Investigation of Reperfusion Injury and Ischemic Preconditioning in Microsurgry

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Abstract

Ischemia/reperfusion (I/R) is inevitable in many vascular and musculoskeletal traumas, diseases, free tissue transfers, and during time-consuming reconstructive surgeries in the extremities. Salvage of a prolonged ischemic extremity or flap still remains a challenge for the microvascular surgeon. One of the common complications after microsurgery is I/R-induced tissue death or I/R injury. Twenty years after the discovery, ischemic preconditioning (IPC) has emerged as a powerful method for attenuating I/R injury in a variety of organs or tissues. However, its therapeutic expectations still need to be fulfilled. In this article, the author reviews some important experimental evidences of I/R injury as well as preconditioning-induced protection in the fields relevant to microsurgery.

Keywords

Introduction

Salvage of devascularized or amputated extremities is one of the most important surgical achievements in the past 40 years. Before the historic accomplishments of Chung Wei Chen and Ronald Malt in 1962, [1, 2] the amputated parts were merely discarded. Today microsurgical replantation of amputations is technically feasible at almost any level of the extremities. Free flap transfer for wound coverage or restoration of function has become a routine surgical procedure.

However, salvage of a prolonged ischemic extremity or flap still remains a challenge for the microvascular surgeon. [3] Sometimes, the replanted limb has to be reamputated in an effort to overcome serious complications such as tissue necrosis. The problem may not necessarily be due to anastomosis failure but rather, it could be due to ischemia/reperfusion (I/R) injury.

Pathophysiology of Ischemia and Reperfusion

During the prolonged ischemia, the elevated glycolysis causes lactic acid accumulation associated with intracellular pH reduction. [4] The reduction of ATP concentration caused by ischemia inhibits Na/K ATPase resulting in the increase of intracellular Na^+ and Ca^{2+}. [5] The depletion of ATP combined with elevated Ca^{2+} could lead to a gradual decline in the cellular

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integrity. 5 If the duration of ischemia lengthens beyond a critical point of tolerance, the extensive necrosis will be certain.6

Reperfusion is the definitive treatment to salvage ischemic tissues from necrosis. However, reperfusion has led to a new pathophysiological condition called "reperfusion injury", a phenomenon which actually provokes a distinct degree of tissue injury directly related to the process of abrupt reperfusion of the ischemic vascular bed. 7 Abrupt reperfusion causes a burst of reactive oxygen species (ROS) production 8 in the post-ischemic tissues (particularly in the vascular endothelial cells) resulting in an inflammatory-like response to occur at the onset of reperfusion, such as, endothelial dysfunction (decreased endothelium-dependent vasodilation), 9 decreased endogenous nitric oxide generation, 10 increased superoxide anion generation, 11 and release of proinflammatory cytokines into the interstitium and vascular space. 12 The main source of ROS in the cell is the mitochondria. 13 Under normal physiological conditions, the mitochondrial inner membrane is impermeable to maintain the membrane potential and pH gradient that drive ATP synthesis through oxidative phosphorylation. However, under conditions of high Ca^{2+} and ROS, a non-specific pore opens in the inner mitochondrial membrane known as the mitochondrial permeability transition pore (MPTP). 14 Halestrap et al. 15 reported MPTP is kept firmly closed under normal physiological conditions and even under the ischemic period, but opens upon reperfusion. Recent studies 16, 17 suggested that ROS burst during early reperfusion is the trigger for MPTP opening which leads to immediate depolarization of mitochondrial membrane potential, further reduction of intracellular ATP concentrations and cell necrosis. Long-lasting MPTP opening results in matrix swelling, outer mitochondrial membrane rupture, releasing of pro-apoptotic molecules such as cytochrome c into the cytosolic compartment, and finally, cell apoptosis via caspase-dependent or - independent mechanisms.18, 19

I/R-induced Microcirculatory Alterations in Microsurgery

When reperfusion occurs after prolonged ischemia in the replanted extremities, releasing the clamps on the feeding artery after the anasomosis may not be sufficient to produce adequate and uniform tissue perfusion because significant impairment to blood flow often occurs at the level of the microcirculation. 20 This phenomenon has been termed as "arterial insufficiency" 20 which actually is the clinical manifestation of vascular endothelial dysfunction (decreased endothelium-dependent vasodilation and endogenous nitric oxide generation). 21 The clinical appearances of arterial insufficiency or endothelial dysfunction are sluggish venous flow, slowed capillary refill, decreased temperature, a bluish and mottled pale color, an empty feeling in the revascularized part, gradual cessation of arterial blood flow followed by thrombosis formation and anastomosis failure. 22 This phenomenon has occurred in 58% of the failures in digital replantations reported by Macleod et al. 3 and in 50% of failures after 4 hours of ischemia in a rat hindlimb experimental model. 22 Even more disturbing is that many patients were experienced an explorative surgery during the first postoperative day. 23, 24 The author has focused on this particular issue for more than 15 years. At the beginning, the author's curiosity concentrated on specific events in the microcirculation after reperfusion following prolonged warm ischemia. We have conducted a series of studies 25⁻31 using a vascular pedicle isolated rat cremaster muscle model (a microcirculation model) and rat gracilis muscle model (a skeletal muscle model) to investigate I/R injury in the microcirculation of skeletal muscle. All studies were designed to simulate the clinic situations, in which free flap and replantations of extremities are denervated and poor reflow can become a critical issue.

We observed (1995) 25 that a short-lived "good perfusion" associated with vasodilatation in the microcirculation of cremaster muscle occurred at the onset of reperfusion after 4h of

warm ischemia. However, this "good perfusion" lasted for only a few seconds to minutes. A significant vasoconstriction in the feeding arterioles (A1, 120 to 160 µm) associated with segmental vasospasm in the branching arterioles (A2, 50 to 120 µm) then occurred. Distal to the segmental vasospasm, the blood flow was sluggish despite systemic blood pressure being within normal range. It was interesting to observe these heterogenic microcirculatory responses. Some areas of muscle sustained an extended time of ischemia during the period of reperfusion, while other areas in the same muscle possessed relatively good perfusion. For example, in some terminal arterioles (A3 and A4; 7 to 49 µm), blood flow was almost stopped or in a status of thrombosis, however, other arterioles were still flowing. It was common to observe many scattered sites of no-reflow capillaries. The average capillary perfusion in the muscle at 2h after reperfusion was only 55% of the pre-ischemic baseline value. Thrombosis and capillary no-flow were definitely present during early reperfusion, but sometimes, were reversible. As reperfusion time continued, some of the thrombosed arterioles or no-flow capillaries gradually recovered to reestablish flow. However, on the other hand, these microcirculatory alterations could last for many hours or could be ended with completely no flow in the entire muscle at any time point during reperfusion. The pathological changes in the capillaries of rat cremaster muscles after I/R have been examined by electron microscopy by Fu-Chan Wei group. Lee et al. (1995) [32] reported that there was endothelial distortion with large vesicles, pseudopod formation and large intraluminal endothelial protrusions after I/R. Capillary lumen diameter was reduced approximately twofold, which is sufficient enough to compromise blood flow because of the resulting 16-fold increase in resistance to flow. We are convinced that the microcirculation is the primary target of I/R injury. [10, 33] The microvasculature, particularly the endothelial cells lining microscopic blood vessels, are especially vulnerable to the deleterious consequences of reperfusion.[34, 35, 36]

Reperfusion-induced Endothelial Dysfunction

The endothelium is a confluent monolayer of thin, flattened cells lining the intimal surface of all blood vessels. The concept that vascular endothelium is a mere barrier between intravascular and vascular smooth muscle and/or interstitial compartments has been completely revised during the last two decades to acknowledge the fact that the endothelium executes important regulatory functions, such as, the regulation of hemodynamics, vascular remodelling, immunoregulation, metabolic, synthetic, anti- and pro-thrombogenic processes. [10, 12] Intact endothelial cells secrete a variety of compounds that reach the bloodstram and/ or diffuse to nearby vascular smooth muscle cells to affect blood flow. [33] For example, endothelial cells synthesize nitric oxide (NO) through the conversion of L-arginine to citrulline by the action of nitric oxide synthetase (NOS). [37] NO diffuses from endothelial cells to underlying smooth muscle cells in the vascular wall and stimulates soluble guanylate cyclase to increase cyclic GMP and cause relaxation of the vascular smooth muscle cell. Due to its abluminal release, the primary function of NO is vasodilatation.[37]

The questions were whether the vasoconstriction we observed during reperfusion in the microcirculation of cremaster muscle is due to the lack of NO or endothelial dysfunction and whether the supplement of exogenous NO during reperfusion can prevent vasoconstriction. To answer these questions, a vascular isolated cremaster muscle in male Sprague-Dawley rats was coupled with local intra-arterial drug infusion as a model to study microvascular responses to IR injury. We reported (1997) [27] that local intra-arterial infusion of acetylcholine chloride (Ach) completely reversed the vasoconstriction caused by topically applied norepinephrine (NP) in non-ischemic cremaster muscle, but was unable to relax the vasoconstriction caused by the reperfusion. Most strikingly, we found that local infusion of a low concentration of sodium nitroprusside (SNP; a donor of NO, an endothelium-independent vasodilator), but not Ach (an endothelium-dependent vasodilator) during

reperfusion prevented reperfusion-induced microcirculatory alterations (including vasoconstriction, thrombosis and capillary no-reflow) and thus improved microvascular blood flow. Our results indicated that I/R-induced endothelial dysfunction is responsible to the microcirculatory alterations we observed during reperfusion. The supplementation of exogenous NO during the early period of reperfusion was definitely helpful for preventing microcirculatory failure at the early stage of reperfusion. However, some concerns can not be ignored since the exogenous NO could be combined with superoxide anions to form the peroxynitrite (a potentially toxic molecule) during reperfusion.[38]

Therefore, it is important to clarify the levels or the concentration of NO as well as the activity of NOSs in the skeletal muscle after ischemia and reperfusion. Electrochemical detection of NO by using porphyrinic sensors was conducted by Hallstrom et al. (2002) [39] who reported that NO was rapidly increased after ischemia and dropped below basal levels at the end of ischemia and then to undetectable levels during the reperfusion. NO was also measured indirectly by using electron paramagnetic resonance (EPR). Lepore et al (1999) [40] found that significant levels of muscle nitroso-heme complexes were detected at 24 hr after reperfusion, but not detected at 0.05, 3, and 8 hr after reperfusion. Nitrites and nitrates are stable metabolites of NO that have been measured by using Greiss assay. Blebea et al. (1996) [41] reported that NO2/ NO3 concentrations were decreased significantly after ischemia and further decreased after 1 hr of reperfusion in a rabbit extremity I/R model. Moreover, eNOS and iNOS expression were examined using immunohistochemical staining in the post-ischemic muscle. Messina et al. (2000) [42] reported that eNOS, which was localized to the endothelium of blood vessels, decreased progressively during ischemia and reperfusion to reach undetectable levels after 16 hr of reperfusion. iNOS was not detectable in the control muscle or during ischemia, however it was first detected after 2 hr of reperfusion, increased further by 8 hr, and remained elevated at 24 hr.

In addition, It should be emphasized that IR-induced endothelial dysfunction could result from the subtle interplay between superoxide and NO levels. Our study (2006) [43] has shown that superoxide generation in the arterial wall (arterial pedicle of cremaster muscle) peaked at first 5 min of reperfusion and declined to near baseline after 60min of reperfusion. Melatonin (an endogenous ROS scavenger) significantly reduced superoxide generation in arterial walls and improved microvascular endothelial dysfunction and increased cell viability in the cremaster muscles. In the conditions of homeostasis, NO is produced at a level far in excess of the superoxide anion, which allows NO to scavenge ROS, regulate vascular tone, prevent platelet aggregation and thrombus formation. However, within minutes of reperfusion, superoxide is produced at a level far beyond the level of NO, which could cause superoxide to quench NO and compromise the endothelium-dependent vasodilatation.[10, 36]

Current literature have clearly demonstrated that the production of NO is dramatically reduced and associated with increased superoxide generation at the early period of reperfusion following a prolonged ischemia in skeletal muscle.[39–42] The supplementation of exogenous NO or ROS scavenger during the early period of reperfusion could be one of the best intervention approaches to interfere with or modulate the pathophysiological processes that are set in motion during reperfusion. Surprisingly, the microcirculatory protection provided by exogenous NO was very similar to the microcirculatory protection induced by ischemic preconditioning in another study [26] conducted in our lab at that time.

Ischemic Preconditioning

Ischemic preconditioning (IPC) is the phenomenon whereby brief episode(s) of ischemia and reperfusion trigger a protective and adaptive mechanism that protects tissues against

injury from a subsequent sustained ischemia and reperfusion. [44] IPC was first described by Murry et al. (1986) [44] who demonstrated that preconditioning of dog myocardium with four, 5 min periods of coronary occlusion interspersed with 5 min periods of reperfusion, significantly reduced the infarct size when the myocardium was subsequently subjected to a 40 min ischemia and 4 days of reperfusion. Following this novel observation, the number of IPC studies on the myocardium has escalated dramatically.[45, 46, 47]

In addition to protecting heart muscle, IPC also protects other organs [48, 49] and tissues including skeletal muscle. [50, 51, 52] For example, Mounsey et al (1992) [50] reported that preconditioning a pig island latissimus dorsi muscle flap with three cycles of a 10 min ischemia and a 10 min reperfusion significantly reduced necrosis of these muscles when subsequently subjected to 4 hr of warm global ischemia and 48 hr of reperfusion. By direct microcirculatory observation _in vivo_ in the rat cremaster muscle, we demonstrated (1996) [26] that vasoconstriction, thrombosis, and capillary no-reflow does not take place in the cremaster muscle if 4 hr of warm ischemia is preceded by IPC. Moreover, IPC-induced protection in skin and myocutaneous flaps has also been documented. [53-62] For example, Zahir et al. (1998) [53] found that the survival areas were two to five times larger in the preconditioned flaps than that of non-preconditioned flaps after ischemia reperfusion in free skin and myocutaneous flaps of rat models. By using a random-pattern myocutaneous rat, Harder et al. [55] found that heat preconditioning induced arteriolar dilation, which was associated with a significant improvement of both arteriolar blood flow and capillary perfusion in the distal part of the flap. Moreover, inhibition of HSP-32 by tin-protoporphyrin-IX completely blunted the preconditioning-induced improvement of microcirculation and resulted in manifestation of necrosis.

Two phases of IPC protection (early and late phases) have been introduced. [63, 64] Most of studies in the literature have focused on the early phase of protection that can be observed immediately after IPC. However, the early phase of protection (only last 2 hr) disappears rapidly [63, 64] because it is developed by rapid posttranslational modification of preexisting proteins through series of signaling cascades. [65] And then, there is a gradual appearance of another phase of protection which is established around 12 to 24 hr after the initial IPC stimuli and lasts for 2 or 3 days. [63] The late phase of protection is mediated by gene expression and by the synthesis of new protective proteins (such as heat-shock proteins, antioxidant enzymes, eNOS, iNOS, etc).[65, 66, 67]

We prefer the late-phase protection because it provides a prolonged period of protection (first 3 days after surgery) when the most microsurgical problems usually arise. The prolonged period of protection, as occurs during the late phase after IPC, could increase the success rate of surgery and decrease the morbidity and mortality associated with I/R injury. Several studies (1999, 2000, 2001, 2002, 2004) [29-31, 68-70] have been conducted in our lab (supported by NIH RO1 research grant) to examine the mechanism of late-phase protection of IPC _in vivo_. Our results can be summarized as follows: 1) IPC, which was performed at 24 hr prior to 4 hr warm ischemia, largely prevented I/R-induced microcirculatory alterations, [29] 2) Microcirculatory protection induced by IPC is even more effective in the late phase than in the early phase, [29] 3) In the absence of IPC, local intra-arterial infusion of adenosine or SNP created a similar microvascular protection to that induced by IPC alone. However, on the other hand, intra-arterial infusion of an adenosine receptor antagonist (8-sulfophenyl-theophylline) or a NOS antagonist (N_ω-nitro-L-arginine) eliminated IPC-induced microvascular protection. [30] 4) Intra-arterial infusion of adenosine combined with NOS antagonist ($N_\omega$nitro-L-arginine) diminished the microvascular protection that was induced by adenosine alone. On the other hand, administration of SNP combined with adenosine receptor antagonist (8-sulfophenyl-theophylline) did not diminish the microvascular protection that was induced by SNP alone. [31] 5) IPC on day 1

significantly enhanced both eNOS and iNOS gene and protein expressions detected on day 2. 70 6) IPC or adenosine-induced late-phase microvascular protection can be blocked by protein kinase c inhibitor (chelerythrine) and mimicked by protein kinase C activator (4-phorbol 12-myristate 13-acetate). 68 Based on the results from our studies, we interpreted that the molecular basis for late preconditioning might consist of an ordered series of events. A brief period of ischemia/reperfusion or IPC rapidly generates vasoactive mediators (such as adenosine and then NO) these may serve as initiators. These initiators then activate a complex signal transduction cascade which may involve protein kinase C and others lead to the activation of transcription factors, cause upregulation of genes, and initiate synthesis of effector proteins (such as eNOS, iNOS, or others). These effector proteins then confer cytoprotection during the second prolonged ischemic stress.

The concept of late preconditioning has relevance to the clinical situation. It could potentially be beneficial in any situation where ischemia can be controlled by the surgeon. For example, free flap transfer for wound coverage or restoration of function has become a routine surgical procedure, IPC or pharmacological preconditioning given at 12 to 24 hr prior to reconstructive microsurgery may help to increase the success rate of free tissue transfer and decrease the complications associated with I/R injury. However, for the salvage of devascularized or amputated extremities where a prolonged ischemia has already occurred or the window of opportunity for preconditioning already closed. In that situation, a maneuver named as the intermittent reperfusion 28 or postconditioning can be applied.71

Intermittent Reperfusion versus Postconditioning

Abrupt reperfusion causes a burst of ROS production in the post-ischemic tissues (particularly in the vascular endothelial cells) resulted in an inflammatory-like response to occur at the onset of reperfusion. 72, 73 This reperfusion-induced pathophysiological condition is called reperfusion injury. If ischemia needs preconditioning, the question was whether reperfusion also needs preconditioning. Therefore, we tested our hypothesis (1998) 28 that a maneuver (we named as intermittent reperfusion which consisted of three 5-min alternate episodes of unocclusion/reocclusion) applied on the vascular pedicle of cremaster muscle at the end of 4 hr of ischemia may produce a similar microcirculatory protection induced by IPC. Four groups were examined: (1) untreated, (2) IPC, (3) intermittent reperfusion, and, (4) IPC plus intermittent reperfusion. Our result showed (1998) 28 that both IPC and intermittent reperfusion are useful techniques for attenuating I/R-induced microcirculatory alterations. IPC seems more powerful than intermittent reperfusion. However, intermittent reperfusion was still very effective ($P < 0.05$) on attenuating reperfusion-induced vasoconstriction (endothelial dysfunction), particularly in the terminal arterioles. Presumably, intermittent reperfusion might modulate the burst of ROS production and oxygen delivery or spread the production of ROS over a longer period of time to allow the clearance by the tissue's own free radical scavenging mechanisms.

Five years after our publication, Zhao et al. applied a similar idea and method of intermittent reperfusion to the heart I/R model, but named this maneuver as ischemic postconditioning. Zhao et al (2003) 71 reported that a maneuver (which consisted of three 30-sec alternate episodes of unocclusion/reocclusion) applied on the left anterior descending (LAD) at the end of 60 min of sustained LAD occlusion significantly reduced myocardial infarction in the canine heart. Since then, postconditioning has been documented (with more than 180 papers searched by the PubMed at June, 2008) as an effective protective strategy in different disciplines and animal models. 74–76 For example, McAllister et al (2008) 74 reported that postconditioning was effective in the salvage of ischemic skeletal muscle from reperfusion injury and the mechanism involves the inhibition of MPTP opening. Moreover, Loukogeorgakis et al. (2006) 75 demonstrated that postconditioning can protect against

endothelial IR injury in the human forearm by modifying the reperfusion phase of I/R. Moon et al. (2008) 76 found that postconditioning attenuated I/R-induced inflammatory cell infiltration and MPO activity in rat adipocutaneous flap.

In my opinion, postconditioning is not a logic term for this maneuver. If the maneuver conduced prior to the sustained ischemia is called ischemic preconditioning, then the same maneuver conduced prior to reperfusion should be called reperfusion preconditioning. This is because the target of preconditioning is the phase of reperfusion or the upcoming event rather than the phase of ischemia or the past event. A more objective term to describe the nature of this maneuver should be named intermittent reperfusion because the real biological effect of this maneuver, so far, is still uncertain and needs to be explored further.

Remote Ischemic Preconditioning

Since the discovery of IPC, the concept of IPC has been expanded to demonstrate that a brief period of ischemia in an organ or tissue not only elicits a local preconditioning effect, but also provides remote protection to other virgin tissues or organs at a distance from later sustained ischemia. 77–83 This phenomenon is called remote ischemic preconditioning (RIPC) which was first demonstrated by Przyklenk et al. (1993) 77 who found that brief episodes of intermittent circumflex artery occlusion protects virgin myocardium, perfused by the left anterior descending coronary artery, from a subsequent sustained ischemic insults. Since then, the phenomenon of RIPC has been documented (with more than 138 papers searched by the PubMed at June, 2008) in different disciplines, protocols, and animals. For example, Addison et al. (2003) 78 found that three cycles of 10-min occlusion and reperfusion in a hindlimb by tourniquet application in the pig reduced the infarction of latissimus dorsi muscle, gracilis muscle, and rectus abdominis muscle flaps by 55%, 60%, and 55%, respectively, compared with their corresponding control when they were subsequently subjected to 4 hr of ischemia and 48 hr of reperfusion. However, the mechanism of RIPC is virtually unknown. Some investigators have hypothesized that RIPC may act through a neuronal mechanism, 79 but others have suggested a humoral mechanism. 80

For examining IPC-induced microvascular protection at a distance and the possible mechanism, we conducted a RIPC study (2004) 81 in an innervated vs. denervated rat cremaster muscle models. RIPC was applied on rat left femoral vessels for a 45 min ischemia followed by 2 hr of reperfusion. After 2 hr of reperfusion in the left lower extremity, 4 hr of ischemia followed by 1 hr of reperfusion was applied in the right cremaster muscle. Microcirculatory responses in right cremaster muscle including terminal arteriole diameter, capillary perfusion, and endothelium function were evaluated. We found that RIPC in the left lower extremity induced a significant microvascular protection in right cremaster muscle against subsequent 4 hr ischemia in both innervated and denervated models, which suggested remote protection is not attributable to the neuronal mechanism. Most strikingly, we found this microvascular protection at a distance was lost not only in sham RIPC group, but also in a fake RIPC group, in which 4 hr of ischemia of right cremaster muscle was instituted before the reperfusion of left femoral artery was begun. This suggested that some activated circulating substance(s) might be released from the left lower extremity during reperfusion after RIPC could contribute to the remote protection in the right cremaster muscle. We interpreted that remote protection induced by IPC is a systemic phenomenon and may act through a humoral mechanism. Recently, Chen et al. (2005) 82 found that brief episodes of ischemia in rat femoral artery significantly reduced myocardial infarction induced by a sustained ischemia. This remote protection was abolished by a free radical scavenger (mercaptopropionyl-glycine) and associated with elevation of heat shock protein and antioxidant enzymes.

The Limitations of Ischemic Preconditioning

Most of the investigations on IPC have been undertaken in the animal model in which ischemia is imposed in the absence of other disease processes. However, it is unclear whether IPC can provide a protection against I/R injury in the animals associated with vascular diseases. For example, diabetes mellitus is often associated with vascular disease in peripheral vessels and endothelial dysfunction in the microvasculature. [84⁻88] If a prolonged ischemia and reperfusion put into effect on the unhealthy vasculature like in diabetic patient, it could result in a disastrous consequence.[89]

Some investigators have examined the effect of early preconditioning in diabetic animal models. [90⁻93] The results from these reports are conflicting. For example, Bouchard JF and Lamontagne D [90] found IPC affords protection to endothelial function in resistance coronary arteries of diabetic hearts. Tatsumi et al. [91] reported that diabetic myocardium benefits more from preconditioning than normal myocardium. However, others studies [92,93] indicate that early preconditioning did not provide protection in the heart of diabetic animal.

Since early preconditioning only provides a short period (2 hr) of protection that could be the reason that early preconditioning fails to provide enough protection in diabetic animal. Therefore, we carried out another experiment (2002) [69] to examine the late phase protection in a microcirculatory model of streptozotocin (STZ)-induced diabetic rats. Our results can be summarized as follows: 1) Four hours warm ischemia followed by reperfusion creates a significant endothelial dysfunction in the cremaster muscle of STZ-induced acute diabetic rats. 2) IPC provides a significant microvascular protection 24 hr later against sustained warm ischemia in the cremaster muscle of normal rats. 3) However, IPC-induced late phase of microvascular protection was completely abolished in the cremaster muscle of STZ-induced acute diabetic rats. Our result suggested that IPC is unable to provide significant microvascular protection in diabetic rats. More recently, more and more studies have reported the negative effect of IPC on the diabetic animals.[94⁻96]

Another important question on IPC is whether IPC can provide protection in humans. Currently, the clinical trials of IPC in the fields of cardiac, hepatic, and pulmonary surgery have been successfully carried out and demonstrated the existence of IPC-induced protection in humans though more studies with greater numbers of patient are needed. [97⁻99] However, so far, there is no report to show the result of clinical trials of IPC in human skeletal muscle, periphery vessels and skin in plastic, reconstructive, orthopedic, vascular, and transplantation surgeries. IPC-induced favorable effects are less evident in diabetic and elderly patients. [100,101] The effect of postconditioning as well as RIPC on clinical outcomes remains to be determined.

Conclusions

Twenty years after the discovery of ischemic preconditioning, there is little doubt that the phenomenon of preconditioning has been recognized as one of the most potent mechanisms to reduce I/R-induced cell necrosis and apoptosis, though its therapeutic expectations still need to be fulfilled. Recently, the research interest in the field of ischemic biology has focused on the mitochondria and the phase of reperfusion protection. As our acquired experience and the increased knowledge of the underlying mechanisms of preconditioning, we expect that the pharmacological preconditioning, by the using of pharmacologic agents that produced by the signal-transduction pathway activated by the preconditioning, should be a more effective interventional strategy to against I/R injury.

Acknowledgments

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