

Remote Ischemic Conditioning: Evolution of the Concept, Mechanisms, and Clinical Application

Pankaj Saxena, M.Ch., D.N.B.,* Mark A.J. Newman, D.S., F.R.A.C.S.,* Jaffar S. Shehatha, F.R.C.S., F.R.A.C.S.,* Andrew N. Redington, F.R.C.P., F.R.C.P.C.,‡ and Igor E. Konstantinov, M.D., Ph.D.*,†

**Department of Cardiothoracic Surgery, Sir Charles Gairdner Hospital, University of Western Australia, Perth, Australia; †Cardiac Surgery Unit, Royal Children's Hospital, Melbourne, Australia; and ‡Department of Cardiology, Hospital for Sick Children, University of Toronto, Toronto, Canada*

ABSTRACT Remote ischemic conditioning is a novel concept of protection against ischemia-reperfusion injury. Brief controlled episodes of intermittent ischemia of the arm or leg may confer a powerful systemic protection against prolonged ischemia in a distant organ. This conditioning phenomenon is clinically applicable and can be performed before—preconditioning, during—perconditioning, or after—postconditioning prolonged distant organ ischemia. The remote ischemic conditioning may have an immense impact on clinical practice in the near future. doi: 10.1111/j.1540-8191.2009.00820.x *(J Card Surg ****;**:**-**)*

The most incomprehensible thing about the universe is that it is comprehensible.

Albert Einstein (1879–1955)

One of the most fascinating mysteries of nature is an innate mechanism by which all living cells protect themselves from the lack of oxygen or an excess of it that occurs during ischemia-reperfusion (IR). IR injury is a widespread phenomenon that occurs in all clinical scenarios when blood supply to tissue is interrupted and then restored. Examples of such scenarios are myocardial revascularization either by angioplasty or by surgery following complete interruption of coronary blood flow, organ transplantation, and various cerebral vascular and peripheral vascular procedures that require transient interruption of blood flow. Paradoxically, it is reperfusion, rather than ischemia per se, that causes major damage to the tissue. There is, however, a powerful innate protective mechanism against the IR injury that has evolved in all mammalian species. Namely, brief transient episodes of ischemia protect against a prolonged period of lethal ischemia. Prolonged lethal ischemia is often referred to as an index ischemia. The types of brief transient ischemia and the timing of its application in relation to index ischemia may vary greatly, yet this brief transient ischemia renders significant protection. These observations evolved into a novel concept. We refer to the concept in which brief transient ischemia protects against prolonged lethal ischemia as *ischemic conditioning*. A clinically applicable remote ischemic conditioning has evolved over the last two decades from the original description of *ischemic preconditioning* (IPC).

Address for correspondence: Igor E. Konstantinov, M.D., Ph.D., Cardiac Surgery Unit, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Melbourne, Australia. Fax: 61-3-9345-6386; e-mail: konstantinov.igor@alumni.mayo.edu

CONCEPT OF ISCHEMIC PRECONDITIONING

Originally, Murry et al. in 1986 identified the concept of IPC in a canine model. The use of four cycles of brief periods of ischemia and reperfusion was followed by 40 minutes of coronary artery occlusion.[1] The size of the myocardial infarct was reduced by 75% in the study animals. The protection against IR injury by brief episodes of ischemia at a remote site from the target organ was first observed in 1993 by Przyklenk et al.[2] and termed *remote* IPC. In dogs, transient ischemia of one coronary artery territory was shown to reduce the effects of subsequent potentially lethal ischemia in the territory of another coronary artery. IPC markedly reduces IR injury in most human tissues and has two phases. An early (also known as *classic* or *first window*) IPC effect occurs within several minutes of the preconditioning stimulus and lasts for approximately six hours. A late (also known as *delayed* or

second window) IPC effect occurs within 24 hours of the preconditioning stimulus and lasts for up to 96 hours. Although the degree of protection against myocardial necrosis is similar, it appears that protection against myocardial dysfunction is greater with the second window effect. The studies to date examining the second window effect have been in animal models of local ischemia.[3] The IPC also alleviates myocardial dysfunction-associated ventricular arrhythmias.[4-6] The duration and timing of the preconditioning stimulus appear to influence the degree of protection.[7-10] Some protection can also be achieved by partial occlusion of a coronary artery prior to total occlusion.[11] Furthermore, some protection can be demonstrated when a brief episode of ischemia is applied locally after prolonged index ischemia—*postconditioning*.[12-15] Thus, early ischemic conditioning has been applied before and after index ischemia clinically.[13-15] Unlike the *early* IPC, the *late* IPC protects not only against myocardial infarction but also against reversible postischemic myocardial stunning.[16] Because of the approximately 50-fold longer duration and the more powerful protection by late IPC, considerable interest has been focused on the late or second window of protection.[17] Unfortunately, clinical application of the *late* phase of local ischemic conditioning is impossible in most patients. Pharmacologic strategies have been explored to mimic the powerful protection by the late phase. A fascinating and more clinically relevant protection can be afforded by a brief ischemia of another organ or skeletal muscle—a phenomenon called *preconditioning at a distance* or *remote* IPC. Ischemic conditioning of the remote organ could utilize the benefits of the late phase.

CONCEPT OF REMOTE ISCHEMIC CONDITIONING

The concept of remote IPC was first described in 1993 by Przyklenk et al.[2] in dogs. Transient ischemia of one coronary artery territory was shown to reduce the effects of subsequent potentially lethal ischemia in the territory of another coronary artery.[2] Further studies in rodent models demonstrated that ischemia of the kidney and intestine may induce myocardial protection.[18,19] Furthermore, a second window of remote protection of the myocardium can be induced in rats and rabbits by applying a short period of preconditioning ischemia to the small intestine.[20-23] Although providing proof of the principle, none of these studies has particular relevance to protection against IR injury in the clinical setting.

Transient limb ischemia as the conditioning stimulus

Transient ischemia of skeletal muscle appears to be a potent preconditioning stimulus in humans and larger animals.[24-26] Four five-minute cycles of occlusion and reperfusion in the hind limb of a pig resulted in significantly decreased myocardial infarction size following subsequent coronary artery occlusion.[27] The degree of protection rendered by brief ischemia of the arm or leg appears to be similar to that by local or other modes of remote conditioning. Induction of this transient limb ischemia, however, is a noninvasive procedure and, as such, is more clinically relevant.

Remote preconditioning

We previously used transient limb ischemia in a porcine model and demonstrated significant protection against cardiopulmonary bypass (CPB)-induced tissue injury.[26] The animals were subjected to 180 minutes of CPB, including 120 minutes of aortic cross-clamping, followed by reperfusion. The parameters monitored were troponin I levels, load independent cardiac indices to assess systolic and diastolic functions, and the measurement of pulmonary resistance and compliance pre- and postbypass. Remote IPC was induced by four cycles of five-minute ischemia alternating with five-minute reperfusion prior to institution of CPB. The study found that preconditioning significantly attenuated myocardial and pulmonary injury. Brief transient limb ischemia also decreased pulmonary leukocyte sequestration and attenuated acute lung injury.[28]

IPC by transient limb ischemia was also shown to enhance the survival of flaps in experimental plastic surgical procedures.[29-31]

Remote perconditioning

Using a porcine model, we have also demonstrated that brief intermittent limb ischemia also provides significant protection *during* evolving myocardial infarction[32] and, thus, introduced a concept of remote ischemic *perconditioning*. In this study, four cycles of five-minute ischemia alternating with five-minute reperfusion were applied to the limb during 40 minutes of the left anterior descending coronary artery occlusion. This intermittent limb ischemia reduced myocardial infarction, preserved global systolic and diastolic function, and protected against arrhythmias during the myocardial reperfusion phase. The process involved adenosine triphosphate (ATP)-dependent potassium (KATP) channels.[32]

Remote postconditioning

Kerendi et al.[33] demonstrated that transient episodes of renal IR at the end of a prolonged episode of myocardial ischemia reduced the resultant myocardial infarction size. Subsequently, Andreka et al.[34] found that using a more practical stimulus of transient limb ischemia that is applied *after* induction of myocardial infarction, remote ischemic *postconditioning*, is also protective. In an isolated rat heart model, Galagudza et al. found that ischemic postconditioning had an effective antiarrhythmic action against reperfusion-induced persistent ventricular fibrillation.[35] Remote ischemic postconditioning was demonstrated in humans, and it appears to be as effective as preconditioning.[36] It was concluded that both provide an equally protective effect on the vascular endothelium. Thus, depending upon the timing of brief transient ischemia, protective strategies have been classified as preconditioning, perconditioning, or postconditioning. It is possible that a combination of all three modalities

may have an additive effect and increase the degree of organ protection.

MECHANISMS

We have recently demonstrated that remote IPC may significantly influence coronary blood flow and resistance.[37] Improvement of postischemic blood flow only partially contributes to myocardial protection by remote ischemic conditioning. The effects of the ischemic conditioning go, undoubtedly, far beyond simple improvement of postischemic blood flow. Although the precise mechanisms of ischemic conditioning are unknown, it is now becoming apparent that all modes of such conditioning induce profound changes in gene expression and cellular function, including mitochondrial adaptation to metabolic stress and leukocyte activation.

The most fascinating question, which remains as yet unanswered, is by what *molecular* mechanisms do these brief controlled episodes of intermittent ischemia of the arm or leg confer a protective effect?

We previously attempted to characterize global molecular responses to myocardial IR injury[38] and the remote IPC stimulus.[39,40] Despite our initial studies of the molecular mechanisms of remote IPC, all we can say with confidence at the present time is that gene expression and KATP channels play a crucial role in the protection induced by brief ischemia. It appears that protection from IR injury by remote ischemic conditioning involves a complex activation of different pathways. The mechanisms of ischemic conditioning are multifactorial, and the exact interrelationship between the various signals is not clearly defined. Although adenosine, bacterial lipopolysaccharide (LPS), bradykinin, heat shock proteins (HSP), catecholamines, opioids, reactive oxygen species, tumor necrosis factor (TNF)-α, and a few other triggers may initiate the cascade of conditioning[41-48] and produce a preconditioning-like effect, it appears that a non-specific innate immunity (Fig. 1) may be involved in ischemic conditioning, and the key elements of the process are gene expression, leukocytes, and mitochondria.

Gene expression

It was recently demonstrated that CPB induces a strong genomic response in the rat myocardium.[49] We have previously demonstrated an early modification of myocardial gene expression in the myocardium in response to intraoperative IR in patients undergoing cardiac surgery.[38] We also demonstrated in mice that transient limb IR modifies genomic responses in remote organs, specifically, the expression of genes involved in myocardial responses to inflammatory or oxidation-induced stress.[39] Although transient limb ischemia triggered an impressive global genomic response, expression of some individual genes was of particular interest. For example, the expression of an early growth response gene 1(*Egr-1*) was suppressed. Egr-1 is a master switch that activates transcription of a number of genes involved in the process of ischemic tissue damage.[50]

The nuclear factor kappa-B (NF-κB) is involved in preconditioning.[51] The NF-κB can be activated via multiple pathways including innate immunity pathways and a ubiquitous phosphoinositide-3 kinase (PI3K) pathway[52-54] (Fig. 2). Our previous study demonstrated that changes in gene expression occur in both early and delayed phases of remote IPC.[38] Similarly, Li et al.[55] demonstrated in a murine model that delayed cardioprotection induced by hind limb preconditioning involves signaling through transcription factor NF-κB and inducible nitric oxide synthase (iNOS). Remote IPC is abolished in mice with targeted deletions for the p105 subunit of NF-κB or the iNOS.[55] Thus, gene transcription appears crucial for preconditioning,[51,55] and a nonspecific inhibition of transcription by actinomycin abolishes the protective effect of IPC.[56]

Leukocyte activation

We have previously demonstrated for the first time that remote IPC stimulus achieved by transient forearm ischemia modifies gene expression in circulating human leukocytes.[40] These changes of gene expression correlate with early (first window) and delayed (second window) phases of remote IPC. It appears that remote IPC suppresses leukocyte activation. The genes involved in leukocyte chemotaxis, adhesion, migration, exocytosis, as well as cytokine synthesis and innate immunity signaling pathways were suppressed. This may, in part, explain previous observations that brief transient limb ischemia decreased pulmonary leukocyte sequestration and attenuated acute lung injury.[28] Indeed, innate immunity pathways (Fig. 1) in leukocytes are nonspecific and can be activated by bacterial LPS, HSP, hypoxia, hyperoxia, nitric oxide, TNF-α, and many other nonspecific stimuli. Many of them may produce a preconditioning-like myocardial protection.[57-59] For example, TNF-α plays an important role in the postischemic injury to various organs. Pretreatment with TNF-α results in reduction of IR injury and correlates with an increase in myocardial antioxidant and manganese superoxide dismutase (MnSOD) activity[60,61] via the activation of the NF-κB, particularly in late preconditioning.[51,61,62] Initial TNF-α signaling pathway activation by IPC not only induces protective MnSOD synthesis but also suppresses the gene expression responsible for subsequent TNF-α synthesis and TNF-α signaling pathway restoration. TNF-α plays an important role in leukocyte function and was shown to be involved in the ischemic conditioning. The responses to tissue injury by infection, ischemia, and trauma are remarkably similar. These responses, regardless of the initiating cause, involve innate immunity. Activation of the innate immunity pathways may, in turn, produce local or systemic inflammatory response. One of the key players of the innate immunity pathways is a toll-like receptor 4 (TLR4). The TLR4 is involved in LPS-induced oxidative burst in neutrophils in response to infection and in a similar response initiated by heat shock protein 70 (HSP70) in IR injury[63-65]

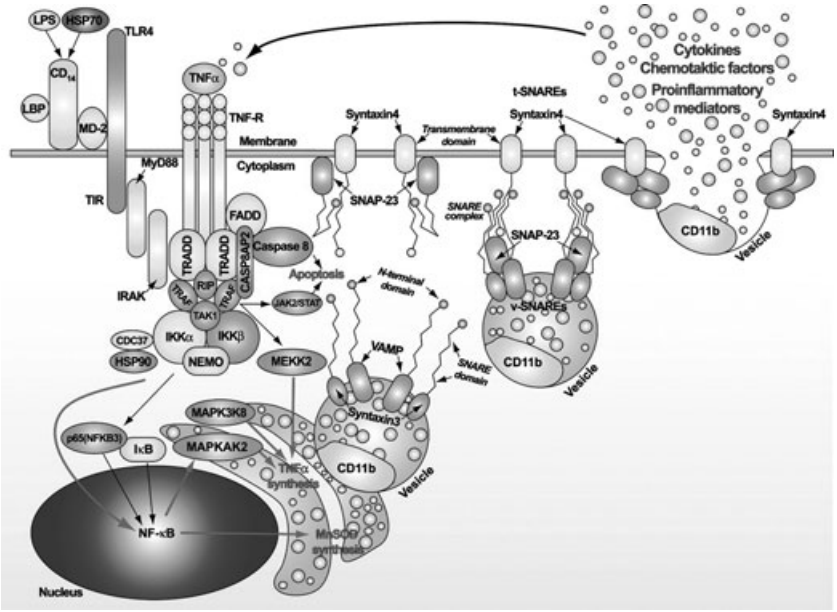

Figure 1. *Innate immunity pathways and NF-κB. Remote ischemic preconditioning differentially regulates gene expression of intracellular inflammatory pathways. HSP = heat shock protein; IKK = I-κB kinase; IRAK = interleukin (IL)-1 receptor-associated kinase; LPS = lipopolysaccahride; LBP = LPS binding protein; MnSOD = manganese superoxide dismutase; NF-κB = nuclear factor kappa-B; SNARE = soluble N-ethylmaleimide-sensitive factor attachment protein receptor; TIR = toll/IL-1/plan R homology domain; TLR = toll-like receptor; TNF = tumor necrosis factor; TRADD = TNF receptor-associated death domain, TRAF = TNF receptor-associated family. (Reproduced from Konstantinov IE, Arab S, Kharbanda RK, et al: The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. Physiol Genomics 2004;19:143-150, with permission of the American Physiological Society.)*

(Fig. 1). For instance, induction of the HSP by glutamine protects against IR injury of local and distant organs.[66]

Multiple stimuli that activate innate immunity pathways may initiate a preconditioning-like response. This fact suggests that leukocytes may play a central role in preconditioning. The central role of leukocytes is also consistent with our observation of significant myocardial protection in the donor heart after transplantation into a preconditioned recipient.[67] This study demonstrated that remote conditioning creates a benign environment in the recipient that protects a denervated donor organ from IR injury. Because the donor heart was not in the body when the recipient underwent a transient limb ischemia, it is plausible to speculate that this protection would involve a circulating factor or suppression of leukocyte activation. Interestingly, the protection of the transplanted heart was abolished by glibenclamide—a sulfonylurea that blocks KATP channels. Thus, whatever be the mechanism of protection in the transplanted heart, it can be abolished by blockade of the KATP channels.

Mitochondrial function

The opening of mitochondrial KATP channels is crucial to all modes of ischemic conditioning. Although both sarcolemmal and mitochondrial KATP channels appear to be involved, it is the mitochondrial channels that are *sine qua non* of the preconditioning effect.[68] It was observed that the selective mitochondrial KATP channel inhibition abolished the cardioprotective effect of both local and remote conditioning.[31,32,68] Interestingly, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) mechanism (Fig. 1) is not only a key element of exocytosis but also may be involved in blocking the KATP channels by syntaxin via the sulfonylurea receptor.[69,70] Our previous observation of decreased gene expression encoding SNARE proteins by the remote IPC[40] may, in part, contribute to functional preservation of KATP channels. The precise molecular mechanism by which opening of these channels provides protection is unknown. It is plausible that opening of the KATP channels in the target organ prior or immediately after sustained ischemia as a result of the transient limb ischemia reduces the rate of ATP hydrolysis[71] or mitochondrial ATPase activity,[72,73] thereby decreasing the rate of ATP depletion during reperfusion.

CLINICAL APPLICATION

Although *local* IPC, induced by transient brief ischemia in the target tissue, has been shown to be of benefit in patients undergoing coronary angioplasty and surgical revascularization in some studies,[74,75] the clinical application of local IPC is limited by the need to induce ischemia in the target organ, a process that itself may induce organ dysfunction. Thus, to date, local IPC has not found a wide clinical application.[76-78] Because of the inherent problems in performing local IPC, the concept of protecting a target organ or tissue by producing ischemia and reperfusion at a *remote* site appeared more attractive and practical.

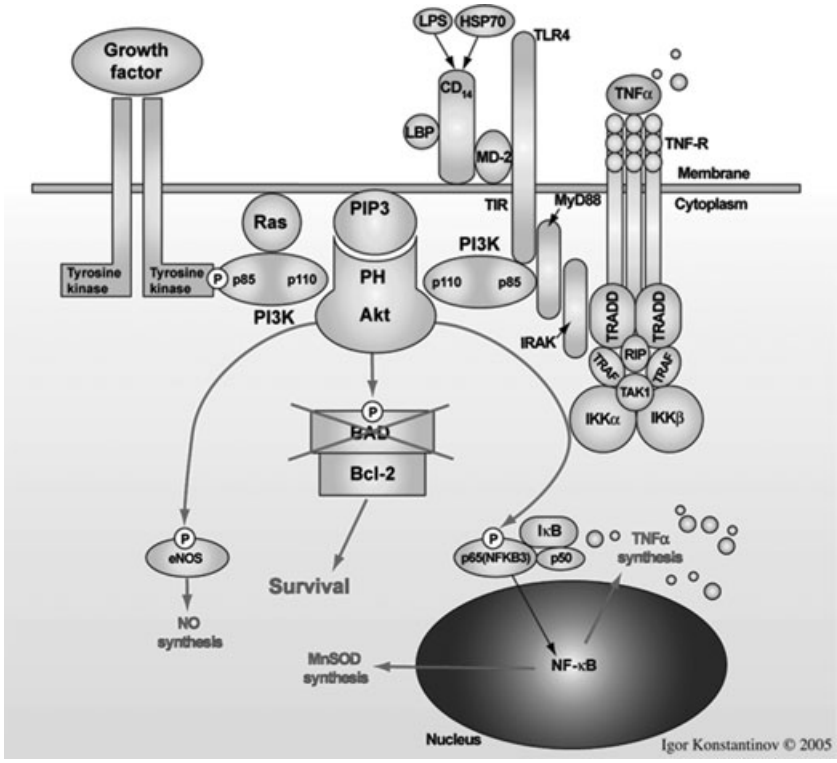

Figure 2. *Schematic of phosphoinositide-3 kinase (PI3K) signaling pathway. Activation of growth factor receptor results in phosphorylation (P) of tyrosine residues. PI3K is activated by binding to tyrosine residues of growth factor receptor with its regulatory subunit (p85). The lipid product of PI3K, phosphadylinositol-3,4,5-triphosphate (PIP3), recruits serine-threonine kinase (Akt) via pleckstrin homology (PH) domain to the membrane, where it is activated. Akt phosphorylation of the apoptosis-inducing protein BAD prevents BAD from binding to Bcl-2, thus releasing it for survival response. Direct binding to Ras protein further stimulates PI3K activity. Stimulation of toll-like receptor (TLR) 4 by bacterial lipopolysaccharide (LPS) or heat shock protein (HSP) results in PI3K activation via the myeloid differentiation factor (MyD) 88. Tumor necrosis factor (TNF) produces PI3K/Akt-mediated phosphorylation of p65 subunit of nuclear factor kappa-B (NF-κB) via the alpha subunit of I kappa-B kinase (IKK). Translocation of NF-κB to the nucleus results in gene expression and synthesis of cytokines, manganese superoxide dismutase (MnSOD), and endothelial nitric oxide synthase (eNOS), which are protective during acute ischemia. (Reproduced from Konstantinov IE, Li J, Redington AN: From mesothelioma to cardiovascular protection via the phosphoinositide-3 kinase pathway: A new vista in cardiothoracic surgery. J Thorac Cardiovasc Surg 2006;131:509-510, with permission of the American Association for Thoracic Surgery.)*

Interestingly, inadvertent systemic effects of local preconditioning were observed in a human study of aortic cross-clamping to precondition the heart before valve replacement. It was noted that the increase in leukocyte numbers, thromboxane B2, and malondialdehyde levels in pulmonary venous blood from the lungs was attenuated by preconditioning of the heart.[28] There was also less lung injury histologically, and a lower lung leukocyte count compared with control patients without myocardial preconditioning. These data may represent inadvertent remote IPC of the lungs and are consistent with our observations of decreased airway resistance postoperatively in a clinical setting[26] and of lower pulmonary vascular resistance and improved lung mechanics in a porcine model of CPB.[26,79] Following preclinical studies,[26,36,39,40,67,79] we successfully applied this concept to clinical practice.[80]

We reported the first clinical application of remote IPC[81] and demonstrated that remote IPC renders significant cardiac and pulmonary protection and attenuates systemic inflammatory response in patients undergoing open heart surgery. Subsequently, Hausenloy et al.[81] demonstrated that remote IPC significantly reduced troponin-T release in patients undergoing coronary artery bypass surgery. Recently, the same remote IPC stimulus was shown to reduce myocardial and renal injury after elective abdominal aortic aneurysm repair.[82] Remote ischemic per- and postconditioning have potential clinical applications as well[32,36] in patients with evolving myocardial infarction.

Another area of potential clinical application of remote ischemic conditioning is transplantation. We demonstrated such potential in a porcine model of orthotopic heart transplantation.[67] The remote IPC of the recipient provided significant protection of the donor heart. Thus, preconditioning of recipient may be, in principle, applicable to transplantation of any organ.

The clinical applications of remote ischemic conditioning could be immense and encompass organ transplantation, protection against stroke, myocardial infarction, acute multiorgan dysfunction, and attenuation of systemic inflammatory response in diverse clinical scenarios.[83,84]

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