculo esquelético em modelo de sepse abdominal em ratos.

Descritores: Sinvastatina. Sepse. Tc99m-sestamibi. Músculo esquelético.

INTRODUCTION

In 1976, the first statin was discovered, mevastatin, isolated from antibiotic derivative compounds. Its major function is inactivating HMG-CoA reductase (enzyme responsible for synthesizing cholesterol)[1], and it was able to influence the appearance of new research for the discovery of other family drugs that promote the reduction of serum cholesterol levels.

Subsequent studies demonstrated pleiotropism of statins, revealing anti-inflammatory action by inhibiting thrombogenesis, and even immunomodulatory effects[2]. The anti-inflammatory effects of statins have been primarily reported in an experimental model of ischemia and reperfusion in normocholesterolemic, hypercholesterolemic and diabetic patterns[3].

These new properties were also illustrated in infection by *Staphylococcus aureus* toxin which elicits a vascular inflammatory response. This study demonstrated that prophylactic clinical doses of simvastatin, 18 hours before endotoxin injection, is

able to reduce the action of this toxin in the rolling and diapede of leukocytes into the mesentery[4]. This occurs through an imbalance in the endothelial-leukocyte cell interaction caused by the drug, regardless of their anti-cholesterolemic actions[5,6].

The persistence of infectious processes, such as those caused by *S. aureus*, is capable of generating a systemic inflammatory response, and may develop into sepsis. This condition may be the cause of muscle loss and this has been observed in mouse models of sepsis experimentally induced by cecal ligation and puncture (CLP), which causes a focus of necrotic tissue and polimicrobial contamination of the peritoneal cavity[7].

Sepsis promotes increased proteolysis of skeletal muscle, a phenomenon that occurs when there is an imbalance between protein synthesis and degradation, as reflected by inhibition of protein synthesis together with the increase of its degradation. The elevated levels of various cytokines such as tumor necrosis factor (TNF-α) and interleukin-6 during sepsis may influence muscle damage and protein degradation[8].

Thus, based on the evidence of the harmful effects of sepsis on skeletal muscles and anti-inflammatory effects of statins on the myocardium in a septicemic context, the present study tested the hypothesis that treatment with simvastatin during sepsis promotes anti-inflammatory effects on the skeletal muscle, using rats as the experimental model.

For this, 99mTc-sestamibi was used as a way to confirm cell viability possibly guaranteed by the anti-inflammatory effects of simvastatin. This marker has been used as scintigraphic agent of myocardial perfusion in the evaluation of coronary artery disease. Moreover, 99mTc-sestamibi has been used to assess the perfusion of skeletal muscle in individuals with peripheral arterial disease[9]. The capture and retention of this component appear to be associated with mitochondrial integrity and cell viability[9,10].

This project aimed at examining the effects conferred by simvastatin which are not related to their lipid-lowering action in an experimental model of abdominal sepsis in rats. It examined the action of simvastatin on the biodistribution of 99mTc-sestamibi in the skeletal muscle in animals with sepsis, and the effect of simvastatin in the survival time of rats.

METHODS

We performed a study with experimental design using 24 Wistar rats randomly separated into two groups. Twelve septic animals and 12 controls (without sepsis) were kept in individual cages with water and standard rodent feed ad libitum (Presence®), after previously experiencing a period of 7 days of acclimation to the laboratory. They

were kept in controlled temperature (22ºC), with light-dark cycles of 12 h and handled in accordance with the provisions of Brazilian Law 11.794/2008. The project was submitted and endorsed by the Ethics Committee on Animal Use (CEUA-UFRN).

Experimental Design

As shown in the diagram below, half of the animals were orally treated with simvastatin and the other half with 0.9%saline solution. Six animals with sepsis and 6 controls were injected with a daily dose of 5 mg/kg/day of simvastatin suspension by gavage for 3 days before induction of peritonitis and 4 hours before surgery (CLP). The remaining animals were orally treated with 1 ml of 0.9% saline solution.

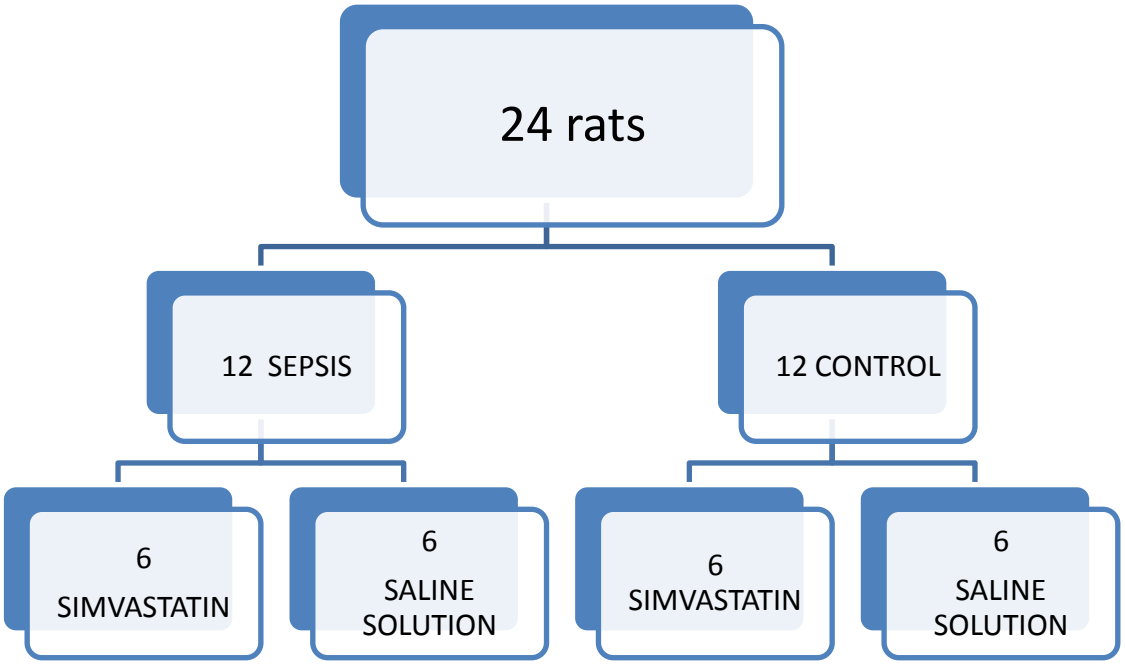

Figure 1: Experimental design

Sepsis Induction

Animals were anesthetized with 20 mg/kg of xylazine and 50mg/kg of ketamine intraperitoneally (IP). Antisepsis of abdominal wall with 70% ethanol was followed by placing fenestrated sterile drapes. A 4 cm laparotomy was done and a cecal ligation and puncture (CLP) was performed through using 3-0cotton thread, at 1 cm of ileum penetration, proceeding 3 punctures in the cecum wall with a 25x8 sterile needle. Then the organ was replaced in the abdominal cavity andclosed in two suture lines with 4-0 nylon. Post-operative pain was controlled with analgesia (i.m. meperidine 10 mg/kg, one daily).

Animals were handled in the operating room of the Experimental Surgery Center, operated on by a surgeon with extensive experience in operating on small animals, and for the observation period they were kept in the post-operative control room of the same facility.

The animals were kept under observation for 24 hours, and survival time was recorded.

Biodistribution of 99mTc-sestamibi in skeletal muscles

All animals were anesthetized with the same technique described above in the 24-hour postoperative observation. The right femoral vein was dissected and 0.1 ml of Tc-99m-sestamibi was administered i.v. corresponding to the introduction of 1.0 MBq (MegaBequerel) in the bloodstream. 30 minutes after, thigh muscle biopsies were performed. The samples were washed in saline, weighed on a precision scale and then introduced into test tubes for the determination of radioactivity, measured with the use of automatic gamma counter WizardCounter, PerkinElmer, Finland. The percentage of radioactivity (ATI%) was calculated by dividing the radioactivity of each muscle segment by the total activity administered in each animal.

Statistical Analysis

Data were analyzed quantitatively and a description including measures of central tendency was performed (mean and standard deviation). Analysis of variance and Tukey test for inferences were performed, with statistical significance of $p<0.05$.

RESULTS

Table 1 – Radioactivity percentage of 99mTc-sestamibi per gram of tissue (ATI%/g) per group.

Organs	*%ATI/g, per group*				p-value[(1)]
	Simvastatin Control	Saline Solution Control	Simvastatin Sepsis	Saline Solution Sepsis	
Skeletal muscles[2]	1.18± 0.31[a]	1.26± 0.24[b]	1.82± 0.21[abc]	1.07± 0.19[c]	<0.001

Average±Standard Deviation
1 – ANOVA analysis p value.
2 – Values followed by at least one similar letter are significantly different, by the multiple comparisons Tukey test, at a significance level of 5%.

There were no significant differences in 99mTc-sestamibi percentage of the activity in skeletal muscle among the control groups treated with simvastatin and with saline solution. The activity percentage of the 99mTc-sestamibi per gram of tissue was

shown to be significantly higher in the muscle samples of sepsis group treated with simvastatin, when compared with the sepsis group treated with saline and control groups (Table 1). Consequently, the muscle perfusion as measured by ATI%/g of 99mTc-sestamibi in the thigh muscle of the animals was significantly higher in the sepsis group pre-treated with simvastatin than in the sepsis group without simvastatin treatment. It was also significantly higher than in animals of the control groups treated with or without simvastatin.

DISCUSSION

Sepsis is one of the most important conditions for morbidity and mortality in intensive care units. This clinical syndrome results from a complex interaction between the host and the infectious agent, leading to systemic activation of the inflammatory response through cytokines, acute phase mediators and coagulation proteins. From these substances, the pathogenesis of sepsis is related to microcirculation disorders, hemodynamic changes and cellular changes capable of altering the balance between blood flow and tissue metabolic needs. Thus, sepsis usually results in severe tissue damage and multiple organ dysfunction[11].

The inflammatory cascade induced by sepsis mainly involves the release and activation of TNF-a and IL-1, substances that induce a strong cellular response and activation of secondary mediators of inflammation, granulocyte chemotaxis and activation. The secondary mediators in turn activate phagocytic cells such as macrophages and monocytes which produce, in sequential fashion, TNF-a, IL-1, IL-6 and IL-8, proliferating an effective ripple[12].

In a study by our group, we evaluated the action of simvastatin in septic rats in relation to dosages of cytokines, and found that there was a significant reduction in the levels of TNF-α, IL-1β and IL-6 in animals with sepsis when simvastatin was administered, demonstrating a significant anti-inflammatory effect this drug[13]. This evidence corroborates the positive results in skeletal muscle sepsis in rats observed in the results of this research.

Liappis et al has shown that the anti-inflammatory effect of statins is probably responsible for the low mortality in patients with bacteremia who received these drugs. It means that the effect of statins originates from various actions (inhibition of the expression of essential adhesion molecules in leukocyte/endothelial connection and control chemotactic proteins, for example)[14]. Importantly, mortality triggered by sepsis is intrinsically related to the inflammatory response and cytokine production[5-17].

In order to evaluate the anti-inflammatory response of simvastatin on skeletal muscle, this study used the radioactive tracer 99mTc-sestamibi, whose tissue penetration depends on the negative plasma potential to be conducted, of the mitochondrial integrity where it should be focused, and of the normal metabolic condition of cells[10,18].

Metabolic and hemodynamic changes caused by the inflammatory process of sepsis can lead to tissue ischemia, which interferes with the K+/ATPase mitochondrial channels, leading to alteration of mitochondrial membrane potential. Thus, Tc99m-sestamibi does not accumulate in this organelle, to the point that it is not significantly detected in ischemic tissue.

Although there is evidence that statins cause side effects related to the skeletal muscle in prolonged use, such as rhabdomyolysis, myalgia, muscle cramps, weakness and muscle pain in patients using long-term simvastatin[19,20], this study evaluated its use in the acute treatment of sepsis, which showed anti-inflammatory relevance in detriment to any deleterious effects.

The data resulting from this study allow us to conclude that the pre-treatment with simvastatin contributed to the biodistribution increase of 99mTc-sestamibi into skeletal muscle tissue, in an abdominal sepsis model in rats. Given that this radiopharmaceutical evaluates muscle perfusion, our results open opportunity for future research aimed at muscle perfusion in sepsis with the use of other assessment methods.

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