Effects of Rosuvastatin on Experimental Infarction in Normal and in Hypercholesterolemic Rabbits

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ABSTRACT

It is well accepted that previous treatment with rosuvastatin may reduce infarct size and improve ventricular dysfunction. Nevertheless, there is no experimental evidence of this action when administered during reperfusion. The objective of the present study was to assess if the administration of rosuvastatin during reperfusion might modify not only the infarct size but also ventricular function recovery after an ischemic episode in normocholesterolemic and hypercholesterolemic rabbits. Isolated and isovolumic rabbit hearts were perfused according to Langendorff technique. Rabbits in group 1 (G1) underwent a 30-minute global ischemia followed by a reperfusion lasting for 120 minutes. Rosuvastatin (50 μM) was administered to rabbits in group 2 (G2) throughout the whole reperfusion. Protocols G1 and G2 were repeated in groups 3 and 4 (G3 and G4), respectively, but in rabbits previously fed for a month with a 1% cholesterol-rich diet. Total cholesterol levels were 59.6 ± 9.3 mf/dl before treatment with the diet, and after a cholesterol-rich diet for 4 weeks, cholesterol levels increased to 185.4 ± 21.4 ($p < 0.05$). No differences among recovery in left ventricle developed pressure (LVDP) or in end-diastolic left ventricle pressure (EDLVP) were reported in normocholesterolemic animals. Nevertheless, the administration of rosuvastatin mitigated systolic and diastolic post-ischemic left ventricular dysfunction. Infarct size in G1 and G3 was 16.6 ± 2.6 y 25.6 ± 2.7, respectively ($p < 0.05$). Administration of rosuvastatin reduced the infarct size in G2 and G4 to 4.5 ± 1.1 y 5.5 ± 1.6 ($p < 0.05$), respectively.
The administration of rosuvastatin since the beginning of reperfusion reduces the infarct size in normocholesterolemic and hypercholesterolemic rabbits, and improves ventricular function only in hypercholesterolemic animals.

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Key words > Myocardial infraction – Rosuvastatin – Pyrimidines – Hypercholesterolemia – Ventricular function

Abbreviations >

eNOS	Endothelial nitric oxide synthase	**LVEDP**	Left ventricular end-diastolic pressure
HDL	High-density lipoproteins	**LVDP**	Left ventricular developed pressure
LDL	Low-density lipoproteins	**IVP**	Intraventricular pressure
L-NAME	G -nitro-L-Arginine-Methyl Ester		

BACKGROUND

HMG-CoA reductase inhibitors (statins) are agents with known hypolipidemic effects that reduce cardiovascular morbidity and mortality. (1, 2) The interest in the study of these drugs has increased during the last years because of their beneficial effects which are independent of their hypolipidemic properties. (3) Statins preserve endothelial function, reduce inflammation, and attenuate vascular smooth muscle cells and fibroblast proliferation. (4, 5) Several studies have been performed with the aim of explaining the intrinsic mechanisms of statins pleitropic effects. (4, 5)

The protective role of statins during myocardial ischemia-reperfusion lesion has been suggested by different experimental studies. (6, 7) In this way, it was demonstrated that previous chronic treatment (during 3 weeks) (6) or previous acute treatment (18 hours before ischemia) (7) with rosuvastatin reduces infarct size and improves post-ischemic ventricular dysfunction in a model of isolated rat heart. In a different study, Jones et al. (8) showed that previous treatment with simvastatin ((18 hs/6 hs/3 hs/1 h before ischemia) reduced infarct size and post-ischemic dysfunction in mice. In the same sense, Tiefenbacher et al. (9) demonstrated that intravenous fluvastatin

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administrated 20 minutes before an episode of regional ischemia reduced the infarct size and improved the recovery of ventricular function. Interestingly, the protective effect of fluvastatin was abolished by L-NAME, meaning that statin protection was achieved through an increase of nitric oxide bioavailability.

Nevertheless, in all these studies the different drugs were administrated before the ischemic event and it might be more important to achieve such a protection during reperfusion. In this sense, Bell et al. (10) administrated atorvastatin during the reperfusion period after 35 minutes of global ischemia in a model of mouse isolated heart. These authors found a significant reduction in the infarct size. However, these studies were performed in normal animals, and as far as we know, no studies have assessed the effect of this drug in animals with comorbidities as hypercholesterolemia. In addition, approximately 30-40% of patients with ischemic heart disease present certain extent of hypercholesterolemia and, in consequence, it would be interesting to assess the effects of statins (rosuvastatin) administered in normal and hypercholesterolemic animals during reperfusion.

The aim of the present study was was to assess if the administration of rosuvastatin during reperfusion might modify not only the infarct size but also the recovery of ventricular function after a 30-minute ischemic episode in normocholesterolemic and hypercholesterolemic animals.

MATERIAL AND METHODS

Male New Zealand rabbits (1.8-2.0 kg) were randomly assigned to two types of diet: 20 animals were fed with a standard diet and 18 rabbits received a 1% cholesterol-rich diet for 4 weeks. Once the diet ended, the rabbits were slaughtered; hearts were removed, isolated and perfused according to Langendorff technique with a Krebs-Henseleit solution composed of NaCl 118.5 mM, KCl 4.7 mM, $NaHCO_3$ 24.8 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM and glucose 10nM, warmed up to 37°C and bubbled with a mixture of 95% O_2 - 5% CO_2 to maintain a pH of 7.45 ± 0.02.

Intraventricular pressure (IVP) was measured by a latex balloon placed in the left ventricle and connected through a rigid polyethylene tube to a Deltram II pressure transducer (Utah Medical System). The latex balloon was filled with an aqueous solution until a left ventricular end-diastolic pressure (LVEDP) of 8-12 mm Hg was attained, and this volume was kept constant during the whole experiment. Changes in LVEDP were assessed as an index of myocardial stiffness. Therefore, if we consider that ventricular stiffness is expressed by the dP/dV ratio, in an isovolumic heart model, LVEDP is an index of ventricular stiffness. Additionally, the contractile state was evaluated by the left ventricular developed pressure (LVDP), which was the difference between LFVDP minus the peak systolic ventricular pressure. Coronary flow and heart rate were kept constant during the whole experiment by means of a perfusion pump and a pacemaker, respectively.

Measurement of infarct size

After completing the assessment of ventricular function, the hearts were perfused for 2 hours and were subsequently frozen. Then they were cut from tip to base and incubated in a 1% 2,3,5-triphenyltetrazolium chloride solution. The infarct areas were measured with computerized planimetry (image analyzer Image Pro Plusâ, version 4.5). The infarct size was expressed as a percentage of the left ventricular area.

Biochemical analysis

Blood samples were obtained before and after the diet. Total cholesterol, LDL cholesterol and HDL cholesterol levels were measured. (8)

Experimental protocols in normal animals

Group 1 (G1; n = 10): hearts of normal animals were subjected to 30 minutes of global ischemia, followed by a 30-minute reperfusion period. When the peristaltic perfusion pump was suddenly closed, coronary blood suddenly ceased and global ischemia was achieved.

Group 2 (G2; n = 10): rosuvastatin (50 μM) was administered to hearts of normal animals for 30 minutes since the beginning of the reperfusion period.

Group 3 (n = 10): the G1 protocol was repeated but in animals previously fed for 4 weeks with a 1% cholesterol-rich diet.

Group 4 (G4; n = 8): the G2 protocol was repeated but in animals previously fed for 4 weeks with a 1% cholesterol-rich diet.

Statistical analysis

Results were expressed as mean ± standard error (SE) and the data were analyzed with a variance analysis followed by the Bonferroni test for multiple comparisons. Differences were considered statistically significant when p value was < 0.05.

RESULTS

In animals fed with a cholesterol-rich diet, total cholesterol levels increased significantly from 61.6 ± 9.3 mg/dl before the beginning of the diet to 284.4 ± 45.4 mg/dl at 4 weeks ($p < 0.05$). LDL cholesterol increased from 21.5 ± 2.3 mg/dl to 158.5 ± 37.2 mg/dl ($p < 0.05$). Finally, HDL cholesterol dis not modify significantly.

Figure 1 shows the values of left ventricular developed pressure (upper panel) and end-diastolic pressure (lower panel), at baseline and during different stages of reperfusion in the group of animals fed with a normal diet. In the control group, LVDP decreased from 94.6 ± 3.6 mm Hg to 37.7 ± 3.8 mm Hg 30 minutes after reperfusion. Rosuvastatin did not modify contractile status impairment and produced a reduction in LVDP from 102.5 ± 3.7 mm Hg to 43.7 ± 7.9 mm Hg, 30 minutes after reperfusion. During reperfusion, a similar increase on LVEDP (myocardial stiffness) was seen in both groups.

Figure 2 shows changes in ventricular function in the groups of animals fed with a rich-cholesterol diet. The upper panel displays a graph showing that LVDP decreases significantly during reperfusion from 85.5 ± 5.9 mm Hg to 26.7 ± 3.9 mm Hg 30 minutes after the reperfusion. Nevertheless, the administration of rosuvastatin attenuated the deterioration of the contractile status which reached a value of 48.3 ± 9.6 mm Hg at the end of reperfusion ($p < 0,05$). Myocardial stiffness is represented at the lower panel by

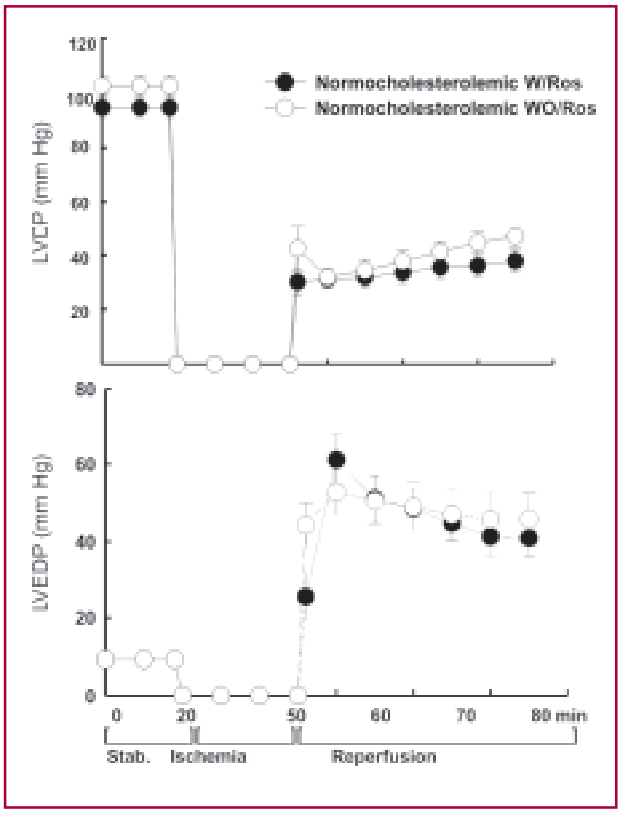

Fig. 1. The upper panel shows left ventricular developed pressure (LVDP) in normocholesterolemic animals. No significant differences were observed in both groups studied. The lower panel displays left ventricular end-diastolic pressure (LVEDP) graph. No differences were either observed in this index of diastolic function. Ros: Rosuvastatin. Stab: Stabilization.

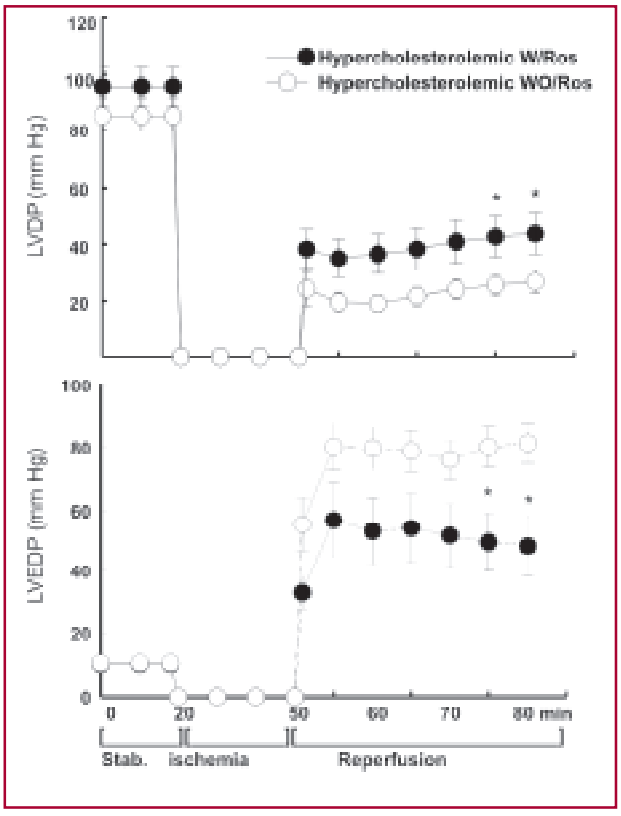

Fig. 2. The upper panel shows left ventricular developed pressure (LVDP) in hypercholesterolemic animals. During reperfusion, a significant improvement of the contractile status is seen in the group treated with rosuvastatin. The lower panel displays a graph of myocardial stiffness (LVEDP); animals treated with rosuvastatin present a lower LVEDP during reperfusion that indicates less myocardial stiffness. Ros: Rosuvastatin. Stab: Stabilization. * $p < 0.05$ versus hypercholesterolemic without ros.

LVEDP. In the control group, animals subjected to 30-minute ischemia presented a significant increase in LVEDP from 10.5 ± 0.3 mm Hg at baseline to 81.5 ± 6.3 mm Hg at the end of reperfusion. Interestingly, rosuvastatin attenuated this increase up to a value of 48.3 ± 9.6 mm Hg, 30 minutes after reperfusion.

Figure 3 shows the infarct size after 30 minutes of ischemia. In normal hearts, the infarct size was 16.6% ± 2.6%, and 25.6% ± 2.7% in hypercholesterolemic animals ($p < 0.05$ normal versus control). Rosuvastatin reduced the infarct size in both groups (normal and hypercholesterolemic rabbits) which reached a value of 4.5 ± 0.9% y 5.5 ± 1.6%, respectively ($p < 0.05$ versus control).

DISCUSSION

The present study demonstrates that the acute administration of rosuvastatin from the beginning of reperfusion, significantly reduces the infarct size in normal and hypercholesterolemic animals. In addition, we have demonstrated that contractile status improves significantly and myocardial stiffness is attenuated only in hearts of hypercholesterolemic animals.

These findings extend the observations performed by other groups (6, 7) who have described the beneficial effects of rosuvastatin on infarct size.

Nevertheless, in those studies the statin was administered before the ischemia. As we have found a similar protection when the drug was administrated at the beginning of the reperfusion, it is feasible to extrapolate our findings to clinical settings. The intervention is applied during the period of reperfusion, once the ischemic event has occurred; thus, this protective mechanism has a potential application in the clinical setting, particularly in patients subjected to reperfusion therapies. However, some authors administrated statins after the ischemic event. Bauersachs et al. (11) failed to demonstrate a reduction in infarct size with the administration of cerivastatin in myocardium of rats, 24 hours after the onset of the infarction.

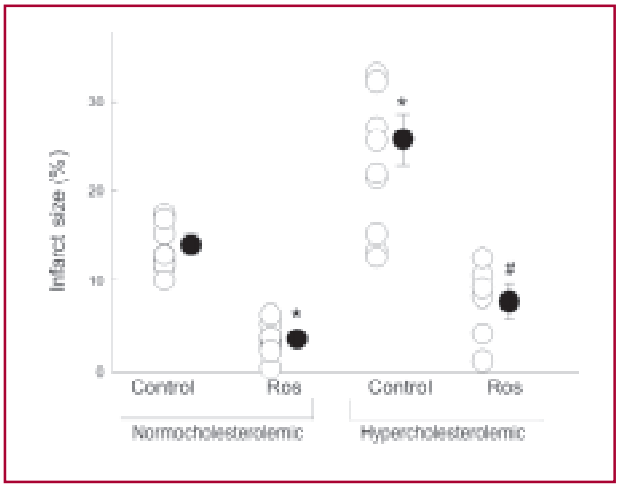

Fig. 3. Infarct size expressed as a percentage of left ventricular area. The administration of rosuvastatin significantly reduced the infarct size in normal and hypercholesterolemic animals. * $p < 0.05$ versus normocholesterolemic control. # $p < 0.05$ versus hypercholesterolemic control. ○: Individual experiments ●: Mean ± standard error in each

Our results are consistent with those reported by Bell et al. (10) who administered atorvastatin in the acute phase, during the period of reperfusion. In this case, the authors used a model of isolated mouse heart and they found a significant reduction in the infarct size after 35 minutes of global ischemia.

The difference of our study with the previous ones is that as we used normal and hypercholesterolemic animals, our results might be extrapolated to a clinical setting. Interestingly, the infarct size of hypercholesterolemic animals was similar to the infarct size of normal animals. (12, 13) This finding, described by other authors (12, 13) has been attributed to several factors, such as the presence of endothelial dysfunction, (12) an increase in endothelial cell adhesion molecules (11) and alterations in calcium homeostasis. (14) We have previously demonstrated that cholesterol-rich diet produces endothelial dysfunction as assessed by acetylcholine infusion. This endothelial dysfunction presents with no histopathology lesions in the coronary arteries; in consequence, this model represents an initial stage of the atherosclerotic process. (15)

Compared to other studies, in ours the improvement in the recovery of ventricular function was only observed in the hearts of hypercholesterolemic animals. Although we have not analyzed yet the possible mechanisms related to this finding, we might pose a hypothesis. Infarct size of hypercholesterolemic animals was greater; in consequence, the percentage of reduction achieved after treatment with rosuvastatin is significantly greater than that of normal animals. Therefore, probably the improvement in the recovery of ventricular function might be related to this reduction in infarct size.

Although we have not assessed the mechanisms involved in the protective phenomenon of rosuvastatin, we can make some speculations.

In this sense, Maron et al. (16) demonstrated that statins increased ventricular nitric oxide (NO) bioavailability. Statins might increase eNOS activity by stimulating PI3-kinase/Akt pathway, leading to eNOS phosphorylation at serine 1177 residue. (17, 18) Thus, eNOS activation through PI3-kinasa/Akt phosphorylation might be responsible for the reduction in the infarct size when the drug is administered during reperfusion, as this mechanism can be activated in minutes. (17)

Atorvastatin and rosuvastatin might share similar protective mechanisms. Nevertheless, these mechanisms deserve further research to explain the protective effects of rosuvastatin.

CONCLUSION

The present study demonstrates that the administration of rosuvastatin, from the beginning of reperfusion, significantly reduces the infarct size in normal and hypercholesterolemic animals. In addition, we have demonstrated that contractile status improves significantly and myocardial stiffness is attenuated only in hearts of hypercholesterolemic animals. As the drug was administrated during the reperfusion period, it is feasible to extrapolate our findings to the clinical setting, in contrast with studies in which the drug was given before the beginning of ischemia.

RESUMEN

Es conocido que el pretratamiento con rosuvastatina disminuye el tamaño del infarto y mejora la disfunción ventricular. Sin embargo, no existe evidencia experimental que demuestre su efecto cuando se administra durante la reperfusión. El objetivo del presente estudio fue evaluar si la rosuvastatina administrada durante la reperfusión modifica el tamaño del infarto y la recuperación de la función ventricular posisquémica en corazones de conejos normocolesterolémicos e hipercolesterolémicos. Corazones aislados e isovolúmicos de conejo fueron perfundidos según la técnica de Langendorff. En el grupo 1 (G1) se realizó una isquemia global de 30 minutos seguidos por 120 minutos de reperfusión. En el grupo 2 (G2) se administró rosuvastatina (50 μM) durante toda la reperfusión. En los grupos 3 y 4 (G3 y G4) se repitieron los protocolos de G1 y G2, respectivamente, pero en conejos alimentados durante un mes con una dieta rica en colesterol al 1%. El colesterol total antes de iniciar la dieta fue de 59,6 ± 9,3 mg/dl y luego de la alimentación durante 4 semanas con una dieta rica en colesterol se incrementó hasta un valor de 185,4 ± 21,4 ($p < 0,05$). No hubo diferencias en la recuperación de la presión desarrollada (PDVI) ni en la presión diastólica final del ventrículo izquierdo (PDFVI) en los animales normocolesterolémicos. Sin embargo, en G3 y G4 la administración de rosuvastatina atenuó la disfunción ventricular posisquémica sistólica y diastólica. El tamaño del infarto de G1 y G3 fue

de 16,6 ± 2,6 y 25,6 ± 2,7, respectivamente ($p < 0,05$). La administración de rosuvastatina redujo el tamaño del infarto en G2 y G4 a un valor de 4,5 ± 1,1 y 5,5 ± 1,6 ($p < 0,05$), respectivamente.

La rosuvastatina administrada desde el inicio de la reperfusión disminuye el tamaño del infarto en conejos normales e hipercolesterolémicos y mejora la recuperación de la función ventricular sólo en los animales hipercolesterolémicos.

Palabras clave > Myocardial Infarction - Rosuvastatin - Pyrimidines - Hypercholesterolemia - Ventricular Function

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