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INVITED REVIEW

The RISK pathway and beyond

Xavier Rossello[1,2] · Derek M. Yellon[1]

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Abstract Research on cardioprotection has attracted considerable attention during the past 30 years following the discovery of ischemic preconditioning with great advances being made in the field, particularly in the description of the molecular signalling behind this cardioprotective intervention. In a time when basic research is struggling to translate its findings into therapies in the clinical setting, this viewpoint has the intention of presenting to clinical and basic scientists how the reperfusion injury salvage kinase pathway has been described and dissected, as well as highlighting its relevance in cardioprotection.

Keywords Cardioprotection · Ischemia/reperfusion injury · Ischemic preconditioning · RISK pathway

Abbreviations

AMI	Acute myocardial infarction
IPC	Ischemic preconditioning
IRI	Ischemia/reperfusion injury
IS	Infarct size
MPTP	Mitochondrial permeability transition pore
RIC	Remote ischemic conditioning
RISK	Reperfusion injury salvage kinase
SAFE	Survivor Activating Factor Enhancement

✉ Derek M. Yellon
d.yellon@ucl.ac.uk

[1] The Hatter Cardiovascular Institute, University College London, 67 Chenies Mews, London WC1E 6HX, UK

[2] Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain

The clinical problem in acute myocardial infarction

Time is muscle in patients undergoing acute myocardial infarction (AMI): the less time the coronary artery is occluded, the smaller the infarct size (IS) and the better the outcome for the patient [9, 17]. Although myocardial reperfusion is essential to salvage viable myocardium, it comes at a price in terms of myocardial reperfusion injury which paradoxically also damages the vulnerable post-ischemic myocardium. Studies in animal models of AMI suggest that reperfusion injury may account for a significant contribution to the final myocardial IS [45]. Therefore, targeting myocardial injury using therapies aimed to protect the heart against ischemia/reperfusion injury (IRI), known as cardioprotective therapies [33], remains one of the top ten unmet clinical needs in cardiology [7].

Ischemic preconditioning: the starting point

Murry et al. published a seminal study demonstrating that several short cycles of non-injurious ischemia and reperfusion significantly protected from a subsequent sustained ischemic insult [29]. This phenomenon, whereby the myocardium can endogenously be protected from lethal IRI, was defined as "ischemic preconditioning" (IPC). This finding, firstly described in dogs, has been subsequently replicated in numerous pre-clinical studies [41], as well as in other organs [42] and in man [43]. The concept of IPC has evolved into "ischemic conditioning", a broader term that encompasses a number of related endogenous cardioprotective strategies, applied either to the heart (ischemic preconditioning or postconditioning) or from afar (remote ischemic pre-, per- or postconditioning).

Although the translational potential of IPC is inevitably limited by the necessity to apply the intervention before the index ischemia, which is unpredictable in AMI patients, this observation was still of significant importance for two main reasons: (1) infarct size was demonstrated to be potentially modulated through an endogenous mechanism, still considered the most powerful cardioprotective therapy to date, and (2) IPC triggered more than three decades of research [11] in which significant advances have been made in our understanding of the mechanisms underlying IRI and IPC and therefore in the potential development of cardioprotective therapies.

Origins of the finding of the RISK pathway: necrosis vs apoptosis

During the 90s, the focus of the research on cardioprotection attributed to different types of cellular death resulting from IRI, namely—both apoptosis and necrosis. This was evidenced by terminal deoxynucleotidyl transferase dUTP nick-end labelling and triphenyl tetrazolium chloride staining, respectively [26]. Briefly, necrosis is the form of cell death that occurs following severe cellular damage and includes uncontrolled disruption of organelles, membrane rupture, and does not require adenosine 5′-triphosphate (ATP) [21]. On the other hand, apoptosis is an ATP-dependent programmed cell death that involves cytochrome-c release from injured mitochondria or autocoid cell-surface receptor (Fas Ligand) activation, followed by the downstream propagation of the signal via caspases and other signalling proteins. These in turn cause the formation of nonselective pores in the outer mitochondria or the opening of the mitochondrial permeability transition pore, as well as in DNA cleavage and nuclear degradation [21]. Unlike necrosis, apoptosis does not result in the release of cellular content into the extracellular milieu.

From this early period, pro-apoptotic proteins were the subject of study to try and develop new targets against IRI based upon the hypothesis that it would be possible to salvage cardiomyocytes already committed to die when the signal of programmed cell death is potentially interrupted. It was therefore demonstrated that inhibiting caspases, at the time of reperfusion, limited infarct size in animal models [28]. Besides reducing cell death through the inhibition of pro-apoptotic caspases, the focus was also on using growth factors to antagonize the apoptotic process through the activation of pro-survival proteins—most notably the PI3K and ERK 1/2 pro-survival kinases as a means of protecting ischemic and reperfused myocardium [2, 44].

This leads to the so-called "Reperfusion injury salvage kinase (RISK) pathway" which was first described by Yellon's group in 2002 whilst assessing the mechanisms underlying the cardioprotective effect induced by urocortin [34]. The use of this growth factor reduced myocardial infarct size and increased the phosphorylation of ERK 1/2 when administered upon reperfusion, these effects being abolished by the co-administration of PD98059 (ERK 1/2 inhibitor) also at reperfusion. The RISK pathway, which is actually a combination of two parallel cascades, PI3K-Akt and MEK1-ERK1/2, was thoroughly dissected through a series of subsequent pharmacological studies where the protective effect of several interventions was blocked with the co-administration of both PI3K and ERK inhibitors at different time-points [14]. In its broadest term, the RISK pathway refers to a group of pro-survival protein kinases, which confer cardioprotection when activated specifically at the time of reperfusion [14, 34].

Relevance of the RISK pathway

The importance of this pathway is based upon three concepts:

1. The short-term activation of its kinases is protective

The recruitment of pro-survival kinases are protective when *acutely* activated, whilst their chronic activation would be considered to be harmful due to their growth-inducing effect; the chronic activation of the PI3K-Akt cascade is deleterious, as it induces cardiac hypertrophy [30]. Furthermore in the clinical setting, ERK and Akt have been demonstrated to be chronically activated in the failing heart [10]. Importantly, the heart seems to have developed an ability to control phosphorylation of these kinases by the activation of PTEN which has been described as an important "switch" for controlling the growth-induced pathways [27, 31]. To put this in context, acutely activating this survival pathway is ideally suited to the setting of AMI patients who would only require a one-off intervention, at reperfusion, to protect the heart from IRI.

2. This pathway must be activated at the time of early reperfusion for a given cardioprotective therapy to protect against IRI

Following an IPC stimulus, the activation of the RISK pathway was demonstrated to occur at two time-points, following a biphasic pattern response [33, 45]: (1) during the preconditioning cycles, prior to the index ischemic episode, this phase known as the "trigger phase", and (2) during the onset of reperfusion, known as the early phase of reperfusion. It is believed that the underlying target for protection against reperfusion injury is the mitochondrial permeability transition pore (MPTP) which opens within the first 15 min of

reperfusion [12]. Importantly, the link between the RISK pathway and the MPTP has been shown to occur in rat myocytes [5]. Equally relevant is the lack of protection observed when the MPTP is targeted following 15 min of reperfusion with agents that directly affect the pore such as cyclosporin, sanglifehrin and insulin [12, 20]. The importance of the activation of intracellular mediators at the onset of reperfusion was unknown 15 years ago and has substantial clinical implications—this molecular signalling can potentially be mimicked by pharmacological agents to produce benefits for patients undergoing myocardial IRI.

3. The RISK pathway is a universal signalling cascade for cardioprotection

The RISK pathway may be recruited not only by ischemic conditioning, but also by other pharmacological agents such insulin, bradykinin adenosine or statins [33, 45]. It is therefore considered a universal signalling cascade, or a common pathway, shared by most cardioprotective therapies [15].

RISK and other important pro-survival pathways

Most of the experimental evidence involving the RISK pathway have been carried out in small rodent models of AMI, whereas its central role in large animals is less well-established [8, 36]. Whilst the link between IPC and RISK activation has been demonstrated in human atrial trabeculae [35], its role in remote conditioning is still at an early phase in both large animal models [13, 37] and humans [18].

In addition to the RISK pathway, other signalling cascades have been suggested to mediate the IPC-induced protective effect [16]: the Survivor Activating Factor Enhancement (SAFE) and the NO/PKG pathway. Lecour et al. demonstrated that the administration of TNF-α before index ischemia (used as pharmacologic IPC-mimetic) was cardioprotective without involving the RISK signalling cascade [25]. Four years later, they described that the administration of TNF-α at reperfusion was recruiting an alternative pathway, coined as the SAFE pathway [22–24], and they also linked the activation of this pathway with preconditioning [39]. In humans, it seems that STAT5, instead of STAT3, may play a relevant role in cardioprotection [6, 18]. A third signalling cascade based on the protein kinase G (PKG) and involving nitric oxide has been also proposed to mediate cardioprotection [4].

The ability to manipulate and up-regulate pro-survival kinase cascades during the early reperfusion phase provides a potential approach to limiting IRI-induced cell death. Indeed, the use of pharmacological agents targeting such pathways is a feasible intervention which can be applied at the onset of myocardial reperfusion for patients presenting with an ongoing AMI*, either in the ambulance or the cath lab. Therefore, s*trategies enhancing these pro-survival pathways are an attractive target to develop adjuvant therapies to be used alongside cardiac catheterization. The synergistic activation of several pathways using combination

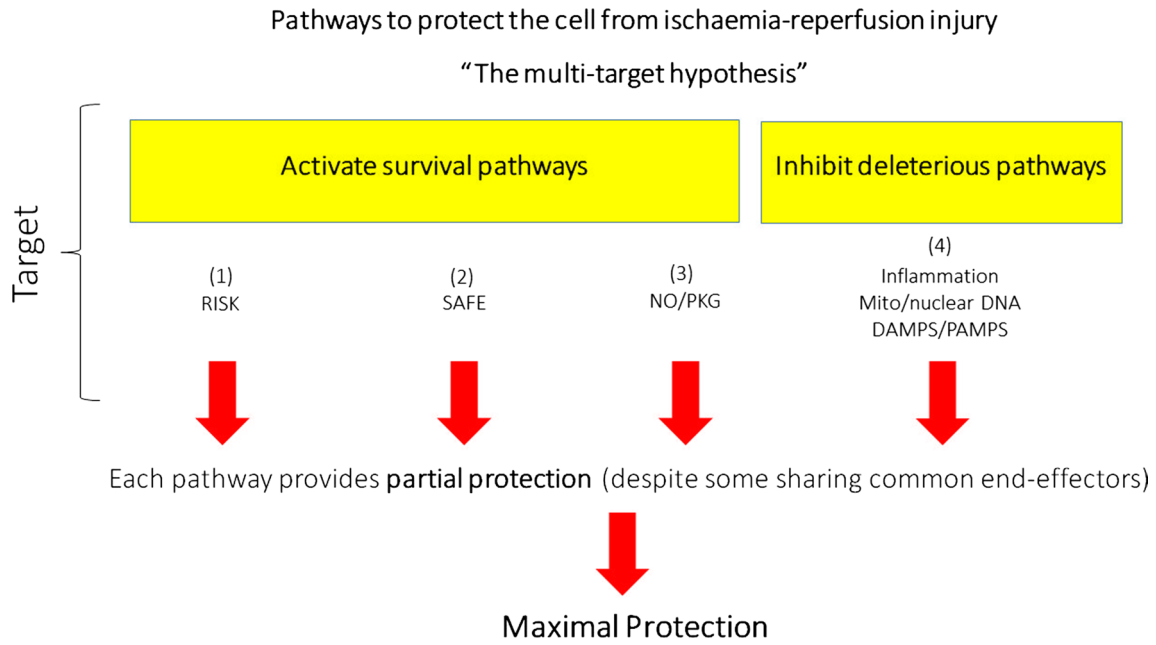

Fig. 1 The multi-target hypothesis. *DAMP* danger-associated molecular patterns, *PAMP* pathogen-associated molecular patterns, *RISK* reperfusion injury salvage kinase, *SAFE* Survivor Activating Factor Enhancement

therapies represents an attractive target in this context. In the experimental setting, matrix metalloproteinase inhibition has been demonstrated to be protective against IRI through an MPTP-independent pathway, and more importantly, to provide an additive effect to the protection observed following inhibition of MPTP opening [3]. Based on the assumption that insulin and exenatide activate cardioprotective pathways different from those of remote ischemic conditioning (RIC), both therapies have also demonstrated to provide additive effects with RIC on infarct size reduction in pigs [1]. Others have demonstrated that targeting two specific isoforms of PKC (a combined treatment with an ε-PKC activator before ischaemia and δ-PKC inhibitor at the onset of reperfusion) has been proved to induce greater protection against IRI than the treatment with each peptide alone [19]. Moreover, we believe that the RISK pathway as well as the other survival pathways (and their combinations) are by no means the singular route to cardioprotection. To obtain maximum protection, there is still a need to assess complementary and parallel pro-survival mechanisms that can potentially be targeted simultaneously—i.e. mitochondrial and Genomic DNA and the activation of the inflammasome complex, which would target other types of cell death including pyroptosis and necroptosis, both of which are forms of inflammatory cell death [38, 40]. The figure illustrates this "multi-target hypothesis" (Fig. 1).

As such, future research should focus not only on improving the potency of the RISK pathway kinases (i.e. assessing the impact of the relevant isoforms for each kinase [32]) but also defining alternative pathways and new mechanisms to study the synergistic effect of combination therapies which gives the cell its best possible chance of survival.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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