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Hyperlipidemia does not prevent the cardioprotection by postconditioning against myocardial ischemia/reperfusion injury and the involvement of hypoxia inducible factor-1α upregulation

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Hyperlipidemia is regarded as an independent risk factor in the development of ischemic heart disease, and it can increase the myocardial susceptibility to ischemia/reperfusion (I/R) injury. Ischemic postconditioning (Postcon) has been demonstrated to attenuate the myocardial injury induced by I/R in normal conditions. But the effect of ischemic Postcon on hyperlipidemic animals is unknown. Hypoxia inducible factor-1 (HIF-1) has been demonstrated to play a central role in the cardioprotection by preconditioning, which is one of the protective strategies except for Postcon. The aim of this study was to determine whether Postcon could reduce myocardial injury in hyperlipidemic animals and to assess whether HIF-1 was involved in Postcon mechanisms. Male Wistar rats underwent the left anterior descending coronary occlusion for 30 min followed by 180 min of reperfusion with or without Postcon after fed with high fat diet or normal diet for 8 weeks. The detrimental indices induced by the I/R insult included infarct size, plasma creatine kinase activity and caspase-3 activity. Results showed that hyperlipidemia remarkably enhanced the myocardial injury induced by I/R, while Postcon significantly decreased the myocardial injury in both normolipidemic and hyperlipidemic rats. Moreover, both hyperlipidemia and I/R promoted the HIF-1α expression. Most importantly, we have for the first time demonstrated that Postcon further induced a significant increase in HIF-1α protein level not only in normolipidemic but also in hyperlipidemic conditions. Thus, Postcon reduces the myocardial injury induced by I/R in normal and hyperlipidemic animals, and HIF-1α upregulation may involve in the Postcon-mediated cardioprotective mechanisms.

Keywords hyperlipidemia; ischemic postconditioning; myocardial infarction; hypoxia inducible factor-1

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Introduction

Ischemic heart disease is the leading cause of death at present. It is well known that heart is an organ that consumes lots of oxygen and is absent of anaerobic glycolysis. Therefore, when myocardium underwent ischemia, it would induce serious myocardial injury and lead to contraction dysfunction due to the oxygen disequilibrium between supply and consumption. In patients with acute myocardial infarction, rapid restoration of blood flow (reperfusion) is the most effective treatment for myocardial salvage. However, it is now clear that reperfusion has the potential to induce additional lethal injuries, which is reperfusion injury. For this reason, it is very critical to develop strategies to limit the reperfusion injury.

Growing evidence has suggested that ischemic preconditioning (Precon) [1] and postconditioning (Postcon) [2] are both very important cardioprotective strategies against the ischemia/reperfusion (I/R) injury. However, as for protection, Precon must be applied before an ischemic event, thus limiting its clinical applicability. While Postcon has the advantage that it may be applied after sustained ischemia and therefore, it is more

clinically predictive and relevant. Moreover, the extent of myocardial injury reduction achieved by Postcon is similar to that obtained by Precon. Although Precon and Postcon confer remarkable cardioprotection in a variety of species, including humans, it is important to note that at present the effectiveness of them is mostly under normal conditions, which is far from the clinical reality. Several studies have reported that the effectiveness of Precon may be influenced in some disease states such as hypertension, diabetes, heart failure, hyperlipidemia and atherosclerosis, etc. [3]. But results remain conflicting. However, very little is known about the effect of Postcon under the pathological conditions.

Among these disease conditions, hyperlipidemia, especially hypercholesterolemia, is regarded as an independent risk factor in the development of ischemic heart diseases including myocardial infarction. Epidemiological studies showed that there was a strong relationship between the elevation of serum total cholesterol (TC) concentration and the morbidity and mortality of myocardial infarction. Although most studies have shown that hyperlipidemia inhibits the cardioprotective effect of Precon, there is a controversy whether the infarct size-limiting effect of Precon is lost in hyperlipidemia or not [4–6]. Additionally, very little is known about the effect of Postcon in hyperlipidemia. Iliodromitis *et al*. [7]. have shown that the infarct size-limiting effect of Postcon is lost in rabbits with experimental hyperlipidemia and atherosclerosis. But there is a contradictory report that demonstrated that Postcon reduces infarct size in hypercholesterolemic rabbits [8]. The discrepancies may be attributed to the substantial differences in hyperlipidemic models (such as species, duration of hyperlipidemia diet, presence/absence of significant coronary sclerosis). In the present *in vivo* study, we used hyperlipidemic rat models without substantial development of atherosclerosis, in order to test the effectiveness of Postcon in limiting the myocardial injury induced by I/R.

A critical component of myocardial ischemia is hypoxia, and hypoxia inducible factor 1 (HIF-1) is the principal transcription factor involved in the regulation of transcriptional responses to hypoxia [9,10]. Under hypoxic conditions, HIF-1α (a key subunit of HIF-1) is stabilized and accumulates in the nucleus, thus to promote transcription of multiple genes and trigger cellular protection and metabolic alterations [11]. It has been reported that an increase in HIF-1α is one of the first adaptive responses, at a molecular level, of human myocardium to myocardial ischemia or infarction [12]. Eckle *et al*. [13] demonstrated that HIF-1 plays a central role in the Precon-mediated cardioprotection. It suggested that the increase in HIF-1 in both expression and activity might be related to the cardioprotection. Our previous studies observed for the first time that Postcon enhanced the HIF-1 expression in the I/R rat models fed with normal diet. Moreover, there was a negative correlation between HIF-1α protein level and the degree of myocardial injury. This result suggested that HIF-1 upregulation may confer cardioprotection by Postcon against I/R injury.

Hyperlipidemia is a major risk factor for atherosclerosis and ischemic heart disease, and it has been reported to influence the expression of HIF-1α. Lee *et al*. [14] demonstrated that HIF-1α was upregulated in the corpus cavernosum of hypercholesterolemic rats, and this expression change may play a protective role. Zhu *et al*. [15] reported that hypercholesterolemia upregulated myocardial HIF-1α and its downstream gene, vascular endothelial growth factor, which led to myocardial neovascularization in pig models fed with high cholesterol diet for 12 weeks. So it was presumed that hyperlipidemia might increase HIF-1 expression. Nevertheless, does hyperlipidemia affect HIF-1 expression in myocardium induced by I/R? Does Postcon change HIF-1 expression in myocardium under hyperlipidemic conditions? These questions still remain to be clarified. Therefore, in the present study, we prepared a hyperlipidemic rat model by feeding high fat diet, to examine the effects of hyperlipidemia on the Postcon-mediated cardioprotection against I/R injury and the expression of HIF-1.

Materials and Methods

Animals and diet

The experimental procedures were conducted in adherence to the 'Guiding Principles in the Use and Care of Animals' published by the National Institutes of Health (NIH Publication No. 85–23, Revised 1996), and approved by the Institutional Animal Care and Use Committee of Shanxi Medical University.

Sixty male Wistar rats weighing 120 ± 10 g were randomly assigned to two different dietary groups: animals in the control diet group ($n = 30$) were fed with normal diet, whereas those in the high fat diet group ($n = 30$) received diet enriched with 1% cholesterol, 10% egg yolk powder, 5% lard, 0.5% sodium cholate, and 83.5% normal feedstuff for 8 weeks, and this formula was modified based on that reported previously [16]. At the end of 8-week feeding period, blood samples were taken

from the rats' vena caudalis for determination of plasma levels of cholesterol and triglycerides in order to judge the success of hyperlipidemic models.

Surgical preparation of animals

All the rats were anesthetized by intraperitoneal injection of urethane (1 g/kg). After endotracheal intubation, the rats were mechanically ventilated with oxygen-enriched room air using a small animal respirator at a rate of 50–60 breaths per minute and a tidal volume of approximately 10 ml. The chest was opened via a left thoracotomy through the fourth intercostal space, and the pericardium was opened to expose the heart. A 6-0 silk suture was passed under the left anterior descending coronary artery (LAD) just below the left atrial appendage. The suture was threaded through a small plastic tube to create a snare. The snare was tightened or released to produce ischemia or reperfusion.

General experimental protocol

Two groups of animals were assigned to one of the three subgroups: (i) sham ($n = 10$): animals underwent the same surgical procedures except that the suture passed under the LAD without being tightened; (ii) I/R ($n = 10$): animals was subjected to 30 min of LAD occlusion followed by 180 min of reperfusion and (iii) Postcon ($n = 10$): at the onset of reperfusion, three cycles of 10 s reperfusion and 10 s LAD re-occlusion preceded the 180 min of reperfusion.

Determination of area at risk and infarct size

At the end of reperfusion, LAD was re-ligated at its original site. Evans blue dye was then injected from femoral vein into the aortic root to stain the normally perfused region blue and outline the AAR. The atria and right ventricle were excised and discarded, and the left ventricle was cut into transverse slices. In each slice, the AAR was then separated from the non-ischemic zone and incubated in 1% triphenyltetrazolium chloride (TTC) solution at 37°C for 15 min to differentiate necrotic (pale) from non-necrotic (red) area at risk (AAR). Tissue samples for analysis of infarct size were saved after TTC staining. The AAR and infarct size were determined by image analysis. Briefly, each slice was scanned and planimeted using Image-Pro Plus software. For each slice, the AAR and infarct area were delineated and calculated in both sides of the section. Cumulative areas for all sections from each heart were used for comparisons. The AAR was expressed as a percentage of the left ventricular area (AAR/LV), and the infarct size as a percentage of the AAR (infarction/AAR).

Criteria for inclusion was that AAR was >15% and <45% of the LV area [17].

Determination of plasma creatine kinase activity

Arterial blood samples were collected at the end of reperfusion in all groups. Samples were centrifuged at 450 *g* for 10 min and the plasma was drawn off. The plasma supernatants were analyzed spectrophotometrically (SoftMax pro software, Molecular Devices, Sunnyvale, USA) for creatine kinase (CK) activity according to the manufacturer's instructions.

Measurement of caspase-3 protease activity

The substrate Ac-DEVD-pNA was used to determine the caspase-3 protease activity according to the manufacture's instructions (BIOMOL, Houston, USA). Myocardial tissue from the AAR at the end of reperfusion was homogenized in ice-cold lysis buffer and then centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant was harvested and 50 μl of it incubated with buffer containing 10 mM dithiothreitol and 5 μl Ac-DEVD-pNA (the final concentration is 200 μM) at 37°C for 1.5 h. Activity of caspase-3 was determined using a spectrophotometer at 405 nm (Molecular Devices) and the results were expressed as folds of the sham group.

Detection of HIF-1α protein expression by western blot analysis

Myocardial tissue samples from the AAR at the end of reperfusion were saved for analysis of HIF-1α protein using western blot analysis. Briefly, cardiac tissue (50 mg) was lysed, then homogenated to extract the total protein. Protein concentration in the supernatant was detected using BCA kit. Total protein (20 μg) was loaded on an 8% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE), electrophoresed (80 V for 30 min followed by 120 V for 90 min), and transferred onto nitrocellulose membrane (100 V, 350 mA for 55 min). The membranes were then blocked with 3% bovine serum albumin for 2 h at room temperature and followed by incubation with primary antibody at 4°C overnight. After repeated washings, the membranes were incubated at room temperature for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000 polyclonal). The primary antibodies used were rat polyclonal anti-HIF-1α (1:200) and anti-β-actin (1:1000; loading control), both purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Specific antibody binding was detected using

DAB staining. The stained membranes were scanned and the density of the scanned protein bands was quantified by densitometry (Image-Pro Plus image analysis software), and the results were presented as %change of the loading control.

Detection of HIF-1α mRNA expression by real-time reverse transcription PCR

Analysis of gene expression was studied using real-time quantitative RT-PCR with SYBR Green (Sigma, St. Louis, USA) detection in the Mx3005 real-time PCR System (Stratagene, Cedar Creek, USA). Total RNA was extracted from the AAR of cardiac tissue using the Trizol reagent (Invitrogen, Carlsbad, USA). Total RNA (3 μg) was reversely transcribed into cDNA. The thermal profile for SYBR Green real-time PCR was 95°C for 2 min, followed by 45 cycles of 94°C for 50 s, 54°C for 50 s, and 72°C for 50 s. The primer sequences were shown in **Table 1**. Samples were normalized against β-actin expression to ensure equal loading. The specificity of the amplified product was monitored by its dissociation curve. The results, expressed as the folds difference in the number of HIF-1α copies relative to the number of β-actin gene copies, were determined by the relative quantitative $2^{-\Delta\Delta Ct}$ method [18] using the following equations: $\Delta\Delta Ct = \Delta Ct$ (HIF-1α) − ΔCt (β-actin) and ΔCt (HIF-1α) = Ct (experimental) − Ct (control) and ΔCt (β-actin) = Ct (experimental) − Ct (control).

Table 1 The primer sequences of HIF-1α and β-actin

Gene	Accession No.	Primer (5′→3′)
HIF-1α	NM_024359	S: ACTGATTGCATCTCCACCTTCT
		A: TCGCTTCCTCTGAGCATTCT
β-actin	NM_031144	S: GGCTACAGCTTCACCACCAC
		A: TCAGGAGGAGCAATGATCTTG

S, sense; A, antisense.

Statistical analysis

Data were expressed as the mean ± SD. One-way ANOVA followed by Tukey-Kramer procedure for multiple comparisons were used to determine the statistical differences between treatments. Differences were considered significant at $P < 0.05$.

Results

Sixty rats were initially included in the study. There were four exclusions in the I/R group: two rats in the high fat diet group died due to ventricular fibrillation during reperfusion and the other two in normal diet group due to an AAR less than the inclusion threshold. Three rats were excluded in the Postcon group: one in the normal diet group died due to conduction blockade, and the other two belonged to high fat diet group due to an AAR less than the inclusion threshold. Data from 53 rats are included in the final analysis: 20 in the sham group, 8 in the normal diet-I/R group, 8 in the high fat diet-I/R group, 9 in the normal diet-Postcon group, and 8 in the high fat diet-Postcon group.

The levels of plasma lipid

There were no significant differences in plasma lipid concentration among the two groups at the beginning of the 8-week feeding period (**Table 2**). After the 8-week feeding period, plasma TC, total triglyceride (TG) and low-density lipoprotein (LDL) levels were markedly increased in high fat-fed rats than in normally fed rats (**Table 3**, $P < 0.01$).

Hyperlipidemia enhanced the susceptibility of myocardium to I/R injury

Hyperlipidemia further increased the myocardial infarct size induced by I/R. The AAR was comparable in all groups [**Fig. 1(A)**], averaging between 29 and 40%, indicating that a comparable degree of ischemia was induced in these groups. Infarct size in the I/R group was greatly increased than that in the sham group in both the normal

Table 2 Baseline of plasma lipid level

Group	Number	TC (mM)	TG (mM)	LDL (mM)
Normal diet group	20	2.82 ± 0.5	0.64 ± 0.15	0.88 ± 0.19
High fat diet group	22	2.92 ± 0.47	0.64 ± 0.21	0.90 ± 0.16

Rats in normal diet group will be fed with normal diet for 8 weeks, and rats in high fat diet group will be fed with high diet for 8 weeks. The plasma levels of TC, TG, and LDL showed no differences in all groups at the beginning of the 8-week feeding period.

Table 3 The plasma lipid levels of rats after fed with high fat diet for 8 weeks

Group	Number	TC (mM)	TG (mM)	LDL (mM)
Normal diet group	20	2.70 ± 0.96	1.01 ± 0.08	1.0 ± 0.19
High fat diet group	22	4.10 ± 0.61*	1.36 ± 0.39*	1.73 ± 0.57*

Normal diet group, rats fed with normal diet for 8 weeks. High fat diet group, rats fed with high diet for 8 weeks. The levels of plasma TC, TG, LDL in rats after feeding with high fat diet for 8 weeks were significantly increased compared with those in normal diet group. It suggested that the models of hyperlipidemia had been prepared successfully. *$P < 0.01$ vs. normal diet group.

and high fat diet groups (33.38 ± 1.4% vs. 3.05 ± 0.99% and 39.54 ± 1.16% vs. 4.98 ± 0.83%, $P < 0.01$) [**Fig. 1(B)**]. Hyperlipidemia increased the extension of myocardial infarct size (4.98 ± 0.83% vs. 3.05 ± 0.99%), but there was no significant difference. However, hyperlipidemia amplified the increase in infarct size induced by I/R than in the control normolipidemia ($P < 0.05$).

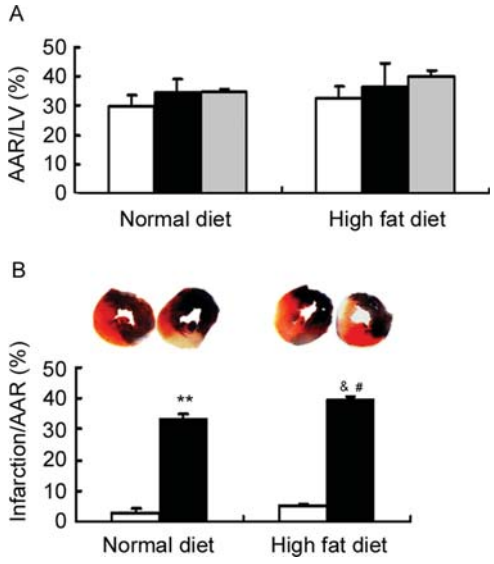

Figure 1 The effects of hyperlipidemia and I/R on myocardial infarct size (A) AAR expressed as a percentage of LV, showing a comparable degree of ischemia was induced in all groups. (B) Infarct size was expressed as percentage of the AAR (infarction/AAR). Upper panel, representative images of infarcts (white area) by Evans blue and TTC staining. Low panel, bar graph showing the effects of hyperlipidemia and I/R on myocardial infarct size. Open bars represent sham, a ligature was placed under the LAD but not tightened serving as sham-operated control. Closed bars I/R, occlusion of LAD for 30 min followed by 180 min reperfusion. Gray bars Postcon, 10 s of reperfusion and 10 s of re-occlusion repeated for three cycles applied at the onset of prolonged reperfusion. **$P < 0.01$ vs. sham in normal diet group, $^{\&}P < 0.01$ vs. sham in high fat diet group, $^{\#}P < 0.05$ vs. I/R in normal diet group.

Hyperlipidemia reinforced the increase of plasma CK activity by I/R. I/R increased the plasma CK activity, which was a surrogate measure of myocardial morphological injury, in both the hyperlipidemic and normolipidemic groups ($P < 0.01$, respectively) [**Fig. 2(A)**]. But hyperlipidemia further reinforced the increase of CK activity induced by I/R than normolipidemia (0.56 ± 0.06 U/ml vs. 0.47 ± 0.04 U/ml, $P < 0.01$) [**Fig. 2(A)**]. This suggested hyperlipidemia remarkably increased the degree of injury induced by I/R.

Hyperlipidemia further augmented myocardial caspase-3 activity induced by I/R. Apoptosis plays a key role in the formation of infarct area. Caspase-3 has been identified as being a key protein in the final pathway of cell apoptosis. Therefore, in the present study, we performed caspase-3 activity assay. As shown in **Fig. 2(B)**, the activity of caspase-3 significantly increased in the I/R group compared with that in the sham group in both hyperlipidemia and normolipidemia. However, hyperlipidemia further augmented the increase of caspase-3 activity induced by I/R (4.63 ± 0.42 vs. 2.31 ± 0.27, $P < 0.01$).

These results suggested that hyperlipidemia led to significant aggravation of myocardial I/R injury.

Hyperlipidemia did not influence the effectiveness of Postcon on the myocardial injury induced by I/R

Postcon attenuated the myocardial infarct size in both hyperlipidemic and normolipidemic rats. As shown in **Fig 3(A)**, in both the hyperlipidemic and normolipidemic groups, Postcon attenuated the myocardial infarct size induced by I/R (29.65 ± 3.48% vs. 39.54 ± 1.16% in hyperlipidemic group, $P < 0.01$; and 24.76 ± 1.17% vs. 33.28 ± 1.4% in the normolipidemic group, $P < 0.05$). There were no differences in the degree of the attenuation by Postcon between the hyperlipidemic and normolipidemic groups.

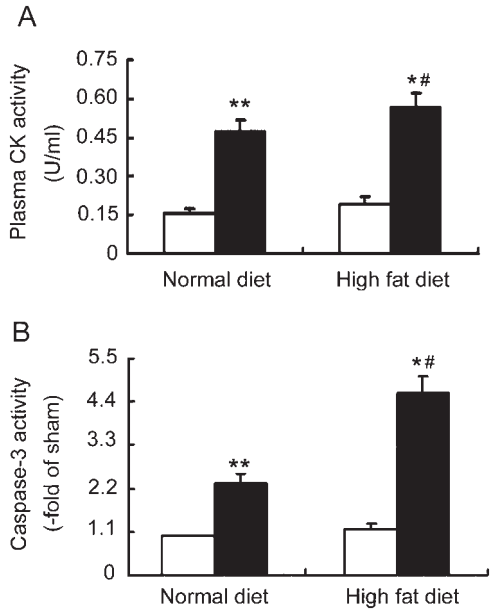

Figure 2 The effects of hyperlipidemia and I/R on plasma CK activity and caspase-3 activity (A) Plasma CK activity was measured at the end of reperfusion. (B) Changes in the activity of caspase-3 at the end of reperfusion. Note that I/R significantly increased the myocardial injury both in the normal diet rats and in the hyperlipidemic rats. $^{**}P < 0.01$ vs. sham in normal diet group, $^{*}P < 0.01$ vs. sham in high fat diet group, $^{\#}P < 0.01$ vs. I/R in normal diet group. Open bars represent sham; closed bars represent I/R.

Plasma CK activity was decreased by Postcon in both hyperlipidemic and normolipidemic groups. Postcon significantly reduced CK activity up to 0.38 ± 0.06 U/ml in the normolipidemic group and 0.43 ± 0.05 U/ml in the hyperlipidemic group ($P < 0.01$, vs. normolipidemia-I/R and hyperlipidemia-I/R groups) **[Fig. 3(B)]**. This result confirmed the changes of infarct size measured by TTC staining.

Postcon attenuated myocardial caspase-3 activity induced by I/R in both the hyperlipidemic and normolipidemic rats. As the effects of Postcon on the infarct size and plasma CK activity, Postcon attenuated the increase of caspase-3 activity induced by I/R in both the normolipidemic and hyperlipidemic groups (1.40 ± 0.05 vs. 2.32 ± 0.27; and 1.94 ± 0.21 vs. 4.63 ± 0.42, $P < 0.01$) **[Fig. 3(C)]**.

All these results suggested that hyperlipidemia did not limit the cardioprotective effects of Postcon against the I/R injury. Moreover, Postcon significantly reduced the I/R injury in the hyperlipidemic animals to the same degree as in the normolipidemic group.

Hyperlipidemia increased myocardial tissue HIF-1α expression after I/R and Postcon

Effects on HIF-1α protein expression. As shown in **Fig. 4**, in normal diet rats, I/R extremely increased the HIF-1α protein level, while Postcon further enhanced the increase of HIF-1α protein expression induced by I/R. But under the hyperlipidemic condition, HIF-1α protein level was much higher in both the sham and I/R groups than in the similarly treated normal diet groups (1.34 ± 0.22 vs. 0.64 ± 0.16 in sham group, $P < 0.05$; and 2.41 ± 0.41 vs. 1.82 ± 0.13 in I/R group, $P < 0.01$). Postcon also markedly increased the HIF-1α protein level up to 4.05 ± 0.18 compared with that of the I/R group in the hyperlipidemic rats ($P < 0.01$).

Effects on HIF-1α mRNA expression. To determine whether the changes in the HIF-1α protein expression during I/R and Postcon in both the normal and high fat diet rats were regulated at a transcriptional level, steady-state mRNA level of HIF-1α was assayed by real-time PCR. As shown in **Fig. 5(A1, A2)**, there were no

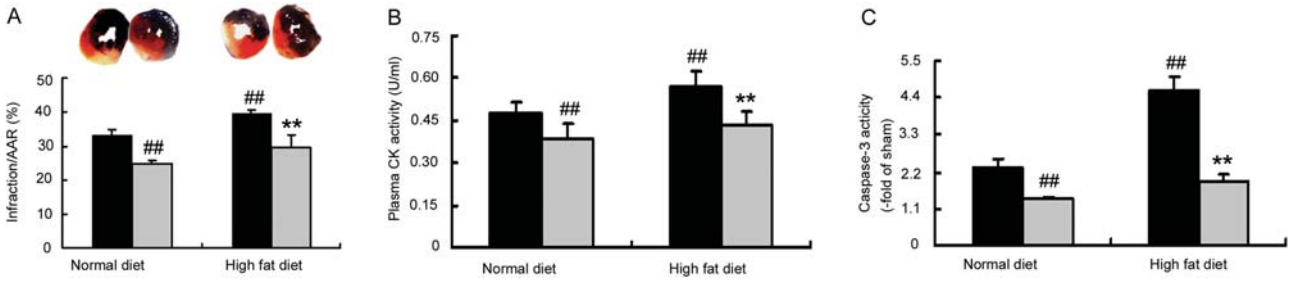

Figure 3 Postcon attenuated myocardial injury induced by I/R both in normal diet and in hyperlipidemic rats (A) Effect of Postcon on infarct size. (B) Changes in plasma CK activity. (C) Changes in caspase-3 activity. Postcon weakened the myocardial injury including infarct size, plasma CK activity and caspase-3 activity induced by I/R both in normal and in hyperlipidemic situation. Gray bars represent Postcon and closed bars I/R. $^{\#\#}P < 0.01$ vs. I/R in normal diet group, $^{**}P < 0.01$ vs. I/R in high fat diet group.

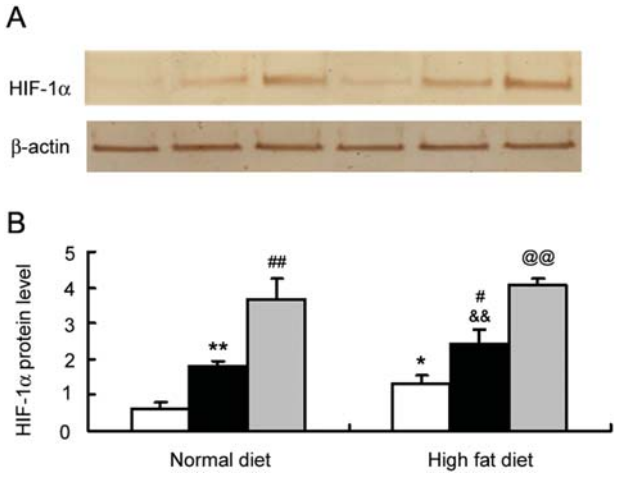

Figure 4 Effects of Postcon and hyperlipidemia on the expression of HIF-1α protein level (A) A representative western blot image for HIF-1α. (B) Bar graph showing the effects of Postcon and hyperlipidemia on HIF-1α protein expression. Equal loading of samples was verified by staining with β-actin-specific monoclonal antibody. HIF-1α protein level was upregulated by I/R and hyperlipidemia, and the degree in the hyperlipidemia-I/R was higher than that in the normolipidemia-I/R. Moreover, Postcon further enhanced HIF-1α protein expression both in normal and in hyperlipidemic situation. Open bars represent sham, closed bars I/R and gray bars Postcon. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. sham in normal diet group, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ vs. I/R in normal diet group, $^{\&\&}P < 0.01$ vs. sham in high fat diet group, $^{@@}P < 0.01$ vs. I/R in high fat diet group.

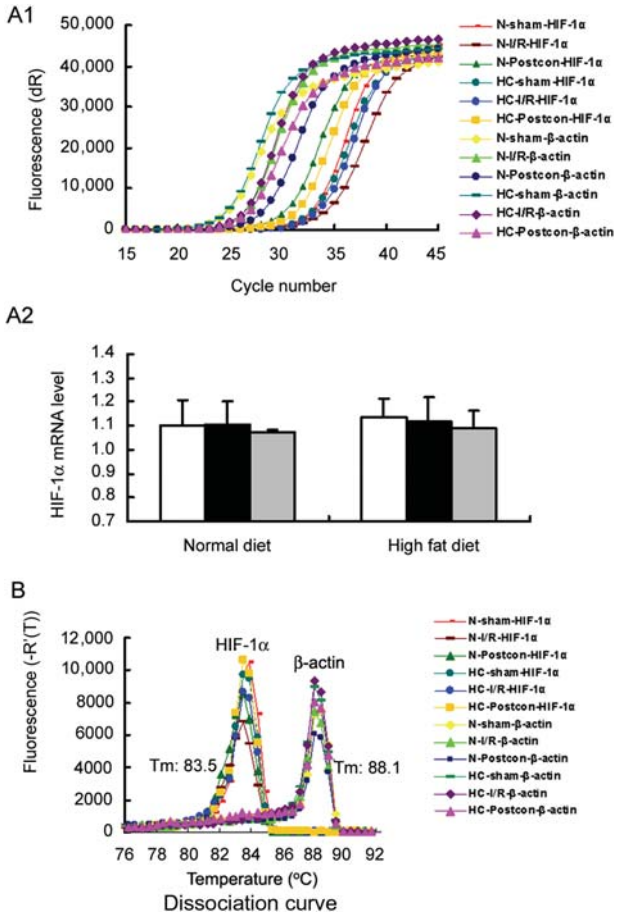

Figure 5 Effects of Postcon and hyperlipidemia on the expression of HIF-1α mRNA level (A1) Representative amplification profile by real-time PCR. (A2) Bar graph showing the effects of Postcon and hyperlipidemia on HIF-1α mRNA expression. Open bars represent sham, closed bars I/R and gray bars Postcon. (B) Dissociation curves by real-time PCR. The values from experimental groups have been normalized to match the β-actin measurement and then expressed as a ratio of normalized values to that in the sham group. No differences in HIF-1α mRNA level were detected in all groups.

significant changes in the HIF-1α mRNA expression in all groups investigated.

Since SYBR Green dye can bind indiscriminately to dsDNA, identification of an intended product was achieved by monitoring its dissociation curve [**Fig. 5(B)**]. The dissociation curve showed a single peak at 83.5 and 88.1°C for HIF-1α and β-actin, respectively. This result demonstrated the integrity of DNA used for the amplification as well as the specificities of amplification product.

These results suggested that the changes in the HIF-1α expression by I/R, Postcon and hyperlipidemia were regulated at its post-translational level but not at a gene level.

Discussion

In this study, we demonstrated that, firstly, hyperlipidemia significantly enhanced the myocardial susceptibility to the I/R injury, not only by increasing the infarct size and plasma CK activity, but also by increasing the activity of caspase-3. Likewise, Postcon attenuated the myocardial injury induced by I/R in normal diet rats as well as in hyperlipidemic rats. Secondly, we detected that hyperlipidemia upregulated myocardial tissue HIF-1α expression, and further increased its protein level after undergoing I/R. Moreover, in the present study we have for the first time demonstrated that Postcon remarkably enhanced the expression of HIF-1α both in the normal and hyperlipidemic rats.

Hyperlipidemia, especially hypercholesterolemia, is regarded as an independent risk factor in the development of ischemic heart disease. It has been reported that hyperlipidemia augmented the extension of myocardial infarction and aggravated heart dysfunction [19]. The present study also confirmed in the hyperlipidemic rat models induced by 8-week feeding with high fat diet

that hyperlipidemia enhanced the myocardial susceptibility to the I/R injury.

Precon [1] and Postcon [2] are two accepted cardioprotective mechanisms. In brief, short periods of ischemia separated by short reperfusion intervals applied before the index ischemia is defined as Precon, while short bouts of ischemia and reperfusion applied at the beginning of reperfusion is defined as Postcon. Although the effectiveness of the two mechanisms has been verified under normal conditions in a variety of species, there are several concerns regarding its effectiveness under pathological circumstances. Dworakowski *et al*. [6] have shown that Precon improves the myocardial contractibility and decreases cardiomyocyte apoptosis in hypercholesterolemic animals, although there are some opposing reports [20]. But very little is known about the effect of Postcon on the myocardial injury under hyperlipidemic condition. Donato *et al*. [8] have recently shown that Postcon reduces infarct size in hypercholesterolemic animals through activation of A1 and K^{+}_{ATP} channels. But it is very interesting that Iliodromitis *et al*. [7] have found that the infarct size-limiting effect of Postcon is lost in rabbits with experimental hyperlipidemia and atherosclerosis. However, it must be considered that these authors evaluated an advanced stage of atherosclerosis that may influence the effects of Postcon, and most of these studies are confirmed in isolated hearts. In the present study, we exposed rats to dietary cholesterol for 8 weeks without development of atherosclerosis because rats do not develop significant atherosclerosis from consumption of a high cholesterol diet [21]. We detected in the *in situ* hyperlipidemic rat models that the infarct size in the I/R group with hyperlipidemia was significantly greater than that in the normolipidemic group. However, ischemic Postcon remarkably reduced the myocardial injury in the hyperlipidemic animals to the same degree as in the normolipidemic group. Therefore, it may be presumed that the reduction in the I/R injury by Postcon was greater in the hyperlipidemic animals than that in the normolipidemic ones.

A critical component of myocardial ischemia is hypoxia, while HIF-1 is the principal transcription factor involved in the regulation of transcriptional responses to hypoxia. HIF-1 is a dimeric transcriptional complex composed of HIF-1α and HIF-1β subunits, and has been recognized primarily for its role in the maintenance of oxygen homoeostasis [22]. The expression and function of HIF-1α are precisely regulated by cellular oxygen concentration, whereas HIF-1β subunit is constitutively expressed and maintained at constant level regardless of oxygen availability, therefore, HIF-1α is regarded as the key determinant of the HIF-1 activity [23]. Under normoxic conditions, HIF-1α is rapidly degraded through the ubiquitin-proteasome pathway, whereas under hypoxic conditions, HIF-1α is stabilized and accumulates instantaneously [24]. Eckle *et al*. [13] demonstrated that HIF-1 played a central role in the cardioprotection by ischemic Precon. Our previous researches have for the first time demonstrated that the cardioprotective role of ischemic Postcon in the normal rat models is also intimately associated with the enhanced expression of HIF-1α. In the present study, we detected that hyperlipidemia itself induced HIF-1α expression, which was consistent with the previous reports [15,25]. However, the mechanism is unknown. Zhu *et al*. [15] presumed that hyperlipidemia increased the expression of HIF-1α protein may reflect the myocardial tissue hypoxia. While Khatri *et al*. [26] suggested that increased HIF-1α did not reflect necessarily the existence of a hypoxic condition, but it could rather be part of the compensatory cellular response to the increased oxidative stress. I/R significantly enhanced HIF-1α protein expression not only in normolipidemic animals but also in hyperlipidemic ones. Moreover, the level of HIF-1α protein in hyperlipidemic conditions was higher than that in the normolipidemic conditions. In addition, we have for the first time demonstrated that *in situ* Postcon treatment resulted in significantly much more increase in the HIF-1α protein level compared with the I/R insult not only in the normolipidemic animals but also in the hyperlipidemic ones. In our *in vivo* experimental models, Postcon treatment showed a remarkable cardioprotective role against the I/R injury in the hyperlipidemic rats. These results suggest that it may exist as 'bigger window' for the HIF-1α expression necessarily to elicit the beneficial effect of Postcon-mediated protection. Associated with the enhanced expression of HIF-1α, it presumed that HIF-1α may be involved in the cardioprotective mechanisms by Postcon under hyperlipidemic conditions. It is clear that more studies are needed to further demonstrate how HIF-1α could be altered by Postcon and how it participated in the protective mechanisms.

In summary, this work suggests that hyperlipidemia does not prevent the cardioprotection by Postcon against the I/R injury, and provides an insight into the key role of HIF-1α involving in the cardioprotection by Postcon. Normoxic upregulation of HIF-1α expression using pharmacological agents may further increase the protective effects by Postcon.

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