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***Experimental Study** / Deneysel Çalışma*

Investigation the effects of cilostazol and rosuvastatin on kidney and heart: An experimental acute kidney and heart injury model

Silostazol ve rosuvastatinin böbrek ve kalp üzerine etkilerinin araştırılması: Deneysel akut böbrek ve kalp hasarı modeli

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ABSTRACT

Background: In this study, we aimed to assess the effects of preoperative cilostazol and rosuvastatin therapy on kidney ischemia/reperfusion injury and remote cardiac reperfusion injury in an experimental model.

Methods: A total of 35 female Sprague-Dawley rats were randomly divided into five groups (n=7). Median laparotomy and a 45-min bilateral kidney ischemia were performed. Oral medications were administered three days before the surgical intervention (20 mg/kg cilostazol, 10 mg/kg rosuvastatin and 20 mg/kg cilostazol + 10 mg/kg rosuvastatin). Blood samples and kidney and heart tissue samples were extracted one day after surgery.

Results: Immunohistochemical examination of the kidney samples revealed that tumor necrosis factor-alpha and hypoxia-inducible factor-1 alpha immunoreactivities in the cilostazol, rosuvastatin, and cilostazol + rosuvastatin groups were found to be significantly lower, compared to ischemia/reperfusion injury group ($p<0.05$). Immunohistochemical examination of the heart samples revealed that tumor necrosis factor-alpha immunoreactivity was significantly lower in the cilostazol group, compared to ischemia/reperfusion injury group. Hypoxia-inducible factor-1 alpha immunoreactivities were significantly lower in the cilostazol, rosuvastatin, and cilostazol + rosuvastatin groups, compared to ischemia/reperfusion injury group ($p<0.05$). Serum urea, creatinine, creatine kinase-muscle and brain, and troponin levels were significantly lower in the cilostazol, rosuvastatin, and cilostazol + rosuvastatin groups, compared to ischemia/reperfusion injury group ($p<0.05$).

Conclusion: Cilostazol and rosuvastatin have protective effects on kidney ischemia/reperfusion and remote cardiac reperfusion injury, and the protective effect can be augmented with cilostazol monotherapy, compared to combined therapy.

Keywords: Heart; ischemia/reperfusion injury; kidney.

ÖZ

Amaç: Bu çalışmada, deneysel bir modelde ameliyat öncesi silostazol ve rosuvastatin tedavisinin böbrek iskemi/reperfüzyon hasarına olan etkisi ve uzak kalp reperfüzyon hasarı üzerine olan etkisi araştırıldı.

Çalışma planı: Toplam 35 adet dişi Sprague-Dawley sıçan rastgele beş gruba ayrıldı (n=7). Median laparotomi yapılarak, her iki böbreğe 45 dakika süren iskemi uygulandı. Cerrahi girişimin üç gün öncesinden başlayarak oral tedavi uygulandı (20 mg/kg silostazol, 10 mg/kg rosuvastatin ve 20 mg/kg cilostazol + 10 mg/kg rosuvastatin). Cerrahi girişimden bir gün sonra ise kan örnekleri ve böbrek ve kalp doku örnekleri alındı.

Bulgular: Böbrek dokularının immünhistokimyasal değerlendirmesinde tümör nekroz faktör-alfa ve hipoksi ile indüklenebilir faktör-1 alfa immünreaktivitesinin silostazol, rosuvastatin ve silostazol + rosuvastatin grubunda, iskemi/reperfüzyon hasarı grubuna kıyasla anlamlı düzeyde daha düşük olduğu tespit edildi ($p<0.05$). Kalp dokularının immünhistokimyasal değerlendirmesinde tümör nekroz faktör-alfa immünreaktivitesi, iskemi/reperfüzyon hasarı grubuna kıyasla silostazol grubunda anlamlı düzeyde daha düşüktü. Hipoksi ile indüklenebilir faktör-1 alfa immünreaktivitesi, silostazol, rosuvastatin ve silostazol + rosuvastatin grubunda, iskemi/reperfüzyon hasarı grubuna kıyasla, anlamlı düzeyde daha düşüktü ($p<0.05$). İskemi/reperfüzyon hasarı grubuna kıyasla, silostazol, rosuvastatin ve silostazol + rosuvastatin grubunda, serum üre, kreatinin, kreatin kinaz-kas ve beyin ve troponin düzeyleri anlamlı düzeyde daha düşüktü ($p<0.05$).

Sonuç: Silostazol ve rosuvastatinin böbrek iskemi/reperfüzyon ve uzak kardiyak reperfüzyon hasarı üzerinde koruyucu etkileri olmakla birlikte, bu etki kombine tedaviye kıyasla silostazol tedavisi ile artabilir.

Anahtar sözcükler: Kalp; iskemi/reperfüzyon hasarı, böbrek.

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Acute kidney injury is an important problem for post-cardiotomy and intensive care unit patients.[1] Renal tubular damage and perioperative inflammation are critical factors which affect the development of kidney injury. Renal ischemia-reperfusion (I/R) injury activates inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α) and interleukins (ILs). Activation of the inflammatory mediators leads to leukocytes activation and leukocyte endothelial adhesion. This inflammatory activation process leads to remote organ injury, particularly heart injury with high morbidity and mortality.[2-4] Medical treatments which reduce renal and heart I/R injury may be beneficial reducing the morbidity and mortality.

Cilostazol, which is a phosphodiesterase inhibitor which increases the activity of cyclic adenosine monophosphate, is in use worldwide for the treatment of lower extremity peripheral arterial disease with arterial vasodilator effects and antiplatelet activities. It also exerts effects on the inflammatory systems by inhibiting them.[5] Its anti-inflammatory activity occurs by inhibition of the inflammatory mediators such as TNF-α, and this effect results in a protective activity. Statins are other agents which improve the endothelial function and decrease leukocyte adherence to the endothelium. They are frequently used for their anti-hyperlipidemic effects; however, it is currently well-established that they improve endothelial functions by upregulating endothelial nitric oxide synthase, increasing nitric oxide, and reducing endothelial leukocyte adherence.[6,7] Rosuvastatin is a highly potent member of the statins. There are data showing that cilostazol and statins improve the effects of each other's against I/R injury and endothelial damage.[5,8]

In this experimental study, we aimed to investigate the protective effects of oral cilostazol, rosuvastatin, and their combination against either renal ischemia or renal I/R-induced remote cardiac reperfusion injury using immunohistochemical and biochemical indicators.

MATERIALS AND METHODS

In this study, 35 female Sprague-Dawley rats (weight: 190-250 g; age 3.5-4 months) were used. The experiments were conducted at Animal Research Laboratory, after the approval of Animal Care and Use Committee. The included rats were randomly divided into five groups (n=7): control group, I/R group, I/R + cilostazol (I/R-CIL) group, I/R + rosuvastatin (I/R-ROS) group, and I/R + rosuvastatin + cilostazol (I/R-CIL+ROS) group. The rats were followed in the lab and kept at 20-22 °C during the study with a 12-hour light and dark rhythm. For the feeding of rats, unlimited tap water and standard rodent feed were used ad libitum. The animals used in this study were maintained in accordance with the guidelines of the Committee on Animals and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council [DHEW publ. no. (NIH) 85-23, revised 1985].

One week before starting the experiments, the animals were allowed to adapt to the ambient conditions. Preoperatively, anesthetic ketamine HCl (Ketalar®, Pfizer, Istanbul, Turkey) 40 mg/kg and xylazine (Rompun®, Bayer, Istanbul, Turkey) 5 mg/kg combination were administered intramuscularly. If necessary, during the experiment, the administration of one additional dose of ketamine HCl was planned. During the procedure, anesthesia was delivered to maintain spontaneous respiration of the rats. The rats were lied down in the supine position on the heated table and under the heated lamp. Venous entry site was opened on the tail vein. After entry sites on the skin of all rats were aseptically prepared, a midline laparotomy incision was made starting immediately below xyphoid and extending up to 0.5 cm above the pubis. After laparotomy, the intestines of the rats were wrapped into wet sterile gauze and deviated to the upper side. During the experiment, 10 mL/kg 0.9% NaCl was given through the tail vein (Perfusor Compact, B. Braun®, Melsungen, Germany) for fluid resuscitation. Atraumatic micro-vascular clamps were placed on bilateral renal arteries (Nova clip® 12 mm Angle, Plymouth, USA). Following clamping, disappearance of arterial pulsation and blanching the kidney color indicated renal ischemia, and reperfusion defined as the emergence of arterial pulsation and changing the kidney color to red again after removal of the clamp. Following clamping, intestines were put in back, and nearly 5 mL warm physiological saline was sprayed into the peritoneal cavity. To prevent fluid loss, a laparotomy incision was approximated with three separate 4/0 silk sutures. After 45 minutes, the clamps were removed and the laparotomy incision was sutured with continuous 3/0 polypropylene sutures. Twenty-four hours after the first surgical intervention, using the same anesthetic protocol, laparotomy and median sternotomy were performed. Blood samples were taken from the right atrial space using 5-mL syringe, and the heart and bilateral kidneys were, then, extracted.

In the control group, no drug study medication was administered to the rats during preoperative period.

Renal arteries were explored via laparotomy and renal arteries were not clamped after exploration. The laparotomy incision was sutured. Twenty-four hours after the operation, blood samples were taken, organs were extracted, and the rats were sacrificed.

In the I/R group, no drug study medication was administered to the rats during preoperative period. Renal arteries were clamped. Forty-five minutes after the clamping period, the clamps were released and laparotomy incision was sutured. Twenty-four hours after the operation, blood samples were taken, organs were extracted, and the rats were sacrificed.

Starting three days before the surgical intervention, daily cilostazol dose of 20 mg/kg (Cilostazol, Otsuka Pharmaceutical Co., Tokushima, Japan), rosuvastatin 10 mg/kg (Crestor, Astra Zeneca, IPR Pharmaceuticals Inc., Porto Riko), and cilostazol 20 mg/kg + rosuvastatin 10 mg/kg were given with gastric gavage at the same time each day to the I/R+CIL group, I/R+ROS group, and I/R+CIL+ROS group, respectively. On the fourth day, the first surgical intervention was performed. Renal arteries were occluded, and after 45 minutes of renal ischemia, the clamps were removed. Abdominal layers were re-approximated. Twenty-four hours after the operation, blood samples were taken, organs were extracted, and the rats were sacrificed.

Bilateral kidneys and heart tissue samples were stored in 10% formaldehyde solution until immunohistochemical examination. A pathologist blinded to the study performed histopathological examinations using a light microscope (Olympus BX51).

Tumor necrosis factor-alpha ([4E1]: sc-130349; Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA) and hypoxia-induced factor-1α (HIF-1α; [H1alpha 67]: sc-53546; Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA) antibodies were used for immunohistochemical examinations of the left ventricle muscle and kidney tissue cross-sections. Density and intensity of dye uptake of the TNF-α antibody was evaluated, as described by Khandoga et al.[9] The TNF-α was evaluated as no staining (-), slight staining (+) and intense staining (++). Immunohistochemical findings according to the groups were calculated as 0 points for no staining (-), 1 point for slight staining (+) and 2 points for intense staining (++). The HIF-1α was evaluated as (+) for 1 to 25% staining, (++) for 26 to 50% staining, (+++) for 51 to 75% staining, and (+++) for 76 to 100% staining, and they were given degrees as 0,1,2,3, and 4, respectively. For both antibodies, staining in the left ventricle muscle fibers and kidney section were evaluated. Cytoplasmic staining for TNF-α and, nuclear and cytoplasmic staining for HIF-1α was accepted as positive.

Blood samples were centrifuged at 4000 r/min for four min at room temperature for 30 minutes, and the rat plasma samples were, then, stored at -80 °C. The Cobass 6000 analyzer (Roche Diagnostics, USA) and c-501 biochemical module was used to measure the followings: serum urea (UV calorimetric method), creatinine (kinetic colorimetric method), creatine kinase-muscle and brain (immunological UV method), troponin-I (enzyme-linked fluorescent assay method).

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean, standard deviation, median, frequency, and percentage. The Kruskal-Wallis and Mann-Whitney U tests were used to analyze significant differences between the groups.

RESULTS

The immunohistochemical examination of renal cross-sections revealed that TNF-α and HIF-1α immunoreactivities in I/R+CIL ($p=0.003$, $p=0.001$), I/R+ROS ($p=0.003$, $p=0.001$) and I/R+CIL+ROS ($p=0.003$, $p=0.002$) groups were significantly lower, compared to I/R group ($p<0.05$). Kidney TNF-α immunoreactivity in I/R+CIL+ROS ($p=0.035$) group was significantly higher, compared to control group. These result showed that combined therapy was not beneficial as monotherapy. However, TNF-α immunoreactivities between I/R+CIL ($p=0.227$) and I/R+ROS ($p=0.096$) groups and HIF-1α immunoreactivites among I/R+CIL, I/R+ROS and I/R+CIL+ROS groups were found similar, compared to control group ($p>0.05$) (Tables 1 and 2; Figures 1 and 2).

Serum urea and creatinine levels were significantly lower in I/R+CIL ($p=0.002$, $p=0.002$), I/R+ROS ($p=0.004$, $p=0.002$) and I/R+CIL+ROS ($p=0.009$, $p=0.003$) groups, compared to I/R group ($p<0.05$). There was no significant difference in serum urea levels among I/R+CIL, I/R+ROS and I/R+CIL+ROS groups ($p>0.05$). There was no significant difference in serum creatinine levels between the control group and I/R+CIL group ($p>0.05$). However, serum creatinine levels of the IR+CIL group were significantly lower, compared to I/R+ROS ($p=0.034$) and I/R+CIL+ROS ($p=0.035$) groups ($p<0.05$) (Table 3).

The immunohistochemical examination of myocardial cross-sections revealed that TNF-α

Table 1. Tumor necrosis factor-alpha scores for kidney and myocardial sections in all groups

	Kidney TNF-α					Myocard TNF-α				
	(-)	I(+)	II(+)	Mean±SD	Median	(-)	I(+)	II(+)	Mean±SD	Median
Control	6	1	0	0.1±0.4	0.0	6	1	0	0.1±0.4	0.0
I/R	0	0	7	2.0±0.0	2.0	0	3	4	1.6±0.5	2.0
I/R+CIL	4	2	1	0.6±0.8	0.0	4	2	1	0.6±0.8	0.0
I/R+ROS	3	3	1	0.7±0.8	1.0	2	3	2	1.0±0.8	1.0
I/R+CIL+ROS	2	4	1	0.9±0.7	1.0	0	6	1	1.1±0.4	1.0
				0.001					0.005	

Kruskal-Wallis, Mann-Whitney U test; SD: Standard deviation; TNF-α: Tumor necrosis factor-alpha; I/R: Ischemia/reperfusion; I/R+CIL: Ischemia/reperfusion + cilostazol; I/R+ROS: Ischemia/reperfusion + rosuvastatin; I/R+CIL+ROS: Ischemia/reperfusion + cilostazol + rosuvastatin; CK-MB: Creatine kinase-muscle and brain.

immunoreactivities in I/R+CIL (p=0.025) group was significantly lower, compared to I/R group (p<0.05), indicating that there was no significant difference between I/R+CIL and control groups (p>0.05), indicating no significant difference among I/R (median=2.0), I/R+ROS (median=1.0) and I/R+CIL+ROS (median=1.0) groups (p>0.05) groups. Although there was not statistical significant difference, the median values of these groups were lower, compared to I/R group; therefore, immunoreactivity of the I/R+ROS and IR+CIL+ROS groups were slightly low, compared to I/R group (Table 1). The HIF-1α immunoreactivities of I/R+CIL (p=0.023), I/R+ROS (p=0.011), and I/R+CIL+ROS (p=0.015) groups were significantly lower, compared to I/R group. There was no significant difference among I/R+CIL, I/R+ROS, and control group (p>0.05); however, HIF-1α immunoreactivities of I/R+CIL+ROS group were significantly higher, compared to the control group (p=0.037) (Table 2; Figure 3 and 4).

Serum CK-MB and troponin levels in I/R+CIL (p=0.003, p=0.004), I/R+ROS (p=0.018, p=0.009), and I/R+CIL+ROS (p=0.048, p=0.030) groups were significantly lower, compared to I/R group (p<0.05). Serum CK-MB levels in I/R-CIL group was significantly lower, compared to I/R+ROS (p=0.018) and I/R+CIL+ROS (p=0.025) groups (p<0.05); however, there was no significant difference in serum troponin levels among I/R+CIL, I/R+ROS, I/R+CIL+ROS groups (p>0.05) (Table 3).

DISCUSSION

Currently, it is well-established that systemic inflammatory activation and multi-organ failure are the major causes of the mortalities.[10] This activation mostly occurs after cardiac surgical interventions and may cause acute kidney injury after cardiac surgery, particularly due to the deleterious effects of cardiopulmonary bypass. In addition, inflammatory activation which increases with acute kidney injury has deleterious effects on remote organs, particularly on heart.[10,11]

There are many studies investigating direct or indirect renal and myocardial I/R injury. Some authors investigated the effects of agents such as niacin,

Table 2. Hypoxia induced factor-1 alpha scores for kidney and myocard sections in all groups

	Kidney HIF-1α						Myocard HIF-1α					
	I(+)	II(+)	III(+)	IV(+)	Mean±SD	Median	I(+)	II(+)	III(+)	IV(+)	Mean±SD	Median
Control	7	0	0	0	0.1±0.0	1.0	6	1	0	0	1.1±0.4	1.0
I/R	0	1	3	3	3.3±0.8	3.0	0	3	3	1	2.7±0.8	3.0
I/R+CIL	6	1	0	0	1.1±0.4	1.0	4	2	1	0	1.6±0.8	1.0
I/R+ROS	5	2	0	0	1.3±0.5	1.0	3	4	0	0	1.6±0.5	2.0
I/R+CIL+ROS	4	3	0	0	1.4±0.5	1.0	2	5	0	0	1.7±0.5	2.0
p					0.000						0.005	

Kruskal-Wallis, Mann-Whitney U test; SD: Standard deviation; HIF-1α: Hypoxia induced factor-1 alpha; I/R: Ischemia/reperfusion; I/R+CIL: Ischemia/reperfusion + cilostazol; I/R+ROS: Ischemia/reperfusion + rosuvastatin; I/R+CIL+ROS: Ischemia/reperfusion + cilostazol + rosuvastatin; CK-MB: Creatine kinase-muscle and brain.

furosemide, statins, and diltiazem.[3,12-14] In a study, Tai et al.[12] created an experimental model with bilateral kidney I/R injury and examined cardiac function. They found that kidney I/R injury was associated with cardiac dysfunction and niacin reduced myocardial oxidative stress using indicators such as serum troponin, urea, creatinine, and malondialdehyde. In another study, the effects of sitagliptin and furosemide were examined on renal I/R-induced myocardial injury, and they found protective effects using biochemical and immunohistochemical indicators.[3] In our experimental model, we designed bilateral kidney I/R model and we explored for the first time the protective effects of cilostazol and rosuvastatin on kidney I/R injury and remote cardiac reperfusion injury using biochemical and immunohistochemical indicators.

Cilostazol is an important advance in the treatment of peripheral arterial disease, and there are many studies on muscle ischemia and its indirect reperfusion injury on other organs with cilostazol pretreatment.[15-17] However, recent studies have addressed to the immunomodulatory effects of cilostazol, particularly on kidney and heart. Ragap et al.[18] investigated the effects of cilostazol and pioglitazone on bilateral renal I/R injury in a rat model. They administered 50 mg and 100 mg cilostazol orally for two weeks and after a 45-min bilateral renal ischemia period, they applied 24-hour reperfusion. The authors found that low doses of cilostazol had renoprotective effects via modulating nitric oxide synthase and IL-18. In another study, Gokce et al.[13] investigated the effects of cilostazol and diltiazem therapies on cyclosporine-induced nephrotoxicity. They occluded only left renal artery for 60 min and they gave oral cilostazol and diltiazem therapy for seven days. At the end of the seven days, they found that cilostazol inhibited renal I/R injury. In our study, we administered oral cilostazol and rosuvastatin for three days, and we clamped bilateral renal arteries for 45 min. Then, we extracted both kidneys 24-hours later. Our results showed that

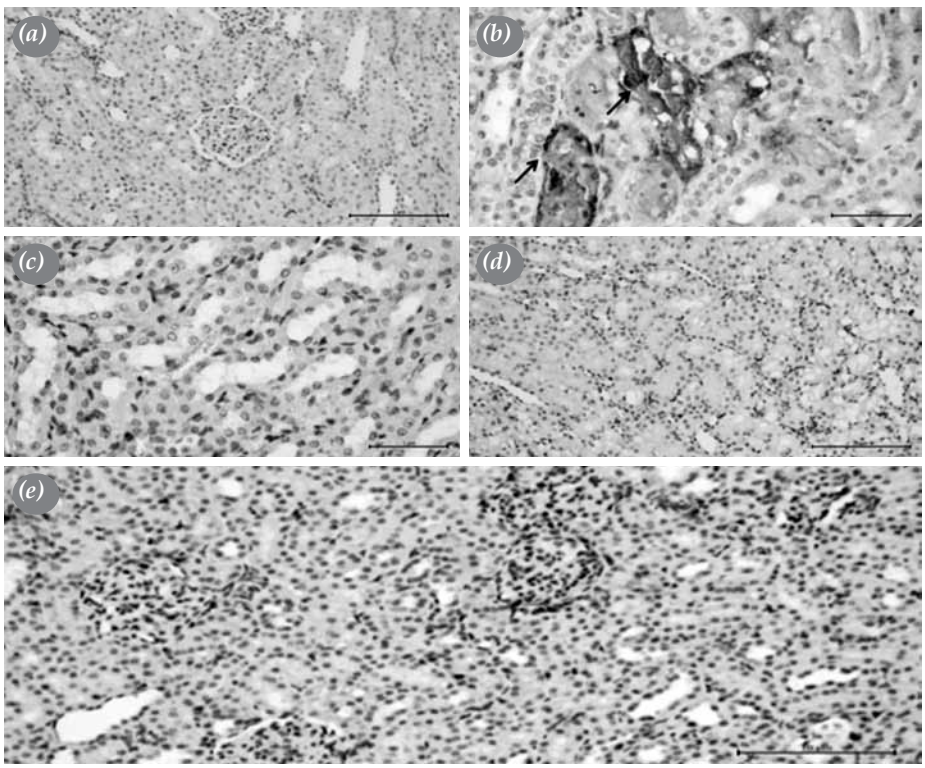

Figure 1. Kidney cross-sections; **(a)** Control group; TNF-α negative cytoplasmic staining on renal glomerulus and tubules (x400), **(b)** I/R Group; Intense cytoplasmic staining of TNF-α on renal tubules (arrows) (x400), **(c)** I/R+CIL Group; No cytoplasmic TNF-α staining on renal tubules (x400), **(d)** I/R+ROS Group; No cytoplasmic TNF-α staining on renal tubules (x400), **(e)** I/R+CIL+ROS Group: No cytoplasmic TNF-α staining on renal tubules (x200).

TNF-α: Tumor necrosis factor-alpha; I/R: Ischemia/Reperfusion; I/R+CIL: Ischemia/Reperfusion + Cilostazol; I/R+ROS: Ischemia/Reperfusion + Rosuvastatin; I/R+CIL+ROS: Ischemia/Reperfusion + Cilostazol + Rosuvastatin.

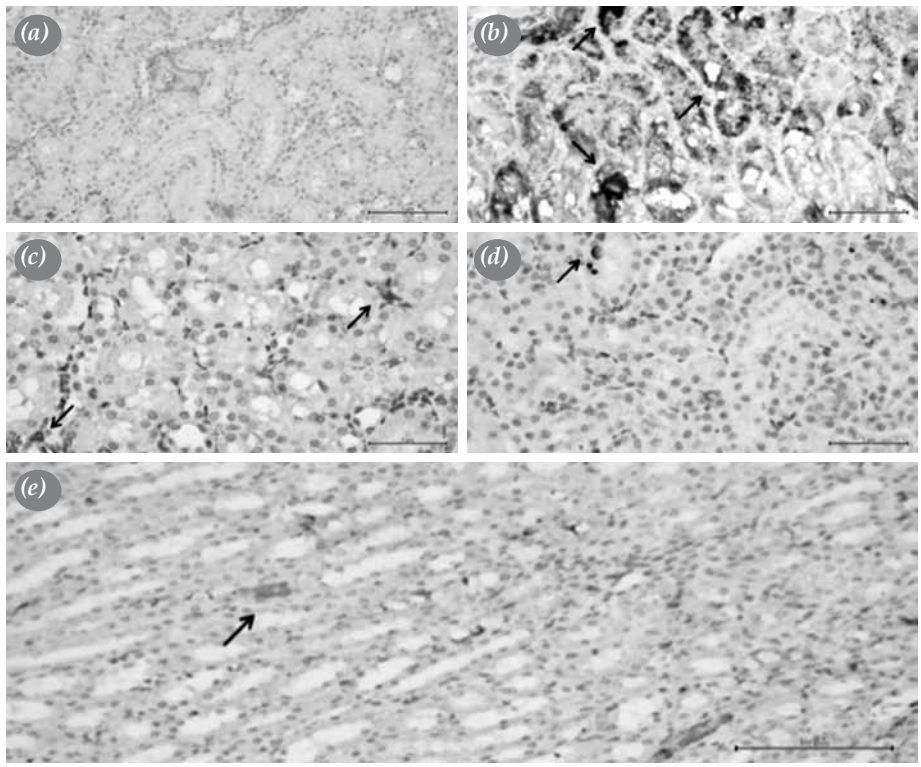

Figure 2. Kidney cross-sections; **(a)** Control group; Normal renal glomerulus and tubules, **(b)** I/R Group; Cytoplasmic and nuclear staining of HIF-1α on renal tubules (arrows) (x400), **(c)** I/R+CIL Group; Focal cytoplasmic HIF-1α staining on renal tubules (arrows), **(d)** I/R+ROS Group; Focal nuclear HIF-1α staining on renal tubules (arrow) (x400), **(e)** I/R+CIL+ROS Group: Focal cytoplasmic and nuclear HIF-1α staining on renal tubules (arrow) (x400).

I/R: Ischemia/Reperfusion; HIF-1α: Hypoxia Induced Factor-1 alpha; I/R+CIL: Ischemia/Reperfusion + Cilostazol; I/R+ROS: Ischemia/Reperfusion + Rosuvastatin; I/R+CIL+ROS: Ischemia/Reperfusion + Cilostazol + Rosuvastatin.

TNF-α immunoreactivity, HIF-1α immunoreactivity, and serum urea and creatinine levels decreased in the medicated groups, compared to I/R group. Hence, we can conclude that cilostazol and rosuvastatin have inhibitory effects on preventing bilateral kidney ischemic injury. However, kidney TNF-α immunoreactivity in I/R+CIL+ROS group was found to be significantly increased, compared to the control

Table 3. Serum urea, creatinine, creatine kinase-muscle brain and troponin levels in all groups

	Control	I/R	I/R+CIL	I/R+ROS	I/R+CIL+ROS	*p*
Urea						0.000
Mean±SD	31.1±6.0	175.6±53.7	64.0±11.2	79.6±25.0	86.6±43.2	
Median	28.0	161.0	61.0	73.0	78.0	
Creatinin						0.000
Mean±SD	0.2±0.0	0.7±0.2	0.3±0.1	0.4±0.0	0.4±0.1	
Median	0.3	0.8	0.3	0.4	0.4	
CK-MB						0.002
Mean±SD	438.8±168.9	1017.9±369.1	386.1±79.7	560.7±193.4	639.7±260.3	
Median	425.5	958.3	400.0	465.2	526.4	
Troponin						0.010
Mean±SD	0.4±0.5	1.9±1.4	0.2±0.2	0.3±0.3	0.5±0.5	
Median	0.1	1.3	0.2	0.1	0.3	

SD: Standard deviation; I/R: Ischemia/Reperfusion, I/R+CIL: Ischemia/Reperfusion+Cilostazol, I/R+ROS: Ischemia/Reperfusion+Rosuvastatin, I/R+CIL+ROS: Ischemia/Reperfusion+Cilostazol+Rosuvastatin.

group. These results indicate that combined therapy or rosuvastatin monotherapy may not be as beneficial as cilostazol. Lower serum creatinine levels in I/R+CIL group, compared I/R+ROS and I/R+CIL+ROS groups, also supported this finding.

Statins have preventive effects on myocardial and kidney injury, following ischemic and inflammatory processes such as acute coronary syndrome, percutaneous coronary intervention, and cardiovascular surgery.[14,19-22] Several studies have shown that atorvastatin and rosuvastatin protects kidney, improving glomerular filtration rate and reducing proteinuria, and atorvastatin may even be more effective in reducing proteinuria.[23] In a study, the rats were subjected to 30 min myocardial ischemia, following an 18-hour intraperitoneal rosuvastatin at a dose of 0.1 to 5 mg/kg.[24] The authors examined the myocardial tissues, and found that myocardial nitric oxide synthase increased and myocardial necrosis decreased. In our study, we found that direct renal I/R injury and myocardial reperfusion injury decreased with rosuvastatin. This protective effect was found to be statistically significant on kidney TNF-α cross-sections and, kidney and myocardial HIF-1α cross-sections for rosuvastatin, but not on myocardial TNF-α cross-sections for rosuvastatin. There was no significant difference in TNF-α immunoreactivities among IR, IR+ROS, and IR+CIL+ROS groups, while HIF-1α immunoreactivity in IR+CIL+ROS group was higher, compared the control group; however, it was lower, compared to IR group. Therefore, we suggest that rosuvastatin may have lower beneficial effects on myocardial protection, which may contradict with

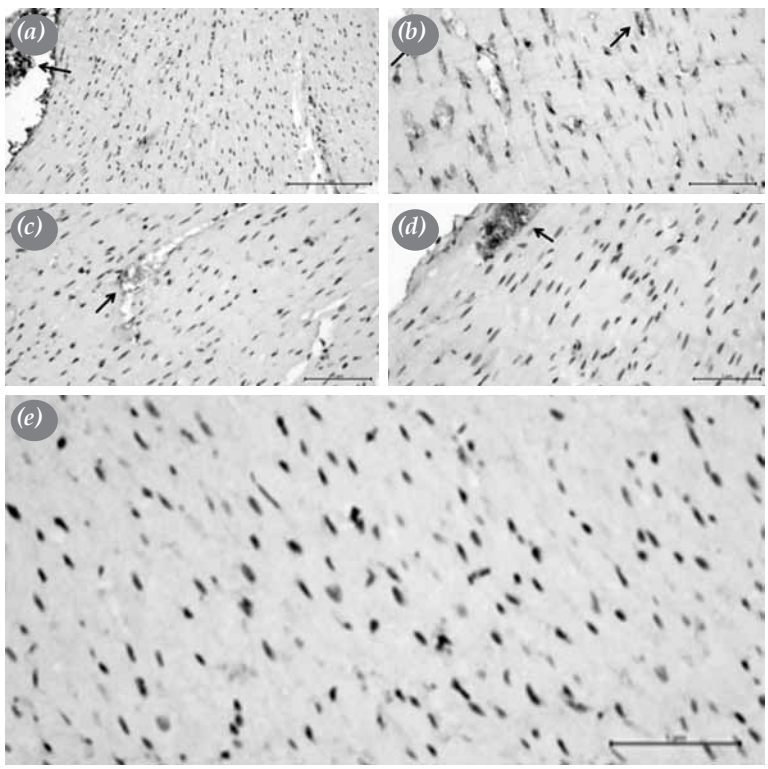

Figure 3. Myocardial cross-sections; **(a)** Control group; TNF-α-negative cytoplasmic immunoreactivity on myocytes and TNF-α-positive staining on erythrocytes in a vascular area (arrow) (x400), **(b)** I/R Group; Intense cytoplasmic staining of TNF-α on myocytes (arrows) (x400), **(c)** I/R+CIL Group; No cytoplasmic TNF-α staining on myocytes and positive staining on erythrocytes in a vascular area (arrow) (x400), **(d)** I/R+ROS Group; No cytoplasmic TNF-α staining on myocytes and positive staining on erythrocytes in a vascular area (arrow) (x400), **(e)** I/R+CIL+ROS Group: No cytoplasmic TNF-α staining on myocytes (x200).

TNF-α: Tumor necrosis factor-alpha; I/R: Ischemia/Reperfusion; I/R+CIL: Ischemia/Reperfusion + Cilostazol; I/R+ROS: Ischemia/Reperfusion + Rosuvastatin; I/R+CIL+ROS: Ischemia/Reperfusion + Cilostazol + Rosuvastatin.

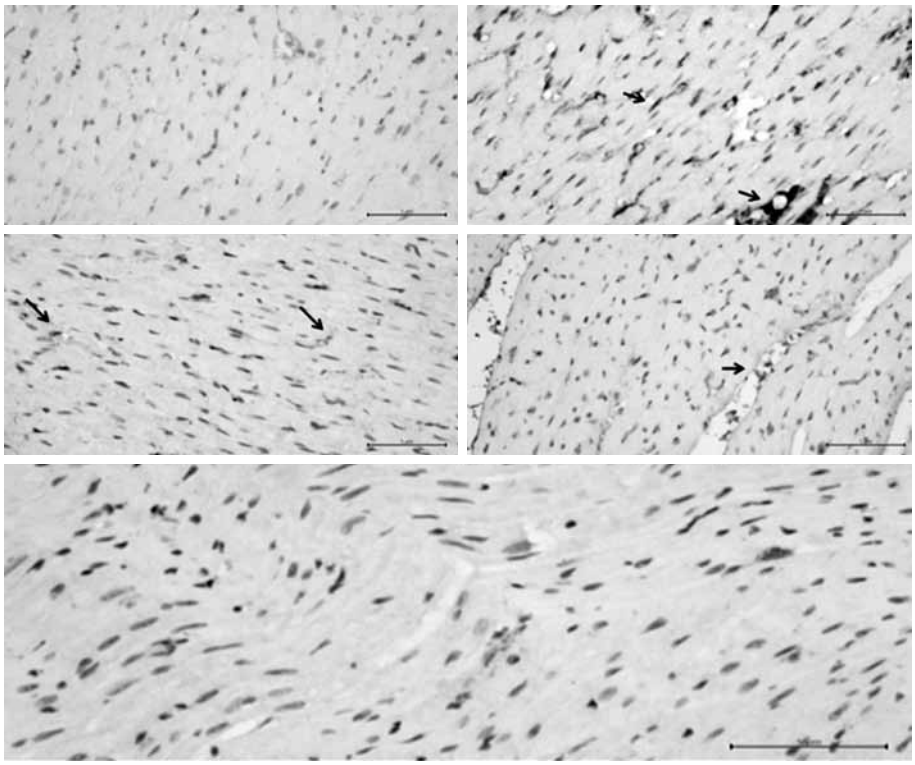

Figure 4. Myocardial cross-sections; **(a)** Control group; Normal myocytes, **(b)** I/R Group; Cytoplasmic and nuclear diffuse staining of HIF-1α on myocytes (arrows) (x400), **(c)** I/R+CIL Group; Focal nuclear HIF-1α staining on myocytes (arrows), **(d)** I/R+ROS Group; Focal nuclear HIF-1α staining on myocytes and positive erythrocyte staining in vascular area (arrow) (x400), **(e)** I/R+CIL+ROS Group: Focal cytoplasmic and nuclear HIF-1α staining.

I/R: Ischemia/Reperfusion; HIF-1α: Hypoxia Induced Factor-1 alpha; I/R+CIL: Ischemia/Reperfusion + Cilostazol; I/R+ROS: Ischemia/Reperfusion + Rosuvastatin.

aforementioned studies. Cilostazol have also a powerful effect, and rosuvastatin may decrease the effect of cilostazol, when combined, on direct renal I/R injury and remote myocardial reperfusion injury. Thus, there is a need to more specific studies investigating how rosuvastatin affects alone and combined treatments, and which factors affect their activity. In addition, the effect of rosuvastatin with other statins should be examined.

Furthermore, Manickavasagam et al.[5] investigated the cardioprotective effects of atorvastatin and cilostazol combination. They administered oral pretreatment for three days and performed 30 min coronary artery occlusion and 4-hour reperfusion. They found that cilostazol, but not atorvastatin, reduced the infarct size. However, the combination of these drugs was more effective. They concluded that cilostazol might augment the effect of statins. In our experimental model, we investigated myocardial protective effects of oral cilostazol, rosuvastatin, and their combination. We found that cilostazol, rosuvastatin, and combined therapy reduced TNF-α and HIF-1α immunoreactivity on myocardial cross-sections. Biochemical indicators also supported this finding. However, our results showed that protective effect of cilostazol monotherapy on inflammation was higher. Rosuvastatin had low beneficial effects and it reduced the protective effect of the combination therapy. These results showed that our study is in consistent with previous studies; however, our results additionally indicated that the combination of rosuvastatin with cilostazol did not augment the effect of each other.

In conclusion, our study results show that cilostazol, rosuvastatin, and combined therapy before bilateral kidney ischemia-reperfusion have protective effects on kidney injury. Remote cardiac reperfusion injury after 24-hour kidney ischemia-reperfusion also decreases with cilostazol, rosuvastatin, and combined pre-medication, and this decrease is higher with cilostazol monotherapy.

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REFERENCES

1. Lafrance JP, Miller DR. Acute kidney injury associates with increased long-term mortality. J Am Soc Nephrol 2010;21:345-52.
2. Burne-Taney MJ, Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. Am J Physiol Renal Physiol 2003;285:87-94.
3. Youssef MI, Mahmoud AA, Abdelghany RH. A new combination of sitagliptin and furosemide protects against remote myocardial injury induced by renal ischemia/reperfusion in rats. Biochem Pharmacol 2015;96:20-9.
4. Gülmen Ş, Doğuç DK, Ceylan BG, Çetin NK, Meteoğlu İ, Okutan H, et al. The effect of beta-glucan on kidney injury in experimental aortic ischemia-reperfusion. Turkish J Thorac Cardiovasc Surg 2011;19:234-41.
5. Manickavasagam S, Ye Y, Lin Y, Perez-Polo RJ, Huang MH, Lui CY, et al. The cardioprotective effect of a statin and cilostazol combination: relationship to Akt and endothelial nitric oxide synthase activation. Cardiovasc Drugs Ther 2007;21:321-30.
6. Hashimoto A, Miyakoda G, Hirose Y, Mori T. Activation of endothelial nitric oxide synthase by cilostazol via a cAMP/protein kinase A- and phosphatidylinositol 3-kinase/Akt-dependent mechanism. Atherosclerosis 2006;189:350-7.
7. Wakida K, Morimoto N, Shimazawa M, Hozumi I, Nagase H, Inuzuka T, et al. Cilostazol reduces ischemic brain damage partly by inducing metallothionein-1 and -2. Brain Res 2006;1116:187-93.
8. Di Napoli P, Antonio Taccardi A, Grilli A, Spina R, Felaco M, Barsotti A, et al. Simvastatin reduces reperfusion injury by modulating nitric oxide synthase expression: an ex vivo study in isolated working rat hearts. Cardiovasc Res 2001;51:283-93.
9. Khandoga A, Enders G, Biberthaler P, Krombach F. Poly(ADP-ribose) polymerase triggers the microvascular mechanisms of hepatic ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol 2002;283:553-60.
10. Martin GS, Becker BN, Schulman G. Cardiac troponin-I accurately predicts myocardial injury in renal failure. Nephrol Dial Transplant 1998;13:1709-12.
11. Hansen MK, Gammelager H, Jacobsen CJ, Hjortdal VE, Layton JB, Rasmussen BS, et al. J Cardiothorac Vasc Anesth 2015;29:617-25.
12. Tai ST, Fu YH, Yang YC, Wang JJ. Niacin ameliorates kidney warm ischemia and reperfusion injury-induced ventricular dysfunction and oxidative stress and disturbance in mitochondrial metabolism in rats. Transplant Proc 2015;47:1079-82.
13. Gokce M, Yuzbasioglu MF, Bulbuloglu E, Oksuz H, Yormaz S, Altınoren O, et al. Cilostazol and diltiazem attenuate cyclosporine-induced nephrotoxicity in rats. Transplant Proc 2012;44:1738-42.
14. Ball T, McCullough PA. Statins for the prevention of contrast-induced acute kidney injury. Nephron Clin Pract 2014;127:165-71.
15. Frias Neto CA, Koike MK, Saad KR, Saad PF, Montero EF. Effects of ischemic preconditioning and cilostazol on muscle ischemia-reperfusion injury in rats. Acta Cir Bras 2014;29:17-21.
16. Frias Neto CA, Koike MK, Saad KR, Saad PF, Montero EF. Effects of ischemic preconditioning and cilostazol on muscle ischemia-reperfusion injury in rats. Acta Cir Bras 2014;29:17-21.
17. Moreira Neto AA, Souza Júnior SS, Capelozzi VL, Parra-Cuentas ER, Schmidt Júnior AF, Francisco Neto A, et al. Effects of cilostazol in kidney and skeletal striated muscle of Wistar rats submitted to acute ischemia and reperfusion of hind limbs. Acta Cir Bras 2012;27:783-8.
18. Ragab D, Abdallah DM, El-Abhar HS. Cilostazol renoprotective effect: modulation of PPAR-γ, NGAL, KIM-1 and IL-18 underlies its novel effect in a model of ischemia-reperfusion. PLoS One 2014;9:95313.
19. Jiang F, Yang J, Zhang L, Li R, Zhuo L, Sun L, et al. Rosuvastatin reduces ischemia-reperfusion injury in patients with acute coronary syndrome treated with percutaneous coronary intervention. Clin Cardiol 2014;37:530-5.
20. Layton JB, Hansen MK, Jakobsen CJ, Kshirsagar AV, Andreasen JJ, Hjortdal VE, et al. Statin initiation and acute kidney injury following elective cardiovascular surgery: a population cohort study in Denmark†. Eur J Cardiothorac Surg 2016;49:995-1000.
21. Qiao B, Deng J, Li Y, Wang X, Han Y. Rosuvastatin attenuated contrast-induced nephropathy in diabetes patients with renal dysfunction. Int J Clin Exp Med 2015;8:2342-9.
22. Weinberg EO, Scherrer-Crosbie M, Picard MH, Nasseri BA, MacGillivray C, Gannon J, et al. Rosuvastatin reduces experimental left ventricular infarct size after ischemia-reperfusion injury but not total coronary occlusion. Am J Physiol Heart Circ Physiol 2005;288:1802-9.
23. Wu Y, Wang Y, An C, Dong Z, Liu H, Zhang Y, et al. Effects of rosuvastatin and atorvastatin on renal function: meta-analysis. Circ J 2012;76:1259-66.
24. Jones SP, Gibson MF, Rimmer DM, Gibson TM, Sharp BR, Lefer DJ. Direct vascular and cardioprotective effects of rosuvastatin, a new HMG-CoA reductase inhibitor. J Am Coll Cardiol 2002;40:1172-8.