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The combined effect of rosuvastatin and ischemic pre- or post-conditioning on myocardial ischemia-reperfusion injury in rat heart

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Abstract. – OBJECTIVE: To investigate the combined effect of rosuvastatin and ischemic preconditioning or postconditioning on ischemia-reperfusion injury in *in vivo* rat heart.

MATERIALS AND METHODS: Ninenty-six male adult Wistar rats were randomly assigned to eight groups: Sham group, ischemia-reperfusion, rosuvastatin preconditioning, rosuvastatin postconditioning, ischemic preconditioning, ischemic postconditioning, ischemic + pharmacologic preconditioning and ischemic + pharmacologic postconditioning groups. Blood samples were taken for creatine kinase evaluation at selected time points. Six rats in each group were separated for either infarct size assessment or immunohistochemical staining with Bcl-2 antibody.

RESULTS: The staining with Bcl-2 was significantly lower in groups Sham, ischemic + pharmacologic preconditioning and ischemic + pharmacologic postconditioning groups which is well correlated with the decrease in infarct size for the same groups. The creatine kinase enzyme levels were also reduced to their lowest levels in ischemic + pharmacologic preconditioning and ischemic + pharmacologic postconditioning groups.

CONCLUSIONS: These findings suggest that enriching the composition of reperfusate with rosuvastatin along with ischemic preconditioning or postconditioning procedures at the opposite sides of ischemia may interact synergistically for protecting ischemic myocardium from reperfusion injury. The combined application of rosuvastatin with ischemic preconditioning or ischemic postconditioning may provide a new therapeutic option in clinical interventions when compared to single treatment with ischemic and rosuvastatin preconditioning or postconditioning.

Key Words:
Preconditioning, Postconditioning, Rosuvastatin, Reperfusion injury.

Introduction

Acute myocardial infarction (AMI) due to prolonged myocardial ischemia without reperfusion is one of the most common cardiac reasons of morbidity and mortality worldwide[1]. Although the immediate restoration of blood supply to the ischemic myocardium is crucial to render myocytes resistant to cell death, it is evident that reperfusion itself is not without risks in clinical settings. Especially in thrombolytic therapy and percutaneous coronary intervention (PCI) reperfusion after ischemia may cause unfavorable effects on the ischemic myocardium. The injury induced by reperfusion can be changed by strategies applied at the time of reperfusion[2-6].

In the last three decades, great efforts have been spent to reduce infarct size and other complications related with ischemia-reperfusion (IR) injury. Ischemic preconditioning[4,7] (IPre) and ischemic postconditioning[2,8] (IPost) are such strategies that are well demonstrated to limit infarct size and preserve cardiac function. In addition, pharmacological options such as carvedilol[7], L-carnitine[9], sodium butyrate[10], TNF-α[11] and recently statin compounds have been tested for limiting IR related injury in various experimental myocardial[12] and cerebral IR models[13,14] so far.

Among these studies[15-19], statins have been shown to exert unique protection against reperfusion injury by limiting inflammatory response, oxidative stress and coronary endothelial dysfunction. This protection is comparable to that of those observed with IPre or IPost and indicates the pleiotropic effects of statins. Recently, antiapoptotic effects of simvastatin and atorvastatin have been pronounced in terms of neuroprotection on cerebral ischemia[20]. However, in all

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those studies, statins were not attempted in a manner of simultaneous administration with IPre or IPost procedures. Therefore, there is a lack of data whether statins provide additional protection against IR injury in terms of limiting infarct size and myocyte cell death.

In the light of this observation, we aimed to investigate and compare the effects of rosuvastatin when given simultaneously with IPre or IPost procedures in a model of IR injury in *in vivo* rat hearts.

Materials and Methods

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) The Experimental protocol was approved by the Dicle University Local Committee on Animal Research Ethics.

Male Wistar rats (weighing 250-300 g) were anesthetized with intraperitoneal administration of thiopental sodium (40 mg/kg). The anesthesia was maintained by additional doses of thiopental (5-10 mg/kg/ i.p.), if necessary. The rats were tracheotomized and after intubation were artificially ventilated (Rodent Ventilator 7025/7125 Hugo Sachs Elektronik-Harvard Apparatus GmbH, D-79232 March-Hugstetten, Germany) with room air supplemented with oxygen (2 ml/stroke) at a rate of 70 strokes per minute. Rats were kept on a feedback-controlled heating system in order to maintain body temperature at 37°C throughout the experimental procedure. Blood pressure and heart rate were monitored with an apparatus (MAY BPHR 9610, Ankara, Turkey) and continuously recorded on MP30 Software (Biopac Systems Inc., Santa Barbara, CA, USA). Right jugular vein was cannulated for blood sampling and left femoral vein was cannulated for infusion of drug (5 mg/kg rosuvastatin) or saline (MAY 9601 Infusion Pump, Ankara, Turkey).

After administration of 200 IU heparin i.v., a left thoracotomy was performed through the fourth intercostal range. Once separated from pericardium, left coronary artery was found and encircled with a 6-0 silk suture about 2 mm from its origin. All rats in study groups, received a 20-min equilibration time for stabilization of blood pressure and heart rate. Coronary occlusion verified by a decrease in blood pressure, akinesis and discoloration of the ischemic zone. Reperfusion was confirmed by change in the color of the myocardium.

Study Groups and Experimental Protocol

Eight groups each containing 12 rats were studied to evaluate the effect of rosuvastatin (Rs) Pre- and Postconditioning with or without ischemic pre- (IPreC) and postconditioning (IPoC) on cardiac creatine kinase myocardial band (CK-MB) enzyme levels, myocardial necrosis and histological changes. Six rats from each group were assessed for infarct size and the rest underwent for immunohistochemical evaluation. The study groups were as follows:

Sham: [150 min perfusion]

IR: [(30 min ischemia (I)) + (120 min reperfusion(R))]

IPreC: [(3 cycles of 5 min I + 5 min R) + (30 min (I)) + (120 min R)]

IPoC: [(30 min I) + (6 cycles of 10 sec R + 10 sec I) + (120 min R)]

RsPreC: [(3 cycles of 5 min **Rs** infusion + 5 min pause) + (30 min I) + (120 min R)]

RsPoC: [(30 min I) + (6 cycles of 10 sec **Rs** infusion + 10 sec pause) + (120 min R)]

IPreC + RsPreC: [(3 cycles of 5 min I + 5 min R with **Rs**) + (30 min I) + (120 min R)]

IPoC + RsPoC: [(30 min I) + (6 cycles of 10 sec **Rs** infusion + 10 sec I) + (120 min R)]

At the end of stabilization, end of ischemia (I), 60th and 120th minutes of reperfusion (R), blood samples of 1 ml were taken for CK-MB evaluation and the same amount of saline replaced via right jugular vein in order to prevent volume excess in each sampling. Blood samples were collected, centrifuged and stored at -20°C until analysis. The samples were analyzed by using standard protocols for CK Assay Kit [The ARCHITECT *STAT* CK-MB Reagent Kit; Abbott Architect c16000 (Abbott Park, IL, USA) chemistry analyser] in accordance with the manufacturers instructions.

Two hours of reperfusion the animals were sacrificed. At 120th minutes of reperfusion, the ligature around the LCA re-tightened and 2 ml of 2% Evans Blue dye was infused via the left femoral vein to estimate the area perfused by the obstructed artery. The area at risk was determined by the negative retention with Evans Blue and the infarcted area was identified as the unstained area among the risk area. The heart was removed from the thorax and separated from auricles and sliced into sections of 2-3 mm thick-

ness. Then, the slices were incubated with 1% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma Chemical Co., St. Louis, MO, USA). The extent of myocardial infarct size was demonstrated as percentage of total left ventricular volume (%LVV) and total left ventricular weight (%LVW) which is previously described[21].

Immunohistochemistry

For immunohistochemistry, in this study Bcl-2 protein was investigated. The sections adhered to positive charged microscope slides (Isotherm, Objektträger, Braunschweig, Germany) and were microwave treated at 760 W in citrate buffer solution for 7 + 5 minutes. Sections were then blocked with 3% H_2O_2 in methanol for 40 minutes at room temperature. They were incubated overnight with Bcl-2 antibody (Santa Cruz, 1/100) at 4°C. Biotinylated secondary antibody (Lab Vision, Thermo Scientific, Fremont, CA, USA) was used before streptavidin peroxidase (Lab Vision, Thermo Scientific, Fremont, CA, USA) proceeding. Visualization of bound antibodies were carried out with DAB substrate kit (Thermo Scientific, Fremont, CA, USA). All incubations were fulfilled in a humidity chamber. At last, sections were counterstain with Harri's Hematoxylin (Bio-optica) and covered with mount medium (Sigma-Aldrich, St. Louis, MO, USA). Negative control were obtained by the omission of primary antibodies that were substituted with phosphate buffered saline (PBS). The immunostaining status was identified as either negative or positive. Immunohistochemistry positive staining was defined as the presence of a brown detection with chromogen, diaminobenzidine (DAB), on the edge of the hematoxylin-stained cell nucleus, distributed within the cytoplasm or plasma membrane of the cells were evaluated by the light microscope. Stain intensity and the proportion of immunopositive cells were also assessed by light microscope. The intensity of staining was scored on a scale of 0-3, according to the following assessment: 0, no detectable staining; 1, weak staining; 2, moderate staining; 3, strong staining. Immunostained slides were blindly evaluated by under light microscope (DM 4000 B, Leica, Wetzlar, Germany) attached to a camera (DFC280 Plus, Leica, Wetzlar, Germany).

Statistical Analysis

Statistical analysis was conducted by using Statistical Package for the Social Sciences for Windows (version 11.0; SPSS Inc., Chicago, IL, USA). All results were shown as means ± standard deviation. For CK-MB and infarct size area variables, One-way ANOVA and post hoc Tukey's test was used for comparisons between groups. A p value < 0.05 was considered as statistically significant.

The immunohistochemistry results for significance between groups were analyzed with Kruskal-Wallis and Mann-Whitney U tests. A p value < 0.05 was considered statistically significant.

Results

The levels of CK-MB at stabilization, the end of ischemia, 60th and 120th min.of reperfusion are shown in Figure 1. CK-MB enzyme activity significantly increased during reperfusion relative to coronary artery occlusion in IR group for each sampling time (7.8 ± 1.5; 7.9 ± 1.6; 7.1 ± 1.6 IU/ml respectively; p<0.05; Figure 1). RsPreC and RsPoC groups significantly lower CK-MB activity at the end of ischemia and 120th min of reperfusion (3.9 ± 0.9; 2.6 ± 0.9 and 4.5 ± 1.8; 2.9 ± 1.5 IU/ml respectively; p<0.05; Figure 1). The lowest values for CK-MB were seen in IPreC + RsPreC and IPoC + RsPoC groups. At the end of ischemia, CK-MB activity in IPreC + RsPreC (2.3 ± 1.1 IU/ml; p<0.05; Figure 1) and IPoC + RsPoC (1.4 ± 1.0 IU/ml; p<0.05; Figure 1) groups were significantly lower than that of observed in IPoC (5.0 ± 1.1 IU/ml; Figure 1) or IPreC groups (5.3 ± 1.6 IU/ml; Figure 1). There was also notable difference at 60th min of reperfusion between groups RsPoC (4.7 ± 1.3 IU/ml; Figure 1) and IPreC + RsPreC (1.7 ± 0.9 IU/ml; p<0.05; Figure 1). On the other hand, pharmacological preconditioning with rosuvastatin was found to reduce CK-MB (3.2 ± 1.6 IU/ml) compared with that in the IR (p<0.05, Figure 1) group at 60th min. of reperfusion, but the difference was not found significant when compared to other groups (p>0.05, Figure 1). There was not notable difference for CK-MB levels in IPoC + RsPoC group at 60th (2.2 ± 0.4 IU/ml) and 120th (1.9 ± 0.5 IU/ml) min. of reperfusion when compared to RsPreC, RsPoC, IPreC (3.9 ± 1.2; 3.5 ± 2.0 IU/ml; respectively), IPoC (3.3 ± 1.4; 4.2 ±1.9 IU/ml; respectively) and IPreC + RsPreC (2.2 ± 0.9 IU/ml at 120th min of R) groups (p>0.05, Figure 1).

Another parameter evaluated in the present study was the determination of necrosis area

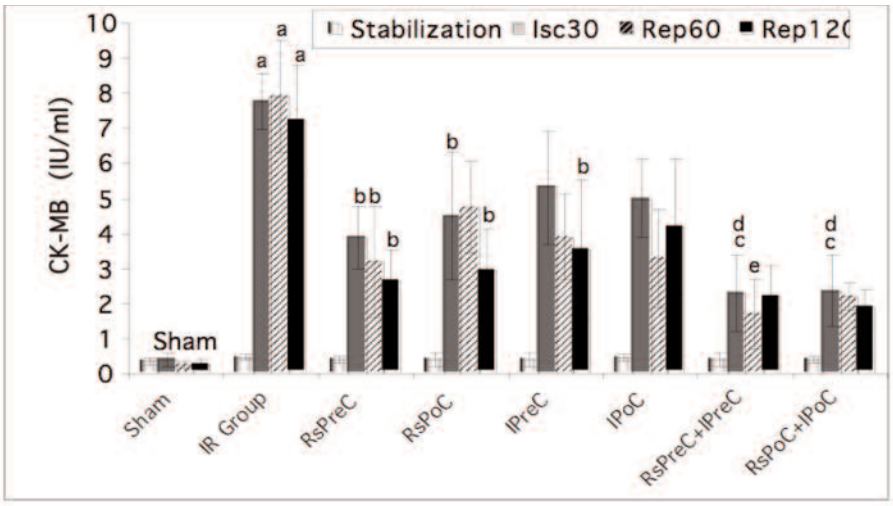

Figure 1. CK-MB enzyme levels at the time of stabilization, end of ischemia, 60th and 120th min. of reperfusion in experimental groups. [a]$p<0.05$ versus Sham at the end of ischemia, 60th and 120th min. of reperfusion. [b]$p<0.05$ versus IR at the end of ischemia, 60th and 120th min. of reperfusion. [c]$p<0.05$ versus IPoC group at the end of ischemia. [d]$p<0.05$ versus IPreC group at the end of ischemia. [e]$p<0.05$ versus RsPoC group at 60th min. of reperfusion. Data represent the mean ± SD. CK-MB: Creatine kinase-Myocardial Band, IR: Ischemia-reperfusion, RsPreC: Rosuvastatin preconditioning, RsPoC: Rosuvastatin postconditioning, IPreC: Ischemic preconditioning, IPoC: Ischemic postconditioning, RsPreC+IPreC: Pharmacologic preconditioning with rosuvastatin and ischemic preconditioning, RsPoC+IPoC: Pharmacologic postconditioning with rosuvastatin and ischemic postconditioning.

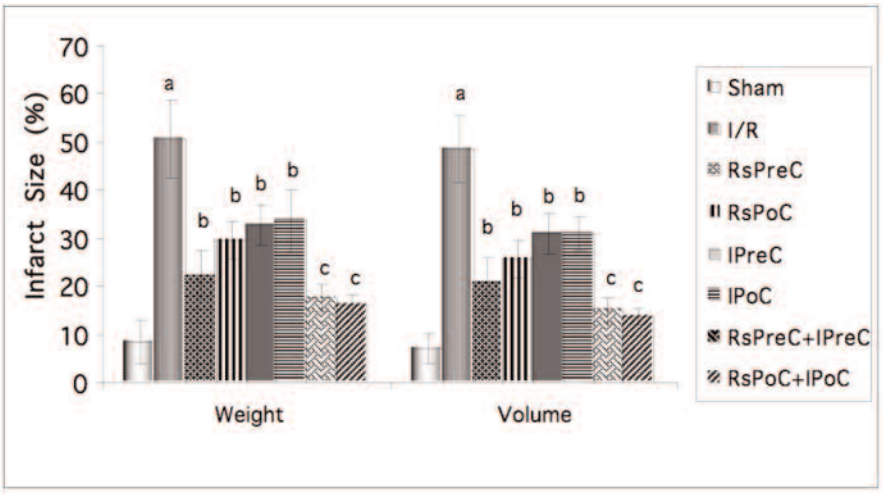

Figure 2. Infarct size data in study groups. Infarct size was evaluated by the weight and volume method. [a]$p<0.05$ versus Sham. [b]$p<0.05$ versus IR group, [c]$p<0.05$ versus IPreC and IPoC groups. Data represent the mean ± SD. IR: Ischemia-reperfusion, RsPreC: Rosuvastatin preconditioning, RsPoC: Rosuvastatin postconditioning, IPreC: Ischemic preconditioning, IPoC: Ischemic postconditioning, RsPreC+IPreC: Rosuvastatin and ischemic preconditioning, RsPoC+IPoC: Rosuvastatin and ischemic postconditioning.

using macroscopic method[21] which is considered as well correlated that of those obtained by microscopic methods. In the IR group, there was an apparent increase in myocardial infarct size, as determined by the weight (50.8 ± 6.5%) and volume (48.5 ± 7.3%) methods, respectively, compared to the Sham group ($p<0.05$). The lowest values for limiting infarct area in terms of infarct size volume were observed in IPreC + RsPreC (19.3 ± 2.9%, 15.6 ± 2.7%; as determined by weight and volume, respectively) and IPoC + RsPoC groups (16.3 ± 3.4%,

13.8 ± 4.0%; as determined by weight and volume, respectively). The myocardial infarction size in IPreC + RsPreC and IPoC + RsPoC groups were signicantly lower than that in the IPreC (32.8 ± 3.8, 31.1 ± 4.3%, respectively) and IPoC group (30.6 ± 6.5, 27.2 ± 3.5%, respectively) in terms of weight and volume variables, respectively ($p<0.05$, Figure 2). On the other hand, there was no notable difference for IPreC, IPoC, RsPreC (22.3 ± 5.1, 20.7 ± 5.5%) or RsPoC (29.6 ± 4.0, 25.6 ± 3.8%) groups when compared to IPreC + RsPreC and IPoC + RsPoC groups ($p>0.05$, Figure 2).

Intensity of immunohistochemical staining in myocardial tissues from Sham (0.2 ± 0.1), IPreC + RsPreC (0.4 ± 0.1) and IPoC + RsPoC (0.38 ± 0.1) groups showed lowest retention for Bcl-2 antibody which is in contrast to that of seen in IR (1.8 ± 1.1), IPreC (2.0 ± 1.3), IPoC (1.7 ± 1.2), RsPreC (2.1 ± 1.2) or RsPoC (1.9 ± 0.8) groups ($p<0.05$). There was no significant difference for paired comparison of the groups Sham, IPreC + RsPreC and IPoC + RsPoC and that of those for IR, IPreC, IPoC, RsPreC or RsPoC groups ($p>0.05$).

Discussion

Restoration of reperfusion to ischemic myocardium may magnify injury[19]. This state, called reperfusion injury and regarded as contractile and vascular endothelial dysfunction of myocardium[5]. In previous studies, in addition to overexpression of endothelial adhesion molecules and inflammation, involvement of oxygen free radicals during early reperfusion has been demonstrated to be major event in the pathogenesis of tissue injury which lead to myocyte cell death[22,23].

Researchers emphasized that myocardium is at risk especially in first few minutes of reperfusion as a consequent of excess free oxygen radical formation[4,8]. Even ischemic but still viable myocardium may undergo severe damage at the onset of reperfusion. Therefore, it is essential to develop therapies that limit the extent of the myocardial injury.

As a general approach, interfering early stages of reperfusion provides effective cardioprotection against reperfusion injury comparable to a pretreatment like preconditioning[24]. In recent studies, it has been shown that as IpreC and IpoC exert their protection against IR with similar mechanism[3].

In IpreC, salvage kinase pathway is shown to be active at early reperfusion[8].In the prevention of reperfusion injury prosurvival kinases phosphatidylinositol 3-kinase (PI3K)-Akt and extracellular signal-regulated kinase (Erk1/2) play an important role and have been shown to be activated by statins[25]. Additionally, an alternative protective mechanism, signal transducer activator of transcription (STAT-3) has also been pronounced for classic IpreC and has been shown to be activated by TNF-α in a rat model of IR. The researchers emphasized that the activation of salvage kinase pathway may not be essential for protection in some cases like pharmacological preconditioning[25]. It is evident that exogenous ligands may exhibit a remarkable protection which is similar to IpreC and IpoC. Hence, acute lovastatin or atorvastatin treatment, but not chronic, has been shown to strengthen the salvage kinase pathway[26].

In many studies, pleiotropic effects of statins have been demonstrated. The antiinflammatory[27], antioxidant[28], antiplatelet[29] and endothelium preserving effects[29] are well known features of statin compounds beyond their lipid lowering activities[30] and are considered as major protective mechanisms in improvement of myocardial blood flow. Currently, lipid lowering effects of statins have been pronounced as a mechanism which may exert an indirect protection against cell death[31]. It has been reported that statins decrease the production of isoprenoid derivatives such as geranylgeranyl pyrophosphate (GGPP) by their inhibition on mevalonate pathway. These isoprenoid derivatives are associated with the biological functions of the cells such as cell motility, membrane transport, and some pathologic events like inhibition of eNOS activity, generation of inflammatory mediators and reactive oxygen species. Rosuvastatin has been shown to prevent intracellular isoprenylation of small GTPases like Rho by reducing the formation of Geranylgeranypyrophosphate (GGPP) which is one of the metabolites of the mevalonate pathway and provide a control on these processes[17,20].

An overall evaluation of the effects of statins, the favourable activities independent of their lipid-lowering activity such as antiinflammatory, antioxidant, antiplatelet and improvement of endothelial function seem to be similar to IpreC and IpoC[3,25]. At this point, it can be suggested that co-application of these procedures may interact in mechanisms involving in myocardial viability and amplify the protection obtained in single implementation of those strategies.

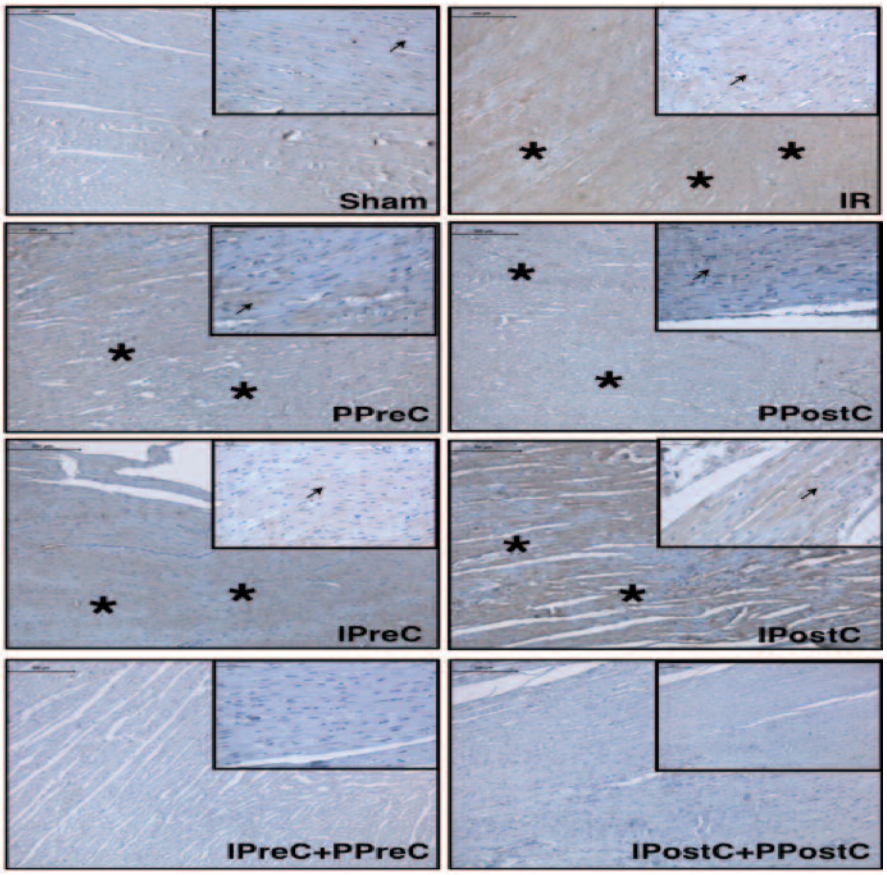

Figure 3. Distribution of Bcl-2 in myocardial tissue were observed in IR[§], PPreC[§], PPostC[§], IPreC[§] and IPoC[§] groups ([§]$p < 0.05$ as compared to Sham, IPreC+PPreC and IPoC+PPoC groups). There was no significant difference for Bcl-2 staining in groups IR, PPreC, PPostC, IPreC and IPoC ($p > 0.05$). Intensity of staining with Bcl-2 in myocardial tissue of Sham[ϕ], IPreC+PPreC[ϕ] and IPostC+PPostC[ϕ] groups showed no significance for paired comparison ($p>0.05$). ↑Arrows: immunreaction in insets (high magnification); [*]Asterisks: immunreaction for low magnification. IR: Ischemia-reperfusion, PPreC: Pharmacologic preconditioning with rosuvastatin, PPostC: Pharmacologic postconditioning with rosuvastatin, IPreC: Ischemic preconditioning, IPoC: Ischemic postconditioning, IPreC+PPreC: Ischemic and Pharmacologic preconditioning, IPoC+PPoC: Ischemic and Pharmacologic postconditioning.

In this study, we also observed such a protection for rosuvastatin. First, we examined the effects of rosuvastatin on creatine kinase (CK) enzyme levels which is considered as a marker for myocardial infarct size[17,19]. Mostly, in IPreC + RsPreC and IPoC + RsPoC groups, CK levels were decreased at their lowest values at each sampling time. The significant difference between IPreC + RsPreC or IPoC + RsPoC groups vs IPreC or IPoC groups for CK indicates the importance of composition of reperfusate[19]. Therefore, enriching the composition of reperfusate with cytoprotective and antiapoptotic compounds may provide further protection against myocardial ischemia-reperfusion injury. Hence, acute intravenous administration of rosuvastatin has already been tested in an experimental stroke model against reperfusion injury and found to be effective as a treatment option[26]. Our results are in line with previous studies[19,32].

Second, we determined myocardial infarct size by the weight and volume method. The most apparent infarct size limiting activity was observed in IPreC + RsPreC and IPoC + RsPoC groups. Group differences for infarct size were consistent with those observed in CK levels. This demonstrates that the addition of rosuvastatin to IPreC or IPoC protocols may decrease the extent of myocardial necrosis when myocardium is reperfused after coronary artery occlusion.

Third, we detected an unusual protection with regard to immunohistochemical examination in IP-

reC + RsPreC and IPoC + RsPoC groups. After Bcl-2 staining, we observed very weak staining along with almost normal myocyte cell morphology in myocardial tissue sections. This view was significantly different from IPreC, IPoC, RsPreC and RsPoC groups. This finding suggests a synergistic protective interaction of IPreC or IPoC with rosuvastatin which lead to an inactivation of apoptotic process at very early stages[33]. The inflammation and oxidative stress associated with ischemia-reperfusion injury[34] have been defined to occur especially in the initial phase of reperfusion and are considered as triggers of apoptotic cell death[20]. The researchers suggest that apoptosis is reversible and likely to be stopped by effective anti-apoptotic agents when given at the onset of reperfusion. Recently, atorvastatin has been reported to inhibit apoptotic signaling pathways via antiinflammatory effects[20]. Such a comment has also been made for rosuvastatin. Thus, rosuvastatin has shown to control cell death pathways in cardiac myocytes and endothelial cells and exerts cytoprotective effects[35].

The beneficial effects of rosuvastatin, i.e., reduction of infarct size after IR, might be due to its antiinflammatory and myocyte-protective effects which can be attributed to enhanced activity of coronary endothelial NO. Hence, the antiinflammatory effect of statins has been shown to be related to inhibition of isoprenoid production and blocks inflammatory response which is not associated with changes in LDL cholesterol levels. Statins also inhibit the expression of transcription factors like NF-κB and adhesion molecules like E-selectin[5]. Moreover, myocyte protective effects of statins have been pronounced to be independent of their lipid lowering and antiinflammatory activities and have been demonstrated to be associated with involvement of cellular protective enzyme systems, salvage kinases pathway (RISK) and AMP-activated kinase (AMPK)[36] which are similar to the protective effects of IPreC and IPoC. While RISK pathway activation increases NO production, AMPK promotes energy balance of the ischemic myocyte. Researchers have indicated that NO production by enhancing the activity of PI3 Kinase-Akt2-eNOS is a physiological response[35] leading cell protection and have been proven to be strengthened by acute statin therapy[36]. These mechanisms can explain how statins succeeded in limiting CK elevation[36] and the extent of myocardial necrosis[27] and confirm the data which we observed in this study.

The prevention of myocyte cell death may require further protective mechanisms in addition to what is mentioned above. Thus, researchers have indicated that statins can inhibit the release of endothelin. Also, statins can prevent oxidation of tetrahydrobiopterin (BH_4) which is a cofactor of eNOS by their antioxidant effects and maintain coronary endothelial NO release. Moreover, statins may exert fibrinolytic activity via decreasing plasminogen activator inhibitor-1 and increasing the levels of tissue plasminogen activator antigen. More recently, high mobility group box 1 protein (HMGB1) has been shown to play an important role in myocardial apoptosis in IR injury as a proinflammatory cytokine[10]. Researchers emphasize that agents exerting antioxidant and antiinflammatory effects may suppress HMGB1 expression. Thus, Du et al[32] have demonstrated the protective effect of rosuvastatin postconditioning in IR injury via inhibition of HMGB1. Additionally, in a cerebral IR model, atorvastatin has significantly reduced p38MAPKs, which involve in inflammation and cell apoptosis[20].

This ultimate protection with IPreC + RsPreC or IPoC + RsPoC combinations in this study may be because of interaction and/or potentialization in inhibiting the cascades involving in different phases of apoptotic pathway.

Conclusions

IPreC may have clinical applications in only selective cases such as percutaneous coronary angioplasty or coronary artery bypass surgery[25]. However, in patients with risk of MI, therapeutic preconditioning can only be applied after the onset of infarction, which limits the success of strategy as compared to animal models of "planned" myocardial infarction[25]. For our point of view, the hyperacute delivery of intravenous forms of statins, with IPreC prior to elective procedures like PCI which has a risk regarded as anticipated myocardial ischemia[36] or with IPoC strategies in clinical cases like AMI might provide an evident therapeutic benefit.

The results of the present study show that salvage of ischemic myocardium can be accomplished when the heart is pre- or post-conditioned along with rosuvastatin. Instead of evaluating the molecular mechanisms underlying this interaction, we preferred to investigate whether such combinations like IPreC + RsPreC or IPoC + RsPoC provide further protection against IR injury in terms of limiting infarct size, cardiac enzyme level increase and ultrastructural chan-

ges. In our point of view, the next step should be to evaluate the molecular mechanisms and interactions in such co-administrations and develop new administration routes for rosuvastatin on behalf of clinical benefits for PTI, surgical revascularization and such interventions.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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