Non-invasive approach to mend the broken heart: is "remote conditioning" a promising strategy for application in humans?

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Non-invasive approach to mend the broken heart: is “remote conditioning” a promising strategy for application in humans?

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Short title: Heart “conditioning” approach and clinical application

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Abstract

Currently, there are no satisfactory interventions to protect the heart against the detrimental effects of ischemia-reperfusion injury. Although ischemic preconditioning (PC) is the most powerful form of intrinsic cardioprotection, its application in humans is limited to planned interventions, due to its short duration and technical requirements. However, many organs/tissues are capable of producing “remote” PC (RPC) when subjected to brief bouts of ischemia-reperfusion. RPC was first described in the heart where brief ischemia in one territory led to protection in other area. Later on, RPC started to be used in patients with acute myocardial infarction, albeit with ambiguous results. It is hypothesized that the connection between the signal triggered in remote organ and protection induced in the heart can be mediated by humoral and neural pathways as well as via systemic response to short sublethal ischemia. However, although RPC has a potentially important clinical role, our understanding of the mechanistic pathways linking the local stimulus to the remote organ remains incomplete. Nevertheless, RPC appears as a cost-effective and easily performed intervention. Elucidation of protective mechanisms activated in the remote organ may have therapeutic and diagnostic implications in the management of myocardial ischemia and lead to development of pharmacological RPC mimetics.

Key words: ischemia/reperfusion, myocardial infarction, remote preconditioning, innate cardioprotection, cell signaling
Introduction

Ischemic heart disease (IHD), in particular, its most serious manifestation, an acute myocardial infarction (AMI), still remains the number-one cause of death in the modern world. Early restoration of blood flow in the ischemic myocardium is a pre-requisite for salvaging viable myocardium. However, revascularization may paradoxically induce ischemia/reperfusion (I/R) injury and accelerate cardiomyocyte death in terms of greater MI and post-AMI myocardial dysfunction later progressing into heart failure (Bulluck et al. 2016; Hausenloy and Yellon 2015a; Yellon and Hausenloy 2007). Development of interventional cardiology (primary percutaneous coronary intervention, PPCI) and/or thrombolytic approaches, such as a new generations of antiplatelet drugs and antithrombotic agents (Bulluck et al. 2016; Li et al. 2014a) have been a major step forward. However, until now, neither pharmacological therapies, nor non-pharmacological interventions have been ultimately successful in protecting the myocardium against AMI or in preventing the adverse effects of reperfusion injury (Burns et al. 2002; Heusch et al. 2014; Larose et al. 2010). Thus, there is a substantial unmet need to develop novel approaches, ideally those that specifically address regeneration of damaged and/or lost myocardium (given limited endogenous repair of cardiomyocytes).

Ischemic preconditioning (PC) is a very robust form of intrinsic cardioprotection observed in all animal species including humans. Unfortunately, due to technical requirements (chest opening to get access to coronary arteries) and short-term duration, its clinical application is limited to planned interventions including PPCI or coronary artery bypass grafting (CABG) surgery (Bulluck et al. 2016; Hausenloy and Yellon 2008; Wu et al. 2000).

On the other hand, a powerful protection against I/R injury can be rendered by other “conditioning” interventions. A strategy introduced by Przyklenk et al. (1993) termed “remote” PC (RPC) revealed that short ischemic episodes of the circumflex branch of left coronary artery reduced the size of infarction induced by occlusion of the left anterior descending (LAD) branch of the coronary artery, i.e., in a different territory of the myocardium. In addition, McClanahan et al. (1993) demonstrated that the size of AMI in rabbits may be decreased by occlusion and reperfusion of a renal artery. Further research supported the hypothesis that the phenomenon of RPC can be induced by ischemia in distant organs/tissues (either cardiac or noncardiac), such as the small intestine (Gho et al. 1996), kidney (Weinbrenner et al. 2004) and other organs, and evoke systemic protection against acute I/R (Przyklenk and Whittaker 2011). Furthermore, the concept of RPC confirmed that
interorgan communication may afford protection against I/R not only in the heart but also in the spinal cord, brain, liver, intestine or kidney (Haapanen et al. 2016; Hausenloy and Yellon 2008). Moreover, RPC has been shown to be evoked by numerous other stimuli, such as surgical trauma (Song et al. 2016), peripheral nerve stimulation (Reddington et al. 2012) or by local release of autacoids (Schulz et al. 1998, Anttila et al. 2016) as depicted in Fig. 1. Thus, it is evident that RPC may represent a general phenomenon of distant cardioprotection.

However, many of these methods were still invasive. Interestingly, Birnbaum et al. (1997) demonstrated that brief limitation of blood flow in the hind limb muscle in rabbits prior to longer lasting occlusion of the coronary artery reduced the size of an AMI by 65%. Furthermore, Oxman et al. (1997) induced RPC in rat hind limb by means of a tourniquet, the efficacy of this was later confirmed in the experiments in pigs (Kharbanda et al. 2002). In the important study by Kharbanda et al. (2001), an RPC protocol was applied in humans using a pressure cuff (placed on the upper extremity), and three cycles of 5-min inflation (200 mmHg)/5-min deflation. This successfully attenuated I/R-induced endothelial dysfunction in forearm blood vessels (assessed as an improved post-I/R forearm blood flow in response to acetylcholine). RPC performed on limbs was termed “limb ischemic preconditioning” (LIPC) (Wu et al. 2011). Figure 2 (left panel) illustrates the application of RPC on a right hind limb of a rat, where verification of femoral artery occlusion/reperfusion was confirmed by magnetic resonance imaging. Furthermore, protection of the heart by LIPC has been demonstrated in children who underwent surgery with cardiopulmonary bypass for congenital heart disease (Tapuria et al. 2008). This protocol of RPC is being used now both in clinical situations, e.g, in patients undergoing PPCI for ST-elevation myocardial infarction (STEMI) (Liu et al. 2016) and in animal experiments (Anttila et al. 2016) including ischemic models of other organs, such as the spinal cord (Haapanen et al. 2016).

The major advantage of limb RPC, compared with other strategies of endogenous cardioprotection, was the possibility to attenuate ischemic injury noninvasively using a standard blood pressure cuff placed on the upper or lower limb. Moreover, as shown in Fig. 2, RPC could be effectively applied in the settings of pre-, per- and post-conditioning (i.e., before and during ischemia, as well as prior to the onset of reperfusion) extending thus a potential window of cardioprotection in contrast to classical ischemic PC (Hausenloy and Yellon 2008; Hausenloy and Yellon 2015a; Heusch et al. 2015; Tapuria et al. 2008). The latter makes RPC more attractive from the clinical point of view. An important breakthrough in the clinical applicability of the limb preconditioning was a study by Günaydin et al. (2000) who provided biochemical evidence (reduced cellular enzyme release) that RPC applied on
the upper limb in patients undergoing coronary artery surgery increased ischemic tolerance during myocardial I/R and protected the heart by enhancing anaerobic glycolysis.

Since initial clinical trials in this area focused on the application of RPC in IHD, and since negative outcomes or absence of positive results were reported as well, this article briefly reviews cardioprotection induced by RPC in the healthy and “diseased” myocardium, its mechanisms and the factors modulating the effectiveness of RPC.

**Timing of protection, potential mechanisms, and RPC signal transmission**

Similar to other forms of preconditioning, RPC exerts a biphasic phenotype, as confirmed in all organ systems in animal models and in human studies (Heusch 2015; Loukogeorgakis et al. 2005; Tapuria et al. 2008). The early phase of RPC (also termed the first window) known to be mediated by posttranslational modification of existing proteins is initiated within 5-30 minutes after the final cycle of preconditioning ischemia. Besides, RPC stimulus simultaneously initiates a complex genomic and proteomic response executed during the late phase of protection that reappears 24 hrs after the initial stimulus and persists during next 3-4 days, albeit modest in its magnitude (Heusch et al. 2015; Przyklenk and Whittaker 2011). Known as a second window of protection (Cai et al. 2012), this phase is attributed to the synthesis of new proteins (Bhuiyan and Kim 2010; Kageyama et al. 2015). Interestingly, both phases of RPC-induced protection against endothelial ischemia-reperfusion injury in humans were mediated neuronally as both were abrogated with an autonomic ganglion blocker (Loukogeorgakis et al. 2005).

**Triggering mechanisms and pathways from remote organ to target protection**

Mechanisms of protection induced by RPC represent a complex cascade of initial triggering in the remote tissue, communication between the distant and the target organ, and end-effects responsible for the induction of the protective phenotype. Figure 1 shows a simplified scheme of RPC induction in different organs by different stimuli and proposed pathways of signal transfer from the conditioned organs to a target organ/tissue.

It has been hypothesized that initial triggers of both ischemic PC and RPC as powerful forms of short-term adaptation, may be universal and able to induce protection either in distant or host organs (Dickson et al. 1999). The mechanisms involved in RPC have been studied extensively; however, the triggering molecules are still a matter of controversy. Possible candidates for RPC triggers include autacoids, such as adenosine and bradykinin,
released from cardiomyocytes, endothelium, and interstitial cells during the preconditioning I/R cycle(s), opioids, angiotensin-1, reactive oxygen species (ROS), catecholamines, nitric oxide (NO), or calcitonin-gene related peptide (Heusch 2015; Kanoria et al. 2007; Tapuria et al. 2008; Wolfrum et al. 2005). In a study of Liem et al. (2005), four cycles of 15-min coronary artery occlusion (CAO) followed by two cycles of 15-min adenosine-dependent CAO induced preconditioning protection in rats. However, four cycles of 15-min CAO followed by three cycles of adenosine-independent remote 3-min mesenteric artery occlusion (MAO) elicited cardioprotection, suggesting utilizing of alternative pathways to maintain this protection.

The triggering stimulus can originate not only from local I/R, but also from peripheral nociception associated with surgical incision (Jones et al. 2009), local surgical trauma (Song et al. 2016), local activation of sensory fibers by capsaicin or electrical nerve stimulation (Redington et al. 2012).

The aspect of RPC-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 via systemic response (Bousselmi et al. 2014).

**Humoral hypothesis**

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 a preconditioning cascade (Hausenloy and Yellon 2008; Rassaf et al. 2014).

Among many circulating substances, some recent findings pointed out to the less known molecules, such as microRNAs (miRNAs) that might be involved as RPC-induced mediators of cardioprotection. MiRNAs are small (about 22 nucleotides) evolutionary conserved segments of RNA that have important regulatory roles in cell biology and cardiovascular pathophysiology (McManus and Freedman 2015; Weiss et al. 2012). It has been demonstrated that, in particular, miRNA-144 is involved in the mechanisms of RPC, most probably due to the downregulation of the mammalian target of rapamycin promoting improved cell survival, and it is also associated with enhanced RISK (reperfusion injury salvage kinase) signaling (Heusch et al.; 2015, Li et al. 2014b). The effects of miRNAs appear to differ between RPC, IPC and ischemic postconditioning. While IPC is associated with a rise in miRNA-1 and miRNA-21 in the rat heart, RPC and ischemic postconditioning reduces
miRNA-1 in the myocardium and has no effect on miRNA-21 (Duan et al. 2012). It has been also demonstrated that in patients undergoing CABG with prior RPC, miRNA-338-3p levels in right atrial tissue samples were higher than in the controls (Slagsvold et al. 2014).

**Neural hypothesis**

The neural hypothesis implies that substances released locally in the remote ischemic area activate neural afferent pathways which in turn activate various efferent pathways that induce cardioprotection in the heart (Donato et al. 2016). It 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 (spinal cord or femoral nerve section) or efferent (blockade of muscarinic cholinergic receptors) pathways abolished RPC-induced cardioprotection (Donato et al. 2013; Donato et al. 2016; Redington et al. 2012; Steensrud et al. 2010). Recently, Abdul-Ghani et al. (2017) provided further evidence of the changes in the autonomous nervous system activity immediately after the protocol of hind limb RPC in mice that was followed by protection against I/R in Langendorff-perfused hearts of mice. Besides the involvement of the parasympathetic system (Donato et al. 2013; Redington et al. 2012), there is also evidence supporting a role for sympathetic adrenergic stimulation in RPC-induced cardioprotection (Jones et al. 2009; Taliyan et al. 2010).

**Systemic response**

Currently, it is suggested that RPC triggers not only humoral and neuronal signaling leading to an increased resistance to I/R in a distant organ/tissue, but also acts via activation of a systemic response as well (anti-inflammatory, anti-apoptotic, anti-oxidative effects, or changes in gene transcription) (Hausenloy and Yellon 2008; Heusch et al. 2015; Shimizu et al. 2010). This was supported, e.g., by up-regulation of IL-10 in mice exposed to RPC 24 hrs prior to myocardial I/R (Cai et al. 2012) or by reduced pro-inflammatory gene expression in circulating leukocytes in humans (Konstantinov et al. 2004).

Currently, it is believed that transmission of the RPC 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).

**Intracellular mediators of conditioning**

It is suggested that cellular signaling pathways mediating a protective signal to an end-effector in the target organ may be similar to those involved in the mechanisms of ischemic
PC or postconditioning. A number of these key mediators include protein kinase C (PKC), NO synthase, phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), MAP-kinases, signal transducers and activators of transcription (STAT) proteins, and reactive oxygen species (ROS) (Hausenloy and Yellon 2008; Heusch et al. 2015; Rassaf et al. 2014). These substances act through multiple receptors (mostly G-protein-coupled receptors [GPCR]), receptors with tyrosine kinase activity (TKR), receptors for tumor necrosis factor α (TNFR) or they activate downstream “survival” pathways via non-receptor-mediated mechanisms (Heusch 2015) (Fig. 2, right panel).

Postreceptor mechanisms involve the RISK pathway through PI3K/Akt, extracellular signal-regulated kinase (ERK1/2), PKCɛ, glycogen synthase kinase 3β (GSK3β) and mitochondrial permeability transition pore (mPTP) (Hausenloy and Yellon 2008), as well as the survivor activating factor enhancement (SAFE) pathway (TNFα-IL-10-STAT3-mitochondrial ATP-dependent K⁺ channels (mitoKATP) (Lecour et al. 2009). Opening of mitoKATP channels is considered as one of the crucial mechanisms in different cardioprotective interventions coupled with an increased production of ROS activating prosurvival signaling pathways, such as PI3K/Akt, PKCɛ, and/or GSK3β (Forbes et al. 2008), leading to functional recovery and IS-limitation. Activation of mitoKATP has also been shown to be involved in the delayed effect of hind limb RPC in rats (Wu et al. 2011).

Potential end-effectors of RPC in the heart

All aforementioned transduction systems appear to converge on the mitochondria (Murphy and Steenbergen 2007). Modulation of their function is linked to changes in 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 and Yellon 2008; Heusch et al. 2015). This is in line with the study of Zhang et al. (2006), in which a specific mPTP activator, atractyloside, abrogated the IS-limiting effect of RPC. These mechanisms of RPC-induced cardioprotection are also briefly summarized in Figure 2, right panel.

RPC and nuclear receptors PPAR

Peroxisome proliferator-activated nuclear receptors (PPARs) are members of a nuclear hormone receptor superfamily regulating expression of genes involved in diverse aspects of
lipid metabolism and cardiac energy production, during different pathological conditions including I/R injury, diabetes and heart failure (Cheng et al. 2004; Huss and Kelly 2004). Members of the PPAR subfamily, namely, PPARα, PPARβ/δ and PPARγ isoforms are all expressed in the heart tissue of many species including rodents and humans (Bishop-Bailey 2000). Moreover, it has been shown that the exogenous ligands of these transcription factors (hypolipidemics or antidiabetic drugs) may exert preconditioning-like genomic and non-genomic effects including antiapoptotic, anti-oxidative and antiinflammatory effects with subsequent myocardial protection against acute I/R (Barlaka et al. 2016; Ravingerová et al. 2015; Yue et al. 2003). In addition, this protection was associated with activation of PPAR downstream pro-survival targets (PI3K/Akt-eNOS) (Bulhak et al. 2009; Ravingerová et al. 2012b) and MMP-2 inhibition (Barlaka et al. 2013). Although the role of PPARs in the mechanisms of RPC-induced protection is less understood, Lotz et al. (2011) have shown the involvement of PPARα and PPARγ isoforms in antiinfarct protection in rabbits conferred by RPC. This effect of RPC was associated with increased heart tissue levels of endogenous PPAR ligand, 15d-prostaglandin J2, PPAR DNA binding activity and gene expression of inducible nitric oxide synthase. In addition, the protective response elicited by RPC, as well as the associated molecular changes, were blunted by inhibition of both PPARα and PPARγ by their respective antagonists. In line, Li et al. (2011) have demonstrated that the myocardial protection afforded by RPC induced by transient limb ischemia is mediated via the PI3K/Akt/GSK3β signaling pathway, activation of which is associated with cytosolic and nuclear accumulation of β-catenin and increased expression of the β-catenin target genes E-cadherin and PPARβ/δ.

Our recent (unpublished) data confirmed the involvement of PPARα in cardioprotection conferred by 3 cycles of hind limb RPC in rats (Figure 2, left panel). This significantly increased gene expression (real time RT-PCR) of PPARα (Figure 3A) in the left ventricular myocardium and was associated with enhanced protein levels of PKCε (Figure 3B). Protection against sustained I/R was manifested in isolated Langendorff-perfused hearts by an improved recovery of systolic function (LVDP, Figure 4A), attenuated diastolic dysfunction (LVEDP recovery, Figure 4B), reduced size of infarction (Figure 5) and decreased arrhythmogenesis (lower total number of ectopic beats and shorter duration of ventricular tachycardia) at the onset of reperfusion (Table 1). Moreover, pretreatment of animals in vivo with PPARα antagonist MKK-866 prior to RPC abrogated its cardioprotective effects and reversed PPARα activation and PKCε levels suggesting the role of PPARα-PKCε pathway in the mechanisms of RPC-induced cardioprotection.
Potential clinical applications of remote preconditioning

The first successful application of RPC in clinical conditions was reported by Cheung et al. (2006) in children undergoing cardiac surgery for a congenital heart defect, which was very often associated with high mortality of patients. These authors found that 4 cycles of 5-min RPC by inflation/deflation of a pressure cuff placed on the 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 I/R were able to reduce the degree of myocardial injury (reduced troponin T release) in adults undergoing CABG surgery. Since that time, numerous research teams have been investigating the effect of RPC under different clinical conditions. Most of the studies were designed to reveal whether RPC was able to protect human heart against I/R injury, in particular, under conditions of cardiac surgery, PPCI or during management of AMI (Lim and Hausenloy 2012). Numerous studies, indeed, demonstrated a positive effect of RPC applied in these patients (Ahmed et al. 2013; White et al. 2015; Zografos et al. 2014). Thus, it was shown that transient limb ischemia prior to PPCI (stenting) led to a reduction in adverse cardiac events in a group of RPC patients compared with control subjects who experienced adverse side effects (Hoole et al. 2009).

However, other studies failed to demonstrate any benefit in these conditions. One of the last big clinical trials was the ERICCA study (Hausenloy et al. 2015). These results failed to demonstrate positive effects of RPC in patients that underwent CABG. Another clinical trial (Meybohm et al. 2015) also did not reveal any relevant benefit of RPC in patients after cardiac surgery. However, all aforementioned studies were focused on clinical outcomes, and were not exploring molecular mechanisms of RPC in humans (Moscarelli et al. 2015). To elucidate the controversies, it will be necessary to consider the molecular basis of RPC in animals and humans and properly design clinical trials taking into consideration potential comorbidities, comedications (including anesthesia) or confounders (Heusch et al. 2015).

Factors modulating the efficiency of RPC

Responses to myocardial ischemia and cardioprotective interventions were originally studied in young and healthy experimental animals, mostly males. In these settings, coronary circulation, per se, is intact and is only subject to controlled occlusion and reperfusion. However, this is not a proper model for the studies of RPC effects in humans. The patients
usually experience myocardial infarction in elder age, when the coronary circulation is often affected by pre-existing atherosclerotic lesions. On the other hand, brief episodes of coronary occlusion and reperfusion that occur spontaneously, before sustained ischemia, may provide 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 PC (Abete et al. 1997). However, numerous comorbidities, such as modern lifestyle-related risk factors, e.g., stress, chronically elevated blood pressure, cardiac hypertrophy, metabolic disorders and obesity, as well as confounders and comedinations (including anesthesia with propofol), have a negative impact not only on the heart as response to ischemia per se but may blunt the protective effect of ischemic PC, postconditioning and RPC (Andreadou et al. 2017; Balakumar et al. 2009; Ferdinandy et al. 2014; Gricsova et al. 2015; Heusch et al. 2015; Ledvenyiova et al. 2013; Murphy and Steenbergen 2007; Ravingerova et al. 2012a).

Hypertension and innate cardioprotection

On the other hand, even the pathologically altered myocardium does not completely lose its adaptive potential. Although hypertrophied hearts are generally more susceptible to ischemic injury (Friehs and del Nido 2003), and in hypertensive (SHR) rats, postischemic recovery of contractile function and lethal injury (infarction size) are altered as compared with their normotensive counterparts (Ravingerova et al. 2011), their hypertrophied hearts were able to respond to ischemic PC, albeit with a lower magnitude. Other studies also demonstrated the persistence of the cardioprotective phenotype of preconditioning in remodelled myocardium (Speechly-Dick et al. 1994) and even in the aged hypertensive animals (Dai et al. 2009). This was confirmed in our recent (unpublished) studies, which demonstrated the IS-limiting effect of hind limb RPC (Figure 2, left panel) evaluated in the Langendorff-perfused hearts isolated from SHR rats. The results demonstrate that despite a larger infarction in both non-preconditioned and preconditioned groups of hypertensive animals as compared with those in the respective normotensive groups, the IS-limiting effect of RPC was preserved in the hearts of hypertensive animals (Figure 5). In addition, the IS-limiting effect was abolished in the hearts of both normotensive and hypertensive animals that were pretreated with the PPARα antagonist MKK-866 prior to RPC protocol. This further supports an important role of PPARα activation in the mechanisms of RPC, and not only in the healthy hearts but also in the pathologically altered myocardium. Furthermore, this effect was associated with significantly enhanced Akt activation (phosphorylation) in the left
ventricular tissue of both normotensive and hypertensive groups of animals (Figure 6A), as well as with elevated protein levels of PKCɛ (Figure 6B) in the hearts of preconditioned rats of both strains. These findings not only support the idea concerning the retainment of the protective phenotype in the pathologically altered heart, but offer a potential explanation related to the activation of RISK cascade in the heart triggered by the RPC mechanisms.

**Effect of aging on conditioning-induced cardioprotection**

With respect to aging as one of confounding factors of classical ischemic PC, the results of clinical and experimental studies are not unequivocal. Thus, while Abete et al. (1997, 2011) reported the loss of antistunning protection by PC in senescent rats as well as attenuated efficiency of a clinical equivalent of PC (preinfarction angina) in aged patients with AMI, no loss of the efficacy of PC was found in middle-aged and older rabbits in vivo (Przyklenk et al. 2001). The impact of aging on the efficiency of RPC is not yet completely elucidated, and the results are controversial as well. The antiinfarct effect of bilateral LIPC in the *in vivo* rats showed its complete abrogation in the aged animals (Behmenburg et al. 2016). In addition, RPC failed to protect the heart of neonatal rabbits against I/R, whereas in adult animals, the IS-limiting effect of RPC was present (Schmidt et al. 2014). Interestingly, more information about age-dependency of RPC effects was gained from the clinical studies. Positive effects of limb RPC 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 RPC effects was also reported (McCrindle et al. 2014; Pavione et al. 2012). On the other hand, although in elder patients the benefit of RPC 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 RPC in adult and old patients when applied prior to coronary interventions (Kono et al. 2014; White et al. 2015). This may indicate the possibility to reactivate reduced protective potential by modification of the intensity of the preconditioning stimulus or by using an additional alternative mode of cardiac adaptation, such as exercise training and/or caloric restriction. These interventions reversed the loss of PC protection in aged animals and elder patients with AMI (Abete et al. 2011).

In line, the effect of RPC may be augmented when this intervention is applied not only in the acute setting of several bouts of limb ischemia, but when it is being administered as repeated cycles of limb I/R in the long-term (Wei et al. 2011). The latter approach increases the efficiency of RPC: reduces the extent of ventricular remodelling and mortality over 28 days after AMI in a rat *in vivo* model. Similarly, repeated RPC increased endothelium-
dependent vasodilatation in healthy humans and in patients with chronic heart failure (Kimura et al. 2007; Kono et al. 2014). Thus, it may be assumed that this intervention will enhance the positive effect of RPC and suppress the negative impacts of chronic myocardial ischemia leading to the failure of cardiac function.

**Conclusions**

Activation of innate adaptive mechanisms of the heart might represent a promising therapeutic strategy under conditions of chronic heart ailments. Moreover, multifactorial targeting conferred by alternative forms of conditioning is a way to prevent post-infarction development of heart failure. This approach may increase the arsenal of non-invasive interventions that could be potentially implemented in human medicine. Despite controversial outcomes of some clinical trials, RPC has shown positive results, and recent experimental studies performed in animals challenged with several pathologies indicate its promising potential to protect the ischemic heart in clinical conditions. The main advantage of this strategy is that it appears to be safe, non-invasive, and easily performed in humans. This cost-effective cardioprotective tool can be applied in scenarios in which ischemic damage is expected but the occurrence of myocardial infarction is unpredictable or not confirmed, and even in STEMI patients before their admittance to the hospital and throughout their further management. Furthermore, identification of molecules involved in RPC cascades may have therapeutic and diagnostic implications in the prevention and treatment of myocardial ischemia, especially in an elder generation. This could also lead to the development of diverse pharmacological RPC mimetics in accordance with the stage of coronary heart disease, the patients' gender and age, the planned intervention and/or the coexistence of prospective comorbidities or comediations.

**Conflicts of Interest**
The authors declare no conflict of interest associated with this study.

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All original results are based on animal experiments approved by the State Veterinary Administration of the Slovak Republic, legislation No 289/2003, the Animal Research and Care Committee and the Ethical Committee of Institute for Heart Research. The experiments were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 85-23, revised 1996).

References


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Figure captions

Figure 1.
Schematic presentation of remote preconditioning in distant organs/tissues, their stimuli and pathways of protective signal transduction to the target organs.

Figure 2.
Remote ischemic preconditioning (RPC) applied on the hind limb of rat in vivo (left panel) and simplified scheme of induction of cardioprotection in a distant organ (heart) exposed to ischemia (right panel).


Figure 3.
Effects of 3 cycles of hind limb remote ischemic preconditioning (RPC) in vivo on the mRNA levels of PPARα (A) and protein levels of PKCε (B) in the left ventricular myocardium of rats exposed to RPC and non-preconditioned controls (C).

RPC was evoked by 5-min pressure cuff inflation (200 mmHg)/5-min deflation in anaesthetized rats with or without PPARα antagonist MKK886 (MK, 3 mg/kg i.p., given prior to RPC). Occlusion/reperfusion of femoral artery was verified by magnetic resonance image.
Data were normalized to the respective levels of GAPDH. Results are the Means ± SEM of 4-5 hearts per group. * - P < 0.05 vs C, # - P < 0.05 vs. RPC.

Figure 4.
Effects of 3 cycles of hind limb remote ischemic preconditioning (RPC) in vivo and prior administration of PPARα antagonist MKK-866 (MK) on postischemic recovery of heart function in isolated Langendorff-perfused subjected to 30 min of global ischemia and 120 min of reperfusion. C – control non-preconditioned hearts.

A – maximal recovery of left ventricular developed pressure (LVDP) expressed in % of baseline values, B – timecourse of recovery of left ventricular end-diastolic pressure.
(LVEDP) expressed in mmHg. Results are the Means ± SEM of 10-12 hearts per group. * - $P < 0.05$ vs C, # - $P < 0.05$ vs. RPC.

**Figure 5.**
Effects of remote ischemic preconditioning (RPC) and PPARα antagonist MKK-866 (MK) on the size of myocardial infarction in the hearts of normotensive and hypertensive rats. IS – infarct size expressed in % of area at risk (AR) size. Results are the Means ± SEM of 10-12 hearts per group. * - $P < 0.05$ vs C, # - $P < 0.05$ vs. RPC, & - $P<0.05$ vs. normotensives.

**Figure 6.**
Effects of remote ischemic preconditioning (RPC) on the protein kinase B (Akt) phosphorylation (activation) (A) and protein levels of protein kinase Cε (PKCε) (B) in the left ventricular tissue of the hearts from normotensive and hypertensive rats. Data were normalized to the respective levels of GAPDH. Results are the Means ± SEM of 4-5 hearts per group. * - $P < 0.05$ vs C, # - $P < 0.05$ vs. normotensives.
Table 1. The effects of remote ischemic preconditioning and PPARα antagonist MKK-886 on reperfusion-induced ventricular arrhythmias in isolated rat heart.

<table>
<thead>
<tr>
<th>Groups</th>
<th>C</th>
<th>RPC</th>
<th>RPC+MK</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td>763 ± 115</td>
<td>355 ± 58*</td>
<td>825 ± 264*</td>
</tr>
<tr>
<td>VTD (sec)</td>
<td>217 ± 43</td>
<td>52 ± 15*</td>
<td>172 ± 69#</td>
</tr>
</tbody>
</table>

RPC – remote ischemic preconditioning, MK - PPARα antagonist MKK-886. PVC - premature ventricular complexes (total number); VTD - duration of ventricular tachycardia (sec). Results are expressed as means ± SEM (n=10-12 hearts per group; * - P<0.05 vs. control, # - P<0.05 vs. RPC). Arrhythmias were determined after 30 min lasting global ischemia during the first 10 min of reperfusion.
Pre-conditioning
RPC: hind limb pressure cuff inflation (200 mmHg) / deflation

Per-conditioning
Ischemia
Reperfusion

Verification of femoral artery occlusion (MRI)
3 cycles of 5-min I / 5-min R

Post-conditioning
Ischemia

RPC signal

TNFα
Adenosine
Bradykinin
Opioids, CAT
Growth factor
Hormones

STAT3
PKCε
ERK1/2
PI3K/Akt

ROS
NO
eNOS

Caspase-3, GSK-3β
BAD

mPTP

Bcl-2

Cardioprotection
(a) 

**PPARα**

![Bar chart showing relative PPARα mRNA levels for different conditions.]

(b) 

**PKCε**

![Bar chart showing PKCε/GAPDH levels for different conditions.]

* C  
* RPC  
* RPC+MK  

129x67mm (300 x 300 DPI)
(a) LVDP recovery

(b) LVEDP recovery

116x58mm (300 x 300 DPI)
Size of infarction

- **C**
- **RPC**
- **RPC+MK**

![Graph showing size of infarction in normotensive and hypertensive conditions with different treatments.](image)

79x47mm (300 x 300 DPI)