Int J Clin Exp Med 2015;8(11):20946-20952
www.ijcem.com /ISSN:1940-5901/IJCEM0008994

Original Article

Remote ischemic precondition prevents radial artery endothelial dysfunction induced by ischemia and reperfusion based on a cyclooxygenase-2-dependent mechanism

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Received April 11, 2015; Accepted June 20, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: Ischemic preconditioning (IPC) and remote ischemic precondition (RIPC) are resistance to ischemia-reperfusion (IR) injury. They have common protective mechanism. Cyclooxygenase (COX)-2 participate in the mechanism of IPC. So, the purpose of this study was to determine whether RIPC protects endothelial function of radial artery in human against IR and whether COX-2 involves in this effect. Endothelial IR injury was induced by arm ischemia (20 min) and reperfusion. Flow-mediated dilation (FMD) of the radial artery was measured before and after IR. RIPC (three 5-min cycles of ischemia of the contralateral arm) was applied immediately and 24 h before IR. All volunteers received the COX-2 inhibitor celecoxib (200 mg orally twice daily) for 5 days. On day 6, all subjects experienced the same studies as described. FMD was reduced by IR without administration of RIPC (P<0.0001). RIPC prevent this impairment of FMD immediately (P=NS) and at 24 h (P=NS). Nevertheless, the COX-2 inhibiter abolished protective effect of RIPC at 24 h (P=NS), but not immediately (P=0.001). After administration of the COX-2 inhibiter, post-IR FMD after RIPC performed immediately had significant increase than after RIPC performed at 24 h (P=0.001) and without administration of RIPC (P=0.003). The COX-2 inhibiter made post-IR FMD evidently decrease after RIPC performed at 24 h (P=0.002). RIPC prevents radial artery endothelial dysfunction induced by IR. This protective effect of RIPC in the late phase is mediated by a COX-2-dependent mechanism.

Keywords: Remote ischemic preconditioning, endothelial function, ischemia and reperfusion, cyclooxygenase-2

Introduction

Reperfusion measures using either thrombolytic therapy or primary percutaneous coronary intervention (PPCI), timely and effectively reopening the infarct-related coronary artery, limit the infarct size and improve the prognosis in patients with ST-elevation myocardial infarction (STEMI). However, these myocardial reperfusion can themself induce further cardiomyocyte injury, known as ischemia and reperfusion (IR) injury contribute to attenuate the full benefits of myocardial reperfusion [1, 2]. Ischemic precondition (IPC) reduces the susceptibility of myocardium and vascular endothelium for IR injury and decreases infart size in STEMI [3, 4]. Nevertheless, it is difficult for IPC to be appied in unexpected cardiovascular events in high-risk patients. Remote ischemic precondition (RIPC) also can protect vascular endothelial function and reduce infarct size against IR injury in STEMI. In particular, the RIPC induced by limbs is easy and simple in clinical application [5, 6]. Moreover, RIPC resembles IPC that its protection has two phases [5-8]: the early one and the late one, sharing common triggers and signal transduction pathway [9]. Thus, IPC and RIPC may have the same mechanism in protecting myocardium and vascular endothelial function against IR injury, but the protective mechanism of RIPC is not fully elucidated at present. The late protection of IPC partly depends on

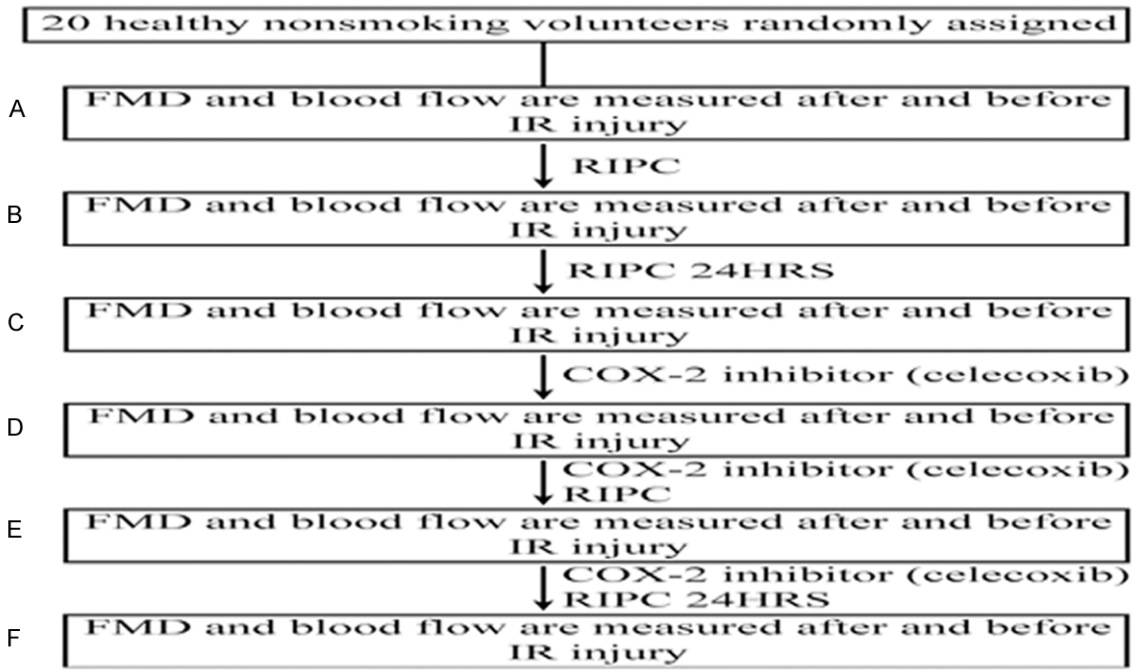

Figure 1. Trial profile. Flow-mediated dilation (FMD) of the radial artery was assessed before 15 min of arm ischemia reperfusion (IR) and at 15 min of reperfusion (A~F). Remote ischemic preconditioning (RIPC) was applied immediately (B, D) and 24 h (C, F) before IR. The COX-2 inhibitor celecoxib (200 mg orally twice daily) was administered for 5 days (D~F).

activating celecoxib-2 (COX-2). Whether COX-2 invovle in the mechanism of RIPC remain uninvestigated.

Methods

Subjects

There were twenty healthy nonsmoking volunteers (32.7 ± 8.3 age) to be enrolled at random. All subjects gave informed consent. The local research ethics committee approved this study. All studies were performed in a temperature-controlled laboratory (24°C to 26°C). On admission, sitting blood pressure was measured and peripheral venous blood was obtained for baseline lipid analysis in all subjects. All subjects participated in all studies and repeated in studies were at least seven days apart.

Experimental design

All subjects were divided into baseline group, RIPC group, RIPC 24 h group, Celecoxib + baseline group, Celecoxib + RIPC group, and Celecoxib + RIPC 24 h group according to administration of IR injury before RIPC or after RIPC at immediate or 24 h moment as well as whether or not administering COX-2 inhibitor. IR injury was performed and flow-mediated dilation (FMD) before and after IR injury was measured in subjects in every group. Subjects in Celecoxib + baseline group, Celecoxib + RIPC group, and Celecoxib + RIPC 24 h group were administered celecoxib, a selective COX-2 inhibitor, 200 mg twice daily for 5 days, then experiencing the measurement of FMD before and after IR injury (**Figure 1**).

Induction of IR and RIPC

A pneumatic cuff placed above the antecubital fossa in the nondominant forearm was inflated to 200 mmHg for 20 min to induce IR injury and then deflated for 15 min of reperfusion before FMD was measured again.

RIPC was induced by three cycles that inflated a pneumatic cuff placed above the antecubital fossa in the contralateral arm to 200 mmHg for 5 min (ischemia) and followed by a 5 min deflation.

Assessment of radial artery function

It was required for subjects to rest for at least 10 minutes in the suprine position before mea-

Table 1. Radial Arterial Diameter (mm)

	Pre-IR		Post-IR	
	Baseline	Change in Diameter After Wrist Cuff Deflation	Baseline	Change in Diameter After Wrist Cuff Deflation
Baseline	2.36 ± .35	.145 ± .033	2.39 ± .30	.102 ± .027*
RIPC	2.37 ± .34	.147 ± .028	2.37 ± .28	.139 ± .035†,‡
RIPC 24 h	2.35 ± .31	.145 ± .026	2.38 ± .29	.136 ± .028†,‡
Celecoxib + Baseline	2.39 ± .31	.157 ± .036	2.38 ± .29	.108 ± .034*
Celecoxib + RIPC	2.42 ± .31	.164 ± .040	2.40 ± .30	.143 ± .032†,‡
Celecoxib + RIPC 24 h	2.38 ± .32	.167 ± .038	2.40 ± .31	.113 ± .038*

*P<0.0001 corresponding value in the baseline group. P<0.01 †versus corresponding value in the Celecoxib + Baseline group, p value in the baseline group versus corresponding value in the RIPC group; ‡P<0.05 versus corresponding value post-IR in the Celecoxib + RIPC 24 h group.

surements were started. FMD was measured before and after IR at 15 minutes, as previously described [10, 11].

Radial artery images were taken with an Acuson Sequoia 512 with a 7- to 12-MHz linear array transducer. Longitudinal, electrocardiogram-gated, end-diastolic B-mode images of radial artery were acquired at 5-second for offline analysis. Baseline radial artery diameter was averaged from 6 separate images taken. Subsequently, a pneumatic cuff placed at the level that was distal to the site of radial artery measurement in the wrist was inflated to 250 mmHg for 4 minutes, 30 seconds. after wrist-cuff deflation, FMD was calculated according to percent maximum increase of radial artery diameter measured starting at 30 seconds and ending at 3 minutes, 30 seconds. Radial artery diameter was calculated semiautomatically using a modified version of the Image J software and custom-designed software.

Baseline blood flow and postischemic blood flow (reactive hyperemia) in radial artery were measured using pulsed-wave Doppler as average velocity-time integral for the first 5 cardiac cycles after cuff deflation [11].

Calculations and statistics

All data are presented as mean ± SE unless otherwise stated. Radial artery diameter was measured in millimeters and dilation expressed as percentage increase from baseline diameter. Data were compared using the Student paired t test, as appropriate. In all cases, P<0.05 was considered statistically significant. SPSS version 17.0 (SPSS Inc, Chicago, IL) was used for all statistical analyses.

Results

Baseline characteristics

All subjects tolerated the procedures without any complications. There was no significant differences in resting radial artery diameter before and after IR injury among groups (**Table 1**). The change of radial diameter between pre-IR and post-IR have significant difference in baseline group (0.145 ± 0.033 vs. 0.102 ± 0.027, P<0.0001), Celecoxib + Baseline group (0.145 ± 0.026 vs. 0.108 ± 0.034, P<0.0001), and Celecoxib + RIPC 24 h group (0.167 ± 0.038 vs. 0.113 ± 0.038, P<0.0001). Compared with baseline group, there were distinct difference in RIPC 24 h group (0.102 ± 0.027 vs. 0.136 ± 0.028, P<0.0001), Celecoxib + RIPC group (0.102 ± 0.027 vs. 0.143 ± 0.032, P<0.0001) and RIPC group (0.102 ± 0.027 vs. 0.139 ± 0.035, P=0.001) for the change of radial diameter after IR injury. Likewise, compared with Celecoxib + baseline group, there were evident difference in RIPC group (0.108 ± 0.034 vs. 0.139 ± 0.035, P=0.008), RIPC 24 h group (0.108 ± 0.034 vs. 0.136 ± 0.028, P=0.008), and Celecoxib + RIPC group (0.108 ± 0.034 vs. 0.143 ± 0.032, P=0.002) for the change of radial diameter after IR injury. In addition, the change of radial diameter after IR injury significantly increased in RIPC group (0.113 ± 0.038 vs. 0.139 ± 0.035, P=0.033), RIPC 24 h group (0.113 ± 0.038 vs. 0.136 ± 0.028, P=0.039), and Celecoxib + RIPC group (0.113 ± 0.038 vs. 0.143 ± 0.032, P=0.011) than Celecoxib + RIPC 24 h group (**Table 1**).

There were no significant differences in baseline blood flow and reactive hyperemia before and after IR injury among groups (**Table 2**).

Table 2. Radial blood flow

Blood Flow (ml/min)	Pre-IR		Post-IR	
	Baseline	Reactive Hyperemia (%)	Baseline	Reactive Hyperemia (%)
Baseline	33.6 ± 15.0	481 ± 162	35.3 ± 17.8	468 ± 223
RIPC	36.4 ± 21.0	472 ± 156	38.2 ± 19.6	488 ± 220
RIPC 24 h	34.3 ± 16.9	478 ± 160	37.6 ± 19.4	500 ± 214
Celecoxib + Baseline	40.8 ± 15.6	463 ± 156	39.2 ± 19.4	480 ± 200
Celecoxib + RIPC	36.5 ± 15.5	480 ± 130	38.9 ± 17.8	474 ± 112
Celecoxib + RIPC 24 h	38.6 ± 14.7	466 ± 121	37.2 ± 16.7	486 ± 100

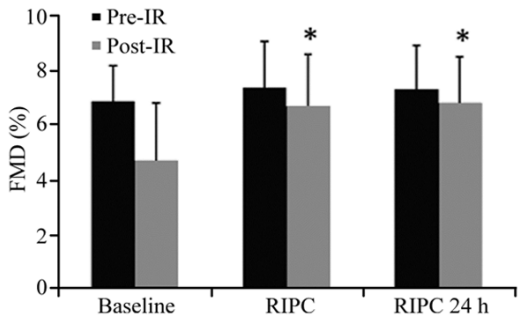

Figure 2. Effect of remote ischemic preconditioning (RIPC) on ischemia-reperfusion (IR) induced endothelial dysfunction. In the Baseline group, Flow-mediated dilation (FMD) was significantly blunted post-IR. This effect was prevented by RIPC applied immediately and 24 h. *P<0.01 versus FMD post-IR in the baseline group.

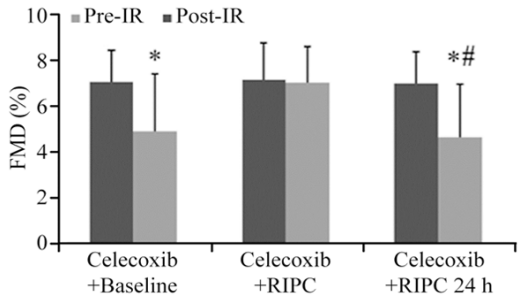

Figure 3. Effect of Celecoxib on FMD after remote ischemic preconditioning (RIPC). In the Celecoxib + baseline and Celecoxib + RIPC24 h groups, Flow-mediated dilation (FMD) were significant decrease in post-IR, but was'nt in the Celecoxib + RIPC group. *P<0.01 versus FMD post-IR in the Celecoxib + RIPC group. #P=0.002 versus FMD post-IR in the RIPC group.

Effect of RIPC on endothelial function induced by IR injury

IR injury significantly blunted FMD in the baseline group (pre-IR: 6.8 ± 1.3%; post-IR: 4.7 ± 2.1%, P<0.0001) (**Figure 2**). On contrary, RIPC protected endothelial function immediately (FMD pre-IR: 7.4 ± 1.7%; post-IR: 6.7 ± 1.9%, P>0.05) and at 24 h (FMD pre-IR: 7.3 ± 1.6%; post-IR: 6.8 ± 1.7%, P>0.05) (**Figure 2**). FMD in the baseline was lower than that in the RIPC group (post-IR: 4.7 ± 2.1%; post-IR: 6.7 ± 1.9%, P=0.003) and in the RIPC 24 h group (post-IR: 4.7 ± 2.1%; post-IR: 6.8 ± 1.7%, P=0.001).

Effect of celecoxib on FMD after RIPC

Likewise, after administering celecoxib, IR injury significantly reduced FMD in the baseline + baseline group (pre-IR: 7.1 ± 1.4%; post-IR: 4.9 ± 2.5%, P=0.002) (**Figure 3**). RIPC also protected endothelial function immediately (FMD pre-IR: 7.2 ± 1.6%; post-IR: 7.0 ± 1.6%, P>0.05), but didn't do endothelial function at 24h (FMD pre-IR: 7.0 ± 1.4%; post-IR: 4.7 ± 2.3%, P=0.001) (**Figure 3**). In comparison with Celecoxib + baseline group (4.9 ± 2.5% vs. 7.0 ± 1.6%, P=0.003) and Celecoxib + RIPC 24 h group (4.7 ± 2.3% vs. 7.0 ± 1.6%, P=0.001), Celecoxib + RIPC group deceased in FMD post-IR (**Figure 3**).

After IR injury, FMD in Celecoxib + RIPC 24 h group had more significant decrease than in RIPC 24 h group (4.7 ± 2.3% vs. 6.8 ± 1.7%, P=0.002) (**Figure 3**).

Discussion

The present study demonstrates that COX-2 participate in the mechanism that RIPC protect endothelial function of radial artery free from IR injury. This COX-2-dependent mechanism occurs in the late phase of RIPC protection.

At present, although the process of myocardial reperfusion in STEMI has maken great progresses, due to myocardial IR injury in STEMI causing damage to the endothelium, exacertion of myocardial reperfusion [12] and worse clinical outcomes [13] the benefit obtained by these process is limited in patients with STEMI.

Human study [5] demonstrates that RIPC prevents vascular endothelial dysfunction induced by IR. This protective role has an early phase of protection lasting four hours after the RIPC

stimulus, and followed 24 h later by a late phase of protection lasting for up to 48 h. In the present study, RIPC also protects vascular endothelial function against IR injury, which has an early phase of protection and a late phase of that, resembling the above mentioned study. In some recent studies, RIPC not only provide perioperative myocardial protection and a decrease in all-cause mortality in patients undergoing elective coronary artery bypass graft (CABG) surgery [14], but significantly improves endothelial function [15] increases myocardial salvage [6], and reduces major adverse cardiac and cerebrovascular events (MACCE) in patients with STEMI experiencing primary PCI [16]. In addition, in the CRISP stent trial, RIPC reduced the MACCE rate at 6 years after elective PCI [17]. Therefore, RIPC can protect endothelial function and improve long-term clinical prognosis by preventing or attenuating IR injury.

Nevertheless, up to now, the protective mechanism of RIPC has not been expounded clearly. RIPC protects vascular endothelium and myocardial by triggers (including adenosine, bradykinin, and opioids.) and upregulation of a spectrum of established prosurvival kinases (including the e-isoform of protein kinase C, extracellular signal-related kinase, Janus kinase, and phosphatidylinositol 3-kinase/protein kinase B) [9], which of these triggers and kinases also are activated in the protective effect of IPC [18]. Moreover, some studies demonstrated that the late protection of RIPC and IPC have common signaling pathway and transcriptional factors, including ERK 1/2, PI3K/Akt, JAK-STAT, and Akt/eNOS pathway as well as NF-κB and iNOS [19-26]. So, RIPC and IPC may have an identical mechanism in protenting myocardium and vascular endothelium.

In animal model study, the cardioprotective effects in the late phase of IPC resulted in increase in COX-2 protein expression and in the myocardial content of prostaglandin (PG) E2 and PGI2. Whereas, the increase in myocardial tissue levels of PGE2 and PGF2 was abolished, while COX-2 selective inhibitor completely blocked the cardioprotective effects of late IPC [27]. Guo *et al*. demonstrated that targeted disruption of the COX-2 gene completely abrogates the cardioprotection of the late IPC, which provide unequivocal molecular genetic evidence for a crucial role of the COX-2/PGI2 receptor axis in the cardioprotection of the late IPC [28]. In addition, late IPC upregulate COX-2 via protein kinase C (PKC)-epsilon in the cardioprotection [19]. Furthermore, COX-2 is located downstream of iNOS in the protective pathway of late IPC, which is modulated by iNOS via cGMP-independent mechanisms [26]. The upregulation of COX-2 also requires upregulated iNOS and iNOS-derived NO via a JAK1/2-STAT1/3 pathway [29]. Moreover, COX-2 achieves the regulation in the protection of late IPC through PGE2 and PGI2 [27]. In another study, PGI2 is evidently upregulated in the cardioprotection of late IPC and COX-2 inhibitor can completely abolish the protective effect of late IPC [30]. Therefore, COX-2 and PGI2 might be the most main participants in the mechanism of the cardioprotection of late IPC. Meanwhile, many studies have confirmed that IPC and RIPC share with common mechanism in their late protection [19-26]. Likewise, COX-2 might involve the mechanism in the late protection of RIPC. In present study, COX-2 inhibitor suppressed the late protection of RIPC in the radial artery, which show for the first time that COX-2 participate in the late protection of RIPC. Hence, COX-2 might involve in the late protection of RIPC.

In a recent study, the specific prostaglandin receptors (IP), downstream of the COX-2/prostanoid pathway, is a key mediator of the late IPC [28]. STAT3 plays an obligatory role by increasing the expression of cardioprotective (COX-2 and Heme Oxygenase-1) and anti-apoptotic proteins in the cardioprotection of the late IPC [31]. But, IP mediates STAT via stimulation of STAT3 Tyr (705) and Ser (727) phosphorylations [32]. Therefore, IP not only may adjust the signal transduction for COX-2, but play the part of promotor for feedback enhancement in multiple pathways mediating the late IPC. Nevertheless, the detailed mechanism of COX-2 in the late phase of RIPC may be analogous to the role of that in the late phase of IPC. However, the study about this aspect hitherto is little reported, and that is also the direction of study in future. If these key role of IP in protection of late RIPC are verified, the receptor of IP will become the most important determinant for achieving the protection of late RIPC. Thus, the selective and specific agonist of IP would be an appealing pharmacological approach to mimic the late phase of RIPC. If this targeted drug is screened or found, it would be applied to clinical treatment by simulating pharmacologic cardioprotection of late RIPC.

In our study, there are some limitations. Firstly, the data of the present study were obtained in healthy volunteers and in radial artery, the latter is different from the coronary circulation and needs to be validated. Secondly, the assessment of IR-induced changement in endothelial function was implemented in radial artery and not in the distal microcirculation that is crucial in clinical IR injury. Thirdly, IR injury only resulted in endothelial function stuning and not in endothelial function necrosis. Additionally, COX-2 protein expression and the correlative trigger and kinase of COX-2 in the cardioprotection of late RIPC also aren't investigated. So, these result need to be further verified in animal study.

Conclusions

In present study, RIPC protected endothedial function of radial artery against IR injury. COX-2 inhibitor completely abrogated the protective effect of late RIPC, evidence that showed that activation of COX-2 mediated the protection of RIPC in the late phase.

Acknowledgements

The authors of this manuscript have demonstrated that they abide by Principles of Ethical Publishing in the International Journal of Cardiology.

Disclosure of conflict of interest

None.

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