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Remote ischemic preconditioning for myocardial protection: update on mechanisms and clinical relevance

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Abstract Ischemic heart disease is the leading cause of death for both men and women worldwide, accruing 7.4 million deaths in 2012. There has been a continued search for better cardioprotective modalities that would reduce myocardial ischemia–reperfusion injury. Among these attempts, a more convenient model of ischemic preconditioning, known as remote ischemic preconditioning (RIPC) was first introduced in 1993 by Przyklenk and colleagues who reported that brief regional occlusion–reperfusion episodes in one vascular bed of the heart render protection to remote myocardial tissue. Subsequently, major advances in myocardial RIPC came with the use of skeletal muscle as the ischemic stimulus. To date, numerous studies have revealed that RIPC applied to the kidney, liver, mesentery, and skeletal muscle, have all exhibited cardioprotective effects. The main purpose of this review article is to summarize the new advances in understanding the molecular mechanisms of RIPC during the past 5 years, including those related to capsaicin-activated C sensory fibers, hypoxia-inducible factor 1α, connexin 43, extracellular vesicles, microRNA-144, microRNA-1, and nitrite. In addition, we have discussed results from several recent human clinical trials with RIPC. Taken together, the emerging clinical evidence supports the concept that the effectiveness of RIPC paired with its low-cost and non-invasive features makes it an ideal treatment before reperfusion after sustained ischemia. More carefully designed studies are warranted to fully exploit the clinical benefits of RIPC and its potential implications in patients with cardiovascular disease.

Keywords Ischemia–reperfusion · Cardioprotection · Remote preconditioning · Myocardial infarction · Translational therapy · Signal transduction

Introduction

Ischemic heart disease (IHD) is the leading cause of death for both men and women worldwide, accruing 7.4 million deaths in 2012 (http://www.who.int/mediacentre/factsheets/fs310/en/). The detrimental effects of acute ischemia–reperfusion injury on myocardial tissue continue to plague IHD patients. Myocardial infarct size is a major determining factor of prognosis and current approaches to improve survival are directed at achieving early myocardial reperfusion with fibrinolytic therapy or via percutaneous coronary intervention. Paradoxically, even early revascularization leads to tissue damage, a phenomenon known as ischemia-reperfusion injury (IRI). This prompted a search for cardioprotective mechanisms that would reduce the infarct size in acute settings and protect against IRI.

In 1986, Murry et al. discovered that multiple, brief nonlethal ischemic episodes precondition the heart and reduce infarct size caused by a subsequent ischemic insult, a phenomenon known as myocardial ischemic preconditioning (IPC) [1]. Subsequent studies showed that IPC also decreases the incidence and severity of post-ischemic arrhythmias and enhances the recovery of cardiac function

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after global ischemia, attenuating the risk of ischemic reperfusion injury [2, 3].

Przyklenk and Whittaker in 1993, extended the concept of IPC by showing that brief regional occlusion–reperfusion episodes in one vascular bed of the heart render protection to remote myocardial tissue [4]. Since then, researchers began looking into more convenient models of preconditioning, known as remote ischemic preconditioning (RIPC). A major advance in myocardial RIPC came with the use of skeletal muscle as the ischemic stimulus. Kharbanda et al. produced RIPC with tourniquet occlusion of blood flow to one hind limb of an experimental pig model in 2002 [5]. To date, studies have revealed that preconditioning applied to the kidney, liver, mesentery, and skeletal muscle have all exhibited cardioprotective effects [6] (Table 1).

Detailed understanding of the cardioprotective pathways induced by RIPC has been thoroughly discussed by Hausenloy and Yellon [7] in 2008 and Costa et al. [8] in 2013. The present review provides update on the molecular mechanisms responsible for myocardial RIPC and its use in the clinical setting.

Potential mechanisms of remote ischemic preconditioning

While preconditioning has proven valuable in reducing IRI, it has provided unprecedented opportunity to search for novel pharmacological interventions, which trigger similar molecular pathways as RIPC. The actual mechanism through which RIPC works is currently unclear but can be divided into three main components: (1) humoral, (2) neural, and (3) systemic factors of cardioprotection.

Humoral factors in RIPC

The humoral hypothesis posits that the ischemic stimulus leads to the production of substances that enter the circulation and reach the myocardium, where they exert a protective effect. This hypothesis was consolidated by Dickson et al. in 1999 who reported that blood taken from a rabbit that underwent simultaneous IPC of both heart and kidney, could reduce myocardial infarct size by 77 % when transfused into an isolated rabbit [9].

In 2009, Shimizu et al. identified several important facts related to the humoral mechanism in which they prepared dialysate of remotely preconditioned rabbit and human hearts [10]. The plasma dialysate derived from humans during RIPC conferred protection in rabbits, suggesting that the humoral substance(s) had cross-species effects. Further, they confirmed the presence of transferable hydrophobic <15 kDa cardioprotective factor(s) in plasma from remotely preconditioned rabbits, which demonstrated resistance to freezing, thawing, and denaturing. However, the actual identity of the humoral mediator remains unknown. Other studies have investigated whether endogenous substances such as adenosine [11], bradykinin [12], opioids [13], calcitonin gene-related peptide (CGRP) [14], and endocannabinoids [15], are generated in the remote tissues. It is proposed that these RIPC mediators enter the bloodstream and activate their respective receptors in the myocardium, thereby recruiting various intracellular pathways of cardioprotection. However, the humoral factors responsible for RIPC still remain unclear and further studies are required.

Neural factors of RIPC

The neural hypothesis postulates that substances produced in the remote ischemic territory act locally via afferent neural pathways, activating various efferent pathways that induce cardioprotection. In support of this notion, hexamethonium, a ganglion blocker, abolished the cardioprotective effects elicited by RIPC [16]. The theory was further developed with the proposition that endogenous substances such as adenosine [11], bradykinin [12], CGRP [14], released by the remote preconditioned organ, stimulated afferent nerve fibers, which then relay to efferent nerve fibers terminating on the myocardium to confer cardioprotection.

Ding et al. demonstrated that renal nerve section abolished the cardioprotective effect induced by renal ischemia (preconditioning) stimulus providing supportive evidence of a neural pathway [17]. Further confirmatory evidence implicating adenosine in a neural pathway of cardioprotection was provided by Liem et al. [18]. These findings suggested that adenosine released locally from the mesenteric bed during IRI stimulated the mesenteric afferent sensory nerves, which helps activate myocardial adenosine receptors. Dong et al. demonstrated that dissecting the femoral nerve abolished the myocardial infarct-limiting effect of remote hind limb preconditioning, suggesting that an intact neural pathway is required for the sensory afferent neural signaling from the preconditioning limb [19].

Schoemaker and van Heijningen reported that bradykinin administration was able to reduce myocardial infarct size, but was sensitive to hexamethonium [12]. Furthermore, the cardioprotective effect was abolished when HOE-140, a bradykinin B2 specific receptor antagonist, was administered prior to brief mesenteric artery occlusion–reperfusion. Similar to the studies on adenosine, these results indicate that bradykinin generation, through mesenteric RIPC, may stimulate afferent nerves, which then activate cardioprotective mechanisms in myocardial tissue. Wolfrum et al. not only confirmed these results but also showed that PKC-ε activation was blocked by HOE-140 and hexamethonium,

Table 1 Summary of the key studies from which the insights are derived on the cellular and molecular mechanisms of remote ischemic preconditioning (RIPC)

Authors	Species	Mechanism studied	Further insight
Gho et al. [16]	Rats	Neural pathway involvement in RIPC as opposed to IPC	Ganglion blocker abolished RIPC cardioprotective effects but did not abolish IPC effects
Schoemaker and van Heijningen [12]	Rats	Bradykinin: up-regulation reduces myocardial infarct size	Sensitive to hexamethonium. Bradykinin, like adenosine, may be released from preconditioning organ to activate neural pathway
Ding et al. [56]	Rabbits	Adenosine activation of renal afferent nerve. Blocking adenosine receptor reduced discharge rate of renal afferent, and abolished cardioprotection	Adenosine released from preconditioning organ may activate the neural pathway of cardioprotection
Weinbrenner et al. [28]	Rats	PKC: up-regulated in protection. Reduced infarct size	Neural ganglion blocker could not abolish protection; exclude neural pathway
Patel et al. [13]	Rat	Opioid: increased signaling and opioid receptor activation yields decreased infarct size	Naloxone, opioid receptor antagonist, attenuated the protective effects
Liem et al. [18]	Rats	Adenosine: up-regulation confers equal protection as RIPC, reducing infarct size from 68–48 %	Hexamethonium (ganglion blocker) administration 5 min into reperfusion has no effect, but 8-SPT administered 5 min in abolished cardioprotection
Konstantinov et al. [25]	Humans	CD11B expression decreased	Suppression of pro-inflammatory and pro-apoptotic gene transcription in protection
Wolfrum et al. [21]	Rats	CGRP: up-regulation reduces IS by 57 %. RIPC increases CGRP plasma levels	CGRP activates PKC-epsilon through neuronal transmission. CGRP also may be transported via bloodstream as well to confer protection
Konstantinov et al. [31]	Pigs	K_{ATP} channel activation induces cardioprotection	Neural pathway excluded
Zhang et al. [57]	Rats	κ-Opioid receptor: activation yielded protection; mPTP: inhibition yielded protection	mPTP inhibition lies downstream of κ-opioid receptor activation
Heidbreder et al. [37]	Rats	MAPK: up-regulated in the preconditioning organ but not the target organ during RIPC	Yet to be studied if MAPK is up-regulated during reperfusion as part of RISK pathway
Jones et al. [24]	Mice	Neurogenic role to cardioprotection via capsaicin, PKC-ε, and K_{ATP} signaling	Topical application of capsaicin activates C sensory fibers in the skin, which was able to significantly reduce myocardial infarction
Davidson et al. [58]	Rats	SDF-1α binding to CXCR4: up-regulated in protection. Based on the effects of its antagonist (AMD3100), SDF-1α seemed to have intermediate effects. However, it may have been due to off-target effects	SDF-1α acts through a Gα1-dependent mechanism and activation of the PI3, MAPK, PKC, and JAK/STAT
Albrecht et al. [26]	Humans	Cytokines: IL-8, IL-1β, and TNF-α levels increased in RIPC group	These inflammatory regulators are up-regulated in protection
Albrecht et al. [26]	Humans	HIF-1α: accumulation in right atrial tissue during RIPC	Involved in cardioprotection; mechanism unknown
Rassaf et al. [35]	Mice	Nitrite: up-regulated in protection. Reduced infarct size, mitochondrial respiration, and ROS formation	Nitrite is reduced to nitric oxide by cardiac myoglobin, to yield its effects
Brandenburger et al. [41]	Rats	Connexin 43: preservation of Cx43 protein expression and phosphorylation after RIPC has a protective role	I/R caused a strong decrease of relative Cx43 protein expression in the AAR that was partly abolished by RIPC
Li et al. [43]	Mice	MicroRNA-144: microarray studies established that RIPC increases, and IR injury decreases MicroRNA-144 levels in mouse myocardium	MicroRNA-144 increased P-Akt, P-GSK3β and P-p44/42 MAPK, decreased p-mTOR level and induced autophagy signaling, and induced early and delayed cardioprotection

individually [20]. They later studied CGRP, a neurotransmitter released from capsaicin-sensitive sensory nerves, as a potential mediator of RIPC [21]. Remote intestinal preconditioning generates nitric oxide which stimulates capsaicin-sensitive sensory nerves in the intestinal vasculature, releasing CGRP into the bloodstream then carried to the heart where it activates myocardial PKC-ε [22, 23].

An interesting study by Jones et al. further elucidated a neurogenic role to cardioprotection via capsaicin, PKC-ε, and K_{ATP} signaling. Instead of IPC, these authors studied

the effects of "nonischemic" preconditioning by surgically producing an abdominal slit in mice in order to activate sensory nerves under the skin [24]. It was observed that skin nociception activated cardiac sensory and sympathetic nerves to elicit cardioprotective effects. Bradykinin, a known hormone and neurotransmitter, is released from sympathetic nerves in the heart and triggers a cascade that ultimately activated PKC-ε and inactivated PKC-δ in cardiomyocytes. In addition, topical application of capsaicin-activated C sensory fibers in the skin which significantly reduced IRI.

Systemic factors

In 2004, Konstantinov et al. sought to determine the effects of RIPC in inflammatory gene transcription in humans. Using microarray technology, they found that within 15 min of preconditioning, genes encoding proteins involved in cytokine synthesis, leukocyte chemotaxis, adhesion and migration, exocytosis, innate immunity, signaling pathways, and apoptosis were all suppressed—even more so after 24 h. Leukocyte CD11b expression also decreased significantly after 24 h, showing that RIPC suppressed pro-inflammatory gene transcription in human leukocytes, helping to confer the protective role of RIPC against IRI [25]. However, Albrecht et al. presented some contradictory results, showing that serum cytokines were actually elevated within the first phase of RIPC [26]. It is expected that during reperfusion, both pro- and anti-inflammatory molecules will act together to restore organ function while preventing tissue damage. A recent study showed an improvement in effective healing and cardioprotection due to the increase in the number of neutrophils just after bypass in the right atrial tissue [26]. This was also corroborated by an increase in the levels of IL-8, IL-1β, and TNF-α—major inflammatory regulators—in the RIPC group versus the control group.

Molecular mechanisms

Based on experimental studies, it has been widely understood that mechanisms underlying RIPC mirror that of classic IPC and postconditioning. These include binding of ligands to G-protein cell surface coupled receptors such as adenosine [11], bradykinin [12], opioids [13], angiotensin [27], and endocannaboids [15], nitric oxide (NO), activation of intracellular kinases such as PKC-ε [20], transcription factors, generation of reactive oxygen species (ROS) [28], and opening mitochondrial K_{ATP} channel [11]. These mechanisms are reviewed as follows.

ATP-sensitive potassium channel (K_{ATP})

In IPC studies, K_{ATP} channels have demonstrated to be a key trigger in conferring cardioprotection. Within the IPC model, it is proposed that the signal transduction cascade terminates at the mitochondria, causing opening of the mitochondrial K_{ATP} channels. This, in turn, generates ROS, which can mediate cardioprotection by up regulating pro-survival kinases or inhibiting the opening of the mitochondrial permeability transition pore (mPTP) [29]. mPTP is a high-conductance channel of the inner mitochondrial membrane, whose opening in the first few minutes of myocardial reperfusion mediates cell death through ATP depletion and mitochondrial swelling [30]. Specifically in RIPC, Konstantinov et al. showed that the cardioprotective effects in myocardial tissue act through a mitochondrial K_{ATP} channel pathway [31].

Nuclear factor kappa B (NFκB)

Diwan et al. investigated the potential role of NFκB in erythropoietin-mediated cardioprotection by employing a selective NFκB inhibitor [32]. They concluded that erythropoietin preconditioning and remote renal RIPC triggered similar signaling mechanisms for activation, i.e., NFκB activation followed by opening of mitochondrial K_{ATP} channels.

Nitric oxide (NO) and nitrite

NO has been implicated as a major mediator of cardioprotection in IPC [29]. However, the potential role of NO in RIPC cardioprotection has yielded opposing results, i.e., the protective effect of RIPC was not abolished by NO inhibition [33]. Xiao et al. reported that intestinal ischemia upregulated CGRP levels, but administration of an nitric oxide synthase inhibitor abolished RIPC-induced cardioprotection [23]. These authors suggested that the delayed cardioprotection yielded by CGRP occurs via NO-dependent pathway. While there is a strong likelihood for other factors to influence the level of CGRP and other compensatory mechanisms, further studies are needed to elucidate the role of NO in the RIPC cardioprotection paradigm.

When the heart is subjected to ischemia, nitrite is reduced by deoxymyoglobin to form NO in the cardiomyocyte, limiting cellular injury and infarction [34, 35]. Rassaf et al. in 2014 reported that circulating nitrite derived from shear stress-dependent stimulation of endothelial nitric oxide synthase (eNOS) at the remote site of remote IPC contributed to cardioprotection during IRI. Interestingly, pharmacological and genetic inhibition of NO and nitrite generation by eNOS at the remote site or nitrite bioactivation by myoglobin within the target organ abrogated the

cardioprotection by RIPC. Transfer experiments of plasma from healthy volunteers subjected to RIPC identified plasma nitrite as a cardioprotective agent in isolated Langendorff mouse heart preparations exposed to IRI [35].

Protein kinase C

PKC is a well-known mediator of cardioprotection [29]. The activation of PKC in the heart by CGRP, adenosine, and bradykinin B2, appears to be one of the earliest events in the myocardial mechanisms of cardioprotection [29]. The IPC cardioprotective mechanism is mediated by kinases including PI3 kinase, ERK/MAPK, PKC, and JAK/STAT [36]. These kinases prevent opening of mPTP, preservation of ATP thereby facilitating mitochondrial and myocardial protection. IPC also activates pro-survival kinases, resulting in the inhibition of the mPTP, but whether RIPC also activates these pro-survival kinases is unclear [36]. Heidbreder et al. demonstrated that mitogen-activated protein kinases (MAPKs) were activated within the intestines, but not within the cardiac tissue following intestinal ischemia–reperfusion [37]. It was not clear if the MAPKs were activated later as part of the RISK pathway during reperfusion.

New emerging mechanisms

Hypoxia-inducible factor 1α (HIF-1α)

Albrecht et al. recently demonstrated the involvement of HIF-1α in RIPC-induced cardioprotection in 32 patients undergoing cardiopulmonary bypass [26]. During four 5-min cycles of transient upper limb ischemia–reperfusion, HIF-1α accumulation and activation began in right atrial tissue. This was associated with reduced activities of caspases 3 and 7 (two markers for cell apoptosis) and significantly reduced serum levels of troponin T (a marker for cardiomyocyte necrosis) in the RIPC patients as compared with 29 control patients during the first 48-h post-operative period. While a causative link between increased HIF-1α levels and cardioprotection by RIPC is not conclusive, it is likely that the induction and/or stabilization of HIF-1α in the heart may be triggered by RIPC-mediated hypoxia-like events and the release of humoral factors, such as several cytokines—IL-8, IL-1β, and TNFα, that eventually reach remote regions including the right atria via blood circulation in these RIPC patients [26]. Various studies have corroborated that HIF-mediated signaling in cardiac tissue regulates myocardial damage, apoptosis, and inflammation, whereas HIF-1α has also been proposed to play a central role in cardioprotection by IPC [38–40].

Connexin 43 (Cx43)

Cx43 is an integral membrane protein that is mainly localized on sarcolemma of cardiomyocytes where six connexin molecules assemble into a connexon or hemichannel. Brandenburger et al. has demonstrated that Cx43 is critically involved in cardioprotective interventions including IPC [41]. These authors investigated the influence of RIPC on the expression patterns of Cx43 after IRI in the rat heart in vivo. IRI caused a strong decrease of relative Cx43 protein expression in the area at risk that was partly abolished by RIPC. Furthermore, RIPC decreased the level of ischemia-induced dephosphorylation of Cx43. Confocal immunofluorescence staining showed that I/R caused a loss of the Cx43 signal at the intercalated disks, while the Cx43 signal at the intercalated disks was partly sustained after RIPC. Thus, preservation of Cx43 protein expression and phosphorylation after RIPC might have a protective role.

Extracellular vesicles

Extracellular vesicles are membrane-bound structures, which contain a high concentration of RNAs and proteins. Since they can be secreted and specifically taken up by other cells, they are prime medium for intercellular signal transfer mechanisms. Giricz et al. demonstrated release of extracellular vesicles from the heart IPC stimuli is increased and these vesicles were responsible for the transmission of remote conditioning signals for cardioprotection [42]. Further cellular and molecular mechanistic studies are warranted to decipher the nature of actual effector factors carried by these vesicles.

Micro RNA-144

In 2014, Li et al. demonstrated a role of microRNA 144 (miR-144) in RIPC-induced cardioprotection. Using microRNA microarray, these authors showed that RIPC increased miR-144, whereas IRI decreased levels in the mouse heart [43]. Moreover, IRI was attenuated by both RIPC and intravenous administration of miR-144 in these studies. The systemic treatment with miR-144 increased phosphorylation of several kinases, i.e., Akt, GSK3β, and p44/42 MAPK. In addition, there was decrease in the phosphorylated mTOR levels and enhanced autophagy signaling. Importantly, systemic administration of a specific antisense oligonucleotide reduced myocardial levels of miR-144 and abrogated cardioprotection by RIPC. These authors also showed that RIPC increased plasma levels of miR-144 in mice and humans. While there was no change in plasma micro-particle (50–400 nM) numbers or their miR-144 content, a ~4-fold increase in miR-144 precursor in the exosome pellet and a significant increase in

miR-144 levels was observed in the exosome-poor serum with associated increase in the miR carriage protein-argonaute-2 levels [43]. These data suggested that systemic release of microRNA 144 plays a pivotal role in the cardioprotection induced by RIPC and plasma miR-144 could potentially serve as a biomarker of the effectiveness of RIPC.

Micro RNA-1

Brandenburger et al. examined the involvement of microRNA 1 (miR-1) in RIPC [44]. In these studies, ischemia alone had no effect on miR-1 expression, whereas RIPC led to a downregulation of miR-1 prior to ischemia as well as after 2 h of reperfusion. However, after 6 h of reperfusion, RIPC caused increase in miR-1. Furthermore, luciferase assays confirmed the interaction of miR-1 with brain-derived neurotrophic factor (BDNF), a protein that exerts cardioprotective effects. However, miR-1 levels did not correlate with protein levels of BDNF, a known target of miR-1 in vivo [44]. The biological significance of changes in miR-1 expression levels and the potential interaction with BDNF in RIPC-induced cardioprotection needs further investigations.

Overall, much of the mechanistic studies in RIPC are still a work in progress. Further studies are needed to establish the direct cause and effect relationship of the various active molecules involved in the cardioprotective effect of RIPC. In such a complex pathway with various factors, it is likely that neither the humoral nor the neural pathways are mutually exclusive.

Translational studies in humans

RIPC has gone from experimental studies with animals to proof of principle studies in humans. The value of RIPC has been evaluated in populations ranging from pediatric patients undergoing cardiac surgery to adult patients undergoing elective abdominal aortic aneurysm repair, coronary angioplasty, and coronary artery bypass surgery [45]. Cardiac surgery is strongly associated with IRI, which leads to myocardial necrosis and mortality. Most, but not all, of these studies showed attenuation in the release of cardiac enzymes in RIPC-treated cohorts versus matched controls [45]. The first clinical trial with RIPC was conducted in 37 pediatric patients who underwent cardiopulmonary bypass during repair of congenital heart defects [46]. Compared to the 20 control group children, 17 RIPC group children that were subjected to four 5-min cycles of lower limb ischemia and reperfusion presented with much lower troponin levels, suggesting reduced damage to the heart postoperatively (Fig. 1). Levels of IL-10 were

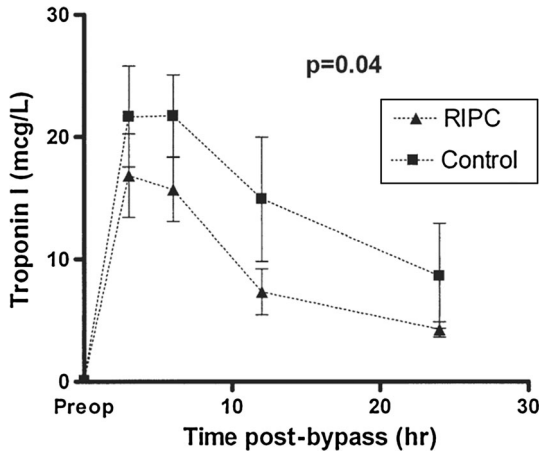

Fig. 1 Pre- and post-operative levels of troponin I in remote ischemic preconditioning (RIPC) and control groups. Adopted with permission from Cheung et al. [46]

significantly increased 3 h postoperatively in the RIPC group, while TNFα was significantly decreased in this group (Fig. 2). In another landmark study by Hausenloy et al., 57 patients who underwent coronary artery bypass graft (CABG), there was a 43 % decrease in the serum troponin T levels in the RIPC group, indicating reduced myocardial damage [47]. To study its safety and efficacy, Thielmann et al. demonstrated that RIPC provided perioperative myocardial protection and improved prognosis in this CABG patient population [48]. In patients undergoing elective percutaneous coronary intervention (PCI), similar results were found: troponin T levels and ischemia-induced chest discomfort were reduced in the RIPC group versus control group [49].

Similar to delayed or late phase of IPC, RIPC-induced cardioprotection has been shown to be a biphasic phenomenon, with an early first phase that lasts for up to 3 h after initial ischemia, followed by a delayed second phase that begins after 12–24 h, and lasts for up to 4 days [50]. A

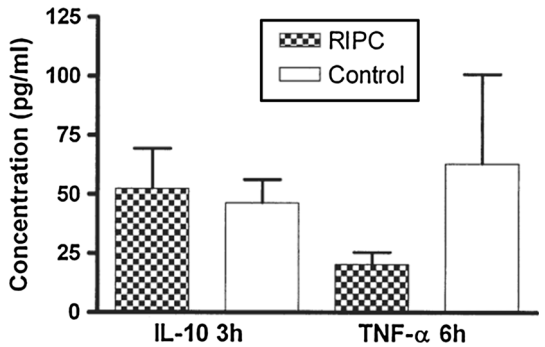

Fig. 2 Levels of interleukin 10 (IL-10) at 3 h and tumor necrosis factor-α (TNF-α) at 6 h postoperatively in remote ischemic preconditioning (RIPC) and control groups. Adopted with permission from Cheung et al. [46]

study by Loukogeorgakis et al. demonstrated that RIPC in humans offers up to 48 h of protection from myocardial reperfusion injury [50]. The most convenient aspect of this phenomenon is that RIPC could be triggered 24 h before cardiopulmonary bypass surgery, angioplasty, or transplantation while providing up to 48 h of resistance to cardiac ischemia–reperfusion injury.

Slagsvold et al., found that after coronary artery bypass surgery, only 14 % of RIPC patients developed atrial fibrillation during the first 3 days versus 50 % in the control group [51]. Interestingly, these authors did not observe a difference in the plasma concentrations of cardiac troponin T or creatine kinase between the RIPC group versus the control. Thus it appears that RIPC induces protection of the human atrium even without the increase in the cardiac markers of injury [50].

Most recently, Yellon's group assessed the effect of RIPC on perioperative myocardial injury (PMI) in 180 patients undergoing elective CABG [52]. They reported that RIPC significantly reduced (1) magnitude of PMI by 26 %, (2) postoperative events of atrial fibrillation by over 50 %, and (3) incidence of acute kidney injury by 48 %. Interestingly, while intravenous glyceryl trinitrate (GTN), a donor of NO and vasodilator, decreased the incidence of PMI in the control group, RIPC was not beneficial to the patients who were administered GTN.

While the use of RIPC may seem irrelevant and impractical for more sudden ischemic episodes such as suffering a ST Segment Elevation Myocardial Infarction (STEMI), an interesting study by Bøtker et al. highlighted its potential merits [53]. Adult STEMI patients, while being transported to the hospital for PCI, were randomized to receive four 5-min cycles of ischemia–reperfusion. The results demonstrated that RIPC before hospital admission increased myocardial salvage compared to the control, and had a favorable safety profile.

Despite these encouraging results, two recent trials reported negative results on RIPC [54, 55]. McCrindle et al. showed that RIPC did not significantly improve clinical outcomes in pediatric patients undergoing cardiopulmonary bypass [54], possibly due to confounding factors such as the use of propofol during anesthesia, which is known to block the effectiveness of RIPC. Kono et al. explored the capability of RIPC to improve coronary microcirculation among healthy subjects and heart failure patients [55]. After 1-week course of RIPC, left ventricular ejection fraction was decreased among heart failure patients and microcirculation improved among healthy subjects without adverse effects. However, no long-term benefits of RIPC were observed in these patients. One of the limitation for this study was its small sample size ($n = 20$).

In summary, the effectiveness of RIPC paired with its low-cost and non-invasive features makes it an ideal treatment before reperfusion after sustained ischemia. Ovize et al. noted that 13 out of the 25 published phase II trials, showed statistically significant positive cardioprotection, 5 showed positive protection (significance not achieved), and 7 demonstrated no benefit or worsened myocardial injury [6]. As such, some enthusiasm has been lost for the effectiveness of RIPC. However, it is important to note that in these studies, the patient population may have contributed to the discrepancy in results. For example, some studies exclusively enrolled stable patients undergoing CABG, whereas others enrolled high-risk patients, who may have had multiple surgeries, which clearly heightens the risk for poor outcomes. Furthermore, the anesthesia regimen is widely varied from study to study, thereby confounding the results and distracting from the potentially invaluable clinical benefit of RIPC. Therefore, more carefully designed studies are warranted to fully explore the clinical benefits of RIPC and its potential implications in patients with cardiovascular disease.

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