THE PRESENT AND FUTURE

STATE-OF-THE-ART REVIEW

Remote Ischemic Conditioning

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ABSTRACT

In remote ischemic conditioning (RIC), brief, reversible episodes of ischemia with reperfusion in one vascular bed, tissue, or organ confer a global protective phenotype and render remote tissues and organs resistant to ischemia/reperfusion injury. The peripheral stimulus can be chemical, mechanical, or electrical and involves activation of peripheral sensory nerves. The signal transfer to the heart or other organs is through neuronal and humoral communications. Protection can be transferred, even across species, with plasma-derived dialysate and involves nitric oxide, stromal derived factor-1α, microribonucleic acid-144, but also other, not yet identified factors. Intracardiac signal transduction involves: adenosine, bradykinin, cytokines, and chemokines, which activate specific receptors; intracellular kinases; and mitochondrial function. RIC by repeated brief inflation/deflation of a blood pressure cuff protects against endothelial dysfunction and myocardial injury in percutaneous coronary interventions, coronary artery bypass grafting, and reperfused acute myocardial infarction. RIC is safe and effective, noninvasive, easily feasible, and inexpensive. (J Am Coll Cardiol 2015;65:177–95) © 2015 by the American College of Cardiology Foundation.

Remote ischemic conditioning (RIC) is the intriguing phenomenon whereby brief, reversible episodes of ischemia and reperfusion applied in one vascular bed, tissue, or organ confer global protection, rendering remote tissues and organs resistant to ischemia/reperfusion injury. Its discovery 2 decades ago in the heart (1) was not serendipitous, but evolved from a mathematical model developed by Whittaker and Przyklenk (2–4), in which brief episodes of pre-conditioning ischemia in one coronary bed were predicted to trigger activation, release, or transport of one or more unknown "protective factors" throughout the myocardium. To test this hypothesis, anesthetized dogs underwent 4 episodes of 5-min ischemia applied in the left circumflex coronary territory, followed by a 1-h sustained ischemic insult in the left anterior descending coronary artery bed. As anticipated, compared with control subjects that underwent left anterior descending occlusion alone, animals that received brief antecedent episodes of circumflex occlusion before sustained left anterior descending occlusion displayed a robust reduction of infarct size (1).

HISTORICAL BACKGROUND AND CONCEPT OF RIC

EVOLUTION OF THE PARADIGM. Although this first report of "intracardiac" RIC was provocative and met with considerable skepticism (4), the concept also engendered curiosity and raised the question: can the RIC paradigm be extrapolated to other remote triggers?

Spatial evolution: from intracardiac to interorgan RIC. During the past 2 decades, multiple variations on the theme of RIC have been investigated, encompassing both in vitro and in vivo models. Cardioprotection by collection and transfer of perfusate among isolated buffer-perfused hearts is a notable example (5–8). Specifically, coronary effluent released from donor rabbit hearts throughout a standard, conventional pre-conditioning stimulus (3 cycles of 5-min global ischemia with 10-min reperfusion) or a time-matched control period was collected, reoxygenated, warmed, and used as the perfusate for 2 cohorts of naïve, acceptor hearts. All 4 groups of hearts then underwent 40 min of sustained global ischemia. Infarct sizes were significantly smaller in both, donor hearts subjected to brief pre-conditioning ischemia and naïve acceptor hearts that received the effluent from pre-conditioned donors, versus donor and acceptor control subjects. There was no difference in the magnitude of the infarct-sparing effect seen in donor- and acceptor-pre-conditioned groups, implying that the efficacy of cardioprotection triggered by RIC was comparable to that achieved by conventional ischemic pre-conditioning (5). This general strategy, involving transfer of effluent or perfusate, has been refined to include collection of serum following brief pre-conditioning ischemia applied in vivo and its administration to either isolated hearts or cultured cells subjected to a sustained ischemic or hypoxic insult (9–11). This strategy also provided evidence of cross-species protection by RIC, including treatment of isolated buffer-perfused rabbit hearts with human serum (9,11).

It could be argued that intracardiac RIC or cardioprotection achieved by transfer of perfusate between hearts is a laboratory curiosity providing mechanistic insight, but of limited translational relevance. Accordingly, the observation of interorgan RIC was a pivotal pre-clinical advance (12). Initial evidence revealed that brief episodes of ischemia/reperfusion in kidney and mesentery rendered the heart resistant to infarction (12–15). Moreover, a number of studies documented RIC-induced attenuation of ischemia/reperfusion injury in brain, lungs, liver, kidney, intestine, skin, and other tissues (reviewed in Candilio et al. [16]). However, the first reported seminal extension of interorgan RIC in a clinically-relevant, large-animal (swine) model (17), which demonstrated that brief episodes of peripheral limb ischemia, achieved by simple tourniquet occlusion of one hindlimb, was sufficient to evoke a profound reduction in myocardial infarct size, accelerated subsequent implementation of phase II trials aimed at establishing efficacy in patients (17).

Conceptual evolution: from ischemic to non-ischemic triggers. In the aforementioned studies, intercardiac and interorgan RIC were (by definition) initiated by a brief ischemic stimulus. However, accumulating evidence from a spectrum of in vivo and in vitro models (some involving perfusate transfer among models) suggests that transient ischemia or interruption of blood flow is not a requisite trigger for remote protection. Multiple alternative triggers capable of recapitulating the infarct-sparing effect of RIC have been proposed, including peripheral nociception (initiated by skin incisions made on the abdomen and termed "remote pre-conditioning of trauma"), direct peripheral nerve stimulation, and noninvasive transcutaneous nerve stimulation and electroacupuncture (18–23). Perhaps the most attractive, for its potential as a clinical cardioprotective strategy, is nontraumatic peripheral nociception instigated by chemical stimulation of sensory C-fibers in the skin (18,21). A >70% reduction in infarct size was reported in mice treated with 0.1% capsaicin cream, applied topically to a 2 cm² area of skin along the abdominal midline 15 min before the onset of coronary artery occlusion, compared with untreated control subjects (18). In spite of its inherent appeal, this concept has not yet been translated to clinical investigation.

Temporal variants: remote pre-, per-, and post-conditioning. In all studies discussed thus far, the remote conditioning stimulus was administered prophylactically in the ~30- to 40-min period before the onset of sustained myocardial ischemia. However, pre-treatment is not a requirement for RIC-induced cardioprotection: reduction of infarct size has also been described with concurrent application of the remote ischemic stimulus during sustained coronary occlusion (remote ischemic *per*-conditioning) or at the time of reperfusion (remote ischemic post-conditioning) (24,25).

The first documentation of infarct size reduction with remote per-conditioning utilized brief renal ischemia/reperfusion as the trigger, applied during

the final minutes of coronary artery occlusion (26). This approach provided proof-of-principle, but has obvious practical limitations as therapy. However, evidence from the swine model demonstrated a significant infarct-sparing effect of 4 5-min cycles of intermittent limb ischemia administered during a 40-min period of left anterior descending coronary occlusion (27), providing the rationale for the landmark clinical trial in which limb ischemia was applied during transport of patients with suspected acute myocardial infarction (AMI) to the hospital (28). Cardioprotection with remote ischemic postconditioning was first demonstrated in swine (29) and subsequently corroborated in other models, including rabbit and rat (8,30). In each case, the protective stimulus was initiated immediately upon relief of the sustained myocardial ischemia.

THE PRE-CLINICAL CONSENSUS. A general consensus regarding RIC has emerged: with rare exceptions (31), there is consistent evidence among diverse models and species that brief ischemia/reperfusion applied in a remote tissue or organ confers cytoprotection against ischemia/reperfusion injury. When the heart is the target organ, the gold standard of RIC-induced protection is reduction of myocardial infarct size. However, remote ischemic pre-conditioning (RIPC) protects the myocardium, but also other parenchymal organs (16) and, notably, the vasculature. Endothelial dysfunction from ischemia/reperfusion can serve as a surrogate for studies on cardioprotection by RIC in healthy humans, but it is unclear whether extrapolation from preservation of peripheral vasomotion to cardioprotection is also true mechanistically (32).

Among the multiple variants of RIC, is any option superior for evoking cardioprotection? Interorgan (rather than intracardiac) conditioning, achieved via intermittent limb ischemia or, potentially, via nontraumatic peripheral nociception, is among the more appealing and practical strategies. Studies where peripheral limb ischemia is the RIC stimulus have mostly employed 3 or 4 episodes of 5-min arm and/or leg ischemia interspersed with 5-min reperfusion periods. However, these are empiric choices, the optimal algorithm has not been identified, and it has been postulated that "hyperconditioning" (i.e., an as-yet undefined, excessive number of conditioning episodes) may be deleterious (33,34). With regard to timing, outcomes of the limited number of head-to-head comparisons revealed no apparent difference in efficacy of RIPC, remote pre-conditioning, and post-conditioning (35,36). The paradigms of remote ischemic per-conditioning and post-conditioning may be particularly relevant, as they expand the potential scope for clinical translation of RIC.

SIGNAL TRANSDUCTION OF RIC

NEURONAL SIGNAL TRANSFER FROM THE REMOTE ORGAN TO THE HEART.

Signal transduction to the heart from the remote organ where the RIC protocol initiates protection appears to involve the somatosensory system, the spinal cord, and the autonomous nervous system **(Central Illustration)**. The stimulus can originate not only from local ischemia/reperfusion injury in an organ other than the heart (e.g., mesentery [12,14,37] or limb [35,38–42]), but also from local surgical trauma (18–20,41); local activation of sensory fibers by capsaicin (18,21,35), bradykinin (14,20), or adenosine (39); and local electrical nerve stimulation (21,23). Accordingly, local anesthesia with lidocaine (18) or a sensory nerve blocker (21) and transection of the peripheral nerve (21,35,39,40) abrogated protection by RIPC, although femoral nerve transection did not abrogate protection by limb RIPC in mice in one study (42).

The local release mechanism in response to a nociceptive stimulus involves protein kinase Cγ in rats (20) and is inhibited by a nitric oxide donor (39). Whereas the causal involvement of peripheral nociceptive sensory nerves is unequivocal, the nature and transfer to the heart of the released transmitter molecule through neuronal or humoral pathways remains ambiguous. A blood-derived dialysate was able to transfer protection to a recipient bioassay heart after local peripheral adenosine or capsaicin administration, peripheral nerve stimulation, or RIPC (21,39), supporting the notion of humoral transfer of a neuronally-released signal molecule. This is also suggested by studies in humans, where the dialysate from diabetic subjects after RIC provided protection only in the absence of diabetic neuropathy (11).

Abrogation of protection by RIPC with spinal cord transection at T7-T10 (18,40) or intrathecal spinal opioid receptor blockade with naloxone (41) and infarct size reduction by spinal cord stimulation by C8-T2 (43) favor a spinal reflex response. The efferent pathway appears to involve the autonomous nervous system. The ganglionic blocker, hexamethonium, abrogated protection by local bradykinin administration or RIPC in most (12,14,18), but not all (38) studies. Another ganglionic blocker, trimetaphan, also abrogated RIPC's protection from ischemia/reperfusion-induced endothelial dysfunction in humans (44). Cardiac sympathetic nerves are involved in attenuation of the observed infarct size reduction upon spinal cord stimulation, and this effect is attenuated by the α1-blocker, prazosin, and the ß-blocker, timolol (43). Another ß-blocker, propranolol, also abrogated

CENTRAL ILLUSTRATION Signal Transduction of Remote Ischemic Conditioning
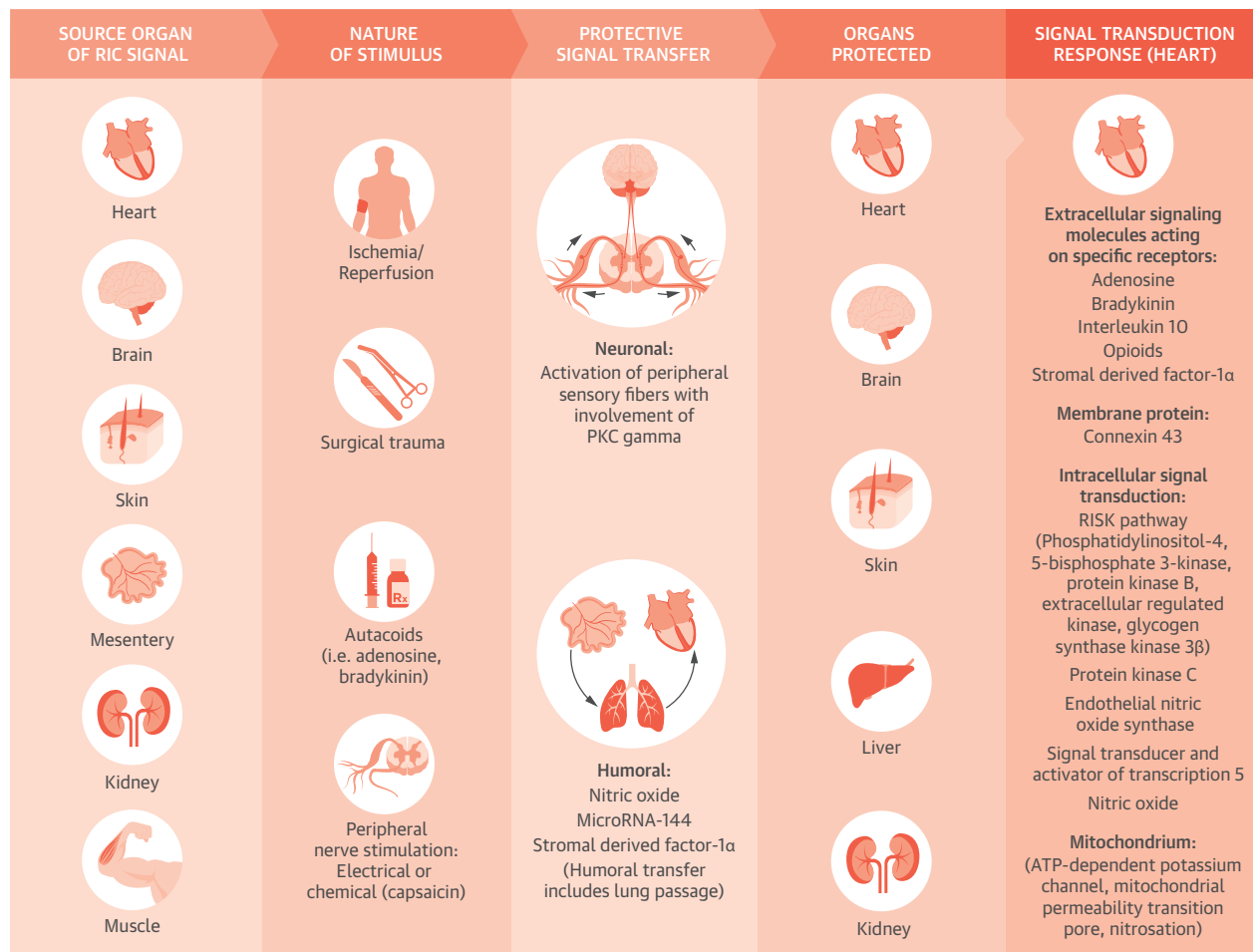
Heusch, G. et al. J Am Coll Cardiol. 2015; 65(2):177–95.

ATP = adenosine triphosphate; microRNA = microribonucleic acid; PKC = protein kinase C; RIC = remote ischemic conditioning; RISK = reperfusion injury salvage kinase.

protection by peripheral surgical trauma (18). Vagotomy (35,40) or atropine (40,45) abrogated the protection by limb pre-conditioning (35,40).

In conclusion, local injury during remote organ pre-conditioning activates nociceptive fibers, which release an unidentified molecule into the blood and/or signal through the spinal cord to activate both cardiac vagal and sympathetic efferents to release cardioprotective substances. Most of the previously discussed data originate from rodent models of RIC or from studies with transfer of dialysate to rodent hearts. However, neuronal involvement in protection by RIPC in humans undergoing coronary artery bypass grafting (CABG) or aortic valve surgery is

suggested by its abrogation with propofol, but not isoflurane anesthesia (46–48).

HUMORAL SIGNAL TRANSFER FROM THE REMOTE ORGAN TO THE HEART. In early studies of local pre-conditioning, coronary effluent from a pre-conditioned heart induced cardioprotection in a naïve acceptor heart (5). The presence of a circulating cardioprotective factor after RIPC was first demonstrated in a porcine transplant model (49), where RIPC of the limb in an acceptor pig provided potent cardioprotection to the subsequently transplanted and denervated donor heart. Subsequent studies confirmed the presence of a circulating element and further characterized the nature of the factor(s). In an

isolated rabbit heart model (9), plasma from remotely pre-conditioned animals was cardioprotective when perfused into an isolated naïve heart. The plasma dialysate using a 15-kDa membrane was similarly cardioprotective. When processed over a C18 column, the small hydrophobic molecule eluate provided potent cardioprotection, along with a protective kinase signature. Importantly, when the dialysate was given to isolated fresh cardiomyocytes (excluding neuronal influence), the resistance of cardiomyocytes to simulated ischemia/reperfusion injury mimicked that of a local pre-conditioning stimulus. Subsequent animal studies using such Langendorff bioassays confirmed that RIC induced by femoral nerve stimulation, transcutaneous peripheral nerve stimulation, capsaicin, and even electroacupuncture appear to work, at least in part, via release of cardioprotective factors into the blood (21-23,39).

Langendorff bioassays have also been used to test for the presence of circulating cardioprotective factors in human RIC. Depending on whether peripheral neuropathy was present, dialyzed plasma from diabetic patients subjected to RIC had differential responses, confirming interaction between the neural and humoral components of remote conditioning (11). Whereas plasma from diabetic patients without neuropathy was highly cardioprotective in naïve acceptor rabbit hearts, patients with peripheral neuropathy failed to provide cardioprotective plasma. Most recently, RIPC had no effect on exercise performance in heart failure patients (50). However, in the isolated mouse heart bioassay, plasma from heart failure patients was cardioprotective at baseline, but provided no additional cardioprotection after RIPC. When the results were stratified for the degree of baseline cardioprotection, those with low baseline cardioprotective activity showed significant improvement in their exercise function after clinical RIPC, suggesting that some patients lacking a pre-existing cardioprotective milieu may benefit from RIPC.

Several recent studies identified putative contributors to the humoral response. A recent proteomic study identified multiple potential cardioprotective targets released into the blood after a limb RIC protocol (51). Several specific circulating molecules also were studied in detail: the shear-stress-related release of nitric oxide, secondary to reactive hyperemia induced by transient limb ischemia, is expected to increase plasma nitrite in the blood, which is known to be cardioprotective (52). Conversely, RIPC was inactive in genetically-modified animals deficient in endothelial nitric oxide synthase (42).

Pre-treatment with the nitrite scavenger sulfanilamide abrogated the cardioprotective effect of plasma obtained after limb RIC in human volunteers, when used to perfuse naïve mouse hearts in a Langendorff bioassay. The clinical effect of nitrite is less certain. The NIAMI (Nitrites in Acute Myocardial Infarction) investigators (53) studied 229 patients with acute ST-segment elevation myocardial infarction (STEMI) randomized to receive an infusion of sodium nitrite or placebo. Nitrite failed to modify either myocardial infarction size or any of the secondary endpoints (e.g., troponin, creatine kinase, or left ventricular function), suggesting that the clinical effect of RIC is beyond that of nitrite alone.

Stromal-derived factor-1α is a small chemokine that fulfills the criteria for a putative circulating effector (9), and is cardioprotective via its interaction with its chemokine receptor 4 (54). Circulating plasma levels of stromal-derived factor-1α increased in rats subjected to RIPC by limb ischemia/reperfusion, and the cardioprotection of RIPC was partially abrogated by pre-treatment of the animals with a specific inhibitor (55). The lack of complete abrogation in this model suggests involvement of other factors.

Finally, a microribonucleic acid (microRNA) was recently shown to play a role in the pre-conditioning effect of transient limb ischemia/reperfusion. MicroRNA-144 levels were increased in mouse myocardium after RIPC and markedly reduced after ischemia/reperfusion injury. In subsequent experiments, the effect of RIC was completely abrogated by the use of a specific antagomir to microRNA-144. Conversely, intravenous microRNA-144 was cardioprotective, both acutely and 3 days after administration. Importantly, microRNA-144 levels were increased in the plasma of mice and humans subjected to limb RIC. Plasma carriage of microRNAs, to prevent digestion by circulating RNase, has been demonstrated within lipoprotein complexes in association with specific carrier proteins, such as argonaute, and in exosomes (56-59). Interestingly, the total number of exosomes in mouse plasma after RIC did not increase (60), although others observed increased numbers of exosomes following RIC in both rats and humans (61). However, the hairpin precursor of microRNA-144 in the exosome pellet increased 4-fold, and single-stranded microRNA-144 levels increased substantially in the plasma supernatant after RIC. Plasma microRNA-144 colocated with argonaute protein complexes, suggesting that this may be the plasma carriage mechanism after release of microRNA-144 precursor from the exosome. Although the exosome fraction was not tested for

cardioprotective activity in that study, effluent from a pre-conditioned heart was able to protect a second heart unless microvesicles and exosomes were removed, demonstrating that protection depends upon their presence (62). In summary, more work is required to identify whether microRNA-144, other microRNAs, chemokines, and perhaps undiscovered circulating factors may act either alternately or in concert as the humoral signal transferring protection to the heart. Nitric oxide, stromal-derived factor-1α, and microRNA-144 are clearly humoral transfer signals, but they do not fully explain the RIC phenomenon.

SIGNAL TRANSDUCTION OF RIC IN THE HEART. The search for signaling molecules/mechanisms of RIC has largely focused on signals identified in local ischemic pre- and post-conditioning studies (63,64). Early studies using pharmacological antagonists identified the involvement of adenosine (13,26), bradykinin (14,19,65), opioids (37,66,67), epoxyeicosa-trienoic acids (19), reactive oxygen species (66), and adenosine triphosphate (ATP)-dependent potassium channels (13,27), but could not dissect whether these molecules/mechanisms were involved in signal generation within the remote organ, transfer of the signal to the heart, cardioprotective signaling in the heart, or any combination of these steps. To attribute signaling to the heart, the signal must be demonstrated to localize in the myocardium or an antagonist must be given in the transfer fluid obtained after a RIC protocol in a donor organism, then administered to an isolated recipient and target heart. However, isolated bioassay hearts contain a number of different cellular compartments in addition to cardiomyocytes including innervation, vasculature, interstitial cells, and matrix with resident leukocytes/immune cells. Also, most signaling molecules/mechanisms have thus far only been determined in rodent hearts, and translation to larger mammals or humans cannot be taken for granted.

With these caveats in mind, there is solid evidence for a causal involvement of the ligands adenosine (10,68), bradykinin (18), interleukin-10 (in delayed RIPC) (69) and stromal-derived factor-1α (55) in the heart. Adenosine acts on its A$_1$ receptor, which, in turn, interacts with δ and κ opioid receptors (68); bradykinin acts on its B$_2$ receptor (18); and stromal-derived factor-1α acts on chemokine receptor 4 (55). Adenosine receptor activation results in improved mitochondrial function, as evidenced by better respiration and reduced formation of reactive oxygen species (10). Bradykinin B$_2$ receptor activation results in protein kinase Cϵ activation (18). The action of interleukin 10 results in increased phosphorylation

of protein kinase B (Akt) and endothelial nitric oxide synthase (69). RIC consistently results in activation of the reperfusion injury salvage kinase (RISK) pathway, that is, activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (70), Akt (8,69–72), extracellular-regulated kinase 1/2 (71), and glycogen synthase kinase 3ß (73). RISK activation was also confirmed by abrogation of infarct size reduction with the respective pharmacological antagonists (8,70,71) and was not only seen in rodent, but also in pig hearts (70), in which RISK activation was previously not found important for protection by ischemic post-conditioning (74). However, the study with RISK activation by remote ischemic pre- and per-conditioning in pigs was confounded by the ambiguous finding that an adenosine antagonist abrogated RISK activation, rather than protection (70). RIC also consistently (18,38,43,65) results in activation of protein kinase C, a key molecule in cardioprotection (75) with a somewhat ambiguous role (76,77); in rodent hearts, protein kinase Cϵ is classically activated and shifted from the cytosolic to the particulate fraction (18,65). The role of hypoxia-inducible factor (HIF)-1α in RIC is controversial; in 1 study, infarct size reduction by limb RIPC was abrogated in heterozygous knockout mice (72), but in another study, HIF-1α expression was increased by limb RIPC in wild-type mice, but was not a prerequisite for protection (73). HIF-1α protein expression is also increased in right atrial tissue of patients undergoing cardiac surgery under cardiopulmonary bypass with RIPC, but its causal involvement in the observed attenuation of troponin T release remains unclear (78). Late RIPC in rats increased heme oxygenase-1 protein expression, and its inhibition by zinc protoporphyrin abrogated protection (79). As in local ischemic pre-conditioning (80), limb RIPC in rats not only reduced infarct size, but also preserved connexin-43 phosphorylation and localization at intercalated disks (81); the role of mitochondrial connexin-43 in RIC has not been addressed.

An unbiased (mass spectrometry) proteomic search for phosphorylated proteins revealed that limb RIC increased expression of several phosphoproteins related to the sarcomeric Z-disc (82). A comprehensive immunoblotting approach for established cardioprotective proteins in right ventricular tissue of children undergoing repair of Fallot's tetralogy revealed no differences in their phosphorylated forms without or with RIPC (83). In left ventricular biopsies from adult patients undergoing CABG, tyrosine-phosphorylated signal transducer and activator of transcription 5 was the only protein among more than 30 established cardioprotective proteins that

was increased by RIPC (84). Autophagy appears to have no role in human RIPC (85).

Mitochondria are clearly involved in cardioprotection by RIC. Human plasma from healthy volunteers undergoing an RIC protocol had increased nitrite concentration and increased the concentration of myocardial nitrite when transferred to an isolated mouse bioassay heart. Myocardial nitrite was converted to bioactive nitric oxide by myoglobin and reduced infarct size. In parallel mouse experiments, the same nitrite-nitric oxide pathway was activated by RIPC, induced S-nitrosation of mitochondrial proteins, and reduced complex I respiration and reactive oxygen species formation (42). In rabbits with limb RIPC, blockade of the mitochondrial aldehyde dehydrogenase-2 by cyanamide abrogated protection; in parallel experiments in humans with a functionally inactive enzyme polymorphism, endothelial protection by RIPC was eliminated, supporting the concept that mitochondrial function is essential in RIC (86). Better preservation of mitochondrial respiration was also seen in right atrial tissue of patients undergoing CABG with RIPC, who also had lower incidence of post-operative atrial fibrillation (87). Apart from mitochondrial function, RIPC increases myocardial glycolytic flux in adult, but not in neonatal rabbit hearts along with reduced infarct size in adult, but not in neonatal hearts (88). In isolated hearts from rats that underwent a RIPC protocol, myocardial microRNA-1, microRNA-2, heat shock protein-70, and programmed cell death protein expression were decreased (89). In right atrial tissue of patients undergoing CABG with RIPC, microRNA-388-3p expression was increased (87). The biological meaning of these changes in microRNA expression is unclear.

Apparently, the intracardiac signal transduction of RIC largely resembles that of local ischemic pre- and post-conditioning, with significant involvement of nitric oxide, protein kinase C, the RISK pathway, and mitochondrial function. The data on myocardial signal transduction of RIC have not yet been integrated into a more complex and comprehensive scheme. Surprisingly, the role in RIC of the survival activating factor enhancement pathway, including signal transducer and activator of transcription 3, has not been addressed.

CLINICAL EVIDENCE FOR RIC

EFFECTS OF RIC ON THE HEART: ELECTIVE ISCHEMIA/REPERFUSION. Patients undergoing elective CABG and percutaneous coronary intervention (PCI) change as the demographics of the general population alter. The percentage of patients age ≥75 years at the time of operation increased from 17% in 1999 to 29% in 2005 (90). Operated patients had more comorbidities, with increased rates of hypertension (from 43.7% in 1999 to 68.9% in 2007) and obesity (from 13% to 17.5% in the same period) and worse functional and cardiac status (reduced ejection fraction, hemodynamic instability, and shock) (90). Improvement in anesthetics and surgical and perioperative treatments allows surgeons to accept patients for operation who, only a few years ago, would have been refused. Left ventricular ejection fraction <30% remains the most important determinant of outcome after isolated CABG (91). In elective CABG and PCI, adverse intermediate and long-term outcomes relate to periprocedural myocardial injury, including reduction of left ventricular ejection fraction; hence, the importance of cardioprotection beyond cardioplegia and off-pump surgery. Pre-conditioning by intermittent cross-clamping of the ascending aorta is invasive and has recently been comprehensively reviewed (64,92).

The first (very small) clinical study evaluating the effect of RIPC on creatine kinase-myocardial band release in CABG patients was negative (93). Translation of RIPC's protective potential to forearm endothelium-dependent vasomotion (17) initiated exploration of the cardioprotective potential of this approach using biomarkers as an endpoint in cardiac surgery, such as pediatric cardiac surgery, CABG, and combined CABG and valvular surgery. Most studies, including one small pilot study of high-risk patients (93-102), demonstrated cardioprotective potential (46,84,87,103-116) (**Table 1**), with similar findings for elective PCI (117-125) (**Table 2**). Many studies only included a few patients. Type 2 error might explain the discrepant results and confounding factors, including age, comedication, anesthesia, comorbidity, and risk factors, may also have influenced the efficacy of RIC (126). Concomitant therapy with beta-blockers (127,128) and statins (129) is cardioprotective, as is an anesthetic regimen using propofol or volatile anesthetics (46,48,128), and may interfere with the cardioprotective effect of RIC. The interference of propofol, which is cardioprotective per se, with further protection by RIC contrasts with the inherent cardioprotective effect of isoflurane, which does not interfere with RIPC (46,48), suggesting a specific interaction of propofol with neuronal transfer of the protective RIPC signal. Although in experimental studies, diabetes mellitus attenuated the effect of local ischemic pre-conditioning (130), the degree of cardioprotection may depend on stimulus intensity (131) and diabetes duration (132), and the

TABLE 1   Clinical Studies of RIC in Cardiac Surgery

First Author, Year (Ref. #)	Patients, n (Control/RIC)	Type of Surgery	RIC Regimen	Endpoint	Outcome
Günaydin et al., 2000 (93)	4/4	CABG	Upper limb 2 cycles I/R (3/2 min)	CK (Sampled via coronary perfusion catheter 5 min after declamping)	No effect
Cheung et al., 2006 (103)	20/17	Pediatric cardiac surgery	Upper limb 4 cycles I/R (5/5 min)	TnT (AUC 24 h after surgery) Post-operative inotropic need Lung function	Reduced TnT; reduced inotropic score; reduced airway resistance
Hausenloy et al., 2007 (104)	30/27	CABG	Upper limb 3 cycles I/R (5/5 min)	TnT (AUC 72 h after surgery)	43% reduction of TnT
Venugopal et al., 2009 (105)	22/23	CABG (cold blood cardioplegia)	Upper limb 3 cycles I/R (5/5 min)	TnT (AUC 72 h after surgery)	42% reduction of TnT
Hong et al., 2010 (106)	65/65	CABG (off-pump)	Upper limb 4 cycles I/R (5/5 min)	TnI (AUC 72 h after surgery)	26% reduction of TnT, NS
Rahman et al., 2010 (96)	82/80	CABG	Upper limb 4 cycles I/R (5/5 min)	TnT (AUC 48 h after surgery)	No effect
Thielmann et al., 2010 (107)	26/27	CABG (crystalloid cardioplegic arrest)	Upper limb 3 cycles I/R (5/5 min)	TnI (AUC 72 h after surgery)	45% reduction of TnI
Li, 2010 (108)	27/26	Valve replacement	Lower limb 3 cycles I/R (4/4 min)	TnI (AUC 72 h after surgery)	40% reduction of TnI
Zhou (2010) (109)	30/30	Pediatric cardiac surgery	Upper limb 2 cycles I/R (5/5 min) 24 and 1 h prior to operation	CK-MB Inflammatory biomarkers (plasma levels 2, 4, 12, and 24 h after surgery) Lung function	Reduced CK-MB and inflammatory biomarkers Improved post-operative lung function
Wagner et al., 2010 (110)	34/32	CABG (cold crystalloid cardioplegia)	Upper limb 3 cycles I/R (5/5 min) 18 h prior to operation	TnI (AUC 24 h after surgery)	Reduced TnI
Ali et al., 2010 (111)	50/50	CABG	Upper limb 3 cycles I/R (5/5 min)	CK-MB (plasma levels 8, 16, 24, and 48 h after surgery)	Reduced CK-MB
Karuppasamy et al., 2011 (97)	27/27	CABG	Upper limb 3 cycles I/R (5/5 min)	TnI (AUC 48 h after surgery)	No effect
Wu et al., 2011 (112)	25/25	Mitral valve replacement	Upper limb 3 cycles I/R (5/5 min) and 3 cycles I/R (5/5 min) + 2 cycles I/R (10/10 min)	TnI (plasma levels 4, 8, 12, 24, 48, and 72 h after surgery)	Reduced TnI with 3 cycles I/R (5/5 min) + 2 cycles I/R (10/10 min) but not 3 cycles I/R (5/5 min)
Kottenberg et al., 2012 (46)	19/20	CABG Propofol vs. isoflurane anesthesia	Upper limb 3 cycles I/R (5/5 min)	TnI (AUC 72 h after surgery)	Reduced TnI with isoflurane, but not with propofol anesthesia
Young et al., 2012 (94)	48/48	Cardiac surgery (high-risk CABG and valve surgery)	Upper limb 3 cycles I/R (5/5 min)	TnT (plasma levels 6 and 12 h surgery)	No effect
Heusch et al., 2012 (84)	12/12	CABG	Upper limb 3 cycles I/R (5/5 min)	TnI (AUC 72 h after operation) Phosphorylation of STAT5	Reduced TnI STAT5 activation
Lee et al., 2012 (98)	28/27	Pulmonary hypertensive infants receiving ventricular septal defect repair	Lower limb 4 cycles I/R (5/5 min)	TnI AUC 24 h after surgery	No effect
Pavione et al., 2012 (99)	10/12	Pediatric cardiac surgery	Lower limb 4 cycles I/R (5/5 min)	TnI (plasma levels 4, 12, 24, and 48 h after surgery)	No effect
Lucchinetti et al., 2012 (100)	28/27	CABG Opioids and propofol for induction and isoflurane for maintenance anesthesia	Lower limb 4 cycles I/R (5/5 min)	TnT (plasma levels 24, 48, and 72 h after surgery)	No effect
Xie et al., 2012 (113)	35/38	Valve surgery	Upper limb 3 cycles I/R (5/5 min)	TnI (plasma levels 6, 12, 24, 48, and 72 h after surgery)	Reduced TnI
Thielmann et al., 2013 (114)	167/162	CABG (cold crystalloid cardioplegia and cardiopulmonary bypass)	Upper limb 3 cycles I/R (5/5 min)	TnI (AUC 72 h after surgery)	27% reduction of TnI Reduced all-cause mortality
Ahmad et al., 2014 (101)	32/35	CABG On-pump	?	CK-MB (plasma levels 1, 12, 24, and 48 h after surgery)	No effect

TABLE 1 Continued

First Author, Year (Ref. #)	Patients, n (Control/RIC)	Type of Surgery	RIC Regimen	Endpoint	Outcome
Hong et al., 2014 (95)	636/644	Cardiac surgery (cardiac valve surgery, CABG, combined valve and CABG surgery, ascending aorta and aortic arch surgery, and congenital heart defect repair)	Upper limb 4 cycles I/R (5/5 min)— 2 cycles before and 2 cycles after cardiopulmonary bypass	Clinical outcome (composite of: death, myocardial infarction, arrhythmia, stroke, coma, renal damage, respiratory failure, gastrointestinal complications, and multiorgan failure)	No effect
Slagsvold et al., 2014 (87)	30/30	CABG	Upper limb 3 cycles I/R (5/5 min)	Mitochondrial oxidation	Improved mitochondrial respiration
McCrindle et al., 2014 (102)	151/148	Pediatric cardiac surgery	Lower limb 4 cycles I/R (5/5 min)	Duration of post-operative hospital stay	No effect
Holmberg et al., 2014 (116)	23/23	Cardiac surgery (CABG, valve surgery, ascending aorta, myxoma)	Upper limb 3 cycles I/R (5/5 min)	TnT (AUC 72 h after surgery)	25% reduction of TnT, NS
Candilio et al., 2014 (115)	90/90	Cardiac surgery (CABG and/or valve surgery)	Upper and lower limb 2 cycles I/R (5/5 min)	Perioperative myocardial infarction (AUC 52 h after surgery)	26% reduction of TnT Reduction of incidence of AF, renal failure, stay at ICU

AF = atrial fibrillation; AUC = area under curve; CABG = coronary artery bypass grafting; CK = creatine kinase; CK-MB = creatine kinase-myocardial band; I = ischemia; ICU = intensive care unit; NS = not significant; R = reperfusion; RIC = remote ischemic conditioning; STAT5 = signal transducer and activator of transcription 5; Tn = troponin.

attenuation of protection by RIC seems minor in the clinical setting (133,134).

Importantly, recent larger studies have not only relied on surrogate markers of cardioprotection, but also included long-term clinical outcomes and demonstrated a reduction of major cardiovascular events by RIPC up to 4 years after CABG (114) and up to 6 years after elective PCI (119). A recent study randomized 1,280 patients scheduled for elective cardiac surgery to control or RIPC and remote post-conditioning (95). RIC was given as 2 cycles of 5-min ischemia and 5 min of reperfusion on the upper arm before cardiopulmonary bypass or coronary anastomoses in those who had beating heart surgery, and was repeated in the same sequence immediately after bypass. Although the cardioprotective effect was not documented by a reduction of post-operative biomarker release, RIC did not reduce the primary endpoint, a composite of major adverse outcomes including death, myocardial infarction, arrhythmia, stroke, coma, renal damage, respiratory failure, gastrointestinal complications, and multiorgan failure, suggesting that this endpoint may have been too broad. Although RIC is thought to have systemic protective effects on various distal organs, the results are debatable because the composite endpoint differs from other studies yielding beneficial results. Moreover, the heterogeneity of the patient group, including CABG, cardiac valve surgery, and their combination, as well as ascending or transverse aortic surgery and congenital heart defect repair, may have introduced bias.

Recent meta-analyses demonstrated that RIPC reduces biomarkers in patients undergoing CABG (92,135). The 2 follow-up studies with clinical outcomes were single-center trials, not powered to demonstrate definitive answers about clinical outcome (114,120). The consistency of the beneficial clinical outcome in the studies adds credibility to a clinically-relevant benefit of RIC in relation to CABG and elective PCI. However, larger multicenter studies are still required to clarify the extent to which these findings translate into clinical benefit. Future studies should include high-risk patients, who might benefit most from protection by RIC, and preferably avoid propofol in their anesthetic regimen when specific cardioprotective effects are addressed.

EFFECTS OF RIC ON THE HEART: AMI. Although the incidence of AMI is declining in the Western World (136,137), ischemic heart disease is still the leading cause of death worldwide (138). Improvements in treatment have changed the epidemiology after AMI, with markedly improved 30-day survival, but have less favorably influenced long-term survival (136,139). Consistent with this, due to remodeling and heart failure (140), nonfatal ischemic heart disease has increased more than ischemic heart disease deaths since 1990 (138). The declining incidence of heart failure after AMI has not reached the magnitude that we might have expected from clinical trial data (139) and the prevalence is increasing (138). Consequently, one of the potentially most important applications of RIC may be in patients with AMI (28,141-147) (**Table 3**).

TABLE 2   Clinical Studies of RIC in Elective PCI

First Author, Year (Ref. #)	Patients, n (Control/RIC)	RIC Regimen	Endpoint	Outcome
Iliodromitis et al., 2006 (117)	21/20	Upper limb 3 cycles I/R (3/3 min)	TnI (12, 24, and 48 h after PCI)	No effect
Hoole et al., (118)	98/104	Upper limb 3 cycles I/R (3/3 min)	TnI (proportion of patients with TnI <0.04 ng/ml)	Reduction of proportion of patients with elevated TnI Reduced cardiac events 6 months after PCI
Davies et al., 2013 (119)	97/95	Upper limb 3 cycles I/R (3/3 min)	MACCE at 6 years	13% reduction of MACCE
Ahmed et al., 2013 (120)	72/77	Upper limb 3 cycles I/R (3/3 min)	TnT (Plasma level 16 h after PCI)	57% reduction of TnT
Prasad et al., 2013 (121)	48/47	Upper limb 3 cycles I/R (3/3 min)	TnT (proportion of patients with TnT ≥0.03 ng/dl)	No effect
Luo et al., 2013 (122)	104/101	Upper limb 3 cycles I/R (3/3 min)	TnI (plasma level 16 h after PCI)	48% reduction of TnI
Xu et al., 2014 (123)	98/102	Upper limb 3 cycles I/R (3/3 min)	TnI (plasma level 16 h after PCI)	24% reduction of TnI in DM patients age ≥65 years, NS
Liu et al., 2014 (124)	102/98	Upper limb 3 cycles I/R (3/3 min)	TnI (plasma level 24 h after PCI)	75% reduction of TnI Reduction of adverse events at 6 months
Zografos et al., 2014 (125)	47/47	Upper limb 1 cycle I/R (5/5 min)	TnI (increase in TnI from baseline to 24 h after PCI)	79% reduction in post-PCI TnI increment

DM = diabetes mellitus; MACCE = major adverse cardiovascular and cerebral events; other abbreviations as in **Table 1**.

Most clinical studies on infarct size after coronary revascularization have used indirect estimates of tissue damage, such as release of biomarkers and resolution of ST-segment elevation (148,149). Direct visualization of the area-at-risk and final infarct size to calculate the salvage index (proportion of salvaged area-at-risk) can be achieved by myocardial perfusion imaging using ^{99}technetium-sestamibi single-photon emission computerized tomography (150) or cardiac magnetic resonance (CMR) imaging (151-153). CMR

quantification of the area-at-risk poses challenges, because the optimal protocol to quantify edema, thought to represent area-at-risk, is not defined (154-156) and because any cardioprotective intervention that reduces final infarct size also may reduce edema (157,158), potentially underestimating salvage.

The first proof-of-concept study demonstrating that RIC can increase myocardial salvage investigated 333 patients undergoing primary PCI for STEMI, of whom 132 had available imaging data (28).

TABLE 3   Clinical Studies of RIC in AMI

First Author, Year (Ref. #)	Patients, n (Control/RIC)	RIC Regimen	Endpoint	Outcome
Bøtker et al., 2010 (28)	69/73	Upper limb 4 cycles I/R (5/5 min)	Salvage index (SPECT)	20% increase in salvage index
Munk et al., 2010 (141)	110/108	Upper limb 4 cycles I/R (5/5 min)	LVEF at 30 days	5% increase in LVEF in anterior infarcts
Rentoukas et al., 2010 (142)	30/33	Upper limb 3 cycles I/R (5/5 min)	ST-segment resolution	20% increase in proportion of patients achieving full ST-segment resolution
Crimi et al., 2013 (143)	50/50	Lower limb 3 cycles I/R (5/5 min)	CK-MB (AUC 72 h after PCI)	20% reduction of CK-MB release
Prunier et al., 2014 (144)	17/18	Upper limb 4 cycles I/R (5/5 min)	CK-MB (AUC 72 h after PCI)	31% reduction of CK-MB release
Sloth et al., 2014 (145)	167/166	Upper limb 4 cycles I/R (5/5 min)	MACCE at 4 years	12% reduction in MACCE
Hausenloy et al., 2014 (147)	260/260	Upper limb 4 cycles I/R (5/5 min)	TnT (AUC 24 h after PCI)	17% reduction of TnT release
White et al., 2014 (146)	40/43	Upper limb 4 cycles I/R (5/5 min)	CMR	27% reduction of infarct size

LVEF = left ventricular ejection fraction; SPECT = single photon emission computerized tomography; other abbreviations as in **Tables 1 and 2**.

A simultaneous study demonstrated that RIC increases the number of patients achieving complete ST-segment resolution and found a statistically borderline reduction of troponin-T release (142). In the former study, RIC was applied as 4 cycles of 5-min upper arm ischemia and 5-min reperfusion and was initiated in the ambulance during transportation to primary PCI. RIC increased salvage by 36% and tended to reduce final infarct size. In patients with anterior infarcts and patients with occluded culprit artery (Thrombolysis In Myocardial Infarction [TIMI] grade 0 to 1) on admission, infarct size reduction, as measured by single-photon emission computed tomography, was 44% and 31%, respectively, indicating that patients at highest risk benefit more from RIC as an adjunctive therapy to primary PCI. The findings translated into an increment of left ventricular ejection fraction in anterior infarcts (141). Although not powered to evaluate clinical outcome, a follow-up study of the total cohort showed that the beneficial effect of RIC translated into a reduction of major cardiovascular events up to 4 years after the index event (145). In a recent study, 4 cycles of 5-min cuff inflation/5-min deflation on the upper arm reduced myocardial edema and reduced infarct size, reflected by troponin release and CMR (146).

The first window of protection lasts for 2 to 3 h and onset appears to be instant, as RIC initiated immediately prior to revascularization also reduces infarct size in STEMI patients (144). In some protocol algorithms, remote pre-conditioning combined with local post-conditioning reduced infarct size in rats (71). This additive effect was not seen for remote pre-conditioning combined with local post-conditioning in the clinical setting of patients with reperfused AMI (144).

In a recent randomized study of 100 patients, remote post-conditioning also reduced infarct size, as assessed by the area under the curve of creatine kinase-myocardial band release (143). Infarct size was consistently reduced, as reflected by delayed gadolinium-enhancement volume on CMR and ST-segment resolution >50% in twice as many patients in the treatment than in the control group. After 1-year follow-up, 1 patient in the control group (refractory heart failure) and none in the post-conditioning group had died, and cardiovascular events were reduced in the treatment group. The beneficial effect was obtained by 3 cycles of 5-min/5-min blood pressure cuff inflation/deflation of the lower limb initiated at the time of reperfusion by balloon inflation or thrombectomy. Although a recent clinical study suggested that 1 occlusion cycle induces protection during elective PCI (125), experimental data from mice indicate that cardioprotective efficacy is determined by the number and duration of inflations (34).

Present reperfusion therapy is effective in the majority of patients undergoing primary PCI. It may be difficult to demonstrate additional clinical benefit from further intervention because this would require the demonstration of further reduction in small myocardial infarcts and its translation into a clinical benefit. A subgroup of patients undergoing not only primary PCI, but also elective PCI and CABG, develops serious complications, including extensive myocardial injury, which is most frequently vascular in origin. Although pre-clinical human data indicate that RIC may modify thrombogenesis (159,160) and yield cardioprotection beyond an unequivocal reduction of infarct size (e.g. by anti-inflammatory mechanisms) (161), the clinical implications are yet unknown. However, some patients, predominantly those with large anterior infarcts, develop heart failure due to myocardial injury and subsequent left ventricular remodeling several months or years after the infarct, despite optimal medical treatment according to guidelines (140). Because RIC reduces final tissue necrosis, improved clinical outcome must be assessed by reduced post-infarction left ventricular dysfunction and heart failure, combined with mortality reduction (162). To achieve widespread clinical acceptance of RIC, focus should be kept on patients at risk of extensive myocardial injury and global tissue damage. Its potential clinical utility is far from fully explored.

An emerging concept, known as chronic conditioning, is the daily use of RIC for a period of weeks. In rats, RIC administration daily for the first 28 days after myocardial infarction had a dose-dependent effect on cardiac remodeling, heart failure, and even death rate in the absence of a significant reduction of infarct size (163). This effect demonstrates benefits beyond modification of acute ischemic effects. However, in a recent pilot study, this did not immediately translate into improved exercise capacity in heart failure patients (50).

CONFOUNDING FACTORS IN RIC

No recognized effective therapeutic intervention for protecting the myocardium against the detrimental effects of ischemia-reperfusion injury presently exists. A major reason for this unfortunate situation is the inability to take the relevance of confounding factors present in the majority of basic and clinical studies into account; RIC studies are no different in this regard (126).

INFARCT LOCATION/PATIENT SELECTION. Only a quarter of all STEMI patients have infarcts of sufficient size to benefit from adjunctive therapy (164). Patients presenting with right and/or circumflex coronary artery occlusion, where the infarct is relatively small, do not benefit as much from cardioprotective therapy as those presenting with proximal left anterior descending coronary artery occlusion, where the infarct is significantly larger (28,165). "All-comer" trials will lead to the recruitment of far more patients with small infarcts and little additional myocardial salvage, which may actually dilute the positive effect elicited by any novel protective strategy. Alternatively, limiting recruitment to patients with large anterior infarcts is more challenging because they are the most ill (166); however, the benefit of proof-of-concept trials is that demonstration of a significant difference between treatment and placebo requires recruitment of fewer patients (167).

CONTROL OF TIMI FLOW PRIOR TO RIC. Some patients presenting with an AMI have already undergone spontaneous reperfusion prior to interventional reperfusion and are not likely to benefit from a therapy designed to protect against reperfusion injury (168). Therefore, only those patients with TIMI scores <1 should be included in such studies (28).

IMPORTANCE OF CORONARY COLLATERALS. The coronary collateral circulation's ability to influence the size of an evolving myocardial infarction cannot be underestimated. In STEMI patients, substantial collateralization reduces the sizes of the area at risk and the evolving infarct. The extent of collateralization will thus negatively influence the ability to demonstrate an effect of any novel cardioprotective strategy. Patients with visible collaterals (Rentrop grade ≥1) should, therefore, be excluded (169).

DURATION OF CHEST PAIN AND TIMING OF INTERVENTION. Patients presenting with an AMI who receive interventional or thrombolytic reperfusion must do so within 12 h of the onset of chest pain (170,171). Given the crucial events that occur in the first few minutes of reperfusion (oxidative stress, calcium overload, and mitochondrial permeability transition pore opening), any cardioprotective strategy must be applied prior to opening the infarct-related coronary artery. Accordingly, RIC given to patients in the ambulance while in transit to the interventional center have demonstrated a beneficial effect (28).

With late presentation, the infarct will have been completed, and the patient will derive little benefit from either intervention or an adjunct to reperfusion. Early presentation and revascularization will lead to small myocardial infarcts, and this patient will have little advantage from adjunctive therapy. There is a "sweet spot," probably between 3 and 8 h from time of symptom onset to time of reperfusion, for adjunctive therapies to demonstrate maximal benefit.

COMORBIDITIES AND COMEDICATIONS. In preclinical studies, age (172) and comorbid diseases (126), such as hyperlipidemia, diabetes, and hypertension, which require a more robust conditioning signal, raise the threshold for protection. This raised cardioprotective threshold reflects fundamental molecular alterations within the heart, affecting both sensitivity to ischemia/reperfusion injury and response to a particular cardioprotective strategy (126,172–174). Unfortunately, most experimental models use healthy young animals, free of any comorbidities (175). Experimental studies using human atrial muscle from patients undergoing CABG, from aged and diabetic patients and patients with heart failure (176-178) confirmed the effect of comorbidity on the conditioning threshold and demonstrated resistance to various conditioning strategies.

Pharmacological therapy also impacts cardioprotection. Specific sulfonylureas used to treat type 2 diabetes can attenuate the conditioning response (134). Conversely, insulin, metformin, some statins, angiotensin-converting enzyme inhibitors, antiplatelet agents, and opioids can themselves be cardioprotective and raise the threshold for an additional benefit (64,173,179–181). A number of pharmacological agents used during cardiopulmonary bypass surgery interfere with the cardioprotective efficacy of RIC. Volatile anesthetics, such as isoflurane, and the intravenous anesthetic, propofol, either themselves confer cardioprotection or interfere with RIC through downregulation of cardioprotective signaling (46,48). Intravenous nitroglycerine, nitroprusside, and opioid analgesics, each protective in experimental settings, also interfere with the apparent cardioprotective efficacy of a study intervention (173,180).

Taking these confounders into consideration in the design of any clinical study investigating RIC is hugely important; we must either design a study that does not use these agents (which may be impractical) or ensure that it is adequately powered and properly randomized.

EFFECTS OF RIC ON THE BLOOD AND VASCULATURE

Platelet activation is both a consequence and a driver of ischemia/reperfusion injury. Local ischemic preconditioning attenuates platelet activation and aggregation (182). In humans, marked systemic platelet

activation has been demonstrated in patients with acute coronary syndromes (183) or acute limb ischemia (184). In animal models, the extent of platelet activation is related to the extent of subsequent tissue injury after reperfusion (185). Indeed, blockade of platelet aggregation alone can significantly attenuate reperfusion injury. In healthy male volunteers subjected to 20-min forearm ischemia (160), platelet activation (measured by increased circulating monocyte-platelet aggregates) persisted up to 45 min, but was completely abolished in subjects randomized to receive RIPC prior to the ischemic insult. In patients with known obstructive coronary artery disease (186), RIPC prior to exercise stress testing reduced ADP-stimulated platelet aggregation. Similarly attenuated platelet aggregation was seen in patients undergoing ablation for atrial fibrillation when receiving RIPC (187). However, the potential clinical benefit of any of these findings remains to be seen.

Circulating monocytes play a key role in ischemia/reperfusion injury. RIC down-regulated the expression of a broad portfolio of proinflammatory genes in circulating monocytes (161). The functional importance of these gene expression changes was demonstrated by reduced neutrophil adhesion over 10 days of daily RIC (188). Neutrophil phagocytosis was not significantly altered at 24 h, but was suppressed after 10 days of RIC. In patients undergoing CABG (189), RIC was not associated with any difference in circulating markers of inflammation (e.g., interleukins 6, 8, or 10, or tumor necrosis factor-α levels) but neutrophil kinase beta-1 and beta-2 receptor expression was significantly reduced, confirming similar results in healthy human volunteers subjected to RIC (190).

The RIC stimulus is associated with coronary vasodilation in animal models (191) and peripheral vasodilation in the contralateral limb of human subjects undergoing RIC (192). In Kharbanda's original description (17), RIPC by 3 cycles of 5-min ischemia/5-min reperfusion in the forearm provided potent protection against the endothelial dysfunction induced by 20-min ischemia/reperfusion in the contralateral arm. Using the same model, RIPC was not only effective immediately, but also induced a second window of protection against endothelial dysfunction at 24 h (44). When the RIC protocol was performed on the contralateral arm during the ischemia phase, but prior to reperfusion, both RIPC and remote ischemic per-conditioning were blocked by pre-treatment with the ATP-dependent potassium channel blocker, glibenclamide (193). Compared with young volunteers, elderly hypertensive subjects benefitted more from

RIC, whereas basal levels of flow-mediated dilation were significantly greater in the younger population (194). RIC in healthy young subjects, repeated daily for 7 days (195), was associated with progressively improved flow-mediated dilation and cutaneous vascular conductance (as a measure of microcirculatory function), which was sustained at 8 days after the cessation of RIC. In a subsequent study (196), similar beneficial effects persisted after 8 weeks of repeated RIC treatments. This prolonged effect of RIC on endothelial function was also observed in patients with AMI undergoing PCI (197). Endothelial function was tested at baseline, within 3 h, and then on days 2 and 7 post-procedure in 48 patients randomized to PCI with or without RIPC. Endothelial function improved early after treatment and was sustained 7 days after the intervention. Whether this was a primary effect of sustained modification of endothelial function or a secondary phenomenon, resulting from less systemic inflammatory reaction, is unknown. Likewise, 1 week of twice-daily limb RIC improved ATP-recruitable coronary blood flow velocity reserve in a small cohort of healthy volunteers and patients with heart failure (198).

CONCLUSIONS AND PERSPECTIVE

Solid evidence from experimental and clinical studies supports protection by RIC from ischemia/reperfusion injury of the heart and other organs (16). Details of the mechanisms for local release of the protective signal at the remote site and the contributions of neuronal and humoral pathways are not yet clear, not only in signal release, but also in signal transfer to the target organ and protective signal transduction within the target organ. Repeated brief inflation/deflation of a blood pressure cuff at the arm, leg, or both is easily feasible, noninvasive, inexpensive, effective, and safe. Ongoing trials will reveal whether the benefit in clinical outcome reported from small proof-of-concept trials where clinical outcome was not the primary endpoint (199) will really hold true (200,201).

Thus far, translation of cardioprotective strategies from successful experiments to the clinic has been somewhat disappointing, for reasons that have been highlighted elsewhere (64,166,167,202): premature enthusiasm for experimental data that were not unequivocal and not confirmed in larger mammalian models; poor clinical trial design; and lack of consideration for patients' multiple comorbidities and comedications (126). The pharmaceutical industry has, understandably, largely given up on development of cardioprotective agents, because they may

need to be given only once in the situation of acute ischemia/reperfusion, but not as continuous therapy.

It appears reasonable to focus on mechanical protection of the heart and other organs by RIC and to optimize protocols. Apart from RIC algorithm optimization (number/duration of ischemia/reperfusion cycles), a better mechanistic understanding of the underlying signal transduction will be necessary to overcome the confounding impact of comorbidities and comedications. RIC may then, indeed, be the future of cardioprotection (203).

Future investigations should explore the potential benefit of RIC, not only in patients with large evolving myocardial infarctions, but also in patients with cardiogenic shock and severe arrhythmias, including cardiac arrest and threatening global ischemia of the brain, heart, liver and kidney during organ transplantation and extensive cardiovascular surgery.

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