
REVIEW

Remote Preconditioning as a Novel „Conditioning“ Approach to Repair the Broken Heart: Potential Mechanisms and Clinical Applications

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Summary

Remote ischemic preconditioning (RIPC) is a novel strategy of protection against ischemia-reperfusion (IR) injury in the heart (and/or other organs) by brief episodes of non-lethal IR in a distant organ/tissue. Importantly, RIPC can be induced noninvasively by limitation of blood flow in the extremity implying the applicability of this method in clinical situations. RIPC (and its delayed phase) is a form of relatively short-term adaptation to ischemia, similar to ischemic PC, and likely they both share triggering mechanisms, whereas mediators and end-effectors may differ. It is hypothesized that communication between the signals triggered in the remote organs and protection in the target organ may be mediated through substances released from the preconditioned organ and transported *via* the circulation (humoral pathways), by neural pathways and/or *via* systemic anti-inflammatory and antiapoptotic response to short ischemic bouts. Identification of molecules involved in RIPC cascades may have therapeutic and diagnostic implications in the management of myocardial ischemia. Elucidation of the mechanisms of endogenous cardioprotection triggered in the remote organ could lead to the development of diverse pharmacological RIPC mimetics. In the present article, the authors provide a short overview of RIPC-induced protection, proposed underlying mechanisms and factors modulating RIPC as a promising cardioprotective strategy.

Key words

Ischemia/reperfusion • Remote preconditioning • Innate cardioprotection

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Introduction

Ischemic preconditioning (IPC) is a very robust form of cardiac adaptation observed in all animal species including humans. Unfortunately, due to technical reasons (chest opening to get access to coronary arteries) and short-term duration, its clinical application is limited to planned interventions, such as primary percutaneous coronary intervention (PCI) or coronary artery bypass grafting surgery (CABG) (Wu *et al.* 2000, Hausenloy and Yellon 2008).

On the other hand, other conditioning intervention may also confer efficient cardioprotection against ischemia/reperfusion (IR) injury – a strategy introduced by Przyklenk *et al.* (1993) and termed remote IPC (RIPC). In this study, the authors found that short ischemic episodes of the circumflex branch of left coronary artery reduced the size of infarction induced by occlusion of left anterior descending (LAD) branch of

coronary artery. In another study by McClanahan *et al.* (1993) it was demonstrated that occlusion and reperfusion of renal artery in rabbits reduced the size of myocardial infarction. Further research supported the hypothesis that the phenomenon of RIPC can be induced by ischemia of distant organs/tissues (either cardiac or noncardiac), such as small intestine (Gho *et al.* 1996), kidney (Weinbrenner *et al.* 2004) and other organs, and evokes systemic protection against acute IR injury (Przyklenk and Kloner 1998). Moreover, interorgan communication afforded protection against IR in different organs and not only in the heart (Hausenloy and Yellon 2008). Thus, it became evident that RIPC may represent a general phenomenon of distant cardioprotection.

However, these methods were still invasive. Finally, important study by Birnbaum *et al.* (1997) demonstrated that brief limitation of blood flow in the

hind limb muscle in rabbits performed prior to longer lasting occlusion of coronary artery reduced the size of myocardial infarction by 65 %. Furthermore, Oxman *et al.* (1997) induced RIPC on rat hind limb by means of tourniquet, which efficiency was further confirmed in experiments in pigs (Kharbada *et al.* 2002). Most importantly, Kharbada *et al.* (2001) applied RIPC protocol in humans using pressure cuff (placed on the upper extremity), and three cycles of 5-min inflation (200 mmHg)/5-min deflation successfully attenuated IR-induced endothelial dysfunction in forearm blood vessels (assessed as an improved post-IR forearm blood flow in response to acetylcholine). Later on, protocol of RIPC performed on limbs was termed „limb ischemic preconditioning“ (LIPC) (Wu *et al.* 2011). This protocol of RIPC is being used until now both, in clinical situations and in animal experiments.

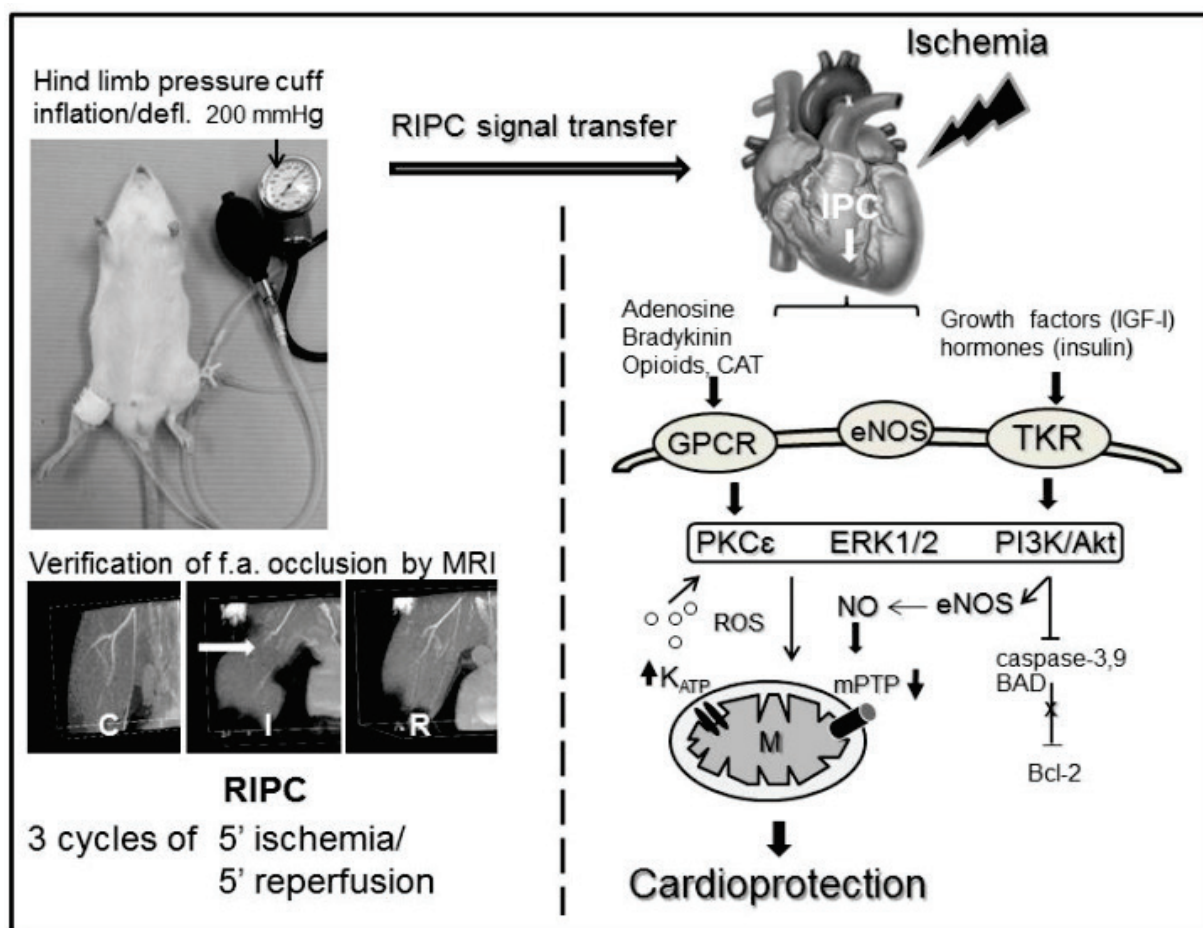


Fig. 1. Remote ischemic preconditioning applied on hind limb of rat and induction of cardioprotection in a distant organ (heart) exposed to ischemia. RIPC – remote ischemic preconditioning, MRI – magnetic resonance image. GPCR – G proteins-coupled receptors, eNOS – endothelial NO synthase, TKR – receptors with tyrosine kinase activity, PKC ϵ – protein kinase C ϵ , ERK1/2 – extracellular signal regulated kinases, PI3K/Akt – phosphatidylinositol-3-kinase/protein kinase B (Akt), ROS – reactive oxygen species, K_{ATP} – mitochondrial ATP-dependent potassium channels, mPTP – mitochondrial permeability transition pore, BAD, Bcl-2 – pro/antiapoptotic proteins.

The major advantage of limb RIPC, compared with other strategies of endogenous cardioprotection, appeared to be the possibility to attenuate ischemic injury noninvasively using a standard blood pressure cuff placed on the upper or lower limb. Figure 1 illustrates application of RIPC on right hind limb of rat, where verification of femoral artery occlusion was confirmed by magnetic resonance technique. Günaydin *et al.* (2000) provided biochemical evidence (reduced cellular enzyme release) that RIPC applied on the upper limb in patients undergoing coronary artery surgery increased ischemic tolerance in the heart and protected the myocardium by enhancing anaerobic glycolysis during cardiac IR. This observation represented an important breakthrough in the clinical applicability of the preconditioning phenomenon. Additionally, it was shown that transient limb ischemia before PCI (stenting) led to a reduction in adverse cardiac events in a group of RIPC patients compared with control subjects who experienced adverse side effects (Hoole *et al.* 2009).

Since initial clinical trials in this area focused on the application of RIPC in ischemic cardiac disease, the present article briefly reviews cardioprotection induced by RIPC, the underlying mechanisms, and the factors modulating effectiveness of RIPC.

Potential mechanisms, phases and transfer of RIPC effect

Similar to other forms of preconditioning, RIPC also exerts a biphasic phenotype, as confirmed in all organ systems in animal models and in recent human studies (Tapuria *et al.* 2008). The ‘first window’ cardioprotection that starts within 5-30 min after the final cycle of RIPC is known to be mediated by modification of existing proteins and completely wanes within 3 h. On the other hand, RIPC stimuli simultaneously initiate a complex genomic and proteomic response that is executed during the late phase of protection that reappears 24 h after initial stimulus and persists during three to four days, sometimes even more. This ‘second window’ of protection is attributed to the activation of transcriptional regulation, protein translation and post-translational changes of newly synthesized proteins, e.g. those with antioxidative properties (Kageyama *et al.* 2015) resulting in longer-lasting protection.

Generally, it is accepted that RIPC represents a complex cascade of initial triggers activated or generated in the remote tissue, mediators of communication between the remote site and the target

organ (heart), and end-effectors responsible for induction of the protective phenotype in the myocardium (Przyklenk and Whittaker 2011).

The aspect of RIPC-induced interorgan communication is still not completely elucidated. Several pathways are proposed to transfer protective signal from the distant organ to the heart: humoral pathways, neural pathways or by means of systemic response (Bousselmi *et al.* 2014).

According to the humoral hypothesis, endogenous substances released from the conditioned organ/tissue are transferred *via* circulation to the target organs, where they activate the respective receptors and trigger cascade of IPC (Hausenloy and Yellon 2008). However, the precise identity of humoral RIPC mediators remains unclear (Gill *et al.* 2015).

Neural hypothesis was based on the findings that ganglion blockers (Gho *et al.* 1996, Loukogeorgakis *et al.* 2005), vagotomy (Lim *et al.* 2010) or other interventions interrupting nervous afferent or efferent pathways abolished RIPC-induced cardioprotection.

The third hypothesis implies that RIPC stimulus induces a systemic response in the organism with subsequent suppression of inflammation and apoptosis (Hausenloy and Yellon 2008), while modifying pro/antiinflammatory gene profile (up-regulation of antiinflammatory genes or suppression of inflammatory genes) (Konstantinov *et al.* 2004, Shimizu *et al.* 2010).

Currently, it is believed that transmission of the RIPC signal to the target organ is multifactorial, requiring a combination of humoral, neuronal and systemic mechanisms, and may be model-dependent (Lim *et al.* 2010). Recent research has revealed that such communication could be also mediated *via* peroxisome proliferator activated nuclear receptors (PPARs) involved in limb RIPC-induced cardioprotection (Lotz *et al.* 2011). Kidney-induced RIPC showed activation of signaling cascades mediated by transcription factor NFκB followed by subsequent opening of mitochondrial ATP-sensitive potassium channels (mitoK_{ATP}) (Diwan *et al.* 2008). These findings confirm an important role of gene transcription in RIPC-mediated effects.

The schematic presentation of potential pathways of signal transfer from conditioned to a target organ is shown in Figure 2. The procedure of RIPC may be applied prior to ischemia (as pre-conditioning), during ischemia (as per-conditioning) or just after ischemia (as post-conditioning) (Kanoria *et al.* 2007, Hausenloy and Yellon 2008, Tapuria *et al.* 2008).

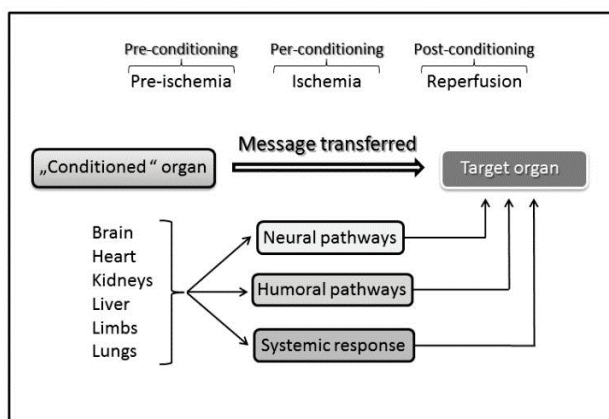


Fig. 2. Schematic presentation of remote preconditioning and pathways of protective signal transduction to distant target organs.

Potential triggers of RIPC

It is hypothesized that initial triggers of both IPC and RIPC may be universal and able to induce protection either in host organs or the organs of recipients of coronary effluent transfer (Dickson *et al.* 1999). The mechanisms underlying RIPC have been studied extensively, however, the exact triggering molecules involved are not completely clarified. Possible candidates involve adenosine, bradykinin-2, opioids, angiotensin-1, reactive oxygen species (ROS), noradrenaline, nitric oxide (NO), heat shock proteins (HSP) and calcitonin gene-related peptide (CGRP) (Heusch *et al.* 2008, Tapuria *et al.* 2008, Rassaf *et al.* 2014). These substances act through multiple receptors (mostly G-protein-coupled receptors [GPCR]) or activate downstream „survival“ pathways *via* non-receptor-mediated mechanisms.

Potential mediators of RIPC

RIPC triggers can activate GPCR-linked pathways that include 1,2-diacylglycerol, protein kinase C (PKC), mitogen-activated protein kinase (MAPK), CGRP, and transcription factors, such as NF κ B (Wolfrum *et al.* 2002, 2005, Li *et al.* 2004) mediating a protective signal to an end-effector.

It is suggested that cellular signaling pathways in the conditioned organ/tissue and in the target one are similar to those involved in the mechanisms of IPC or postconditioning (IPost). A number of mediators are known to belong to these signaling cascades including PKC, NO synthase, phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), MAP-kinases, signal transducers and activators of transcription 5 (STAT5) proteins, ROS,

etc. (Hausenloy and Yellon 2008, Heusch *et al.* 2012, Rassaf *et al.* 2014). Postreceptor mechanisms also encompass cyclic guanosine monophosphate-dependent protein kinase (cGMP/PKG) pathways (Burley *et al.* 2007), the reperfusion injury risk kinases (RISK) pathway involving PI3K/Akt and extracellular signal-regulated kinase (ERK1/2) pathway (Hausenloy and Yellon 2008), and the survivor activating factor enhancement (SAFE) pathway (Lecour *et al.* 2009). Opening of mitoK_{ATP} channels is considered to be one of the crucial mechanisms in different cardioprotective interventions coupled with an increased production of ROS activating prosurvival signaling pathways, such as PI3K/Akt, PKC ϵ , and/or glycogen synthase kinase-3 β (Forbes *et al.* 2008, Matejikova *et al.* 2009), leading to functional recovery and infarct size limitation. Activation of mitoK_{ATP} has been also shown to be involved in the delayed effect of hind limb RIPC in rats (Wu *et al.* 2011). However, it remains unclear whether they act as triggers, mediators and/or end-effectors.

Some recent findings demonstrated less known mechanisms of RIPC-induced mediators of cardioprotection, such as the involvement of hypoxia-induced factor HIF-1 α (Albrecht *et al.* 2013), the role of extracellular vesicles (Gircz *et al.* 2014), connexin 43 phosphorylation (Brandenburger *et al.* 2014a) or microRNA-1 (Brandenburger *et al.* 2014b).

Potential end-effectors of RIPC in the heart

Impairment of mitochondrial function plays a major role in IR injury, and not surprisingly, mitochondrial modulation has been considered to be implicated in the cardioprotective effects of IPC. The fluidity of mitochondrial membranes is a biophysical property that governs cell fate toward survival or death, and is tightly bound with the regulation of diverse adaptive and/or pathological processes including IR (Ziegelhöffner *et al.* 2012). All aforementioned transduction systems involved in the preconditioning protection appear to converge on the mitochondria (Murphy and Steenbergen 2007). Modulation of mitochondrial function is linked to changes in mitochondrial permeability transition pore (mPTP) opening and cytochrome c release, leading to activation or inhibition of proapoptotic cascades in the cytosol (Halestrap *et al.* 2007). The inhibition of mPTP opening *via* RISK is suggested as a final common target through which the signaling pathways can protect the cell against necrosis/apoptosis (Hausenloy *et al.* 2004, Heusch *et al.*

2008). This is in line with the study of Zhang *et al.* (2006), in which specific mPTP activator atractyloside abrogated IS-limiting effect of RIPC. Moreover, it has been recently shown that RIPC applied on rat hind limb improved functional and biophysical properties of cardiac mitochondria (Ferko *et al.* 2014). These mechanisms of RIPC-induced cardioprotection are briefly summarized in Figure 1.

Potential clinical applications of remote preconditioning

One of the first studies in humans was the study by Kharbanda *et al.* (2001). Since that time, numerous research teams have been investigating the effect of RIPC against acute IR under different clinical conditions. Most of the studies were designed to reveal whether RIPC was able to protect human heart against IR injury, in particular, under conditions of cardiac surgery, primary PCI or during the management of acute myocardial infarction (Lim and Hausenloy 2012). First successful application of RIPC in clinical conditions was reported by Cheung *et al.* (2006) in children undergoing cardiac surgery for congenital heart defect, which was very often associated with high mortality of patients. These authors found that 4 cycles of 5-min RIPC by inflation/deflation of pressure cuff placed on lower extremity, prior to surgery, resulted in a reduced extent of myocardial injury (less troponin I release, lower inotropic score). Later on, Hausenloy *et al.* (2007) demonstrated that 3 cycles of IR were able to reduce the degree of myocardial injury (reduced troponin T release) in adults undergoing CABG surgery.

Since then, numerous studies demonstrated positive effect of RIPC applied in patients prior to CABG surgery, primary PCI (Choi *et al.* 2011, Ahmed *et al.* 2013, Zografos *et al.* 2014) or in patients with acute myocardial infarction (Prunier *et al.* 2014, White *et al.* 2015). However, several other studies failed to demonstrate any benefit in these patients (Rahman *et al.* 2010, Karuppasamy *et al.* 2011).

One of the last big clinical trials was ERICCA study (Hausenloy *et al.* 2015), which recent results failed to demonstrate positive effects of RIPC in patients that underwent CABG. Another clinical trial (Meybohm *et al.* 2015) also did not reveal any relevant benefit of RIPC in patients after cardiac surgery. However, all aforementioned studies were focused on clinical outcomes, and were not exploring molecular mechanisms of RIPC in humans (Moscarelli *et al.* 2015). To better

understanding the controversies in the results, it will be necessary to consider the elucidation of molecular basis of RIPC in animals and humans.

Factors modulating the effectiveness of RIPC

RIPC was originally characterized in young and healthy experimental animals. In these settings, coronary circulation, *per se*, is intact and is only subject to controlled occlusion and reperfusion by external or internal devices. This model is not adequate for the studies of RIPC in humans who experience myocardial infarction usually in elder age, and coronary circulation of these patients is influenced by pre-existing atherosclerotic lesions. Ischemic heart disease is characterized by a certain level of limitation of coronary circulation, which is an essential cause of ischemic injury (Ravingerova *et al.* 2012). On the other hand, cycles of coronary occlusion and reperfusion that occur spontaneously, often before sustained ischemia, may thus act as a stimulus of cardioprotection. The state of coronary circulation is, therefore, a major determinant of cardioprotection, and so called pre-infarction angina pectoris is considered as a clinical equivalent of IPC (Abete *et al.* 1997). However, coronary circulation may be affected by numerous comorbidities. Risk factors for cardiovascular diseases, such as stress, chronically elevated blood pressure and metabolic disorders, have a negative impact on the heart exposed to ischemia and may aggravate lethal injury (i.e. myocardial infarction). Moreover, it has been shown that the protective effect of IPC, postconditioning and RIPC is suppressed by comorbidities, such as hypercholesterolemia, hyperglycemia, hypertension, cardiac hypertrophy, aging, obesity, as well as by comedications (Ferdinandy *et al.* 2014). However, pathologically altered myocardium does not completely lose its adaptive potential. Thus, no loss of the efficacy of IPC was shown in middle-aged and older rabbits *in vivo* (Przyklenk *et al.* 2001). The impact of aging on the efficiency of RIPC is not yet completely elucidated, and the results are controversial. While RIPC failed to protect the heart of neonatal rabbits against IR, in adult animals, IS-limiting effect of RIPC was obvious (Schmidt *et al.* 2014). More information about age-dependency of effects of RIPC was gained from the clinical studies. Positive effects of limb RIPC applied prior to cardiac surgery in children have been observed in several studies (Cheung *et al.* 2006, Zhou *et al.* 2010), however, a lack of RIPC effects was also reported (Pavione *et al.* 2012, McCrindle *et al.* 2014). On the other

hand, although in elder patients the benefit of RIPC was often absent (Meybohm *et al.* 2015), especially in those individuals with diabetes mellitus (Xu *et al.* 2014), other authors reported positive effects of different protocols of RIPC in adult and old patients when applied prior to coronary interventions (Kono *et al.* 2014, White *et al.* 2015). This may indicate the possibility of reactivating reduced preconditioning potential by modification of the intensity of the preconditioning stimulus or by use of an alternative mode of cardiac adaptation.

Conclusions

Despite controversial outcomes of some clinical trials, RIPC has shown promising results in recent experimental studies indicating its potential to protect the ischemic heart in clinical practice. The main advantage of this strategy is that it appears to be an effective, safe, noninvasive, easily applicable and cost-effective

cardioprotective tool against IR injury in scenarios in which ischemic damage is suspected but the occurrence of myocardial infarction is unpredictable. Identification of molecules involved in RIPC cascades may have therapeutic and diagnostic implications in the management of acute myocardial ischemia and could lead to the development of diverse pharmacological RIPC mimetics in accordance with the stage of ischemic heart disease, planned intervention and/or prospective comorbidities.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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