Rosuvastatin Given During Reperfusion Decreases Infarct Size and Inhibits Matrix Metalloproteinase-2 Activity in Normocholesterolemic and Hypercholesterolemic Rabbits

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Abstract: There is evidence that statin treatment before ischemia protects myocardium from ischemia/reperfusion injury. The objective is to determine whether rosuvastatin administered during reperfusion modifies infarct size and the recovery of postischemic ventricular dysfunction in normocholesterolemic and hypercholesterolemic rabbits. In addition, we also evaluated the role of matrix metalloproteinase type 2 (MMP)-2 activation. Langendorff-perfused rabbit hearts were subjected to 30 minutes of ischemia and 120 minutes of reperfusion. In group 2, we added rosuvastatin after 30 minutes of ischemia and from the beginning of reperfusion. In group 3, an MMP inhibitor (doxycycline) was administered during the first 2 minutes of reperfusion. Finally, we repeated these groups but in hypercholesterolemic rabbits (groups 4, 5, and 6). The infarct size was 16.6% ± 3.9% in group 1 and 25.6% ± 2.7% in group 4. Rosuvastatin reduced infarct size to 4.5% ± 1.1% and 6.1% ± 1.5% in groups 2 and 5, respectively ($P < 0.05$). Rosuvastatin significantly decreased MMP-2 activity during reperfusion, and doxycycline induced an inhibition of MMP-2 activity and a reduction of infarct size in normocholesterolemic (4.9% ± 0.9%) and hypercholesterolemic animals (8.3% ± 1.6%) ($P < 0.05$). Rosuvastatin reduces infarct size and attenuates MMP-2 activity. These data and the correlation between MMP-2 and infarct size suggest that MMP-2 plays an important role in the mechanisms of cardioprotection afforded by rosuvastatin.

Key Words: myocardial infarction, statins, matrix metalloproteinases

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INTRODUCTION

Several large clinical trials showed that HMG CoA reductase inhibitors (statins) reduce cardiovascular morbidity and mortality.[1,2] Furthermore, several groups have reported that statins are able to exert beneficial cardiovascular effects, independent of cholesterol levels.[3–5]

Bell and Yellon[6] documented beneficial pleiotropic effects of atorvastatin in protecting the murine isolated heart against lethal reperfusion-induced injury. It appears that this effect is conferred through activation of the phosphatidylinositol 3-kinase (PI3K)/AKT prosurvival signaling pathway. Furthermore, Di Napoli et al[7] showed cardioprotective effects when simvastatin was acutely administered before ischemia in the isolated rat heart, and they related it to the effects of the drug on nitric oxide (NO) synthases.

Thus, previous studies in animals demonstrated that statin treatment before the onset of myocardial ischemia reduces ischemia/reperfusion injury.[7–10] Although these studies demonstrate the impact of prophylactic therapy, they do not address the question whether patients with acute myocardial infarction might benefit from an initiation of statin therapy before starting the reperfusion. In this sense, Bauersachs et al[11] failed to demonstrate a reduction in infarct size when the statin was administered 24 hours after onset of myocardial ischemia similar to the treatment protocol of the Myocardial Ischemia Reduction with Acute Cholesterol Lowering trial.[12] The acute administration of rosuvastatin exerts direct vascular and cardioprotective effects in models of ischemia–reperfusion by increasing endothelial NO production or reducing polymorphonuclear leukocyte adherence.[13]

It is important to emphasize that all these studies were performed in normal animals and that the drug was administered before ischemia. To the best of our knowledge, no data currently exist on the effect of rosuvastatin on ischemia/reperfusion injury in animals with comorbidities like hypercholesterolemia.

On the other hand, matrix metalloproteinases (MMPs) behaved as mediators of acute myocardial postischemic dysfunction.[14,15] During reperfusion, MMP-2 is intracellularly activated and it cleaves troponin I[16] and myosin light chain 1.[17] Cardiac postischemic dysfunction correlates directly with activation of MMP-2, which is stimulated by peroxynitrite.[18] Because statins decrease reactive oxygen species generation,[19] rosuvastatin may remove endogenous peroxynitrite, a major stimulus for MMP-2 activation, subsequently reducing infarct size.

We have studied the infarct size–limiting effect of rosuvastatin in isolated rabbit hearts and measured changes in cardiac MMP-2 activity. To examine the link between MMP inhibition and rosuvastatin cardioprotection, we also studied the possible infarct size–limiting effect of pharmacological inhibition of MMPs with doxycycline administered during the first 2 minutes of reperfusion. Doxycycline is a member of the tetracycline family of antibiotics and has been shown to inhibit MMP expression and activity and to preserve cardiac function.[20–22]

MATERIALS AND METHODS

Experimental Protocols

Animals were assigned to 12 different experimental groups.

Myocardial Infarction Measurements

Group 1 (n = 12): 30 minutes of global no-flow ischemia and 2 hours of reperfusion induced myocardial infarction. Global no-flow ischemia was induced by abruptly decreasing the total coronary flow provided by the perfusion pump.

Group 2 (n = 10): The same protocol as in group 1 was performed, but rosuvastatin (30 μmol/L) was administered during the reperfusion.

Group 3 (n = 8): The same protocol as in group 1 was performed, but during the first 2 minutes of reperfusion, the nonselective MMPs inhibitor doxycycline (50 μmol/L) was administered.

Group 4 (n = 10), group 5 (n = 8), and group 6 (n = 6) were similar to group 1, group 2, and group 3, respectively, but the animals were fed a diet enriched with a 1% cholesterol during the 4 weeks period before killing and the rest of procedures.

Ischemic Coronary Vasodilatation Response

Ischemic vasodilatation response was tested after 15 minutes of global no-flow ischemia and 30 minutes of reperfusion. We used 15 minutes of ischemia to discard the influences of myocardial infarction on ventricular function.

Group 7 (n = 8): Vascular dysfunction was induced by 15 minutes of global no-flow ischemia and 30 minutes of reperfusion (stunned).

Group 8 (n = 8): The same protocol as in group 7 was performed, but adenosine (30 mmol/L) was administered during the reperfusion to test coronary vasodilatation response.

Group 9 (n = 4): The same protocol as in group 7 was performed, but adenosine (30 mmol/L) and rosuvastatin (30 μmol/L) were administered during the reperfusion to test coronary vasodilatation response.

Group 10 (n = 8), group 11 (n = 5), and group 12 (n = 4) were similar to group 7, group 8, and group 9, respectively, but the animals were fed a diet enriched with a 1% cholesterol during the 4 weeks period before killing.

Experimental Procedure

New Zealand rabbits (n = 91, 1.8–2.0 kg) were randomly assigned to 2 different dietary groups: 50 were fed standard rabbit food and 41 received a cholesterol-supplemented diet (1% cholesterol) for 4 weeks. The experimental procedures were conducted in compliance with the American Physiological Society's and National Institutes of Health guiding principles.

After 4 weeks of receiving the normal or cholesterol-enriched diet, the rabbits were anesthetized with ketamine (75 mg/kg) and xylazine (0.75 mg/kg) and then killed with a lethal dose of thiopental (35 mg/kg). The thorax was rapidly opened and the heart was excised. Each heart was placed in a perfusion system according to the modified Langendorff technique.

The heart was perfused with a Krebs–Henseleit buffer containing NaCl 118.5 mM, KCl 4.7 mM, $NaHCO_3$ 24.8 mM, KH_2PO_4 1.2 mM, Mg SO_4 1.2 mM, $CaCl_2$ 2.5 mM, and glucose 10 mM, pH 7.4 ± 1.4 and then gassed with 95% O_2–5% CO_2 at 37°C. Two electrodes were sutured and connected to a pacemaker with a constant heart rate of 200 beats/min.

A latex balloon connected to a pressure transducer (Deltram II, Utah Medical System) via a polyethylene cannula was inserted into the left ventricle (LV) for measurement of LV pressure. The latex balloon was filled with water to achieve an left ventricular end-diastolic pressure (LVEDP) of 8–10 mmHg. We also recorded the coronary perfusion pressure (CPP) through a pressure transducer connected to the perfusion line. All hearts were perfused with constant flow. Coronary flow was adjusted to obtain a CPP of 70.5 ± 4.2 mmHg during the initial stabilization period. This flow level was maintained constant throughout the experiment. In a heart perfused at a constant coronary flow, the CPP indicates vascular coronary resistance.[23]

LV pressure and CPP were recorded in real time using a computer with a data acquisition hardware. The left ventricular developed pressure (LVDP) was calculated as the difference between peak systolic pressure and LVEDP. Ventricular function was assessed at baseline and during the first 30 minutes of reperfusion, although the hearts were reperfused during 2 hours to measure infarct size.

Drugs

The dose of rosuvastatin was calculated based on previous studies performed in different animal models (2 $mg \cdot kg^{-1} \cdot day$ intravenous or intraperitoneal) to evaluate its pleiotropic effects.[13,14,21,22] At a temperature of 37°C and under continuous agitation, rosuvastatin completely dissolves in Krebs solution without addition of any solvent.

Doxycycline, a member of the tetracycline family of antibiotics, was used to inhibit MMP-2 activity. The dose was obtained from different authors[20–22] who showed a significant inhibition of MMP-2 activity in coronary effluent.

Adenosine was diluted in Krebs–Henseleit buffer. The dose used has shown a significant vasodilatation effect in isolated rabbit heart.[23]

Infarct Size Measurement

The assessment of the infarct size using triphenyltetrazolium is a validated and widely used method, both in vitro[24,25] and in vivo models,[26,27] and it is used in different animal species. After 2 hours of reperfusion, the hearts were frozen and cut into 2 mm transverse slices from apex to base.

Sections were incubated for 20 minutes in 1% triphenyltetrazolium chloride (pH 7.4, 37°C) and then immersed in 10% formalin. With this technique, viable sections were stained red, whereas the nonstained sections corresponded to the infarct area. Sections were traced to acetate sheets and planimetered (Image Pro Plus, version 4.5). The infarct size was expressed as a percentage of the LV area.

Biochemical Analyses

Blood samples were collected both at the beginning of the protocol and on the day of the killing to determine serum cholesterol levels. Total cholesterol and triglycerides were determined in a Hitachi 727 autoanalyzer (Tokyo, Japan) by enzymatic methods, standardized by Roche (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein cholesterol (HDL-C) was performed by precipitation[28,29] and non-HDL-C was calculated by subtracting HDL-C from total cholesterol. These parameters were determined under good quality control (coefficient of variation routinely <3%).

Zymographic Analysis of MMP Activity

Coronary effluent samples of 4 mL were collected to determine MMP activity during basal state and at different times during the reperfusion period (basal, 2, 5, and 30 minutes). Fixed volumes of effluent samples were concentrated (4 mL concentrated to 0.2 mL approximately) in Amicon Ultra Centrifugal 4mL-30K concentrating vessels (5000 g, 4°C, Millipore, MA). After concentration, proteins were measured by the Lowry method.[30] Metalloproteinase activity was detected with zymography. Sodium dodecyl sulfate–polyacrylamide gels (7.5%) were copolymerized with gelatin 0.1% (G-8150, Sigma). A constant amount of protein (2 μg) was loaded in each well in nonreducing conditions, and gels were run for 3 hours in 25 mM Tris, 192 mM glycine, and 0.1% sodium dodecyl sulfate at 4°C, pH: 8.3, in a Mini Protean-3 (Bio-Rad Laboratories). After running, gels were rinsed with 2.5% Triton X-100 for 30 minutes and incubated 18 hours in 0.15 M NaCl, 10 mM $CaCl_2$, and Tris HCl pH 7.4 at 37°C. After staining with Coomassie blue R-250 (B-0149, Sigma) and distained with acetic acid–methanol–water (1:3:6), enzyme activity was detected as colorless bands against the blue-stained background. The gelatinolytic bands disappeared in parallel zymograms in which the development buffer contained EDTA, confirming the gelatinolytic activity to be caused by metalloproteinases. Individual enzymes [MMP-2, 72 kDa (pro-form) and 64 kDa (active form)] were identified by molecular weight. Conditioned media from the promyelocyte U-937 cell line, kindly provided by Dr A. Jawerbaum (CEFYBO, Buenos Aires, Argentina), were used as activity standard for pro-MMP-2. Our coefficients of variation were 4.8% (intraassay) and 8.6% (interassay). Gels were scanned and band intensities quantified using Scion-Image J software (Scion Corporation), and relative activity was normalized to the internal standard, loaded in each gel, and expressed as a ratio to the basal value.

Statistical Analysis

Data are expressed as mean ± SEM. Intergroup comparisons were performed using analysis of variance and the Bonferroni test for multiple comparisons, and $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows average values for total cholesterol, HDL-C, and non-HDL-C in the plasma of animals before and after 4 weeks of cholesterol-enriched diet. An increase in both total cholesterol and non-HDL-C values was observed in the animals fed with the cholesterol-enriched diet versus control group ($P < 0.05$).

Figure 1 illustrates the LVDP (panels A and B) and LVEDP (panels C and D) at baseline, 2, 5 and 30 minutes of reperfusion in normocholesterolemic and hypercholesterolemic animals. Figure 1A shows that LVDP was significantly lower compared with the basal values at 30 minutes of reperfusion in both normocholesterolemic groups, although there were no significant differences over the course of the procedure related to the rosuvastatin treatment. In the control hypercholesterolemic group (Fig. 1B), LVDP decreased to 26.7 ± 3.9 mmHg after 30 minutes of reperfusion. Rosuvastatin attenuated postischemic ventricular dysfunction, reaching 49.5 ± 5.4 mmHg after 30 minutes of reperfusion ($P < 0.05$ vs. control group). The LVEDP (myocardial contracture) (Fig. 1C) increased significantly during the reperfusion period in both groups, without significant differences among groups. Figure 1D shows that LVEDP increased to 81.5 ± 6.3 mmHg at 30 minutes of the reperfusion phase. Administration of rosuvastatin attenuated the increase of myocardial contracture, reaching 35.6 ± 8.4 mmHg at 30 minutes of reperfusion ($P < 0.05$). Thus, treatment with rosuvastatin attenuated contractile state dysfunction and myocardial contracture after 30 minutes of global ischemia in hypercholesterolemic rabbit hearts. The administration of doxycycline did not modify the recovery of ventricular function in any of the studied groups.

Figure 2 shows the changes in CPP (mmHg) after 15 minutes of ischemia and 30 minutes of reperfusion. The ischemia and reperfusion induced the well-known vasoconstriction effect reflected in CPP increase in both normocholesterolemic (32.2% ± 5.3%) and hypercholesterolemic rabbit hearts (28.1% ± 9.6%). Adenosine administration decreased CPP only in normocholesterolemic rabbit hearts (20.8% ± 1.7%), showing that the coronary vasodilatation response is severely impaired after ischemia/reperfusion in hypercholesterolemic rabbits hearts. The administration of rosuvastatin with adenosine fully recovers coronary vasodilatation in hypercholesterolemic rabbit hearts, decreasing CPP to 27.1% ± 4.1%.

TABLE 1. Biochemical Analysis

	Total Cholesterol (mg/dL)	HDL-C (mg/dL)	Non-HDL-C (mg/dL)
Before cholesterol-enriched diet	59.6 ± 9.3	23.7 ± 2.6	21.5 ± 2.3
After cholesterol-enriched diet	185.4 ± 21.4*	32.1 ± 8.1	148.5 ± 37.2*

*$P < 0.05$ versus before cholesterol-enriched diet.

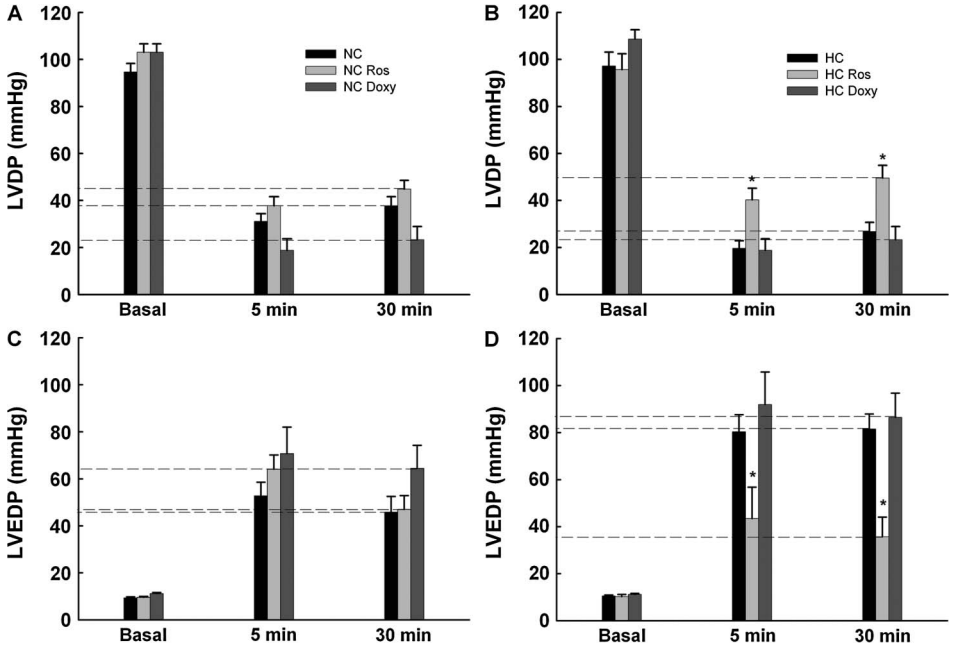

FIGURE 1. Panels A and B show LVDP in normocholesterolemic and hypercholesterolemic animals, respectively. No differences were observed between groups in normocholesterolemic animals. Rosuvastatin-treated animals show significant recovery of contractile state compared with controls (HC). Panels C and D show LVEDP in normocholesterolemic and hypercholesterolemic animals. Administration of rosuvastatin attenuates the increase of myocardial contracture only in hypercholesterolemic animals. Ros, rosuvastatin; NC, normocholesterolemic; HC, hypercholesterolemic. *$P < 0.05$ versus Basal HC.

Infarct size, expressed as a percentage of the LV area after 30 minutes of global ischemia, was 16.6% ± 3.9% in the control normocholesterolemic group and increased to 25.6% ± 2.7% ($P < 0.05$) in animals fed with cholesterol-enriched diet. Rosuvastatin and doxycycline administration reduced infarct size to 4.5% ± 1.1% and 6.1% ± 1.5%, respectively, in normocholesterolemic animals. In hypercholesterolemic animals, both (rosuvastatin and doxycycline) decreased infarct size to 4.9% ± 0.9% and 8.3% ± 1.6%, respectively, (Fig. 3).

Figure 4 shows gelatinolytic activities of MMP-2 in coronary effluents collected during aerobic perfusion (basal) and during 2, 5, and 30 minutes of reperfusion after 30 minutes of global no-flow ischemia. This results demonstrate that rosuvastatin significantly decreased MMP-2 activity ($P < 0.05$) as compared with the control group in normocholesterolemic animals (Fig. 4C). The administration of doxycycline completely abolished the activity of MMP-2 after 2 minutes of infusion. The area under the curve (AUC, arbitrary units) represents MMP-2 activity during the first 30 minutes of reperfusion and was calculated for all experimental groups (Fig. 4B). Rosuvastatin significantly reduces AUC from 1873 ± 286 (control normocholesterolemic) to 441 ± 229 (rosuvastatin normocholesterolemic), representing a 76.4% reduction ($P < 0.05$). Figure 4F shows a similar behavior of MMP-2 activity in hypercholesterolemic animals. Under these conditions, the AUC decreases from 1859 ± 240 in control group to 1015 ± 224 in rosuvastatin group with a significant ($P < 0.05$) 45.4% reduction (Fig. 4E). Figures 4A, D illustrate a representative zymogram, showing gelatinolytic activities in coronary effluent samples from hearts of control and rosuvastatin-treated normocholesterolemic and hypercholesterolemic animals.

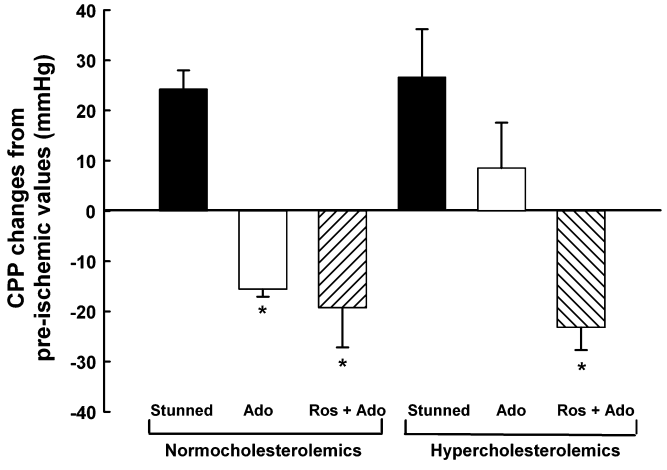

FIGURE 2. Changes in CPP after adenosine administration at 30 minutes of reperfusion period. Rosuvastatin fully recover coronary vasodilatation in hypercholesterolemic rabbit hearts in response to adenosine. *$P < 0.05$ versus stunned. Ado: adenosine; Ros: Rosuvastatin.

Figure 5 (panels A and B) shows the relationship between MMP-2 activity at 2 minutes of reperfusion and infarct size for all experimental groups using individual or mean values, respectively. In both figures, there is a significant positive correlation between these variables. The attenuation of MMP-2 activity during reperfusion and the correlation between MMP-2 and infarct size in control and rosuvastatin-treated groups suggest that MMP-2 plays an important role in cardioprotection mechanisms induced by rosuvastatin during myocardial reperfusion injury.

DISCUSSION

We report in the present study that rosuvastatin decreases infarct size associated with the inhibition of MMP-2 activity induced by ischemia/reperfusion in isolated

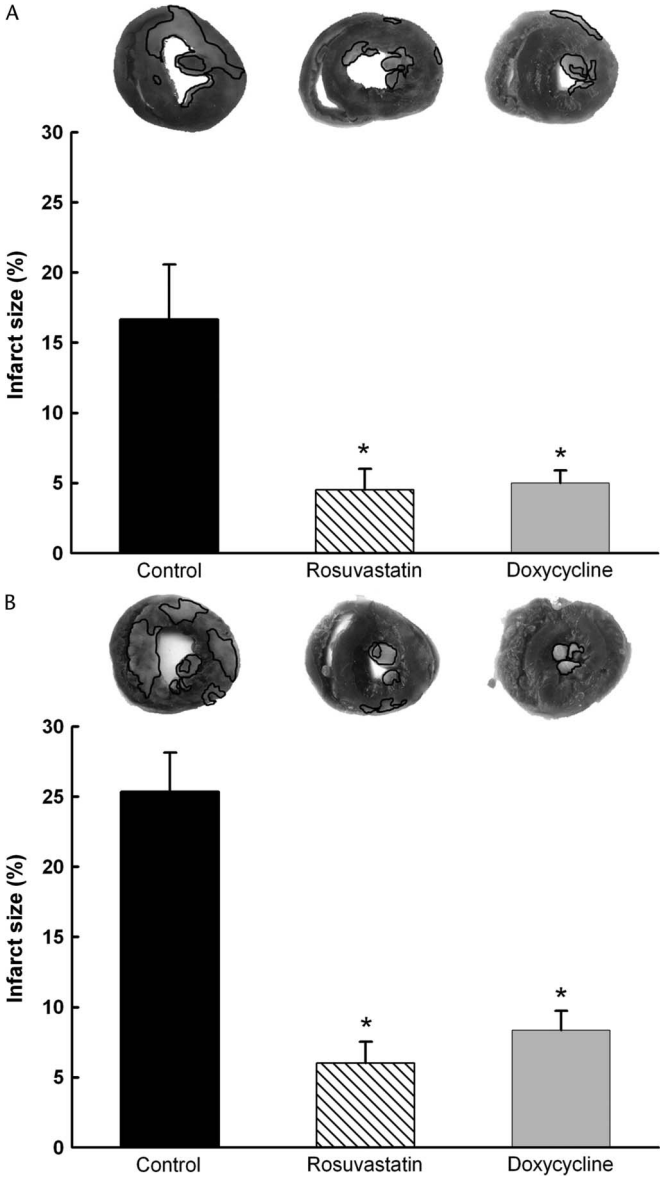

FIGURE 3. Effect of rosuvastatin on the infarct size is observed in normocholesterolemic (panel A) and hypercholesterolemic rabbit hearts (panel B). Infarct size is expressed as a percentage of the total LV area. Infarct size decreased with both rosuvastatin and doxycycline treatments and was larger in the hypercholesterolemic animals. $*P < 0.05$ versus control. Representative slices stained with triphenyltetrazolium and with the marked infarct area of each studied group can be observed at the top of the figure.

rabbit hearts. This beneficial effect was observed in both normocholesterolemic and hypercholesterolemic animals. Furthermore, in hypercholesterolemic rabbit hearts, rosuvastatin induced a significant improvement in postischemic ventricular dysfunction and coronary vasodilatation response.

Previous studies have determined that treatment with statins before the onset of myocardial ischemia reduces ischemia/reperfusion injury.[31,32] Indeed, Jones et al[10] reported that pretreatment of mice with simvastatin led to limitation of infarct size and myocardial dysfunction after 30 minutes of regional ischemia and reperfusion. Consistent with these results, Tiefenbacher et al[32] found that fluvastatin administered only 20 minutes before onset of regional ischemia, followed by a continuous intravenous infusion of fluvastatin throughout ischemia and reperfusion, reduced infarct size and improved regional myocardial function and perfusion. After pretreatment with L-NAME: N - nitro - L - Arginine methyl ester, the fluvastatin effect was completely abolished, indicating that acute application of fluvastatin ameliorates ischemia/reperfusion injury via increasing NO activity.

Although those studies demonstrated the impact of prophylactic therapy, they did not evaluate the possible benefits of the statin therapy initiated at the reperfusion. To our knowledge, few studies have considered the effects of statins administered during reperfusion. Bell and Yellon[6] used isolated mice hearts to demonstrate that atorvastatin administered only during reperfusion, after 35 minutes of global ischemia, significantly limits myocardial infarction. These authors, however, used only healthy animals and did not evaluate either ventricular function or MMP-2 activity.

The results of the current study have revealed a significant reduction in infarct size both in normal and in hypercholesterolemic animals. Interestingly, systolic and diastolic ventricular functions were more impaired in hypercholesterolemic than in normocholesterolemic rabbit hearts. The rosuvastatin treatment improved ventricular function only in hypercholesterolemic rabbit hearts, reaching values similar to those in the normal animals. It is important to mention that in these experimental models, the ischemia/reperfusion injury is influenced by the presence of certain degree of postischemic dysfunction (stunned myocardium), in the viable areas. In this regard, Cohen et al[26] showed that ischemic preconditioning significantly reduces the infarct size; however, approximately 72 hours of reperfusion are necessary to observe the improvement in the ventricular function. In this sense, Satoh et al[33] showed that the administration of rosuvastatin to dogs subjected to 15 minutes of ischemia and 2 hours of reperfusion did not modify postischemic ventricular dysfunction.

On the other hand, in the hearts of hypercholesterolemic animals treated with rosuvastatin, we detected a greater recovery of the ventricular function during reperfusion. In a previous study,[34] we showed experimental evidence that the hearts of hypercholesterolemic animals did not present vasodilator response to acetylcholine infusion, which suggested the presence of endothelial dysfunction. In addition, in the present study, normal and hypercholesterolemic hearts subjected to 15 minutes of global ischemia have a different response to the adenosine infusion during reperfusion. The administration of adenosine during reperfusion induced coronary vasodilatation only in normal but not in hypercholesterolemic animals. The administration of rosuvastatin fully recovers coronary vasodilatation in response to adenosine.

Feigl et al[35] reported that NO mediates the early phase of coronary vasodilatation to hypoxia and that adenosine sustains the vasodilatation initiated by NO. We demonstrated that endothelial dysfunction is present in hypercholesterolemic animals[34] because adenosine-sustained vasodilatation response is

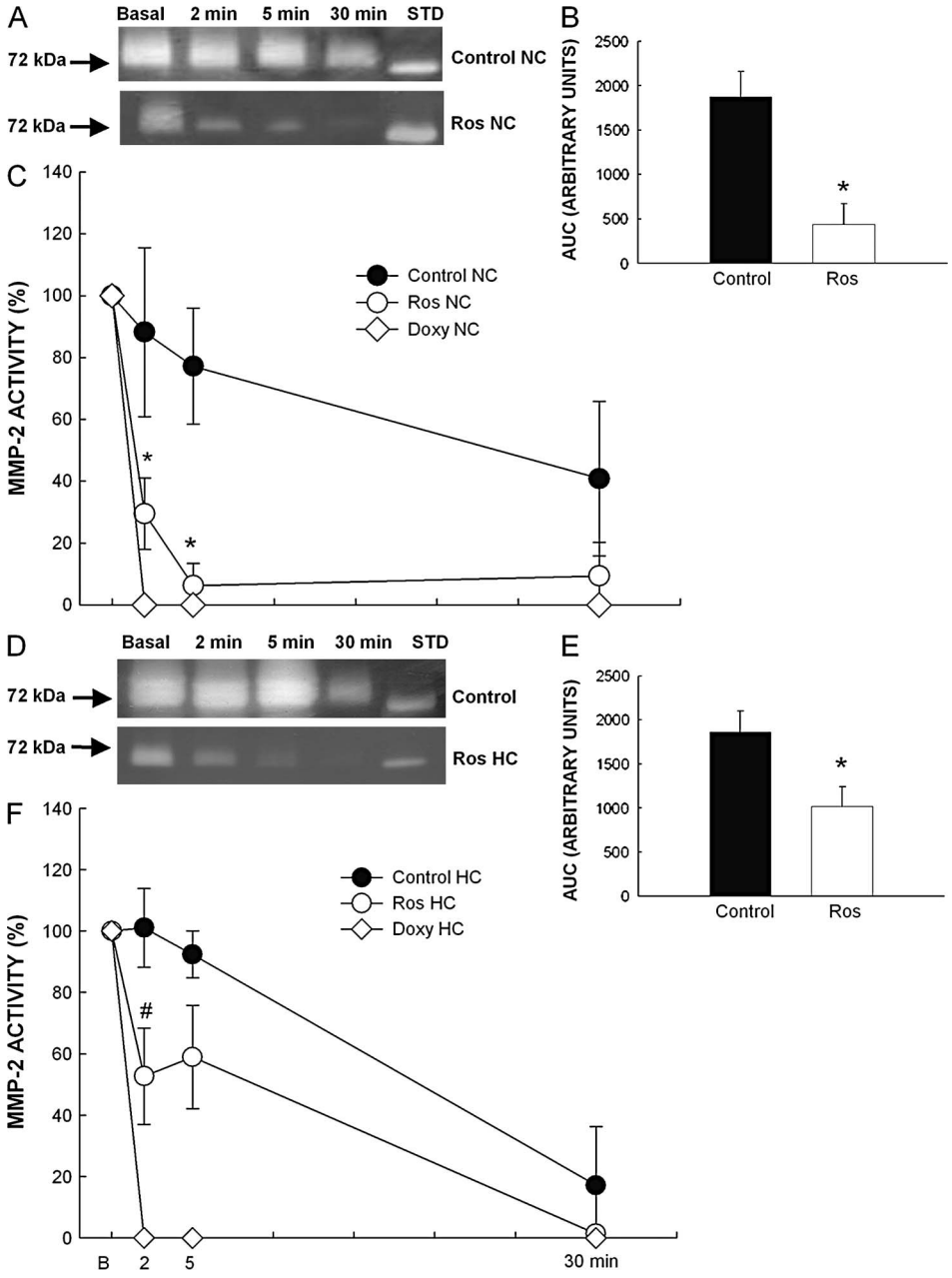

FIGURE 4. Effect of rosuvastatin on the gelatinolytic activities in coronary effluents of normocholesterolemic and hypercholesterolemic rabbit hearts. Panels A and D show representative zymogram of MMP-2 activity in coronary effluents. Summary data for densitometric analysis of MMP-2 gelatinolytic activities in coronary effluents in normocholesterolemic (C) and hypercholesterolemic animals (F). *$P < 0{:}05$ versus control NC and #$P < 0{:}05$ versus control HC. Panels B and E show the AUC (arbitrary units) of MMP-2 activity. There was a significant reduction in the AUC of MMP-2 activity in both experimental treated groups ($P < 0.05$ vs. control).

impaired. In this sense, rosuvastatin was able to restore the adenosine-sustained vasodilatation, improving myocardial perfusion and ventricular function. These actions are independent from its effects on the infarct size.

In the present study, rosuvastatin cardioprotection was achieved when the drug was administered during reperfusion, which simulates the setting of a patient with acute myocardial infarction undergoing percutaneous coronary intervention. Our results are in accordance with Bell and Yellon[6] who observed a significant reduction of infarct size by coperfusion with atorvastatin during reperfusion in isolated mice hearts.

On the other hand, the possible role of MMP-2 as a mediator of myocardial injury was investigated. MMP-2 is a key member of the metalloproteinase family, found in normal cardiac myocytes[36] and cardiac fibroblasts.[37] MMPs are synthesized as proenzymes and are usually activated by proteolytic cleavage of an inhibitory propeptide domain. However, peroxynitrite ($ONOO^-$) has shown the ability to activate this enzyme by oxidizing the sulfydryl bond between a cysteine residue of the prodomain and the Zn^{2+} catalytic center, resulting in an active enzyme. Generation of abundant oxygen free radicals during early reperfusion has been implicated in pathogenesis of tissue injury; moreover, a burst of oxygen-derived free radicals within the first minutes of reperfusion has been described. In this sense, Wang et al[18] showed that infusion of $ONOO^-$ caused a rapid and significant increase of MMP-2 gelatinolytic activity into the coronary effluent, which preceded the myocardial contractile

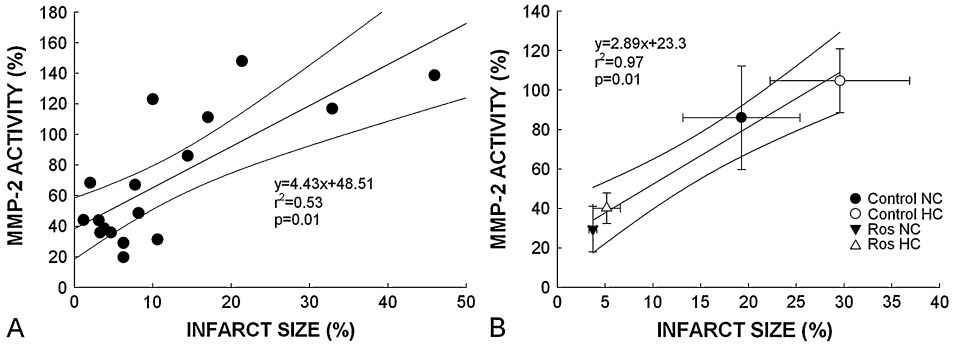

FIGURE 5. Correlation between infarct size and MMP-2 activity for the 4 experimental groups using individual (panel A) or mean values (panel B). The 95% confidence interval was 1.263–4.098 for individual values and 0.5491–0.9998 for mean values.

dysfunction. Detoxification of $ONOO^-$ with glutathione not only prevented the increase in MMP-2 activity but also the myocardial dysfunction.

Interestingly, Lalu et al[15] showed that ischemic preconditioning reduces peroxynitrite formation, removing a major stimulus for MMP-2 activation and resulting in a decrease in the release and activation of MMP-2. As far as we know, no studies have considered the effect of a cardioprotective intervention during reperfusion on MMP-2 activity. Because statins decrease ROS generation through inhibition of prenylation and translocation of cytosolic rac1 to the membrane subunits of Nicotinamide adenine dinucleotide phosphate oxidase,[19] rosuvastatin may remove endogenous peroxynitrite, a major stimulus for MMP-2 activation and, consequently, it can reduce infarct size. Because a significant reduction in infarct size with attenuation of MMP-2 activity in rosuvastatin protocols has been proved, current data support this hypothesis. The relationship between MMP-2 and infarct size was shown by Giricz et al.[38] These authors demonstrated that ilomastat (MMP-2 inhibitor) decreased infarct size, an effect similar to preconditioning. According with Giricz, a significant decrease in infarct size with the administration of doxycycline was found, suggesting that MMP inhibition plays a role in the cardioprotection obtained with rosuvastatin.

It is well known that in the first minutes of reperfusion exists, a burst of ROS and calcium overload, which determines the severity of the reperfusion injury.[39] Furthermore, we found that rosuvastatin administration induced an early inhibition of MMP-2 activity in the coronary effluent. Therefore, in the present study, doxycycline was added only in the first 2 minutes of reperfusion to determine whether the early inhibition of MMP-2 could mimic the beneficial effects of rosuvastatin.

One limitation of the present study is that we did not administer rosuvastatin only in the first minutes of reperfusion. However, the decrease of the infarct size, the inhibition of MMP-2 activity, and the correlation between these 2 variables suggest an involvement of these enzymes in the mechanism of protection. Although these significant acute cardioprotective effects of rosuvastatin administration adjunct to reperfusion could be explained, at least in part, by inhibition of MMPs; other more rapidly inducible pathways that involved activation of the PI3K/Akt signaling cascade,[6] and eNOS phosphorylation[9] cannot be discarded.

Other authors[14,15] have described a significant increase of MMP-2 activity in the first minutes of reperfusion, which has been linked to the postischemic ventricular dysfunction. In our study, we did not observe this effect, probably due to the high basal levels of MMP-2 activity. The increased MMP-2 activity in the coronary effluent, in isolated rabbit hearts, was described by Prasan et al.[40] These authors showed that there is an increase in the gelatinolytic activity of MMP-2 in the coronary effluent of hearts that have been perfused aerobically during 90 minutes. This study showed that after the basal peak of gelatinolytic activity, there is a gradual reduction of enzyme activity that is significant after 30–40 minutes of perfusion. However, in our experimental conditions, an extension of stabilization time would lead to deterioration of the isolated heart. For this reason, we collected the basal samples after 20 minutes of aerobic perfusion. On the other hand, we cannot rule out some degree of ischemia caused by the extraction of the heart, which could induce activation of the MMP. Despite this limitation, we observed significant differences in the activity of MMP-2 during reperfusion among the studied groups.

In conclusion, present results afford the first strong evidence that rosuvastatin given during reperfusion attenuates MMP-2 activity and decreases infarct size in normocholesterolemic and hypercholesterolemic conditions. The reduction of infarct size provided by doxycycline and the correlation between MMP-2 and infarct size in control and rosuvastatin-treated groups suggest that MMP-2 plays an important role in the mechanisms of rosuvastatin cardioprotection during myocardial reperfusion injury.

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