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Research review

Remote ischemic preconditioning—a simple, low-risk method to decrease ischemic reperfusion injury: Models, protocols and mechanistic background. A review

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ABSTRACT

Interruption of blood flow can cause ischemic reperfusion injury, which sometimes has a fatal outcome. Recognition of the phenomenon known as reperfusion injury has led to initial interventional approaches to lessen the degree of damage. A number of efficient pharmacologic agents and surgical techniques (e.g., local ischemic preconditioning and postconditioning) are available. A novel, alternative approach to target organ protection is remote ischemic conditioning triggered by brief repetitive ischemia and reperfusion periods in distant organs. Among the different surgical techniques is so-called remote ischemic preconditioning, a method that applies short periods of ischemic reperfusion to a distant organ delivered during target organ ischemia. Although ischemic reperfusion injury is reduced by this technique, the explanation for this phenomenon is still unclear, and approximately only a dozen reports on the topic have appeared in the literature. In our study, therefore, we investigated the connective mechanisms, signal transduction, and effector mechanisms behind remote preconditioning, with a review on molecular background and favorable effects. In addition, we summarize the various treatment protocols and models to promote future experimental and clinical research.

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1. Introduction

Decreasing the degree of ischemia-reperfusion (I-R) injury has great practical significance, considering that this clinical problem involves a large number of patients. The possibility of severe I-R injury should be considered during acute arterial occlusions (myocardial infarction, percutaneous coronary interventions, ischemic stroke, mesenteric ischemia, and lower limb vascular occlusions), valve replacements, and organ transplantations. Since Jennings *et al* [1] introduced the

idea of reperfusion injury in 1960, several surgical and nonsurgical cardioprotective strategies have been developed to lessen the degree of injury, some more successful than others. Murry *et al* [2] described ischemic preconditioning (IPC) in 1986. The essence of this adaptive surgical method is to induce short periods of local ischemia and reperfusion before target organ ischemia. There is a vast literature on the strong protective effect of IPC, which has been proven by numerous experimental and clinical studies. However, the technique is limited to elective situations in which the onset of ischemia

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can be predicted. Local preconditioning cannot be used in acute clinical settings such as acute myocardial infarction, ischemic stroke, or acute major vascular occlusion. It therefore became necessary to develop new techniques suitable for providing protection against unpredictable ischemic events. One option is modification of the reperfusion period by means of brief coronary artery occlusions and reperfusion applied at the onset of myocardial reperfusion, a phenomenon called ischemic postconditioning (IPOST). Although Na *et al* [3] formulated the seminal idea and terminology, the first easily reproducible experimental results on this topic were published in 2003 by Zhao *et al* [4]. A shortcoming of both preconditioning and postconditioning is the prolongation in operative time, possibly even for a duration of 15–20 min. A further negative aspect is that in the presence of atherosclerosis, these invasive techniques can lead to serious, life-threatening complications, such as plaque rupture.

Reducing the extent of I-R injury via short episodes of ischemia and reperfusion instituted at a remote site is a novel idea. As for local conditioning techniques (IPC and IPOST), remote ischemic conditioning can be applied before target organ ischemia (remote ischemic preconditioning [R-IPC]) or at the onset of reperfusion (remote ischemic postconditioning [R-IPOST]). When the site of conditioning is located remotely, the conditioning cycles can be applied during target organ ischemia—a novel phenomenon known as remote ischemic perconditioning (R-IPER) (Fig. 1).

Przyklenk *et al* [5] originally developed the idea of remote conditioning in 1993. In their study, the authors described the effects of regional preconditioning of the myocardium, with manipulations in the same vascular bed (the territory of the left coronary artery). They demonstrated that transient occlusion of the circumflex artery provided subsequent

protection in the myocardial territory of the left anterior descending artery exposed to sustained, potentially lethal ischemic insult. This finding assumes the presence of certain transportable mediators and other protective signals that are delivered via the circulation to the distant myocardium. Although the protocol used in the study cannot be considered an example of genuine remote ischemic preconditioning, it provided the basis for further research. Investigations over the past 2 decades have demonstrated that short I-R episodes applied to kidney [6], the splanchnic area [7], or limbs [8] may induce organ protection against a prolonged ischemic period. For clinical application, the invasiveness of the procedure and the cost-benefit ratio are the two most important criteria. Obviously, the best clinical approach to treatment is the use of noninvasive or minimally invasive techniques, because the most important goal is to decrease both frequency of complications and the technical risks [9]. Birnbaum *et al* [10] provided the basis for minimally invasive treatments. In 1997, they showed that myocardial infarct size can be reduced by conditioning applied on the flexor muscles of the lower limb. Oxman *et al* [8] reported the first noninvasive technique, carrying out conditioning cycles on the lower limb by the use of an elastic tourniquet. In their experimental model, the authors found a significant decrease in the frequency of reperfusion arrhythmias of the heart in rats.

Unfortunately, however, remote preconditioning shares the major disadvantages of local preconditioning: among others, the restriction to elective ischemic conditions and the prolongation of operative time. Hence, it cannot be applied to a wide range of clinical situations.

Remote ischemic postconditioning [11–14] was developed as a protective strategy bearing similarity to local postconditioning (IPOST). Therefore, it extends the range of application of the conditioning techniques (Fig. 1).

Remote ischemic perconditioning, first proposed in 2007 by Schmidt *et al* [15], has even more beneficial features from a practical point of view. The essence of this strategy is that brief, remote ischemic attacks are applied after the induction of target organ ischemia, but before the onset of reperfusion (Fig. 1).

Whereas the perconditioning technique reduces ischemic reperfusion injury, the explanation for the phenomenon is unclear. The technique could become most feasible to perform in clinical settings; yet, there is scant literature on the subject.

This review summarizes the available literature on R-IPER and discusses the underlying mechanistic pathways and favorable effects involved in the strategy. Furthermore, we attempted to provide guideline for future experimental and clinical settings.

1.1. Terminology

There is much confusion in the literature regarding the concept called “ischemic perconditioning.” Three different terms exist for this strategy, meaning “the induction of brief, repetitive interruptions of blood flow at a distance, concurrently with sustained target organ ischemia”: (1) remote perconditioning [15–21]; (2) remote perconditioning [22,23]; and (3) remote postconditioning [24–27]. The term “R-IPOST” can

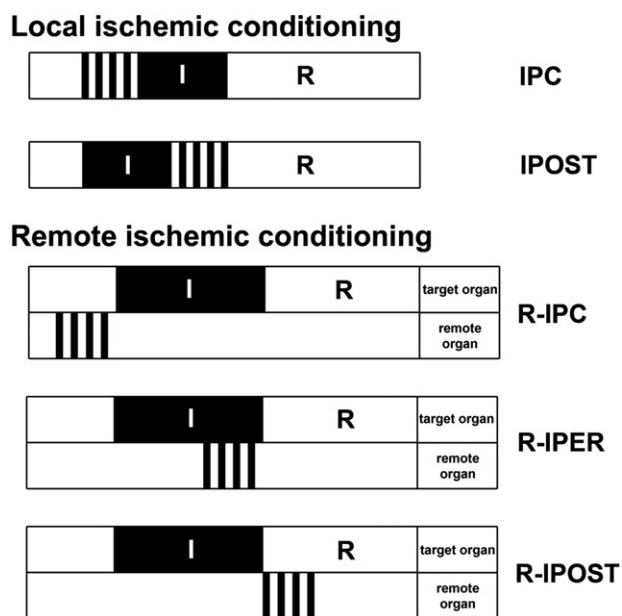


Fig. 1 – Treatment algorithms of local and remote adaptive surgical ischemic conditioning techniques. Black bands = interruption of circulation; white bands = unimpeded circulation.

be excluded first as proper terminology, because it implicates that the conditioning cycles take place after (“post”) the target organ ischemia (Fig. 1). The distinction between per-conditioning and preconditioning is more a semantic rather than a methodical one. The Greek prefix *peri* means “around something in time,” and is found in the expression “perinatal mortality.” In contrast, the meaning of the prefix “per” is “over, through, during the course of something,” such as “perennial rhinitis.” Thus, from a linguistic point of view, the term “per” is suitable for describing this method. Furthermore, Schmidt et al [15], who were the pioneers of this procedure, proposed the term “remote preconditioning,” as well. Accordingly, “perconditioning” seems the suitable term for all cases in which brief ischemic and reperfusion cycles are instituted after the onset of target organ ischemia and completed before reperfusion.

2. Ischemic perconditioning: underlying mechanisms

There is scarce evidence as to how brief cycles of I-R on a distant organ during prolonged target organ vascular occlusion are able to provide organ protection. Here, we discuss the mechanistic background of the perconditioning technique, to understand the mechanistic process. However, it is also necessary to touch on the experimental and clinical data available on other conditioning strategies.

Recently, Hausenloy et al [28] and Lim and Hausenloy [29] gave a detailed review of the underlying mechanisms of R-IPC. According to their theory, three main mechanisms—humoral, neural, and systemic—hypothetically interact with each other

to provide target organ protection from a remote site [28,29]. However, their categorization cannot be applied directly to R-IPER, owing to the lack of sufficient literature. We have therefore attempted to extend the classification on the basis of available data, to provide better understanding of the mechanisms behind remote perconditioning.

According to our hypothesis, there are two main different groups of inter-organ protective pathways: (1), connective mechanisms, which provide linkage between distant and target organs (principally the classical humoral, neural, neurohumoral, and systemic mechanisms); and (2) signal transduction and effector mechanisms, which are mainly molecular, subcellular, and channel-related components (most likely participants of the distal pathways of inter-organ protection). Based on previous studies, most of these are downstream-acting agents compared with the classical connective components (Fig. 2 and Table 1).

These mechanisms are related in a complex manner; hence, neither of these artificial classification models can perfectly reflect *in vivo* pathophysiologic conditions. Nonetheless, a classification is essential to understand this complicated phenomenon.

A further consideration is that the concentration or activation status of the molecular, humoral, and subcellular participants, for instance, may vary significantly among the different remote ischemic conditioning subtypes (R-IPC, R-IPER, and R-IPOST): namely, the timing of these procedures is radically different (before, during, or after target organ ischemia). On the other hand, it is assumed that the main biochemical and signal transduction pathways might be similar among the conditioning techniques, so some comparison between the remote conditioning strategies is inevitable [30].

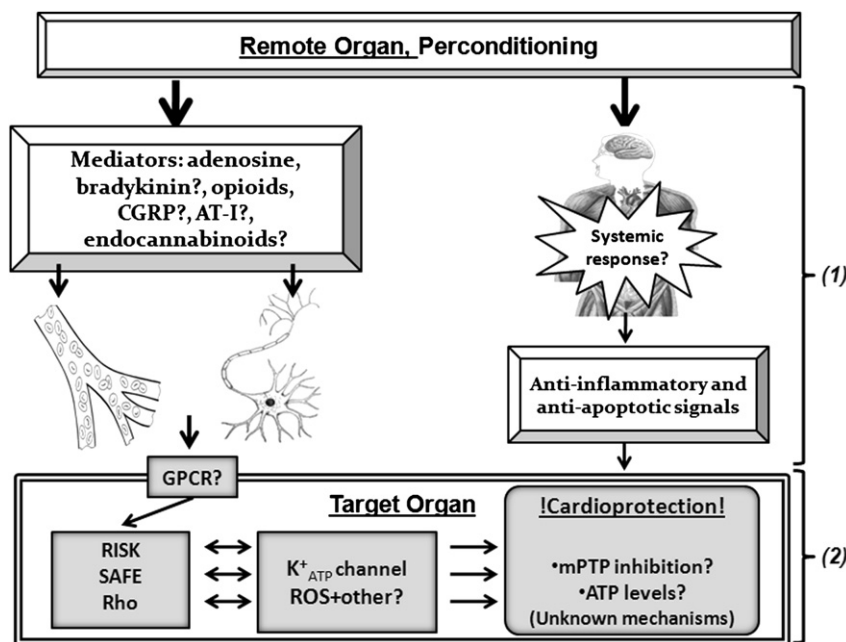


Fig. 2 – Underlying mechanisms. Different components of inter-organ protection. (1) Connective mechanisms between remote and target organ are mostly humoral, neurohumoral, and systemic. (2) Effector and signal transduction components are mostly channel-related, subcellular, and molecular mechanisms. Knowledge gaps and problematic components and relationships between the participants are marked with a question mark.

Table 1 – Experimental and clinical studies available at present regarding remote ischemic preconditioning.

Study	Model	R-IPER protocol	I/R time; tissue, region	End point	Mechanism
Connective mechanisms					
Tsubota et al [25]	Mice	5 min left hind limb I and 5 min reperfusion before right hind limb reperfusion	180 min/24 h Skeletal muscle, right leg	Muscle necrosis reduction 62%	Adenosine receptors
Tang et al [27]	Rabbit	5 min left pulmonary artery occlusion and 5 min reperfusion	30 min/180 min; Myocardium, LAD	Reduction of blood creatinine kinase 23%, and malondialdehyde concentrations 47%. Increased SOD activity by 63%	eNOS activation
Hausenloy et al [21]	Pig	4 × 5 min limb ischemia and 5 min reperfusion	60 min/180 min; Myocardium, LAD	Infarct size reduction 63%	Adenosine receptor–independent mechanism
Signal transduction and effector mechanisms					
Xin et al [16]	Rat	3–4 × 5 min limb ischemia and 5 min reperfusion in combination with IPOST	45 min/120 min; Myocardium, left coronary artery	Infarct size reduction 54%	RISK cascade activation
Tamarelle et al [70]	Rat	10 min limb I and 10 min reperfusion in combination with IPOST	40 min/120 min; Myocardium, LAD	Infarct size reduction 67%	RISK and SAFE cascade activation
Hausenloy et al [21]	Pig	4 × 5 min limb ischemia and 5 min reperfusion	60 min/180 min; Myocardium, LAD	Infarct size reduction 63%	RISK-independent mechanism
Schmidt et al. [15]	Pig	4 × 5 min limb ischemia and 5 min reperfusion	40 min/120 min; Myocardium, LAD	Infarct size 37% and reperfusion arrhythmia reduction	K ⁺ ATP channel activation
Zhao et al [22]	Mini-swine	4 × 5 min limb ischemia and 5 min reperfusion	180 min/120 min; Myocardium, LAD	Infarct size 24%, no-reflow reduction 45%	K ⁺ ATP channel activation via Rho kinase inhibition
Loukogeorgakis et al [24]	Human model	2 × 5 min limb ischemia and 5 min reperfusion	20 min/–; Skeletal muscle, arm	Preventing of endothelial I-R injury (preserved flow mediated dilation) 59%	K ⁺ ATP channel activation
Wei et al [55]	Rat	4 × 5 min limb ischemia and 5 min reperfusion	45 min/28 d; Myocardium, LAD	Improved survival, infarct size reduction 34%, suppression of inflammatory responses	Attenuated inflammatory responses
Further studies with no mechanistic investigations					
Lujan et al [89]	Rat	Partial hind limb occlusion during coronary artery occlusion	Left coronary artery was occluded until onset of ventricular arrhythmia (but no longer than 8 min)	Increased ventricular arrhythmia threshold by 38%	
Li et al [17]	Human patients underwent valve replacement (clinical trial)	3 × 4 min right lower limb ischemia and 4 min reperfusion (by tourniquet)	46.7 ± 16.6 min aortic clamping in preconditioned group (but with no significant differences among groups)	Reduced cardiac troponin-I release by 40%	
Botker et al [94]	Human patients with STEMI underwent primary PCI (clinical trial)	4 × 5 min upper limb ischemia and 5 min reperfusion (by blood pressure cuff)	Chest pain within 12 h of onset	Increased mean myocardial salvage index by 21%	

Munk et al [95]	Human patients with STEMI underwent primary PCI (clinical trial)	4 × 5 min upper limb ischemia and 5 min reperfusion (by blood pressure cuff)	Chest pain within 12 h of onset	Increased ejection fraction by 9% 30 d after intervention in patients with extensive area at risk
Hougaard et al [19]	Human patients with acute ischemic stroke, underwent thrombolysis (clinical trial)	4 × 5 min upper limb ischemia and 5 min reperfusion (by blood pressure cuff)	Symptoms within 4.5 h of onset	Neuroprotective effect (data evaluation in progress)
Hahn et al [20]	Rat	4 × 5 min of hind limb ischemia and 5 min reperfusion (by tourniquet)	120 min middle cerebral artery occlusion, and 24 h reperfusion	Infarct size reduction 41%

PCI = percutaneous coronary interventions; LAD = left anterior descending; RISK = reperfusion injury salvage kinases.

2.1. Connective mechanisms

As a result of remote organ ischemia, soluble mediators are washed into the circulation, which are suspected to be responsible for the protective effect. McClanahan et al [6] reported the first evidence of humoral factors. Since their discovery, numerous models have become available to verify the role of humoral factors [31–37]. Cardioprotection was achieved in untreated isolated hearts by means of blood transfusion from remote conditioned animals to untreated ones [32]. Investigations on denervated hearts—therefore excluding the neural component—were also carried out [38,39]. Among the suspected humoral mediators are bradykinin [40], adenosine [41,42], opioids [43], calcitonin gene-related peptide (CGRP) [44], and endocannabinoids [45]. Regarding the subject of the present review (R-IPER), it appears that adenosine has key role in mice, given the fact that the protective effects of remote lower limb conditioning during sustained contralateral limb ischemia can be abolished by the nonselective adenosine receptor antagonist 8-sulfophenyl theophylline [25]. In contrast to these results, Hausenloy et al [21] were not able to demonstrate this adenosine receptor-dependent effect on pig myocardium, because 8-sulfophenyl theophylline had no influence on preconditioning-induced cardioprotection [21]. The reasons behind these controversial results lay dormant. The differences between the various experimental models could be held accountable (different species and target organs); hence, this complex issue needs further investigation.

Other studies have implicated the role of opioids and nitrogen monoxide (NO) [27]. Gho et al [7] showed that the potential role of remote ischemic conditioning in reducing myocardial infarct size can be diminished by the ganglion blocker hexamethonium, which suggests the presence of neural factors. Further investigations with various receptor blocking agents indicated that afferent neuron stimulation from a remote organ by humoral mediators such as adenosine [41,42], bradykinin [40], and CGRP [42,46] provides the neural component in organ protection.

Calcitonin gene-related peptide release from capsaicin-sensitive sensory neurons is presumably caused by NO generation during remote organ I-R [47,48]. A study by Ren et al [11] confirmed the role of CGRP regarding the protective effect of remote conditioning, which could be delayed by neural inhibition with capsaicin.

To date, there is no available evidence to support the role of neural components in remote ischemic preconditioning. However, the presence of certain neural elements among the components of preconditioning is most likely.

It has been confirmed that target as well as remote organ ischemic conditioning is able to change the gene expression profile of the whole system (including target organ and circulating leukocytes) in an anti-inflammatory and antiapoptotic direction. This is partially due to the down-regulation of specific proinflammatory genes and the up-regulation of specific anti-inflammatory genes [49,50]. The secretion of certain cytokines, the tumor necrosis factor–receptor signaling pathway, and the processes of innate immunity and apoptosis—via repression of caspase-8—are also all suppressed. Anti-inflammatory gene up-regulation also occurs (i.e., in case of heat-shock protein [HSP] 70 or calpastatin [51]). Inhibition of

proinflammatory cytokine secretion results in reduced leukocyte-endothelial adhesion and tissue infiltration, which leads to a milder local inflammatory response [52]. Less leukocyte infiltration results in fewer leukocyte-related reactive oxygen species (ROS) production, which is an important factor in the late phase of reperfusion. This phenomenon suggests a partial explanation for both the observation of Harkin *et al* [53] and our findings [54], according to which lower limb ischemic preconditioning and postconditioning can reduce remote lung injury and leukocyte sequestration after local limb I-R injury. In these cases, however, the dominating factor is presumably the local I-R injury reducing effect of the conditioning strategy, which in turn leads to less remote organ damage. Nonetheless, Wei *et al* [55] reported a remission in inflammatory responses caused by R-IPER, as well. Those authors observed that preconditioning significantly diminished the infiltration of macrophages and neutrophil granulocytes in rat myocardium (Table 1).

2.2. Signal transduction and effector mechanisms

Regarding remote conditioning strategies, the gene regulatory effect is presumably mediated by similar signal transduction cascades: First, the ligand binds to the G-protein–linked receptor; then, the intracellular kinase PKC epsilon [56,57] and other signaling mechanisms (NO, reactive oxygen species [ROS], and mitochondrial K⁺ATP channels) are activated [16,22,41,58]. Similar signal pathways are present both in IPC [59] and IPOST [60].

It is well known that local preconditioning, postconditioning, and remote organ conditioning result in the activation of certain pro-survival kinase elements of the reperfusion injury salvage kinase (RISK) cascade. However, the mechanism of RISK activation during remote conditioning is unknown. The RISK pathway seems to be particularly important, mediating the protective effects of the different conditioning procedures. In view of local ischemic preconditioning, the most clearly defined mechanism—the PI3K-Akt-eNOS-cGMP-PKG pathway—results in the opening of mitochondrial ATP-sensitive K⁺ (K⁺ATP) channels. This, in turn, leads to ROS production and more survival kinase activation (p38, JNK, ERK, PKC, AKT, and TK), so as a consequence, the K⁺ATP channel opening induces further effector mechanisms [57]. A similar consistent kinase chain has not been found so far in any other conditioning procedure.

In the case of remote ischemic preconditioning, Xin *et al* [16] confirmed the activation of certain elements of the RISK pathway. According to their results, R-IPER was able to induce significantly higher Akt and ERK1/2 activation compared with the I-R control group (Table 1). However, the results obtained are controversial, despite the impressive strides made. In their study, Hausenloy *et al* [21] reported that R-IPER can reduce myocardial I-R injury in an unknown manner, independent of RISK activation.

Adenosine triphosphate–sensitive K⁺ channels are actively investigated components of the conditioning strategies, with confirmed roles in every existing technique [15,24,39,61,62]. Regarding the widely explored local preconditioning, both sarcolemmal and mitochondrial channels have an important role in the mechanisms, but “it is the

mitochondrial channels that are sine qua non of the preconditioning effect” [18].

Schmidt *et al* [15] demonstrated that the protective effect of R-IPER was abolished by administration of the K⁺ATP channel inhibitor glibenclamide. Furthermore, Zhao *et al* [22] recognized that during remote preconditioning, K⁺ATP channel activation is achieved by inhibition of Rho-kinase (Table 1). This Rho-kinase inhibition is mediated by protein kinase A (PKA), which Sanada *et al* [63] also confirmed. It is therefore likely for the PKA-induced inhibition of Rho-kinase to result in K⁺ATP channel activation.

Although the exact role of K⁺ATP channel activation in the reduction of I-R injury is unclear, its key role in preserving mitochondrial function is suspected, by way of moderating ATP depletion [63,64] and inhibiting mitochondrial permeability transition pore (mPTP) channel opening [65]. The mPTPs are nonselective channels in the mitochondrial inner membrane. As the consequence of their opening, the selective permeability of the inner membrane disintegrates: Oxidative phosphorylation is disturbed and mitochondrial integration is completely abolished, leading to cell death [66]. The previously mentioned opioids also have a major role in the inhibition of mPTP opening via opioid receptor-mediated signal transduction [26,67,68].

A further well-known participant in the mechanism of the different conditioning strategies is the so-called survivor activating factor enhancement (SAFE) pathway, in which signal transducer and activator of transcription proteins (STATs) are suspected key elements. Boengler *et al* [69] demonstrated that activation of mitochondrial STAT isoforms (STAT 1 and 3) can inhibit mPTP channel opening in mouse myocardium. Based on these data, mPTP channels could represent the final common path elements for both the RISK and SAFE pathways.

Tamareille *et al* [70] recently demonstrated an improvement in cardioprotection with the use of R-IPER in combination with IPOST, as opposed to IPOST alone. The authors associated this phenomenon with the recruitment of the SAFE pathway [70] (Table 1). Their study indicated the existence of a complex cross-talk between the RISK and SAFE pathways. They reported that inhibition of both cascades can abolish the cardioprotective effects of the R-IPER+IPOST combination. They observed decreased activation of the RISK pathway achieved by administering SAFE inhibitors, and vice versa when RISK inhibitors were given. These data suggest that there are many elements in common among the various conditioning methods; yet, the specific role of these common mediators in the different procedures still needs to be investigated.

Several studies report on the key role of ROS production as a second messenger in the mechanism of conditioning procedures during the early phase of reperfusion [71–76]. The protective effects most likely prevail in local preconditioning [77] and postconditioning [78] via activation of the RISK pathways and inhibition of the mPTP channels. However, the positive effects of ROS production are to be sharply separated from the extensive ROS generation observed during reperfusion, which correlates with tissue injury and subsequent cell death [79]. These results are consistent with the findings of Xin *et al* [16], who demonstrated reduction of malondialdehyde—an indicator of lipid peroxidation—and superoxide levels related to

R-IPER. Furthermore, those authors observed increased survival kinase activation, which suggests an inverse correlation with malondialdehyde and superoxide levels (Table 1).

The role of alterations in heat-shock protein (HSP) gene expression as a major factor in the mechanism of the different conditioning procedures has been intensively investigated. Marber *et al* [80] showed that an increase in tissue HSP-70 expression is associated with better ischemic tolerance in a rodent myocardial ischemic reperfusion injury model. Other studies have also verified this observation in different organs such as the liver [81,82] and in the central nervous system [83].

Heat-shock-70 has manifold roles, is possibly a major participant in I-R injuries, and is likely to have a role in the protective subcellular mechanisms of the various procedures. Furthermore, HSP-70 inhibits protein accumulation as a molecular chaperone and facilitates disassembly of the un-reparable proteins [84,85]. In addition, it inhibits the NF- κ B transcription factor [86] and enhances the expression of HSP genes [87].

Nevertheless, the participation of HSPs in relation to R-IPER has not yet been extensively studied, and presents a gap in our knowledge requiring examination.

3. Other favorable effects of remote ischemic preconditioning

Schmidt *et al* [15] reported that remote ischemic preconditioning has a profound effect on the reduction in myocardial infarct size and preservation of left ventricular function. Moreover, a considerable decrease in the frequency of malignant reperfusion arrhythmias was notable in their model. These findings are consistent with those of other studies [16,22,55,88] (Table 1).

A study by Hahn *et al* [20] showed that remote ischemic preconditioning significantly reduced brain infarct size in a rat transient middle cerebral artery occlusion model of acute stroke. These authors observed a potent neuroprotective effect by both remote preconditioning and perconditioning; however, the remote preconditioning treatment resulted in superior neuroprotection compared with R-IPC.

Other studies demonstrated that R-IPER has positive effects on the myocardial no-reflow phenomenon and coronary blood flow during reperfusion [22], ischemia-induced ventricular arrhythmia thresholds [89], vascular endothelial function [24], and cardiac function after valve replacements [17] (Table 1).

4. Models and treatment protocols for remote ischemic preconditioning

The treatment sequence has great importance for research practice. Based on our available knowledge about local preconditioning [90,91] and postconditioning [91], it is assumed that the most important parameters in the preconditioning protocol are timing of the treatment and length of the cycles. Nevertheless, despite the many reports dealing with the length of applied brief I-R cycles, a reliable guideline is still not within reach. An experimental study by Xin *et al* [16] aimed to compare different preconditioning procedures. In their model,

one to two cycles of 10-min remote preconditioning could not provide extra cardioprotection in combination with local postconditioning, similar to treatment performed in four cycles of 1- to 3-min ischemia followed by 1- to 3-min reperfusion. They reported that only three to four cycles of 5-min ischemia followed by 5-min reperfusion resulted in a further decrease in myocardial infarct size. Several other authors confirmed their finding [15,17,22].

The other point at issue concerns optimal timing. Reported treatment protocols involve timing at either the early [17] or late [19,22,25] phase of target organ ischemia, or continuous application during the whole period of the target organ ischemia [15,24]. Each strategy has proved to be effective; yet, the most widely used method is late phase timing, because it appears that during reperfusion, the highest concentration of transportable mediators (with unknown serum half-life) can be achieved by this procedure.

5. Clinical application of R-IPER

As previously discussed, compared with other conditioning techniques, R-IPER does not prolong the operative time. In addition, this noninvasive technique is suitable for application in nonelective ischemic situations, and target organ protection can also be achieved in this setting. Considering the beneficial features mentioned earlier, it is perhaps the most promising protective strategy among the adaptive surgical techniques discussed in this review. The use of invasive exclusion techniques to induce preconditioning—introduced in experimental research—is less acceptable in clinical settings owing to the notable complication rate and risk of large vessel exclusion [92,93]. It can be concluded that the most practical way to induce brief ischemic and reperfusion cycles is to apply simple blood pressure cuffs placed around the proximal part of the limb.

Previous clinical studies in the field of cardiovascular research reported that R-IPER has desirable cardioprotective effects when used as an additional treatment in case of valve replacements [17]. In a blinded, randomized study, Hougaard *et al* [19] aimed to prove R-IPER efficiency in neuroprotection. Their study is currently recruiting patients with acute ischemic stroke at Aarhus University Hospital in Denmark, using R-IPER as an additional therapy combined with regular thrombolysis. Recent studies by Botker *et al* [94] and Munk *et al* [95] confirmed the advantageous properties of this method. In these clinical studies of acute myocardial infarction and ischemic stroke, R-IPER was applied during transport to hospital. In such emergency situations, with the application of this additional procedure, more functionally intact tissue can be spared, with possible better postinterventional outcome and survival (Table 1).

This easy-to-use, low-risk procedure can be applied in every elective as well as unscheduled situation, whenever a greater danger of developing I-R injury exists. Cardiac surgery, coronary bypass surgery, valve replacements, vascular reconstructions, organ transplantations, major liver resections, and invasive cardiology are just a few of the many possibilities for use of this promising method. Remote ischemic preconditioning can be beneficial in organ

transplantation during donor surgery, possibly in combination with remote ischemic postconditioning (or preconditioning), to achieve a higher level of organ protection in the recipient. In their recent study, Wei *et al* [55] described the positive effects of this combination in a rat myocardial infarction model.

6. Conclusions

The protective strategy discussed in this review (remote preconditioning) seems suitable for application in a wide range of clinical settings. Nevertheless, further studies are required before introducing remote preconditioning in everyday clinical practice. The efficiency of this method should also be confirmed by multicentric clinical trials. Further important aspects that need consideration are the fine details of connections between remote and target organs, as well as the subcellular mechanisms required to reach the target points. The end result would be an efficient and practical pharmacologic conditioning technique.

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REFERENCES

- [1] Jennings RB, Sommers HM, Smyth GA, et al. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch Pathol* 1960;70:68.
- [2] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124.
- [3] Na HS, Kim YI, Yoon YW, et al. Ventricular premature beat-driven intermittent restoration of coronary blood flow reduces the incidence of reperfusion-induced ventricular fibrillation in a cat model of regional ischemia. *Am Heart J* 1996;132:78.
- [4] Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol* 2003;285:H579.
- [5] Przyklenk K, Bauer B, Ovize M, et al. Regional ischemic preconditioning protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993; 87:893.
- [6] McClanahan TB, Nao BS, Wolke LJ, et al. Brief renal occlusion and reperfusion reduces myocardial infarct size in rabbits. *FASEB J* 1993;7:A118.
- [7] Gho BCG, Schoemaker RG, vandenDoel MA, et al. Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* 1996;94:2193.
- [8] Oxman T, Arad M, Klein R, et al. Limb ischemia preconditions the heart against reperfusion tachyarrhythmia. *Am J Physiol* 1997;273:H1707.
- [9] Candilio L, Hausenloy DJ, Yellon DM. Remote ischemic conditioning: a clinical trial's update. *J Cardiovasc Pharmacol Ther* 2011;16:304.
- [10] Birnbaum Y, Hale SL, Kloner RA. Ischemic preconditioning at a distance—Reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit. *Circulation* 1997; 96:1641.
- [11] Ren CC, Yan ZM, Wei DT, et al. Limb remote ischemic postconditioning protects against focal ischemia in rats. *Brain Res* 2009;1288:88.
- [12] Andreka G, Vertesaljai M, Szantho G, et al. Remote ischaemic postconditioning protects the heart during acute myocardial infarction in pigs. *Heart* 2007;93:749.
- [13] Jimenez-Navarro MF, Carrasco-Chinchilla F, Munoz-Garcia AJ, et al. Remote ischemic postconditioning: does it protect against ischemic damage in percutaneous coronary revascularization? justification and design of a randomized placebo-controlled clinical trial. *Cardiology* 2011;119:164.
- [14] Xiong J, Liao X, Xue FS, et al. Remote ischemia conditioning—an endogenous cardioprotective strategy from outside the heart. *Chinese Med J* 2011;124:2209.
- [15] Schmidt MR, Smerup M, Konstantinov IE, et al. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a K-ATP-dependent mechanism: first demonstration of remote ischemic preconditioning. *Am J Physiol* 2007;292:H1883.
- [16] Xin P, Zhu W, Li J, Ma SX, et al. Combined local ischemic postconditioning and remote preconditioning recapitulate cardioprotective effects of local ischemic preconditioning. *Am J Physiol* 2010;298:H1819.
- [17] Li L, Luo WJ, Huang LJ, et al. Remote preconditioning reduces myocardial injury in adult valve replacement: a randomized controlled trial. *J Surg Res* 2010;164:E21.
- [18] Saxena P, Newman MAJ, Shehatha JS, et al. Remote ischemic conditioning: evolution of the concept, mechanisms, and clinical application. *J Cardiac Surg* 2010;25:127.
- [19] Hougaard KD, Hjort N, Zeidler D, et al. Remote ischemic preconditioning in acute stroke: An endogeneous model to generate neuroprotection. Presented at the MINDLab AU Symposium, Aarhus, Denmark, 2011.
- [20] Hahn CD, Manlihot C, Schmidt MR, et al. Remote ischemic per-conditioning: a novel therapy for acute stroke? *Stroke* 2011;42:2960.
- [21] Hausenloy DJ, Iliodromitis EK, Andreadou I, et al. Investigating the signal transduction pathways underlying remote ischemic conditioning in the porcine heart. *Cardiovasc Drugs Ther* 2012;26:87.
- [22] Zhao JL, Yang YJ, Pei WD, et al. Remote periconditioning reduces myocardial no-reflow by the activation of K-ATP channel via inhibition of Rho-kinase. *Int J Cardiol* 2009; 133:179.
- [23] Zitta K, Meybohm P, Bein B, et al. Cytoprotective effects of the volatile anesthetic sevoflurane are highly dependent on timing and duration of sevoflurane conditioning: findings from a human, in-vitro hypoxia model. *Eur J Pharmacol* 2010; 645:39.
- [24] Loukogeorgakis SP, Williams R, Panagiotidou AT, et al. Transient limb ischemia induces remote preconditioning and remote postconditioning in humans by a K-ATP channel-dependent mechanism. *Circulation* 2007;116:1386.
- [25] Tsubota H, Marui A, Esaki J, et al. Remote postconditioning may attenuate ischaemia-reperfusion injury in the murine hind limb through adenosine receptor activation. *Eur J Vasc Endovasc Surg* 2010;40:804.
- [26] Xu YC, Xue FS, Liao X, et al. Combined morphine and limb remote ischaemia postconditioning may produce an enhanced cardioprotection. *Med Hypoth* 2009;73:302.

- [27] Tang YH, Xu JJ, Li JX, Cheng XS. Remote postconditioning induced by brief pulmonary ischemia and reperfusion attenuates myocardial reperfusion injury in rabbits. *Chin Med J* 2011;124:1683.
- [28] Hausenloy DJ, Yellon DM. Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc Res* 2008;79:377.
- [29] Lim SY, Hausenloy DJ. Remote ischemic conditioning: from bench to bedside. *Front Physiol* 2012;3:27.
- [30] Shi W, Vinten-Johansen J. Endogenous cardioprotection by ischaemic postconditioning and remote conditioning. *Cardiovasc Res* 2012;94:206.
- [31] Shimizu M, Tropak M, Diaz RJ, et al. Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. *Clin Sci* 2009;117:191.
- [32] Dickson EW, Porcaro WA, Fenton RA, et al. Preconditioning at a distance in the isolated rabbit heart. *Acad Emerg Med* 2000;7:311.
- [33] Dickson EW, Tubbs RJ, Porcaro WA, et al. Myocardial preconditioning factors evoke mesenteric ischemic tolerance via opioid receptors and K-ATP channels. *Am J Physiol* 2002;283:H22.
- [34] Lim SY, Yellon DM, Hausenloy DJ. The neural and humoral pathways in remote limb ischemic preconditioning. *Basic Res Cardiol* 2010;105:651.
- [35] Huffman LC, Koch SE, Butler KL. Coronary effluent from a preconditioned heart activates the JAK-STAT pathway and induces cardioprotection in a donor heart. *Am J Physiol* 2008;294:H257.
- [36] Przyklenk K, Whittaker P. Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities. *J Cardiovasc Pharmacol Ther* 2011;16:255.
- [37] Redington KL, Disenhouse T, Strantzas SC, et al. Remote cardioprotection by direct peripheral nerve stimulation and topical capsaicin is mediated by circulating humoral factors. *Basic Res Cardiol* 2012;107:1.
- [38] Konstantinov IE, Arab S, Li J, et al. The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium. *J Thorac Cardiovasc Surg* 2005;130:1326.
- [39] Kristiansen SB, Henning O, Kharbanda RK, et al. Remote preconditioning reduces ischemic injury in the explanted heart by a K-ATP channel-dependent mechanism. *Am J Physiol* 2005;288:H1252.
- [40] Schoemaker RG, van Heijningen CL. Bradykinin mediates cardiac preconditioning at a distance. *Am J Physiol* 2000;278:H1571.
- [41] Pell TJ, Baxter GF, Yellon DM, Drew GM. Renal ischemia preconditions myocardium: role of adenosine receptors and ATP-sensitive potassium channels. *Am J Physiol* 1998;275:H1542.
- [42] Schulte G, Sommerschild H, Yang J, et al. Adenosine A(1) receptors are necessary for protection of the murine heart by remote, delayed adaptation to ischaemia. *Acta Physiol Scand* 2004;182:133.
- [43] Patel HH, Moore J, Hsu AK, Gross GJ. Cardioprotection at a distance: mesenteric artery occlusion protects the myocardium via an opioid sensitive mechanism. *J Mol Cell Cardiol* 2002;34:1317.
- [44] Tang ZL, Dai W, Li YJ, Deng HW. Involvement of capsaicin-sensitive sensory nerves in early and delayed cardioprotection induced by a brief ischaemia of the small intestine. *Naunyn Schmiedeberg Arch Pharmacol* 1999;359:243.
- [45] Hajrasouliha AR, Tavakoli S, Ghasemi M, et al. Endogenous cannabinoids contribute to remote ischemic preconditioning via cannabinoid CB2 receptors in the rat heart. *Eur J Pharmacol* 2008;579:246.
- [46] Xiao L, Lu R, Hu CP, Deng HW, et al. Delayed cardioprotection by intestinal preconditioning is mediated by calcitonin gene-related peptide. *Eur J Pharmacol* 2001;427:131.
- [47] Wolfrum S, Nienstedt J, Heidbreder M, et al. Calcitonin gene related peptide mediates cardioprotection by remote preconditioning. *Regul Peptides* 2005;127:217.
- [48] Li YJ, Xiao ZS, Peng CF, Deng HW. Calcitonin gene-related peptide-induced preconditioning protects against ischemia-reperfusion injury in isolated rat hearts. *Eur J Pharmacol* 1996;311:163.
- [49] Peralta C, Fernandez L, Panes J, et al. Preconditioning protects against systemic disorders associated with hepatic ischemia-reperfusion through blockade of tumor necrosis factor-induced P-selectin up-regulation in the rat. *Hepatology* 2001;33:100.
- [50] Souza MVP, Loiola RT, Rocha EL, et al. Hind limb ischemic preconditioning induces an anti-inflammatory response by remote organs in rats. *Brazil J Med Biol Res* 2009;42:921.
- [51] Konstantinov IE, Arab S, Kharbanda RK, et al. The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. *Physiol Genom* 2004;19:143.
- [52] Shimizu M, Saxena P, Konstantinov IE, et al. Remote ischemic preconditioning decreases adhesion and selectively modifies functional responses of human neutrophils. *J Surg Res* 2010;158:155.
- [53] Harkin DW, D'Sa A, McCallion K, et al. Ischemic preconditioning before lower limb ischemia-reperfusion protects against acute lung injury. *J Vasc Surg* 2002;35:1264.
- [54] Gyurkovics E, Aranyi P, Turocz Z, et al. Postconditioning attenuates remote organ injury after lower limb arterial occlusion. *Intervent Med Appl Sci* 2010;2:169.
- [55] Wei M, Xin P, Li SA, et al. Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. *Circ Res* 2011;108:1220.
- [56] Wolfrum S, Schneider K, Heidbreder M, et al. Remote preconditioning protects the heart by activating myocardial PKC epsilon-isoform. *Cardiovasc Res* 2002;55:583.
- [57] Hausenloy DJ, Yellon DM. Survival kinases in ischemic preconditioning and postconditioning. *Cardiovasc Res* 2006;70:240.
- [58] Weinbrenner C, Nelles M, Herzog N, et al. Remote preconditioning by infrarenal occlusion of the aorta protects the heart from infarction: a newly identified non-neuronal but PKC-dependent pathway. *Cardiovasc Res* 2002;55:590.
- [59] Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 2003;83:1113.
- [60] Vinten-Johansen J. Postconditioning: a mechanical maneuver that triggers biological and molecular cardioprotective responses to reperfusion. *Heart Failure Rev* 2007;12:235.
- [61] Yang XM, Proctor JB, Cui L, et al. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J Am Coll Cardiol* 2004;44:1103.
- [62] Konstantinov IE, Li J, Cheung MM, et al. Remote ischemic preconditioning of the recipient reduces myocardial ischemia-reperfusion injury of the denervated donor heart via a Katp channel-dependent mechanism. *Transplantation* 2005;79:1691.
- [63] Sanada S, Kitakaze M, Papst PJ, et al. Cardioprotective effect afforded by transient exposure to phosphodiesterase III inhibitors—The role of protein kinase A and p38 mitogen-activated protein kinase. *Circulation* 2001;104:705.
- [64] Dos Santos P, Kowaltowski AJ, Laclau MN, et al. Mechanisms by which opening the mitochondrial ATP-sensitive K+ channel protects the ischemic heart. *Am J Physiol* 2002;283:H284.

- [65] Costa ADT, Jakob R, Costa CL, et al. The mechanism by which the mitochondrial ATP-sensitive K⁺ channel opening and H₂O₂ inhibit the mitochondrial permeability transition. *J Biol Chem* 2006;281:20801.
- [66] Gateau-Roesch O, Argaud L, Ovize M. Mitochondrial permeability transition pore and postconditioning. *Cardiovasc Res* 2006;70:264.
- [67] Zhang SZ, Wang NF, Xu J, et al. Kappa-opioid receptors mediate cardioprotection by remote preconditioning. *Anesthesiology* 2006;105:550.
- [68] Weihrach D, Krolikowski JG, Bienengraeber M, et al. Morphine enhances isoflurane-induced postconditioning against myocardial infarction: the role of phosphatidylinositol-3-kinase and opioid receptors in rabbits. *Anesthes Analg* 2005;101:942.
- [69] Boengler K, Hilfiker-Kleiner D, Heusch G, Schulz R. Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. *Basic Res Cardiol* 2010;105:771.
- [70] Tamarelle S, Mateus V, Ghaboura N, et al. RISK and SAFE signaling pathway interactions in remote limb ischemic preconditioning in combination with local ischemic postconditioning. *Basic Res Cardiol* 2011;106:1329.
- [71] Penna C, Rastaldo R, Mancardi D, et al. Post-conditioning induced cardioprotection requires signaling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K⁺ channel and protein kinase C activation. *Basic Res Cardiol* 2006;101:180.
- [72] Downey JM, Cohen MV. A really radical observation—A comment on Penna et al. in *Basic Res Cardiol* (2006) 101: 180–189. *Basic Res Cardiol* 2006;101:190.
- [73] Penna C, Mancardi D, Rastaldo R, Pagliaro P. Cardioprotection: a radical view. *Free radicals in pre and postconditioning*. *Biochim Biophys Acta Bioenerget* 2009;1787:781.
- [74] Liu Y, Yang XM, Iliodromitis EK, et al. Redox signaling at reperfusion is required for protection from ischemic preconditioning but not from a direct PKC activator. *Basic Res Cardiol* 2008;103:54.
- [75] Hausenloy DJ, Wynne AM, Yellon DM. Ischemic preconditioning targets the reperfusion phase. *Basic Res Cardiol* 2007;102:445.
- [76] Penna C, Mancardi D, Rastaldo R, et al. Intermittent activation of bradykinin B-2 receptors and mitochondrial K⁺-ATP channels trigger cardiac postconditioning through redox signaling. *Cardiovasc Res* 2007;75:168.
- [77] Hausenloy DJ, Duchon MR, Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. *Cardiovasc Res* 2003;60:617.
- [78] Argaud L, Gateau-Roesch O, Raisky O, et al. Postconditioning inhibits mitochondrial permeability transition. *Circulation* 2005;111:194.
- [79] Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* 2004; 61:461.
- [80] Marber MS, Mestril R, Chi SH, et al. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest* 1995;95:1446.
- [81] Kume M, Yamamoto Y, Saad S, et al. Ischemic preconditioning of the liver in rats: implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. *J Lab Clin Med* 1996;128:251.
- [82] Oba M, Suico A, Morino S, et al. Modified mild heat shock modality attenuates hepatic ischemia/reperfusion injury. *J Surg Res* 2010;162:213.
- [83] Nishi S, Taki W, Uemura Y, et al. Ischemic tolerance due to the induction of HSP70 in a rat ischemic recirculation model. *Brain Res* 1993;615:281.
- [84] Jones Q, Voegeli TS, Li G, et al. Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. *Inflamm Allergy Drug Targets* 2011;10:247.
- [85] Giffard RG, Han RQ, Emery JF, et al. Regulation of apoptotic and inflammatory cell signaling in cerebral ischemia —The complex roles of heat shock protein 70. *Anesthesiology* 2008; 109:339.
- [86] Hasegawa A, Iwasaka H, Hagiwara S, Noguchi T. Relationship between HMGB1 and tissue protective effects of HSP72 in a LPS-induced systemic inflammation model. *J Surg Res* 2011; 169:85.
- [87] Kobba S, Kim SC, Chen L, et al. The heat shock paradox and cardiac myocytes: role of heat shock factor. *Shock* 2011;35:478.
- [88] Kregel KC. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Physiol* 2002;92:2177.
- [89] Lujan HL, DiCarlo SE. Partial hindlimb occlusion reduced the susceptibility to sustained ventricular tachycardia in conscious rats. *J Cardiovas Pharmacol Ther* 2009;14:199.
- [90] Wang HG, Li ZY, Liu XL. Effects of various protocols of ischemic preconditioning on rat tram flaps. *Microsurgery* 2008;28:37.
- [91] Skyschally A, van Caster P, Iliodromitis EK, et al. Ischemic postconditioning: experimental models and protocol algorithms. *Basic Res Cardiol* 2009;104:469.
- [92] Banchini E, Villani LG, Capelli P. Dissection of the thoracic aorta following partial clamping. *Int Angiol* 1989;8:157.
- [93] Coelho JCU, Sigel B, Flanigan DP, et al. Arteriographic and ultrasonic evaluation of vascular clamp injuries using an in vitro human experimental model. *Surg Gynecol Obstet* 1982;155:506.
- [94] Botker HE, Kharbanda R, Schmidt MR, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet* 2010;375:727.
- [95] Munk K, Andersen NH, Schmidt MR, et al. Remote ischemic conditioning in patients with myocardial infarction treated with primary angioplasty impact on left ventricular function assessed by comprehensive echocardiography and gated single-photon emission CT. *Circ Cardiovasc Imag* 2010;3:656.