# CONDICIONAMENTO ISQUÉMICO CARDÍACO REMOTO: MECANISMOS DE CARDIOPROTECÇÃO E APLICAÇÕES CLÍNICAS

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## Resumo

Apesar de uma melhoria significativa dos cuidados prestados no âmbito da doença coronária aguda, a mortalidade e morbilidade associadas a esta última continuam significativas. Uma das explicações para esta ainda elevada mortalidade reside no facto de que a própria reperfusão coronária pode paradoxalmente provocar lesão miocárdica adicional, através da denominada lesão de isquemia-reperfusão, comprometendo assim, pelo menos parcialmente, o efeito benéfico da reperfusão miocárdica.

Ao longo das últimas duas décadas, várias intervenções farmacológicas (como o uso de antioxidantes, anti-inflamatórios, magnésio, glicose/insulina/potássio ou a rápida normalização do pH) foram estudadas com o intuito de prevenir a lesão de isquemia-reperfusão. Apesar dos resultados promissores obtidos com a experimentação animal, as tentativas de transposição desses resultados para o ser humano e consequentemente para a prática clínica têm sido desapontadoras.

Por outro lado, o condicionamento isquémico cardíaco é uma intervenção que tem produzido resultados positivos. De forma genérica, o conceito de "condicionamento isquémico" remete para a protecção induzida por curtos períodos de isquemia intercalados por períodos de reperfusão, previamente a um evento isquémico major. Os estímulos isquémicos podem ser aplicados antes (pré-condicionamento), durante (per-condicionamento) ou após (pós-condicionamento) o evento isquémico.

Uma descoberta importante no âmbito da investigação sobre condicionamento isquémico cardíaco, foi a de que a protecção podia ser induzida à distância, introduzindo o conceito de condicionamento isquémico remoto.

No presente trabalho, propusemo-nos rever os mecanismos subjacentes ao condicionamento isquémico cardíaco remoto e abordar as suas aplicações clínicas, considerando mais especificamente o pré- e o per-condicionamento.

## Summary

### Remote cardiac ischemic conditioning: Underlying mechanisms and clinical applications

*Despite a significant improvement in the care of acute coronary disease, mortality and morbidity remain important. One explanation for this lies in the fact that the very coronary reperfusion may paradoxically result in additional myocardial injury, through the so-called ischemia-reperfusion injury, partially mitigating the beneficial effects of myocardial reperfusion.*

*Over the past two decades, numerous pharmacological interventions (such as the use of antioxidants, anti-inflammatory, magnesium, glucose/insulin/potassium, rapid normalization of pH) were studied in order to prevent ischemia-reperfusion injury. Despite the promising results obtained in animal experiments, attempts to transpose these results to humans, and consequently to clinical practice, have been disappointing.*

*On the other hand, cardiac ischemic conditioning is an intervention that has produced positive results. Ischemic conditioning refers to the protection induced by short periods of ischemia followed by reperfusion, prior to a major ischemic event. Ischemic stimulus can be applied before (pre-conditioning), during (per-conditioning) or after (post-conditioning) the major ischemic event.*

*An important finding regarding cardiac ischemic conditioning, was that protection could be induced remotely, introducing the concept of remote ischemic conditioning.*

*In this paper, we proposed to review the mechanisms underlying remote ischemic cardiac conditioning and the possible clinical applications, considering more specifically pre and per-conditioning*

## INTRODUCTION

Coronary heart disease is one of the leading causes of mortality worldwide, accounting for more than 7 million deaths per year (about 12.8% of all deaths)[1]. It is estimated that in Europe every sixth man and every seventh woman will die of acute myocardial infarction (AMI)[2].

However, recent studies and registries have showed a reduction in AMI mortality following the increased use of reperfusion therapy (mainly with percutaneous coronary intervention), newer antithrombotic therapies, and adherence to secondary prevention measures (pharmacological treatment and changes in lifestyle)[3-6]. In the context of AMI with ST segment elevation, rapid restoration of coronary flow is actually the most effective strategy for reducing the infarct size and thus improving prognosis associated with AMI.

Thus, despite a significant improvement in the care of acute coronary disease, mortality and morbidity associated with the latter remain high.

One of the reasons for a mortality rate still so high is that reperfusion itself can paradoxically cause additional myocardial injury, through the so-called ischemia-reperfusion injury (or reperfusion injury), reducing the beneficial effects of myocardial reperfusion[7-9]. Ischemia-reperfusion injury (IRI) has classically been described as causing four main types of cardiac dysfunction: myocardial stunning, the phenomenon of "no-reflow", reperfusion arrhythmia and myocardial necrosis (called "lethal reperfusion injury")[9-12]. IRI may account for up to 40 - 50% of the final infarct size, thereby contributing to an increased mortality and progression to heart failure (Fig. 1)[9]. Several factors have been suggested as contributing to IRI: oxidative stress, changes in pH, calcium overload and acute inflammatory response. These events favour the opening of

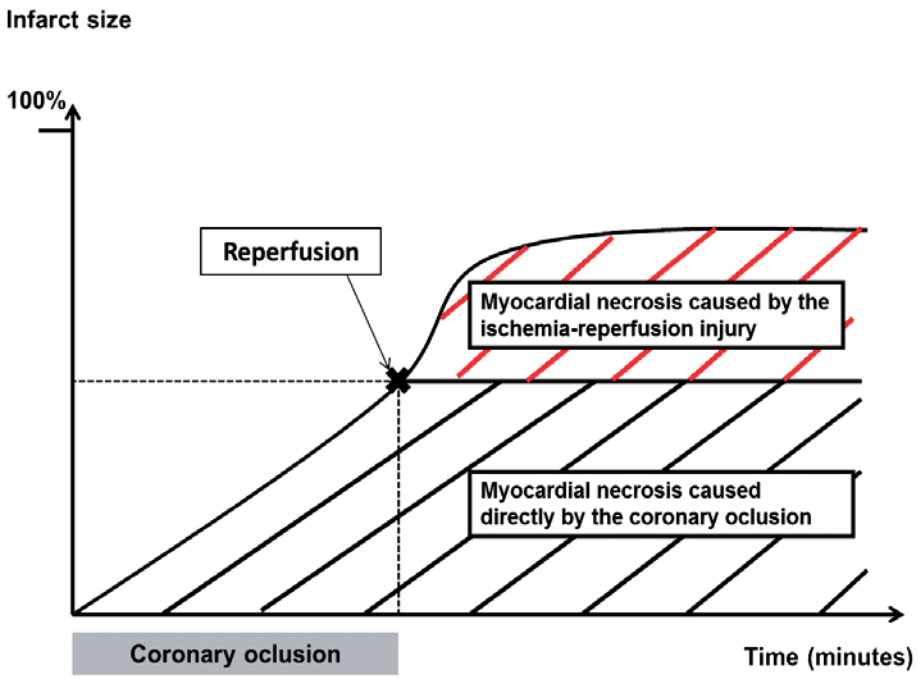


**Figure 1** *Schematic representation of the contribution of ischemia-reperfusion injury to the final size of myocardial infarction.*

the mitochondrial permeability transition pore (mPTP). The mPTP is a non-specific channel located in the inner membrane of the mitochondria whose opening leads ultimately to cell lysis[8, 9, 13-16]. The mPTP is thus a critical determinant of IRI.

Over the past three decades, several pharmacological interventions (use of antioxidants, anti-inflammatory agents, magnesium, glucose/insulin/potassium or rapid normalization of pH) were studied in order to prevent IRI. Despite promising results in animal experiments, attempts to translate those results into humans have been largely disappointing[9,17,18].

On the opposite, cardiac ischemic conditioning is an intervention that has produced positive results, both in animal experiments and in human clinical trials[17-27].

The concept of "ischemic conditioning" refers to the protection induced by short cycles of ischemia and reperfusion prior to a major ischemic event. Various studies have shown that ischemic conditioning is ubiquitous and can be applied in all organs.

Murry et al. first described cardiac ischemic conditioning in a canine model in 1986[28-29]. These authors observed that the application of four 5-minutes cycles of myocardial ischemia (by occlusion of a coronary artery) alternating with 5-minutes of reperfusion could protect the myocardium against a longer period of ischemia. In subsequent years, ischemic conditioning was the subject of intense investigation, both in the description of the underlying cellular mechanisms and the assessment of its effectiveness in various clinical settings[17-27].

Due to the extensive research conducted in the last 25 years, we now know that stimuli may be applied before ischemia (preconditioning - as originally described by Murry et al.), during (per-conditioning) or after (post-conditioning) the major ischemic event, thereby allowing three windows for the application of this therapeutic intervention.

An important discovery about cardiac conditioning was the existence of two windows of cardioprotection. After the initial ischemic stimulus, the protection induced immediately, called "classic ischemic preconditioning", extends over a maximum period of about 1 to 2 hours. Notably, 12 to 24 hours after the initial ischemic stimulus, a second window appears, lasting for 48 to 72 hours, although resulting in a lesser amount of protection[30].

Another remarkable finding about cardiac ischemic conditioning was that cardioprotection could be induced at a distance, introducing the concept of remote cardiac ischemic conditioning.

## DEFINITION

Remote cardiac ischemic conditioning (RCIC) describes the cardioprotection induced by short cycles of ischemia and reperfusion applied to a distant organ before, during or even after a prolonged period of myocardial ischemia.

Przyklenk et al. first described the concept of RCIC (as preconditioning) in 1993[31]. These authors demonstrated, in a canine model, that the application of transient ischemia to the circumflex coronary artery territory lessened the effects of a potentially lethal ischemia subsequently applied to the territory supplied by the anterior descending coronary artery.

Later, in 2002, Kharbanda et al. demonstrated that RCIC could be reproduced non-invasively by applying short periods of ischemia and reperfusion in the forearm, using a blood pressure cuff[32]. Since then, several studies have highlighted the benefits of RCIC in different clinical circumstances[25-27].

## MECHANISMS

Although the mechanisms underlying the immediate cardioprotection of RCIC are not fully understood, studies have shown that some of the signalling pathways activated in cardiomyocytes preconditioned at a distance appear to be similar to the ones recruited in preconditioning and post-conditioning applied directly to the heart[33-36].

However, it is the mechanism linking the remote organ to the heart that is less clear and which constitutes the main difference between the direct and the remote ischemic conditioning[35].

### 1. Communication between the remote organ and the heart

Three main hypotheses, each one favouring a different pathway, were proposed to explain the transmission of a protective signal from the remote organ to the heart.

#### a. Humoral pathway

The observation that a period of reperfusion of the organ submitted to briefs periods of ischemia was necessary for remote cardioprotection to occur, soon raised the suspicion that reperfusion was required to carry a substance previously produced by ischemic preconditioning[37-38]. Later, several studies added arguments to this hypothesis. One study revealed the occurrence of cardioprotection in a rabbit receiving blood from another rabbit previously submitted to local and remote cardiac preconditioning[39]. Another study showed that a pig, receiving a denervated heart and subsequently subjected to ischemia of a limb, continued to benefit from cardioprotection, reinforcing the hypothesis of a humoral factor mediating the cardioprotection[40].

Some of the substances identified as possible humoral factor are opioids, endocannabinoids and adenosine[41-44].

#### b. Neuronal pathway

When considering the neuronal pathway hypothesis, it was proposed that afferent nerve fibres of the remote organ were activated by endogenous substances produced by the ischemic stimulus, with subsequent activation of efferent nerve fibres in the heart. The substances most often involved in the activation of afferent nerve fibres are adenosine, bradykinin and the neurotransmitter CGRP ("calcitonin gene-related peptide")[45-48].

Several studies have pointed the existence of a neuronal pathway for the transmission of the cardioprotective signal and assessed possible candidates for the initial activation of the afferent nerve fibres. In general, the various

studies followed a similar methodology: the role of the nervous system in the signal transmission of cardioprotection was shown by demonstrating its inhibition following the destruction of afferent nerve fibres in the remote organ submitted to the initial ischemic stimulus[45-48].

**c. Systemic pathway**

RCIC has been shown to suppress the inflammatory response and induce a favourable profile of gene transcription in the myocardium, promoting anti-inflammatory and anti-apoptotic responses[49,50]. The understanding of the exact contribution of such responses in the cardioprotection induced by RCIC requires further investigation.

It should be noted that the pathways described above are likely not mutually exclusive but rather interact with each other.

**2. Mechanisms involved in myocardial cardioprotection elicited by remote conditioning**

After the cardioprotective signal has been transmitted from the remote organ to the heart, intracellular mechanisms of signal transduction are recruited to mediate

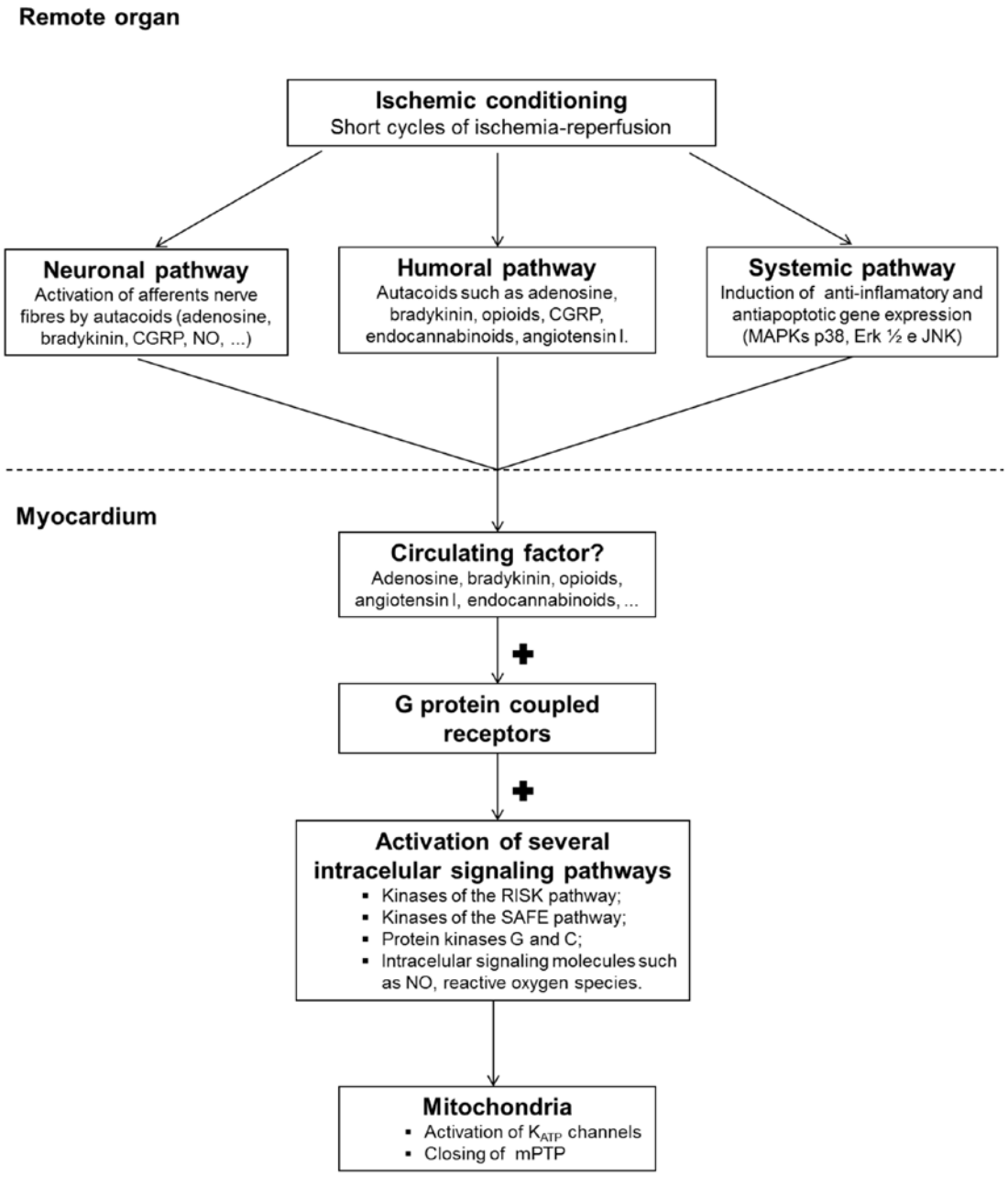


**Figure 2**

*Mechanisms underlying cardiac remote ischemic conditioning.*
*CGRP: calcitonin gene-related peptide; Erk 1/2: extracellular signal-regulated kinases; JNK: Jun N-terminal kinase; KATP channel: ATP-dependent mitochondrial potassium channel; MAPK: mitogen-activated protein kinases; mPTP: mitochondrial permeability transition pore; NO: nitric oxide; RISK: reperfusion injury salvage kinase; SAFE: survivor activating factor enhancement; "+": stimulation / activation.*

cardioprotection. At this point, the mechanisms are similar to the ones underlying ischemic pre and post-conditioning applied directly to the heart[34-36, 51-52].

The first step is the activation of G protein coupled receptors by ligands as adenosine, bradykinin, angiotensin I, opioids and endocannabinoids[41,44,45,53]. The activation of these receptors will in turn lead to the activation of intracellular protein kinases such as protein kinase G, protein kinase C (especially its isoform ε), protein kinases Akt and Erk 1/2 (kinases of the protective RISK pathway - "reperfusion injury salvage kinase") or generation of intracellular signalling molecules such as nitric oxide (NO) or reactive oxygen species[45,54-57]. Another pathway that may also mediate cardioprotection in RCIC, as in classical pre-conditioning, is the SAFE pathway ("survivor activating factor enhancement") that involves the activation of STAT1/3 ("signal transducer and activator of transcription") by TNFα, conferring cardioprotection through induction of anti-apoptotic genes and inhibition of pro-apoptotic ones[58].

The final target of the various intracellular mechanisms seems to be the mitochondria via the activation of the mitochondrial ATP-dependent potassium channel ($K_{ATP}$ channel) and, ultimately, the inhibition of the opening of the mitochondrial permeability transition pore (mPTP)[51,59-62]. As mentioned above, the mPTP is a non-specific channel located in the inner membrane of the mitochondria. Its opening leads to an equilibrium of $H^+$ ions on each side of the membrane with the consequent loss of the membrane potential necessary for ATP production. The mPTP also allows the entry of water into the mitochondria, causing its dilation and ultimately cell lysis. The opening of the mPTP is favoured by increased intracellular concentration of $Ca2^+$, inorganic phosphate and reactive oxygen species, which occurs after reperfusion[9]. The various intracellular signalling pathways mentioned previously inhibit mPTP opening, preventing the cascade of events leading to the death of the myocyte[51,59-61].

## 3. DELAYED CARDIOPROTECTION OR SECOND WINDOW OF PROTECTION

As in cardiac conditioning induced locally, RCIC seems to induce two windows of protection, one immediate and one delayed[33,62].

The mechanisms underlying the delayed response are very similar to those described for the immediate response: communication between the remote organ and the heart, triggers recruiting mediators which in turn lead to the activation of the effector of cardioprotection (most probably the mPTP)[62].

The main difference between the delayed and immediate responses lies in the recruitment of early mediators (protein kinase C, protein tyrosine kinase, MEK 1/2-Erk 1/2,...), which activate transcription factors (STAT 1/3, NFκB ("nuclear factor kappa-light-chain enhancer of activated B-cells"), AP-1 ("activator-protein-1"), Nrf2 ("nuclear factor--like 2 '),...). These transcription factors induce the synthesis of distal mediators 12 to 24 hours later (inducible NO synthase, heat shock proteins, cyclooxygenase 2). These mediators finally recruit the final effectors of cardioprotection (mitochondrial $K_{ATP}$ channel and mPTP)[62].

The intermediate steps described above explain the observed delay of 12 to 24 hours until the beginning of the second window of protection.

The proposed mechanisms underlying RCIC are summarized in Fig. 2.

## CLINICAL APPLICATIONS

When we considered the ischemic preconditioning, the need to apply the stimulus prior to the ischemic heart injury limits its application to situations in which myocardial injury is predictable. Thus, the ideal context for the application of ischemic preconditioning of the heart is elective interventions associated with myocardial injury such as cardiac surgery and percutaneous coronary angioplasty.

On the other hand, ischemic per-conditioning begun to be studied in the last 5 years, as an adjunct to angioplasty in the context of AMI with ST segment elevation.

### 1. Cardiac surgery

Perioperative myocardial injury after cardiac surgery (evidenced by the elevation of biomarkers of myocardial necrosis such as troponin I or T) is associated with worse prognosis. As a result, the prevention of the former is of great importance[63-66].

Cardiac surgery for the correction of congenital defects in children was the first clinical setting in which remote cardiac ischemic pre-conditioning (RCIPreC) has been studied[23]. In the study by Cheung and co-workers, a protocol of remote ischemic preconditioning (four 5-minute cycles of inflation and deflation of a blood pressure cuff applied to the thigh) allowed the reduction of perioperative myocardial injury (demonstrated by the lower elevation of troponin I), need for inotropic support and airway resistance[23].

In the context of coronary artery bypass surgery (CABG) in adults, Hausenloy et al. were the first to demonstrate that RCIPreC (induced by three 5-minute cycles of inflation and deflation of a blood pressure cuff applied to the arm) could lead to a reduction of perioperative myocardial injury[24]. Subsequently, several studies have confirmed the cardioprotective effect of RCIPreC in CABG surgery[67-68].

However, one of the biggest studies performed afterward in the setting of CABG showed negative results, failing to demonstrate beneficial effects of a remote ischemic preconditioning protocol (three 5-minute cycles of inflation and deflation of a blood pressure cuff applied to the arm). Some hypotheses have been evoked to explain the negative results of this study: the inclusion of patients with recurrent angina (unintentionally preconditioned), nitrate use, the anesthetic protocol and inefficiency of remote ischemic preconditioning protocol[69].

Two large trials (ERICCA and RIPHeart), with samples large enough to demonstrate possible benefits of RCIPreC in regard to hard clinical events, are currently under recruitment. These studies will bring more definitive answers regarding the clinical utility of RCIPreC in the setting of CABG.

**2. Elective percutaneous coronary angioplasty**

As shown for cardiac surgery, the occurrence of myocardial injury in the setting of elective percutaneous coronary intervention (PCI) is associated with worse prognosis, making necessary the utilization of effective strategies for its prevention[70-73].

The first clinical study evaluating RCIPreC for elective PCI did not show any benefit, possibly due to an inadequate remote ischemic conditioning protocol[74]. However, in a subsequent study conducted by Hoole et al. (CRISP trial), a protocol of remote ischemic preconditioning (three 5-minute cycles of inflation and deflation of a blood pressure cuff applied to the arm) resulted in a lower troponin T elevation after the procedure[25]. Interestingly, the same authors did not demonstrate benefits of RCIPreC in reducing myocardial dysfunction in patients with coronary heart disease submitted to dobutamine stress echocardiography[75].

We are currently missing large-scale trials to evaluate a possible impact on the prevention of adverse clinical events.

**3. Acute myocardial infarction with ST-segment elevation**

Despite intense investigation on cardiac ischemic conditioning for over two decades, only recently protocols of remote ischemic conditioning have been tested in the setting of AMI with ST segment elevation[26-27].

In 2010, Rentoukas et al. were the first to present the results of a randomized trial testing the effect of a protocol of remote cardiac ischemic per-conditioning (RCIPerC), with or without morphine, in the setting of AMI with ST-segment elevation. In this study, 96 patients were randomized into 3 groups: RCIPerC (three cycles of 4-minute inflations and deflations of a blood pressure cuff placed on the upper arm; inflation to 20 mmHg above systolic pressure and deflation to 0 mmHg), RCIPer with morphine (administered prior to per-conditioning) or control treatment (three cycles of 4-minute inflation to 20 mmHg below diastolic blood pressure of a blood pressure cuff placed on the upper arm and deflation to 0 mmHg). Overall, a complete resolution of ST elevation was achieved more often in patients receiving any form of RCIPerC. The group that received RCIPerC with morphine had a greater reduction in ST elevation and lowest elevation of troponin I in relation to the control group while the patients undergoing RCIPerC alone had a non significant trend for greater reduction of ST elevation and lower elevation of troponin I[26].

In 2010, Botker et al. published the results of the largest randomized study conducted so far to evaluate the effects of RCIPerC in the setting of AMI with ST-segment elevation. In this study, 333 patients were randomized to RCIPerC (four 5-minute cycles of inflation to 200 mmHg and deflation to 0 mmHg of a blood pressure cuff applied to the arm) or control treatment (deflated cuff placed on the arm). Although only 142 of the 333 patients initially randomized were analysed, a higher rate of myocardial salvage was observed among patients undergoing RCIPerC (0.75 vs. 0.55, $p = 0.0333$). The authors also showed that the beneficial effects of RCIPerC were more pronounced in patients presenting with more extensive EAM, particularly those related to the occlusion of the left anterior descending.

Studies of larger scale are needed for assessing the effect of RCIPerC on the incidence of more relevant clinical adverse events.

## CONCLUSION

The mortality associated with coronary heart disease has been declining in recent years as a result of the greater use of revascularization (both percutaneous and surgical) and the improvement of antithrombotic treatment. However, the in-hospital mortality recorded in European countries remains high, ranging between 6 and 14%[2]. One factor that may contribute to this still significant mortality is the IRI. The IRI may contribute to up to 40 to 50% of the final infarct size, thereby facilitating the development of heart failure with the significant mortality and morbidity associated to it[9].

Remote ischemic conditioning is a safe, non-invasive and very low cost intervention with the potential to reduce the morbidity and mortality associated with coronary heart disease. Until now, extensive investigation has been conducted with positive results. In the past 5 years, several studies have evaluated the potential beneficial effects of RCIC in human, once again with encouraging results. However, we are lacking large-scale studies to verify the clinical impact of RCIC, namely for the reduction of clinically relevant adverse events. These studies may confirm RCIC as a clinically useful tool or confine it to laboratory studies.

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