



Simvastatin and acute ischemic renal injury in rats*

Simvastatina e lesão renal aguda isquêmica em ratos

Simvastatina y lesión renal aguda isquêmica en ratas

Claudia Akemi Shibuya Teshima¹, Mirian Watanabe¹, Cassiane Dezoti da Fonseca¹, Maria de Fátima Fernandes Vattimo²

ABSTRACT

Objectives: The study aimed to verify the protective renal action of *simvastatin* in an animal model of ischemia / reperfusion for 30 minutes. **Methods:** Ischemia was obtained by clamping bilateral renal pedicles for 30 minutes, followed by reperfusion. Male Wistar rats were used, weighing between 250-300g, distributed into the following groups: *SHAM* (control, without clamping renal), *Ischemia* (renal ischemia for 30 minutes), *Ischemia + Statin* (simvastatin 0.5 mg/kg, orally for three days). Renal function (creatinine clearance, Jaffé method), urinary osmolality, and urinary peroxides were evaluated. **Results:** The results showed that the statin improved renal function, and reduced urinary osmolality along with excretion of PU. **Conclusion:** In summary, the study confirmed the protective renal effects of statins, with an antioxidant action that protects the kidney.

Keywords: Acute kidney injury; Ischemia; Simvastatin/adverse effects; Rats

RESUMO

Objetivos: O estudo visou verificar a ação renoprotetora da simvastatina em modelo animal de isquemia/reperfusão por 30 minutos. **Métodos:** A isquemia foi obtida por meio do clampamento dos pedículos renais bilaterais por 30 minutos, seguida de reperfusão. Ratos Wistar, machos foram usados pesando entre 250-300g, distribuídos nos seguintes grupos: SHAM (controle, sem clampamento renal); Isquemia (isquemia renal por 30 minutos); Isquemia+Estatina (simvastatina 0,5 mg/kg, via oral durante três dias). A função renal (*clearance* de creatinina, método de Jaffé), a osmolalidade urinária, os peróxidos urinários foram avaliados. **Resultados:** Os resultados mostraram que a estatina melhorou a função renal, a osmolalidade urinária e reduziu a excreção de PU. **Conclusão:** Em síntese, o estudo confirmou o efeito renoprotetor da estatina, com ação antioxidante de proteção renal.

Descritores: Lesão renal aguda; Isquemia; Simvastatina/efeitos adversos; Ratos

RESUMEN

Objetivos: El estudio tuvo como objetivo verificar la acción renoprotectora de la simvastatina en modelo animal de isquemia/reperfusión por 30 minutos. **Métodos:** La isquemia se obtuvo por medio del pinzamiento de los pedículos renales bilaterales por 30 minutos, seguida de la reperfusión. Fueron usadas ratas Wistar, machos que pesaban entre 250-300g, distribuidos en los siguientes grupos: SHAM (control, sin pinzamiento renal); Isquemia (isquemia renal por 30 minutos); Isquemia+Estatina (simvastatina 0,5 mg/kg, vía oral durante tres días). Fueron evaluadas la función renal (*clearance* de creatinina, método de Jaffé), la osmolaridad urinaria y los peróxidos urinarios. **Resultados:** Los resultados mostraron que la estatina mejoró la función renal, la osmolaridad urinaria y redujo la excreción de PU. **Conclusión:** En síntesis, el estudio confirmó el efecto renoprotector de la estatina, con acción antioxidante de protección renal.

Descriptores: Lesión renal aguda; Isquemia; Simvastatina/efectos adversos; Ratas

* Study conducted at the Laboratory of Experimental Animal Models (LEMA - Laboratório Experimental de Modelos Animais) - School of Nursing, University of São Paulo (USP), São Paulo (SP), Brazil.

¹ Masters from the School of Nursing, University of São Paulo (USP), São Paulo (SP), Brazil.

² Associate Professor. Head of the Laboratory of Experimental Animal Models at the School of Nursing, University of São Paulo (USP), São Paulo (SP), Brazil.

INTRODUCTION

The first deceased-donor renal transplant was performed in Brazil in 1964. Currently, renal transplantation has evolved from an experimental surgical procedure to an established treatment for the end stages of chronic renal failure, resulting in longer organ survival and better quality of life for the recipient. The development of new surgical techniques, the use of deceased or living donors, the discovery of new immunosuppressive drugs with fewer adverse effects and national legislation that standardizes the search for and procurement of organs are several factors that contribute to the achievement of a successful transplant⁽¹⁻²⁾.

Performing a renal transplant involves ischemia/reperfusion (I/R) mechanisms in which the level of ischemia is determined by removing the kidney from the donor and implanting the organ into the extraperitoneal space of the recipient. The implantation of the graft results in reperfusion to the organ and reestablishes the conditions necessary for recovery; however, this process triggers a new injury mediated by the activation of the inflammatory response and the release of reactive oxygen species (ROS) into the endothelial microvascular bed of the graft. Graft dysfunction is a clinical manifestation with an immediate reduction in renal function after transplantation, which is an important cause of ischemic acute kidney injury (iAKI)⁽³⁻⁴⁾.

Studies using animal models of I/R allow for the implementation of clinical situations at the bench in an attempt to isolate variables that cannot be studied in humans, allowing us to identify pathophysiological mechanisms and physiological responses to pharmacological or mechanical manipulations, which is the focus of this study. These models, primarily performed using rats or mice, represent approximately 40% of the studies published in *Kidney International* and *Nephrology Dialysis Transplantation* in the last three years⁽⁵⁾.

The model of renal I/R consists of clamping the renal artery or the renal pedicle for various periods between 15 and 60 minutes with subsequent reperfusion, which reproduces the clinical scenario consistent with renal transplantation. The applicability and feasibility of this model is due to the similarity between the inflammatory responses, medullary congestion and tubular damage compared with data obtained from renal biopsies from patients with AKI⁽⁵⁾.

Our hypothesis for this study is that statins have, through their pleiotropic effects and independent of their antilipidemic, anti-inflammatory and antioxidative actions, a renoprotective action in a rat model of I/R. Recent evidence has confirmed that administering statins prevents vascular inflammatory reactions in both clinical studies and animal models⁽⁶⁻⁷⁾; however,

our study presents research advances in examining their antioxidant mechanisms.

OBJETIVE

To assess the renoprotective effect of simvastatin in an animal model of renal I/R for 30 minutes.

METHODS

The procedures in this study were performed according to the Ethical Principles for Animal Experimentation adopted by the Brazilian College of Animal Experimentation and were approved by the Ethics Committee on Animal Experimentation at the Institute of Biological Sciences of the University of São Paulo. All animals were provided food and water ad libitum and were housed in a climate-controlled facility under day-night cycles throughout the experiment.

Adult male Wistar rats weighing between 250-300 g were used. They were divided into the following groups: *SHAM* - surgery control with surgery simulation, *Statin* - animals pre-conditioned for 3 days with 0.5 mg / kg Sinvascor-Baldacci® via gavage (p.o.), *Ischemia* - bilateral clamping of the renal pedicle for 30 minutes, *Ischemia + Statin* - renal ischemia in animals pre-conditioned for 3 days with 0.5 mg / kg Sinvascor-Baldacci® via gavage (p.o.).

The animals were anesthetized with 40-50 mg / kg sodium thiopental intraperitoneally (i.p.) and subjected to laparotomy for the bilateral clamping of the renal pedicle for 30 minutes and for the reestablishment of renal perfusion. The animals were then placed in metabolic cages to collect their urine for 24 hours. After this period, the animals were again anesthetized with 60 mg / kg sodium thiopental for another laparotomy and blood sampling via a puncture of the abdominal aorta, which was used for renal function (RF), tubular function and oxidative stress studies.

RF was measured by the creatinine clearance test, which determined plasma and urinary creatinine using the Jaffe method⁽⁸⁾.

Tubular function was measured by urine osmolality (Uosm) via an osmometer - *Advanced Osmometer*® - model 3D3.

Oxidative stress was measured by urinary peroxide levels via the FOX-2 method. The measurement of urinary peroxide levels is considered to be a biomarker for the generation of hydrogen peroxide and a predictor of the extent of oxidative stress in experimental models in vivo⁽⁹⁾.

We used the GLM method (univariate ANOVA) and multiple comparison tests with a Bonferroni correction for the statistical analyses. P values <0.05 were considered significant.

RESULTS

Table 1 shows the RF data for the various groups, among which the animals that underwent renal ischemia for 30 min had a non-significant increase in urinary flow, reduced creatinine clearance (0.20 ± 0.02 vs. 0.60 ± 0.07 , $p < 0.05$ vs. SHAM) and urinary osmolality (766 ± 188 vs. 1793 ± 191 , $p < 0.05$ vs. SHAM) compared with the SHAM control group, confirming the model of ischemic AKI with reduced glomerular filtration and tubular function. The three-day pre-conditioning with simvastatin for the ischemic group resulted in improvements in RF, with an increase in the glomerular filtration rate (0.49 ± 0.04 vs. 0.20 ± 0.02 , $p < 0.05$ vs. ischemia) and higher Uosm values (1153 ± 404 vs. 766 ± 188 , $p < 0.05$ vs. ischemia) compared with the ischemia group.

Table 1. Overall renal function of the various groups.

Groups	No.	UF (ml/min)	Crcl 100 g (ml/min)	Urinary Osmolality (Uosm)
SHAM	6	0.006 ± 0.001	0.60 ± 0.07	1793 ± 191
Statin	6	0.008 ± 0.002	0.85 ± 0.19	1849 ± 71
Ischemia	7	0.013 ± 0.008	0.20 ± 0.02^a	766 ± 188^a
Isch + Statin	10	0.016 ± 0.011	0.49 ± 0.04^b	1153 ± 404^a

UF – urinary flow, Crcl 100 g – creatinine clearance per 100 grams.

^a $p < 0.05$ vs. SHAM

^b $p < 0.05$ vs. Ischemia

The data are represented as the means \pm standard deviation.

The data in Table 2 show the urinary peroxide values of the animals subjected to renal ischemia for 30 minutes. Higher urinary peroxide excretion values were observed in rats presenting with renal ischemia compared with the sham group, indicating a redox imbalance and the presence of oxidative damage. Simvastatin treatment induced a reduction in the urinary peroxide values, demonstrating the antioxidant effect of the drug.

Table 2. Urinary peroxide values for the different groups

Groups	No.	Urinary Peroxides (nmol/g creatinine)
SHAM	8	5.6 ± 0.9
Ischemia	8	13.5 ± 0.8^a
Isch + Statin	8	7.9 ± 1.0^b

^a $p < 0.05$ vs. SHAM

^b $p < 0.05$ vs. Ischemia

The data are represented as the means \pm standard deviation.

DISCUSSION

Statins, inhibitors of HMG-CoA reductase, exert beneficial effects that are independent of their primary function of reducing blood cholesterol levels, including anti-inflammatory and antioxidant action and vascular protective abilities that are known as their pleiotropic effects⁽⁸⁾.

In clinical practice, the reduction of dyslipidemia with the use of pravastatin in patients with chronic renal disease resulted in a decrease in disease progression and cardiovascular events⁽⁶⁾. Studies that use animal models have also confirmed this finding when administering statins and have observed renoprotection with the preservation of glomerular function, thus preventing the progression of chronic renal disease⁽⁷⁾.

In the present study, pre-conditioning with simvastatin in animals subjected to renal ischemia for 30 minutes resulted in renal protection by increasing the glomerular filtration rate as measured by the creatinine clearance rate and the increase in Uosm, confirming improved tubular function and reduced urinary peroxide levels, indicating lower ROS levels.

Other studies using animal models have reinforced the renoprotective action of statins. Pre-conditioning for 5 days with cerivastatin resulted in renal protection in mice subjected to iAKI⁽¹⁰⁾. In addition, the administration of simvastatin in a model of renal I/R resulted in a reduction in the area of acute tubular necrosis, which was confirmed by histological data and improvement in tubular function⁽¹¹⁾.

Studies have confirmed the power of statins as renoprotective agents due to their anti-inflammatory and antioxidant actions, two mechanisms that are significant in iAKI. The anti-inflammatory effect may also be related to decreased graft rejection via its immunomodulatory effects and the inhibition of factors that activate the inflammatory cascade⁽¹²⁾. The administration of fluvastatin in dyslipidemic patients induces reduced lipid levels and increased antioxidant protection by reducing the markers of oxidative injury⁽¹³⁾.

CONCLUSION

In summary, simvastatin pre-conditioning in animals that are subjected to renal ischemia for 30 minutes conferred renal protection by increasing the glomerular filtration rate, improving tubular function and reducing markers of oxidative injury. These data confirm the renoprotective effects of simvastatin, which are most likely due to its pleiotropic antioxidative effects.

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