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Basic Research

The Role of Macrophage Migration Inhibitory Factor in Remote Ischemic Postconditioning

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ABSTRACT

Background: Remote ischemic postconditioning (RIPostC) could reduce myocardial ischemia/reperfusion injury markedly. However, the mechanism of the protective signal transfer of RIPostC to the heart remains unclear. In this study, we hypothesize that macrophage migration inhibitory factor (MIF) plays an important role in the cardioprotection conferred by RIPostC.

Methods: RIPostC was induced by 4 cycles of 5 min ischemia/5 min reperfusion on the lower limbs of rats immediately after myocardial reperfusion. The plasma level of MIF was compared between the RIPostC and reperfusion injury groups. (S,R)-3-(4-hydroxy -phenyl)-4,5—dihydro-5-isoxazoleacetic acid methyl ester (ISO-1) was used as a potent inhibitor of MIF. 2-methoxyestradiol (2ME2), an inhibitor of HIF-1α (hypoxia-inducible factor-1α) , was used as a tool to inhibit the role of HIF-1α.

Results: We found that a significant elevation in the level of plasma MIF occurred when RIPostC was carried out; this elevation could be blocked by femoral occlusion. The cardiac MIF level decreased significantly after RIPostC stimulus compared with the ischemia/reperfusion (IR) group ($P < 0.01$). In addition, inhibition of MIF by ISO-1 could induce the loss of cardioprotection and aggravate the apoptosis of the heart in RIPostC. RIPostC confers protection against myocardial IR injury via the MIF-AMPK signaling pathway. Finally, inhibition of HIF-1α may result in the reduction of plasma MIF in RIPostC.

Conclusions: MIF plays an important role in RIPostC through the humoral pathway in a HIF-1α–dependent manner, which could activate the cardiac AMP-activated protein kinase (AMPK) pathway to confer powerful cardioprotection.

RÉSUMÉ

Introduction : Le post-conditionnement ischémique à distance (post-CID) pourrait nettement réduire l'ischémie myocardique/les lésions de reperfusion. Le mécanisme qui sous-tend le transfert du signal protecteur du post-CID au cœur reste néanmoins obscur. Dans le cadre de cette étude, nous avons formulé l'hypothèse voulant que le facteur inhibiteur de la migration des macrophages (FIMM) joue un rôle important dans la protection cardiaque conférée par le post-CID.

Méthodologie : Nous avons induit un post-CID chez le rat en alternant 4 cycles d'ischémie/de reperfusion de 5 minutes chacun au niveau des membres inférieurs, immédiatement après la reperfusion myocardique. Nous avons comparé la concentration plasmatique du FIMM entre le groupe soumis à un post-CID et le groupe présentant des lésions de reperfusion. Nous avons utilisé l'ester méthylique de l'acide (S,R)-3-(4-hydroxy-phényl)-4,5—dihydro-5-isoxazoleacétique (ISO-1) comme inhibiteur puissant du FIMM. Le 2-méthoxyestradiol (2ME2), un inhibiteur de l'HIF-1α (facteur-1α induit par l'hypoxie) a été utilisé comme outil pour inhiber le rôle de l'HIF-1α.

Résultats : Nous avons observé une élévation significative de la concentration plasmatique de FIMM lorsque le post-CID a été pratiqué. L'occlusion fémorale a permis de bloquer cette élévation. La concentration cardiaque de FIM a nettement diminué après le stimulus de post-CID, comparativement au groupe IR (ischémie/reperfusion) ($p < 0{,}01$). L'inhibition du FIMM par l'ISO-1 pourrait par ailleurs induire la perte de la protection cardiaque et aggraver l'apoptose des cellules cardiaques en cas de post-CID. La post-CID confère une protection contre les lésions myocardiques d'IR par la voie de signalisation MIF-AMPK. Enfin, l'inhibition de l'HIF-1α pourrait se traduire par une réduction de la concentration plasmatique de FIM en cas de post-CID.

Conclusions : Le FIMM joue un rôle important dans le post-CID, par le truchement de la voie humorale et d'une manière dépendante de l'HIF-1α, ce qui pourrait activer la voie cardiaque de l'AMPK (protéine kinase activée par l'AMP) pour conférer une solide protection cardiaque.

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See page 508 for disclosure information.

Ischemic heart disease (IHD) remains one of the global leading causes of mortality and illness. Reperfusion of the ischemic myocardium, which is currently the most effective treatment of IHD, could reduce the size of an evolving myocardial infarction (MI); however, this treatment may induce injury. Thus, it is necessary to seek a feasible strategy to solve this problem.[1]

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Remote ischemic conditioning (RIC) is the phenomenon whereby brief, reversible episodes of ischemia and reperfusion performed in certain organs or tissues (such as the limbs) could confer powerful cardioprotection to reduce myocardial ischemia/reperfusion (IR) injury markedly.[2-5] In 1993, Przyklenk et al.[6] first reported the cardioprotective role of remote ischemic preconditioning (RIPC), one kind of RIC performed before prolonged ischemia. However, in more than 80% of cases, percutaneous coronary intervention (PCI) is carried out *ad hoc*, promptly after diagnostic coronary angiography, which seriously limits the application of RIPC as accurate information about the timing of index ischemia is uncertain in vasoocclusive emergencies. Therefore, remote ischemic postconditioning (RIPostC), when applied immediately after prolonged myocardial ischemia, overcomes the above limitations of RIPC. This makes the clinical application of RIPostC more likely, especially as it has gained emphasis in recent years.[7-9]

At present, the mechanism of the protective signal transfer of RIC from the limbs to the heart is still unclear. It has been shown that the humoral pathway plays a vital role in this process, as its protective role could be blocked by femoral occlusion.[4,10] Several humoral factors, such as IL-10,[11,12] apolipoprotein A-I,[13] stromal derived factor-1(SDF-1),[14] and MicroRNA-144,[15] are produced during RIC in the limbs and are then transferred to the heart via circulating blood, where such factors activate signal transduction and lead to cardioprotection.

Several proteomic studies have previously shown that the cardioprotective circulating factor in RIPC is usually a protein greater than 3.5 kDa and less than 15 kDa in size.[16,17] However, most existing studies focus on the mechanism of RIPC. It has been reported that the mechanisms of RIPC and RIPostC are not identical.[8] Therefore, it is necessary to explore the mechanism of RIPostC at present.

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine 12.5 kDa in size (between 3.5 kDa and 15 kDa) that plays key roles in many acute and chronic inflammatory diseases.[18-20] A series of clinical studies have shown that MIF plasma levels remain high in patients with acute myocardial infarction (AMI).[21-23] In addition, it was reported that a significant elevation in plasma MIF levels could be found in patients in the early phase of AMI, identifying elevated plasma MIF levels as a potential early predictor of AMI.[24] For comorbidities in patients presenting with AMI , such as metabolic syndrome, diabetes, or chronic kidney disease, the increase of plasma MIF also has been verified.[25,26] MIF may activate the AMP-activated protein kinase (AMPK) pathway in ischemic heart.[27] Moreover, Dake Qi et al. found that MIF exhibits a powerful antiapoptotic cardioprotective role during IR by inhibiting c-Jun-N-terminal kinase (JNK) pathway activation.[28] Studies have demonstrated that (S,R)-3-(4-hydroxy -phenyl)-4,5—dihydro-5-isoxazoleacetic acid methyl ester (ISO-1) is a potent inhibitor of MIF tautomerase activity, which could block MIF-dependent stimulation of AMPK phosphorylation in cardiac ischemic injury.[29-33] It has been reported that hypoxia-inducible factor-1α (HIF-1α) plays a key role in RIC and could result in the increase of plasma IL-10.[12] A number of studies have shown that HIF-1α could also induce the secretion of MIF under hypoxia and inflammatory condition.[34-37] It was reported that 2-methoxyestradiol (2ME2) is an inhibitor of HIF-1α, as a tool to investigate the role of HIF-1α.[38] However, to our knowledge, it remains uncertain whether and how MIF is involved in the phenomenon of RIPostC. Therefore, in this study, we hypothesized that MIF would be an effective circulating factor in RIPostC that may confer protection against myocardial IR injury by activating the cardiac AMPK signal pathway in a HIF-1α—dependent manner.

Materials and Methods

Experimental animals

This study was performed 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 protocol was approved by the Animal Care and Use Committee of Capital Medical University.

Male Wistar rats with body weights of 250 to 300 g were used in this study. Animals were raised under specific pathogen-free conditions at room temperature with a natural light-dark cycle and had free access to food and water. All rats were allowed to acclimate for 1 week prior to experiments.

Animal experiments

For the *in vivo* model of myocardial IR injury, briefly, rats were anaesthetized and core body temperature was maintained at 36.8° to 37.2°C (98.2° to 99°F). Left thoracotomy was performed between the third and fourth ribs, and the chest was opened. After thoracotomy, ventilation was started immediately with room air using a tidal volume of 8 to10 mL/kg at a respiratory rate of 70 to 80 per minute. Ligation of the left anterior descending (LAD) coronary artery was performed at approximately 2 mm below the tip of the left atrium. Successful LAD occlusion was verified when ST elevation was achieved and the anterior wall of the left ventricle became pale. Rats were subjected to 20 minutes of myocardial ischemia followed by 3 hours of reperfusion.

The protocol of RIC was induced by 4 cycles of 5 minutes of lower-limb ischemia followed by 5-minute reperfusion as previously described,[15] The study design for animal grouping was presented as follows (Fig. 1):

Sham: Sham operation was carried out on rats.
RIC: The protocol of RIC was performed on healthy rats.
RIC+V: Femoral vein occlusion was performed prior to RIC on healthy rats.
IR: 20 minutes of myocardial ischemia followed by 3 hours of reperfusion.
RIPostC: RIC was performed at the beginning of myocardial reperfusion.
RIPostC + V: Femoral vein occlusion was performed prior to RIPostC.
RIPostC +ISO-1: ISO-1, (30 mg/kg) was delivered by intraperitoneal (IP) iinjection 15 minutes before RIPostC.[29,31,39]
RIPostC +2ME2: 2ME2, (15 mg/kg) was delivered by IP injection 15 minutes before RIPostC.[38,40,41]

TTC staining

Rats were subjected to myocardial IR. The LAD was then tightened, and 1% Evans blue dye was injected to demarcate

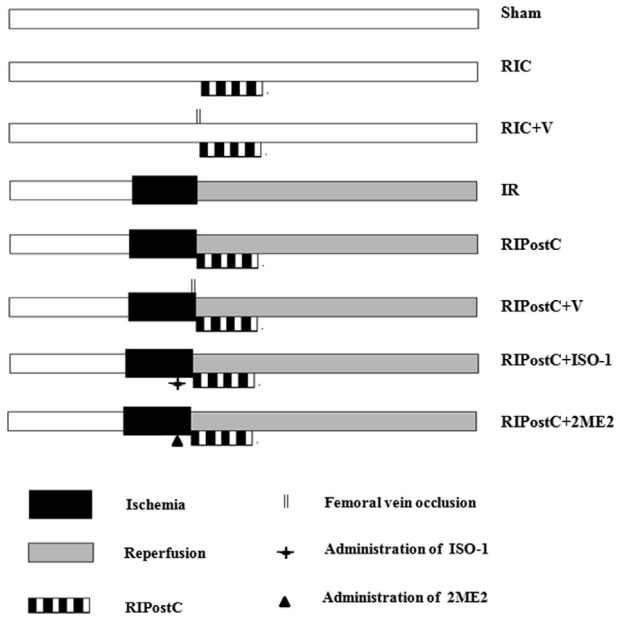

Figure 1. The schematic of the study design.

the risk area. The heart was then excised, frozen, and cut into 5 1- to 2-mm sections from the apex to the occlusion site. The slices were then stained with 1% triphenyltetrazolium chloride (TTC) for 15 minutes at 37°C (98.6°F). The slices were then fixed in 10% neutral buffer formalin and photographed. The risk zone and infarct size were measured by ImageJ software (Imagej Software, NIH, Rockville, Maryland). The risk zone was expressed as the percentage of the left ventricle that was at risk (AAR) and infarct size as a percentage of the risk zone.

Plasma preparation and ELISA

Rat blood was collected in EDTA tubes (Becton Dickinson, Franklin Lakes, New Jersey). This was followed by 1500-g centrifugation for 15 minutes at 4°C (39.2°F). The plasma samples were then used to perform the enzyme-linked immunosorbent assay (ELISA) (ELISA; Cusabio Technology, Houston, TX and Life Diagnostics Inc, West Chester, PA) to determine the level of MIF and troponin I by commercial ELISA kits, according to the manufacturer's instructions.

Apoptosis assay

terminal deoxynucleotidyl dUPT Nick-End Labeling (TUNEL) staining was carried out using the *In Situ* Cell Death Detection Kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Cells with stained nuclei were counted in 10 randomly selected fields with an upright microscope (Olympus BX51; Olympus Corporation, Shinjuku, Tokyo, Japan). Cell counting was performed by 2 independent investigators.

Western blotting

The extract proteins (50 to 100 μg) were subjected to 10% to 12% SDS-PAGE and then blotted onto nitrocellulose membranes that were incubated with antibodies overnight at 4°C (39.2°F). After tris-buffered saline and polysorbate (TBST) washing, blots were incubated with horseradish peroxidase-linked goat anti-rabbit or anti-mouse immunoglobulin G (IgG) antibodies (1:2000; Cell Signaling Technology, Danvers, MA) for 1 hour at room temperature. Immunolabeled bands were visualized using the SuperSignal West Pico Chemiluminescence Kit (Thermo Fisher Scientific, Pittsburgh, PA). Bands were normalized to eukaryotic translation initiation factor 5 (eIF5) expression.

Statistical analysis

All values are the mean ± standard deviation (SD). Tests for differences between experimental groups were performed by Students' *t*-test or analysis of variance (ANOVA) and adjusted with a Bonferroni correction for multiple comparisons. $P < 0.05$ was considered to be statistically significant. The analyses were performed using Statistical Package for the Social Sciences 20.0 software (IBM, Inc, Armonk, NY). Animal sample size was determined by using power analysis and referring to literature in studies on MIF/RIC.

Results

There was a significant increase of plasma MIF in both RIC and RIPostC groups via the humoral pathway in the *in vivo* model.

In the RIC group, plasma MIF was elevated significantly immediately after RIC and at 3 hours after the beginning of RIC, compared with plasma MIF before RIC (4.31 ± 0.24 ng/mL, 8.82 ± 0.55 ng/mL vs 3.06 ± 0.57 ng/mL, respectively, $P < 0.01$, Fig. 2B), which showed that RIC could simulate the elevation of plasma MIF directly. A marked increase in the plasma MIF level was also detected in rats subjected to RIPostC both immediately (6.60 ± 0 .70 ng/mL vs 4.81 ± 0.40 ng/mL, $P < 0.01$) and after reperfusion for 3 hours (18.10 ± 4.23 ng/mL vs 9.77 ± 0.82 ng/mL, $P < 0.01$) compared with the IR group (Fig. 2D). In addition, a significant elevation of plasma MIF did not occur immediately after RIC or at 3 hours after the beginning of RIC in the RIC-V group (2.73 ± 0.68 ng/mL, 3.58 ± 1.32 ng/mL vs 2.82 ± 0.33 ng/mL, $P < 0.01$, Fig. 2C). There was no significant change in plasma MIF levels between the RIPostC+V and IR groups immediately after RIPostC (4.98 ± 0.69 ng/mL vs 4.81 ± 0.40 ng/mL, $P > 0.05$) and after reperfusion for 3 hours (11.05 ± 0.83 ng/mL vs 9.77 ± 0.82 ng/mL, $P > 0.05$) (Fig. 2D). These data indicate that the increase of plasma MIF is via the humoral pathway in RIC and RIPostC.

The expression of MIF in both cardiac and skeletal muscle in limbs after RIPostC

We next examined the effect of RIPostC on cardiac MIF contents using Western blotting. The cardiac MIF level decreased significantly in RIPostC group after reperfusion for 3 hours compared with the IR group (Fig. 3B, C).

We hypothesized that RIPostC induced the release of MIF from the limbs into circulation; thus, we detected the change of MIF expression in limb skeletal muscle. However, no significant changes occurred after RIC (Fig. 4B). Similar results were also observed between the RIPostC and IR group (Fig. 4C, D).

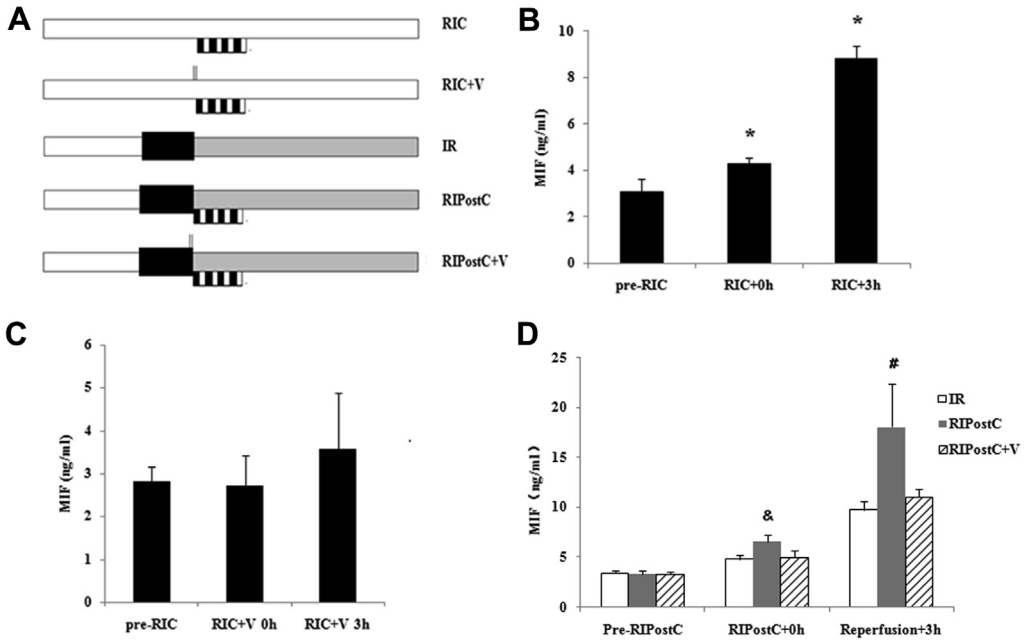

Figure 2. The significant elevation of plasma MIF occurred in RIC and RIPostC via humoral pathway. (**A**) Experimental protocol. (**B**) RIC increased plasma MIF. pre-RIC: prior to RIC; RIC+0h: immediately after RIC; RIC+3h: 3h after the beginning of RIC. (**C**) The elevation of plasma MIF induced by RIC was blocked by femoral vein occlusion. RIC+V 0h: immediately after RIC with femoral vein occlusion; RIC + V 3h: 3h after the beginning of RIC with femoral vein occlusion. (**D**) RIPostC increased plasma MIF via humoral pathway; pre-RIPostC: prior to RIPostC/reperfusion; RIPostC + 0h: immediately after RIPostC (40 min after reperfusion); reperfusion +3h: 3h after reperfusion. * vs pre-RIC $P < 0.01$; & vs IR group at RIPostC + 0h $P < 0.01$; # vs IR group at reperfusion + 3h $P < 0.01$, n = 7 for each group.

Inhibition of MIF could induce the loss of cardioprotection in RIPostC

Rats were subjected to RIPostC at the beginning of myocardial reperfusion. We found that RIPostC reduced infarct size compared with the IR group. Also, the occlusion of the femoral vein could induce the loss of cardioprotection of RIPC, which indicated the importance of the humoral pathway in transferring the protection signal of RIPC. Furthermore, we found that this infarct-limiting effect was blocked by administration of ISO-1, a specific inhibitor of MIF (Fig. 5, B-E), as well as the level of plasma TnI (Fig. 5F). Thus, these results suggest that RIPostC induces protection against IR injury and that MIF plays a key role through the humoral pathway in this process.

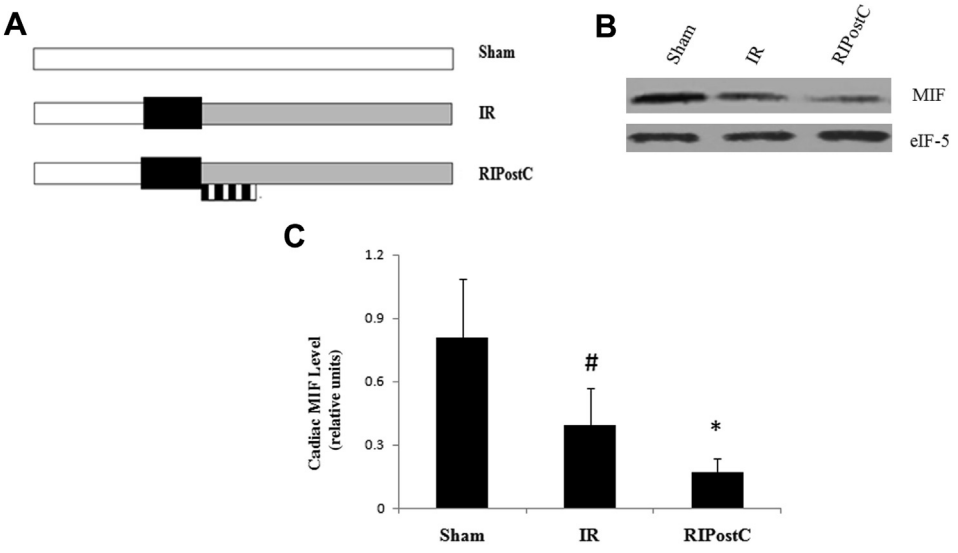

Figure 3. The effect of RIPostC on MIF expression in heart tissue. (**A**) Experimental protocol. (**B, C**) Representative immunoblots and densitometric analysis. # vs Sham $P < 0.01$, * vs IR $P < 0.01$, n = 6 for each group

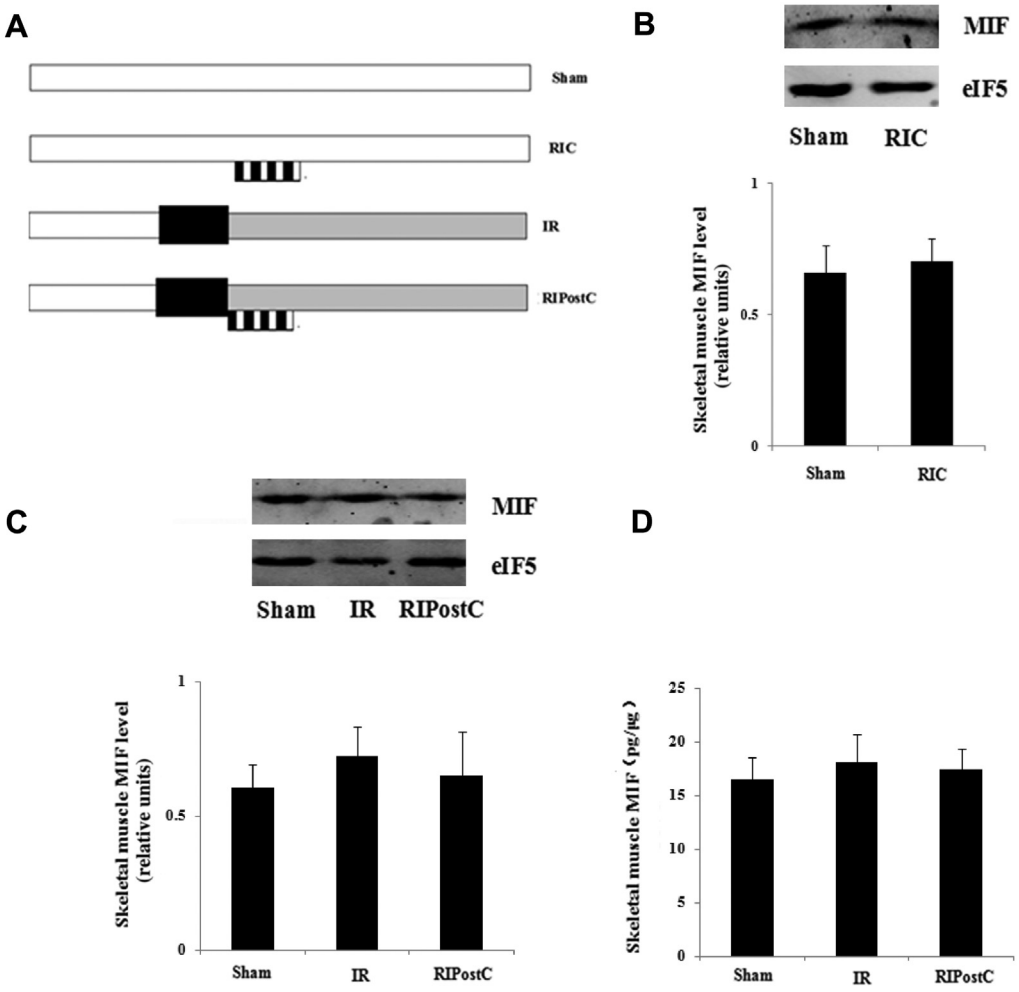

Figure 4. The effect of RIPostC on MIF expression in skeletal muscle in rat limbs. (**A**) Experimental protocol. (**B**) Detection of MIF levels in skeletal muscle after RIC using Western blotting. (**C, D**) Detection of MIF levels in skeletal muscle after RIPostC using Western blotting and ELISA, n = 6 for each group.

Inhibition of MIF may aggravate the apoptosis of the heart in RIPostC

It has been reported that MIF can inhibit apoptosis in cardiac cells during IR. We thus designed experiments to verify the role of MIF in apoptosis in RIPostC using TUNEL staining. The results showed that RIPostC could markedly inhibit apoptosis in the rat heart during myocardial IR. Furthermore, this antiapoptotic role of RIPostC could be inhibited by administration of ISO-1, which suggests that MIF plays an important role in regulating cell apoptosis in RIPostC (Fig. 6).

RIPostC confers protection against myocardial IR injury via the MIF-AMPK signalling pathway

Previous studies have shown that MIF can modulate AMPK signalling in myocardial IR. Thus, we examined whether MIF-AMPK signaling is involved in RIPostC. Using Western blotting, we found that RIPostC could induce myocardial AMPK activation, and this activation could be blocked by ISO-1, which indicates that MIF conferred cardioprotection by modulating AMPK signalling in RIPostC (Fig. 7).

Inhibition of HIF-1α may result in the reduction of plasma MIF in RIPostC

We used the HIF-1α inhibitor, 2ME2, to investigate the role of HIF-1α in the regulation of MIF release. We found that compared to RIPostC group, the level of plasma MIF in RIPostC + 2ME2 group decreased significantly, which suggests that the secretion and release of MIF in RIPostC may be regulated by HIF-1α (Fig. 8).

Discussion

Emerging evidence has shown that MIF is a crucial player against myocardial IR injury.[42] However, the role of MIF in RIPostC is unclear. In this study, we found that RIPostC could markedly reduce MI size and apoptosis during IR injury in which MIF plays a key role. There was a significant change in both plasma and cardiac MIF levels after RIPostC. The cardioprotection of RIPostC may be mediated by MIF through the humoral pathway in a HIF-1α–dependent manner. In addition, the cardioprotective role of RIPostC may be regulated by MIF-AMPK signalling. Therefore, this study provides a theoretical basis for the

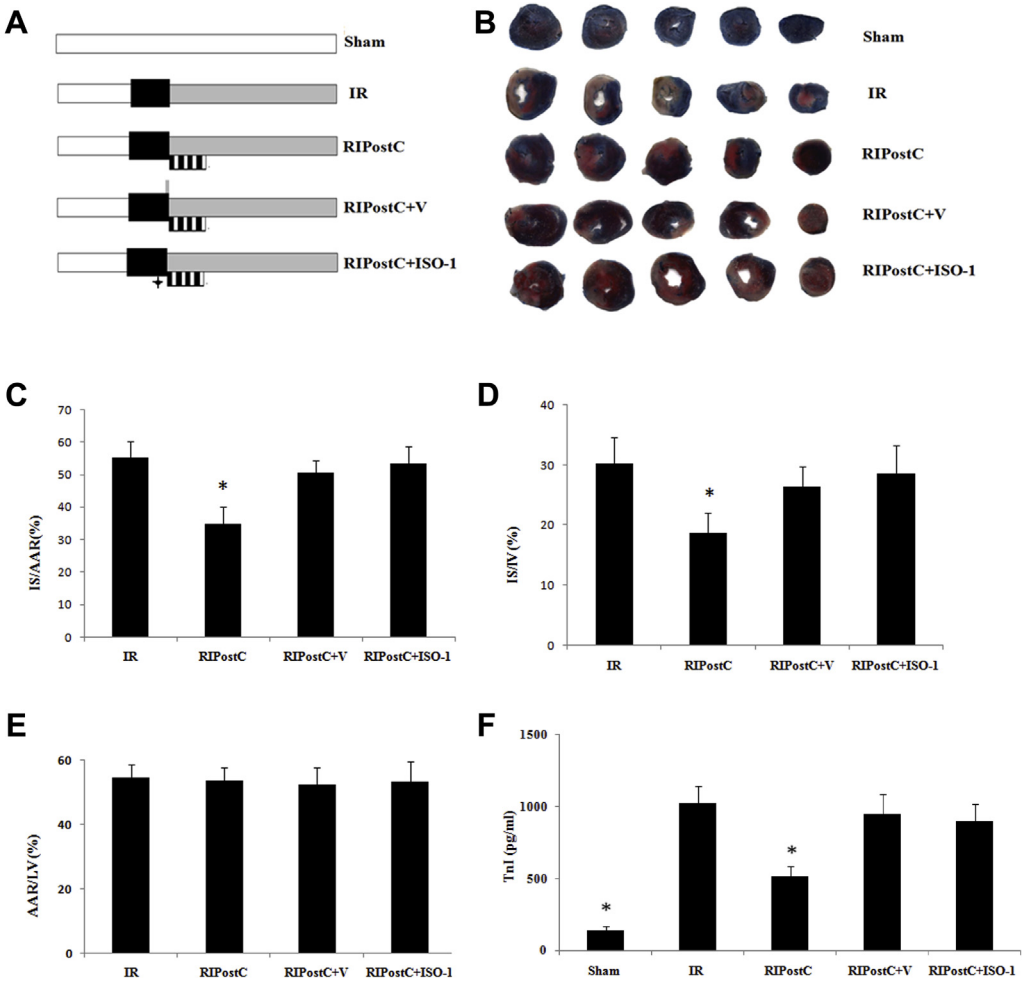

Figure 5. Inhibition of MIF could induce the loss of cardioprotection in RIPostC. (**A**) Experimental protocol. (**B**) Analysis of MI. The hearts were stained with Evans **blue** dye to identify AAR and triphenyltetrazolium chloride to determine IS and then cut into five sections. **C-E** represents IS/AAR, IS/LV, AAR/LV, respectively. (**F**) The level of plasma TnI. * vs IR $P < 0.01$, n = 6 for each group.

application of RIPostC to provide powerful protection against IR injury.

As it was carried out immediately after reperfusion, the timing of RIPostC may be controlled completely by cardiologists.[43,44] Therefore, compared with RIPC, RIPostC is more flexible and has better potential for future clinical application. However, the mechanisms underlying RIPostC have not been fully elucidated to date. Specifically, it is not known how the cardioprotective signal can be transmitted to the heart. Previously, the cardioprotective signal of RIC was hypothesized to be transferred from the remote organ or tissue to the heart by humoral and neuronal transmitter mechanisms; this hypothesis was verified preliminarily by Hausenloy et al. in 2010,[10] who found that the cardioprotective role of RIPC may be blocked by occluding the femoral vein and/or femoral nerve resection.

In the current study, we found that a marked elevation of plasma MIF was detected immediately and at 3 hours after the beginning of RIC. Furthermore, the significant increase of plasma MIF did not occur in RIPostC when the femoral vein was occluded, indicating that the elevation of plasma MIF in RIPostC could be caused by the release of MIF into circulation, which led to the activation of the protective signal in the heart. We may conclude that the cardioprotective signal mediated by MIF was transmitted via the humoral pathway to heart.

ISO-1 is an antagonist of MIF, which may bind to an MIF tautomerase site in a similar manner to p-hydroxyphenylpyruvic acid to inhibit the activity of MIF.[45] In 2013, Wang et al.[31] found that ISO-1 could inhibit MIF-AMPK activation during cardiac ischemic injury *in vitro*. In the current study, we found that RIPostC may reduce apoptosis of myocardial cells significantly, whereas the use of ISO-1 could aggravate the apoptosis and infarct size of the heart by reducing AMPK phosphorylation in RIPostC; this indicates that MIF-AMPK signal transduction plays a key role in the cardioprotection conferred by RIPostC by inhibiting cell apoptosis.

There are 2 main pathways for the secretion of MIF: the increase of MIF expression and the release of MIF from pre-existing MIF stores. Increased MIF expression usually occurs in various chronic disorders such as gastric cancer. The release of MIF from pre-existing MIF stores happens in many acute physiological and pathological processes, such as myocardial IR, as it responds more rapidly to these acute stimuli.[46-48]

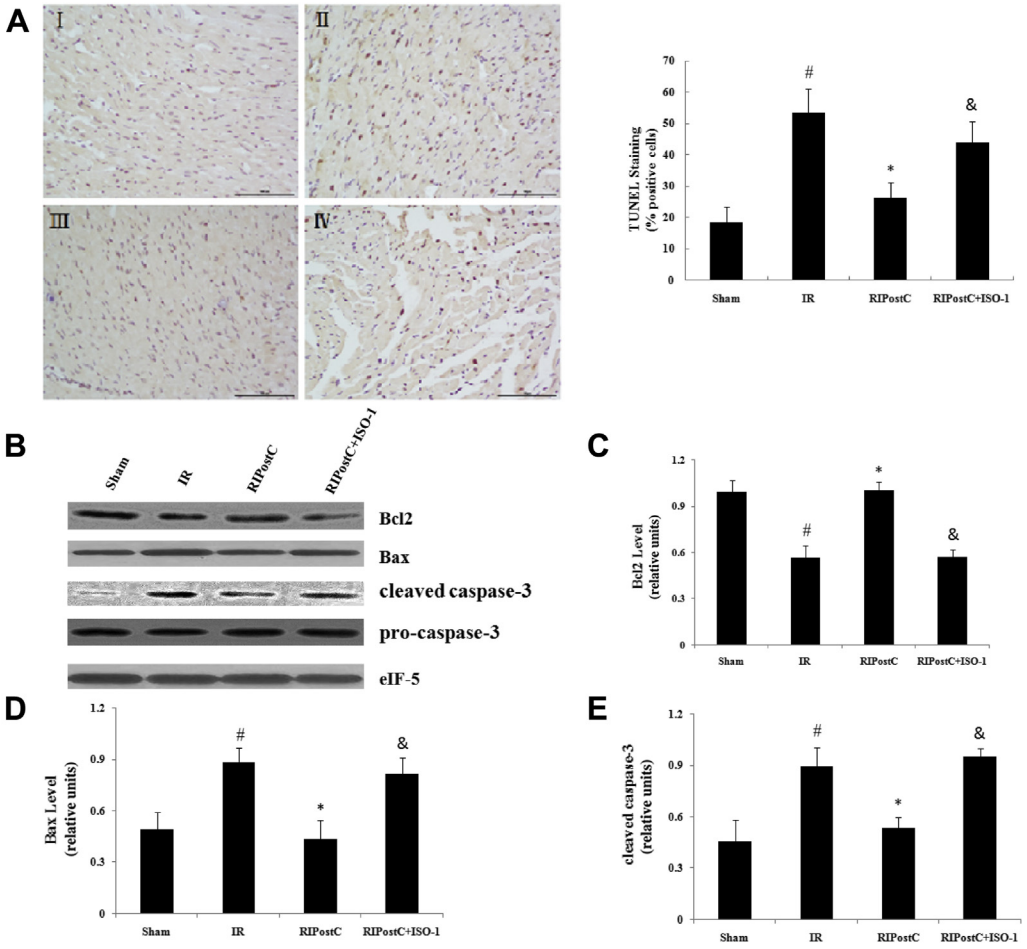

Figure 6. Inhibition of MIF may aggravate the apoptosis of heart in RIPostC. (**A**) **A**poptosis assay. (**B-E**) Detection of Bax, Bcl-2, caspase-3 levels in heart after RIPostC using Western blotting. **I**: Sham **II**: IR **III**: RIPostC **IV**: RIPostC+ISO-1. Scale bar: 50 μm. # vs Sham $P < 0.01$; * vs IR $P < 0.01$; & vs RIPostC $P < 0.01$, n = 8 for each group.

During myocardial IR, cardiac endogenous MIF is secreted from the heart into circulation, leading to the increase of plasma MIF and corresponding decline of cardiac MIF. This release of cardiac endogenous MIF from pre-existing MIF stores into circulation would confer powerful protection via activation of the AMPK pathway.[28,49,50] In this study, we found that the reduction of cardiac endogenous MIF and elevation of plasma MIF also occurred promptly after RIPostC stimulus compared with the IR group. These data indicate that similarly to the mechanism of myocardial IR, RIPostC stimulus might also induce the rapid release of cardiac MIF from pre-existing MIF stores into circulation and result in a

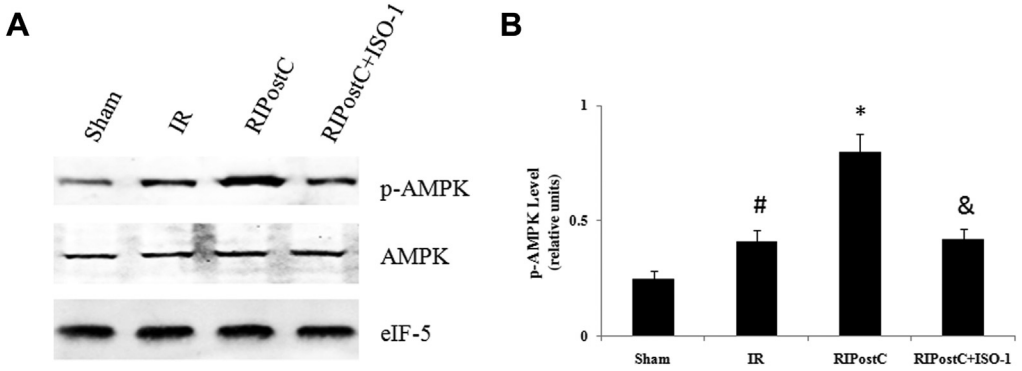

Figure 7. RIPostC confers protection against myocardial IR injury via the MIF -AMPK signaling pathway. (**A, B**) Representative immunoblots and densitometric analysis. # vs Sham $P < 0.01$; *vs IR $P < 0.01$; & vs RIPostC $P < 0.01$, n = 6 for each group.

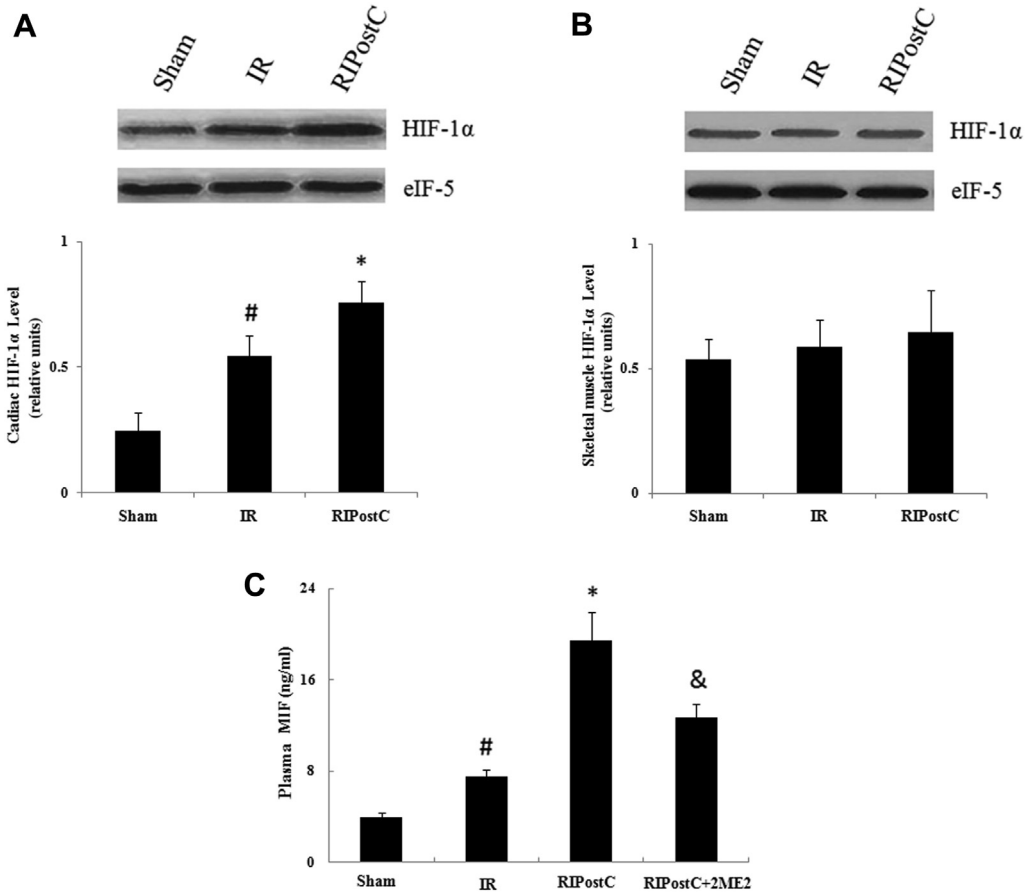

Figure 8. Inhibition of HIF-1α may result in the reduction of plasma MIF in RIPostC. (**A**) Detection of HIF-1α levels in heart tissue after RIPostC using Western blotting. (**B**) Detection of HIF-1α levels in skeletal muscle after RIPostC using Western blotting. (**C**) Detection of plasma MIF levels after RIPostC + 2ME2 treatment using ELISA. # vs Sham $P < 0.01$; * vs IR $P < 0.01$; & vs RIPostC $P < 0.01$, n = 6 for each group.

significant decrease of cardiac MIF to protect against myocardial IR.

Cai et al. found that HIF-1α could induce the marked increase of serum IL-10 and confer powerful cardioprotection; however, IL-10 may not be the only HIF-regulated factor induced by RIC.[12] It has been reported that HIF-1α could trigger the secretion of MIF in many physiological and pathological processes.[34-37] HIF-1α/MIF axis has been investigated in multiple experimental models including cardiomyocyte, smooth muscle cell cultures after induction of hypoxia, and in models of myocardial ischemia/reperfusion injury.[37,51] In our study, we found that the inhibition of HIF-1α could significantly decrease the level of plasma MIF. HIF-1α was increased in heart tissue after RIPostC. These data indicate that the secretion of MIF could be induced by HIF-1α in RIPostC. At present, the mechanism of HIF-1α/MIF axis mediated cardioprotection in RIPostC remains unclear. Further studies should be performed to explore this issue in future.

We can conclude that limb RIPostC could induce increases in the levels of MIF in the blood. Also, MIF plays a crucial role in cardioprotection conferred by RIPostC via activation of AMPK in the heart and through the humoral pathway in a HIF-1α–dependent manner. With the discovery that MIF is involved in RIPostC, it may be helpful to clarify the mechanism of RIPostC and explore optimized RIC/RIPostC for clinical use to reduce myocardial IR injury and greatly improve patients' prognoses.

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Disclosures

The authors have no conflicts of interest to disclose.

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