HIGHLIGHTED TOPIC | Perspectives in Innate and Acquired Cardioprotection

Past and present course of cardioprotection against ischemia-reperfusion injury

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Liem DA, Honda HM, Zhang J, Woo D, Ping P. Past and present course of cardioprotection against ischemia-reperfusion injury. J Appl Physiol 103: 2129–2136, 2007. First published August 2, 2007; doi:10.1152/japplphysiol.00383.2007.—Despite tremendous advances in cardiovascular research and clinical therapy, ischemic heart disease remains the leading cause of serious morbidity and mortality in western society and is growing in developing countries. For the past 5 decades, many scientists have studied the pathophysiology of myocardial ischemia-reperfusion (I/R) injury leading to infarction. With the exception of reperfusion therapy, attempts to salvage the myocardium during an acute myocardial infarction showed disappointing results in directly decreasing infarct size. Nevertheless, the phenomena of ischemic preconditioning and ischemic postconditioning show a consistent and robust cardioprotective effect in every used experimental animal model. As a result, many studies have focused on the intracellular protective signaling pathways that are involved in preconditioning and postconditioning. More recently, it has been suggested that components of the reperfusion injury salvage kinases pathway, protein kinase B, and the extracellular signal-regulated kinases can induce cardioprotection against I/R injury when they are activated during the postischemic reperfusion period. In addition, inhibition of mitochondrial permeability transition during postischemic reperfusion also shows a strong cardioprotective effect against I/R injury. The present mini-review highlights a short summary of the historical and present course of research into cardioprotection against myocardial I/R injury.

postischemic reperfusion injury; mitochondrial permeability transition

ISCHEMIC HEART DISEASE, which results from an occlusion of one of the major coronary arteries, is currently still the leading cause of morbidity and mortality in western society. It is considered a global burden and is expected by the World Health Organization to surpass infection diseases and become the leading cause of mortality in the whole world by the year 2020 (48). Coronary artery occlusion is the main cause of acute myocardial infarction (AMI), which can lead to pump failure of the heart and arrhythmias, often leading to sudden death. Despite impressive strides in diagnosis and management of AMI over the last three decades, AMI continues to be a major public health problem. In the United States, nearly 1.5 million patients annually suffer from AMI. About 20% of total deaths in the US are caused by ischemic heart disease, the majority occurring before the patient is able to reach a hospital. Because of the formation of coronary care units in the 1960s and improved therapeutic interventions, the in-hospital death rate from AMI has declined. However, impaired myocardial contractility and heart failure, which is often the result of severe AMI, have significantly increased morbidity. The main treatment of AMI is reperfusion therapy, either by thrombolysis therapy, angioplasty, or surgical bypass grafting. Nevertheless, reperfusion therapy during AMI is often too late to prevent tissue damage, which often leads to an impaired pump function of the heart due to severe infarct size. Accordingly, the search for means to protect the heart against ischemia during coronary occlusion has been going on for nearly 50 years, both in a clinical setting and in basic research. Over the past decades, basic cardiovascular research into the pathophysiology of myocardial ischemia-reperfusion (I/R) injury and means of limiting infarct size has shifted from a classical physiology approach, using in vivo animal models to study myocardial infarction at a whole organ level, towards a molecular cellular biology and proteomics approach. More currently, many studies are focused on understanding the underlying intracellular mechanisms and finding novel signal transduction complexes of cardioprotection. The current mini-review will concisely describe the early physiology studies of AMI and attempts to limit infarct size. We will further describe the present course and understanding of cardioprotection against I/R injury.

EARLY EFFORTS TO LIMIT MYOCARDIAL INFARCT SIZE

Ischemia in the heart was first described and defined by the German pathologist Rudolf Virchow in 1858, and it is characterized by an imbalance between myocardial oxygen supply...
and demand due to a decrease of blood flow secondary to narrowing of the coronary artery (66). In the second half of the 20th century, cardiovascular scientists became aware that infarct size due to ischemic injury was strongly correlated with mortality and development of heart failure. Accordingly, in 1971 a study by Maroko et al. (43) proposed the at that time revolutionary idea that myocardial tissue during AMI was not predestined to result in cell death at onset of ischemia, but rather could be salvaged by a therapeutic intervention which was applied during ischemia. Consequently, the authors investigated possible strategies that could limit the development of cell death during AMI. In this first study, monitoring epicardial ST segment elevation as measured in an electrocardiogram was used as an indicator of ischemic injury in open chest anesthetized dogs, and Braunwald and Klomer further demonstrated that myocardial oxygen demand, modified by the use of β-blockers, was a major determinant of the severity of ischemic injury (43). Nevertheless, it was not known whether the reduced ST segment sum represents tissue salvage. In addition, Reimer and colleagues proposed the first myocardial infarct size model in the open chest anesthetized dog using a direct measure of cell death after AMI (58, 59). Subsequently, all previous and promising results in the ST segment model suggesting that certain pharmacological agents such as β-blockers could limit ischemic injury failed to show a protective effect on infarct size when myocardial cell death was directly measured (29). Pioneering work in the pathophysiology of ischemia and AMI by Reimer and Jennings and colleagues (58, 59) further revealed that the size of infarction is determined by 1) the duration of ischemia; 2) the level of collateral blood flow; and 3) the area at risk, i.e., the tissue area that was rendered ischemic. In addition, they showed that cell death during AMI starts from the subendocardium, due to a lower collateral flow in this area, and progresses to the outer ventricular wall.

It became apparent that reperfusion of the ischemic myocardium by restoration of the occluded coronary artery was able to significantly limit the total amount of cell death due to AMI. These observations directly resulted in the concept of reperfusion therapy by lysis of coronary thrombi with thrombolytic therapy with streptokinase (14). Two years after the first thrombolytic therapy study, Rentrop et al. (60) disrupted the first coronary thrombus in an acutely occluded artery using a guide wire passed through a catheter. These two studies in the late seventies are the foundation of modern reperfusion therapy of AMI with either thrombolysis or primary percutaneous transluminal coronary angioplastie, which have currently become the standard in-hospital treatment for AMI together with surgical bypass grafting.

**PRECONDITIONING THE HEART WITH ISCHEMIA**

With the exception of reperfusion therapy, most pharmacological attempts to salvage the myocardium during AMI with a therapeutic intervention applied during ischemia have failed to directly show decreased amount of cell death (56). However, the first indication that the heart can adapt itself after repeated ischemic stress was demonstrated in porcine myocardium, where lactate release in a subsequent I/R episode was significantly lower compared with lactate release after the first episode of I/R (65). Accordingly, in 1986 Reimer and Jennings published a landmark article in which they observed in anesthetized dogs that four repetitive 5-min periods of regional ischemia induced an extremely powerful protection against a subsequent lethal ischemic period in anesthetized dogs. Infarct size after 40 min of ischemia was limited from 29% to 7% with preconditioning (Murry et al., 49). The investigators concluded that they had discovered a new phenomenon of myocardial protection against I/R injury and termed it “ischemic preconditioning” (IPC). It was found that IPC is comprised of two separate phases of cardioprotection against lethal ischemia (41, 42). The first window of protection (often referred to as “classical ischemic preconditioning”), as originally reported by Reimer and Jennings, develops very early within a few minutes after the preconditioning stimulus and lasts only 1–2 hours before the effect fades away (49). The second window of protection develops more slowly, 12–24 hours after the preconditioning stimulus, but lasts much longer, for 3–4 days. The underlying mechanisms of these two phases are different. The first window of protection is initiated by posttranslational modifications of proteins that are already present, whereas the second window is mediated by synthesis of new proteins that have a cardioprotective effect (10).

Although IPC was originally described as a protective response of the heart to nonlethal short bursts of ischemia, it was soon also discovered that several other nonischemic (and thus clinically relevant) stimuli can also elicit cardioprotection against I/R injury. These include interventions such as heat stress, ventricular stretch, ventricular pacing, cytokines, and physical exercise, which all have been found to be very cardioprotective (56) and work via similar signaling mechanisms as IPC (56). Furthermore, the elucidation of signaling molecules that mediate IPC allowed for the identification of a significant number of pharmacological agents that can act as preconditioning mimetics. It soon became clear that the underlying mechanisms of cardioprotection by nonischemic stimuli are similar to that of IPC (56).

**MECHANISM OF ISCHEMIC PRECONDITIONING**

During the first window of protection, the short episodes of preconditioning ischemia lead to the release of signaling molecules. One of several triggers is adenosine, which binds to its Gi-coupled receptor protein and transmits its preconditioning signal, eventually leading to translocation of PKC, which then opens mitochondrial ATP-sensitive potassium (mKATP) channels. Opening of mKATP channels can protect the mitochondria from Ca2+ overload and prevent cytochrome c loss (21, 39). In addition, mild uncoupling of mitochondrial respiration may be protective (45). Other released triggers such as bradykinin, acetylcholine, noradrenaline, epinephrine and opioids cause the transactivation of epidermal growth factor receptors, which is followed by the activation of phosphatidylinositol 3-kinase (PI3-K), which in turn causes activation of protein kinase B (Akt) through phosphorylation. Akt activation results ultimately in opening of mKATP channels. As potassium enters the mitochondria, it causes them to release free radicals, known as reactive oxygen species (ROS; see Ref. 19). Although a large burst of ROS is known to lead to cell damage, a moderate release of ROS, which occurs during nonlethal short episodes of ischemia, can play a significant triggering role in the signal transduction pathways of IPC (63). PKC has been considered
as a central key player in the signal transduction pathways of IPC (34). There are several isoforms, but PKCε has been shown to be part of cardioprotective signal transduction, as was shown by pharmacological inhibition and by a functional proteomics approach (53, 54).

The second window of protection (SWOP) is even more complex in its development. The main triggers for the SWOP that are released by the initial ischemia are nitric oxide (NO), adenosine, and ROS, which set in motion a series of signals that lead to synthesis of new cardioprotective proteins such as NO synthase, cyclooxygenase-2, and superoxide dismutase (10). The release of these triggers activates two major signal transduction pathways. One leads to the activation of PKC, and the other to activation of the Janus-activated kinases 1 and 2 and also of STAT. Both major pathways result in the activation and stress-responsive transcription factors such as NF-κB. The complete story is far from being told and is addressed elsewhere in greater detail (10, 69).

More recently, Vinten-Johansen and colleagues demonstrated that brief repetitive ischemic periods of ischemia followed by reperfusion after the lethal ischemic period also results in a robust infarct size limitation, a phenomenon which is termed as “ischemic postconditioning” (IPO; Zhao et al., 75). Several studies have shown that similar pathways and triggers as seen in classical IPC may be involved in IPO. For instance, classical ligands such as adenosine, opioids, and NO have been reported to be triggers in IPO, whereas activating the mKATP channel openers and PKC pathways may be evoked after the trigger phase (76). More recently, the cardioprotection by both IPC and IPO has been associated with important targets during postischemic reperfusion (28).

**POSTISCHEMIC REPERFUSION INJURY**

As reperfusion therapy of the ischemic myocardium became a standard treatment for AMI, it soon became a subject of debate if all tissue injury occurs during the ischemic period, or whether reperfusion of the ischemic area paradoxically is the culprit for tissue injury (12). The effects of reperfusion are complex and may include several deleterious effects (44). The major mediators of postischemic reperfusion injury are ROS, dysregulation of intracellular Ca²⁺ overload, and neutrophil accumulation (44, 52).

ROS can be generated from injured cells within the ischemic zone, but also from activated neutrophils that enter into the ischemic zone. Many studies have supported the notion that ROS can generate cell injury when oxygen is reintroduced during reperfusion after a lethal ischemic period. Soon it was observed that administration of ROS scavengers can improve several aspects of reperfusion injury (70) and can even result in a reduction of infarct size (35). The protective effect could be seen only when ROS scavenger administration was started 15 minutes prior to reperfusion. Several studies have identified that the ROS burst is at its peak at 10–20 s following reperfusion (1, 79). During myocardial I/R injury, the normal cell balance is lost, leading to elevated hydrogen peroxide levels. The main source of mitochondrial ROS are from the mitochondrial inner membrane (complex I–III), the mitochondrial matrix (dehydrogenases), and the outer membrane (monoamine oxidase) (78). Subsequently, hydroxyl radicals can be produced via the Fenton reaction in the cytosol, which uses ischemia-induced free metal ions (30). Hydroxyl radicals are extremely reactive and may cause direct cell membrane damage, lipid peroxidation, and damage to sulfhydryl bonds (25). Most importantly, ROS can also cause damage to proteins, which may lead to destruction of critical enzymes and the interference of ion pumps followed by dysregulation of intracellular and mitochondrial Ca²⁺ levels. In contrast, the levels of ROS seen in cardioprotection is estimated to be 10-fold less (19, 79).

The relative contribution of cell death by necrosis and/or apoptosis during either the ischemic episode and/or reperfusion is still in dispute (20, 36). Necrotic cell death (also known as oncocytic cell death) is thought to result from severe ischemia and usually presents in the central region of the infarct. It is a form of uncontrolled death that does not require energy and is characterized by cell swelling and membrane rupture. Furthermore, cell death by necrosis stimulates inflammation with macrophage infiltration and fibroblast activation (9). Apoptosis, or programmed cell death, was first described in 1972 by Wyllie et al. (73) and has been a subject of intense investigation. Apoptosis progresses to cell death by a genetically programmed series of biochemical events that require energy, leading to cell shrinkage and DNA fragmentation. In contrast to necrosis, there is no inflammation or fibrosis involved, and the neighboring cells remove the apoptotic bodies without any trail (9). Ischemic injury is thought to induce both necrosis and apoptosis; however, in order to have substantial apoptotic cell death, reperfusion seems to be pivotal, and it is therefore to be specifically more associated with postischemic reperfusion (22, 64). The family of cysteine proteases, known as caspases, are key mediators of apoptosis. An extrinsic pathway involving cell surface death receptors leads to the activation of procaspase-8 and procaspase-3, which is followed by proteolysis of cellular substrates and killing of the cell (3). In an intrinsic pathway, intracellular and extracellular death signals are transmitted to the mitochondria through members of the Bcl-2 family (40). This family is composed of antiapoptotic (e.g., Bcl-2 and Bcl-XL) and proapoptotic (e.g., Bax, Bak, Bid, and Bad) proteins that share Bcl-2 homology (BH) regions. Several intracellular stimuli, including oxidative stress, translocate Bax and/or Bak to the mitochondria, leading to dysfunction of this organelle, the release of proapoptotic proteins (such as cytochrome c and Smac), and the activation of caspase-9 (18). This whole process can be opposed by Bcl-2 and Bcl-XL. Moreover, the extrinsic and intrinsic pathways are interconnected via Bid (17). Another important pathway that can lead to apoptosis is the endoplasmic reticulum (ER) pathway. Stress to the ER can also result in apoptosis (72). It has been demonstrated that caspase-12, which is an ER membrane-bound caspase, is a major mediator of ER stress-mediated apoptosis (50). Caspase-12 release from the ER is followed by the processing of downstream caspases such as caspase-3, -7, and -9 (57). Nevertheless, the precise contribution of necrosis and apoptosis during ischemia and/or reperfusion is still not clear.

In recent years, several important findings have promoted for the existence of postischemic reperfusion injury. First of all, several pharmacological agents that were given during reperfusion have been shown to induce cardioprotection against lethal ischemia and limit infarct size. Adenosine that was directly infused upon reperfusion into the coronary arteries of anesthetized dogs after 90-min total coronary occlusion was
shown to reduce infarct size and improve ventricular function (51, 55). Furthermore, Gross et al. (24) have demonstrated that δ-opioid receptor agonists (but not κ-opioid receptor agonists) can limit infarct size when administered upon reperfusion. Similar to IPC and IPO, the cardioprotective effect of opioid agonists that are administered at onset of reperfusion can be abolished by pharmacological inhibitors (24). As aforementioned, ROS scavengers that were administered during postischemic reperfusion have been shown to preserve left ventricular function and a reduction in infarct size (1, 35, 79). A very strong evidence of the existence of postischemic reperfusion injury is IPO, during which a robust infarct size limitation can be induced after a lethal ischemic period (75). The cardioprotection by both IPC and IPO have been associated with activation of the prosurvival PI3-K-Akt pathway and the ERK1/2 pathway, since pharmacological inhibition of these pathways completely abrogated their protective effect (76). Both the PI3-K-Akt and ERK1/2 have been referred to as the “reperfusion injury salvage kinases” (RISK) pathway, as they were uncovered in the myocardium as important targets during postischemic reperfusion (28). Subsequently, several observations have implicated that pharmacological activation of PI3-K kinase and ERK1/2 during postischemic reperfusion strongly protects the heart from I/R by its antiapoptotic effect (28). Most interestingly, pharmacological inhibition of RISK pathway components at the time of reperfusion completely abrogates the cardioprotective effects of both IPC and IPO, suggesting that the RISK pathway is essential during the postischemic reperfusion period in order to manifest cardioprotection (28). Further prosurvival signaling kinases that have been implicated in RISK are endothelial nitric oxide synthase, PKCe, MAPKs (other than ERK1/2), mammalian target of rapamycin, p70s6K kinase, and GSK-3β (27).

MITOCHONDRIAL PERMEABILITY TRANSITION

For many years mitochondria have been regarded as central regulators of cell survival in myocardial I/R injury. Recently, great interest has been focused on mitochondrial permeability transition (MPT) pore opening as an important determinant of cell death (31, 47, 71). The MPT pore is hypothesized as a voltage-dependent, high-conductance multiprotein complex in the inner mitochondrial membrane. Under normal circumstances, the inner mitochondrial membrane must remain relatively impermeable to maintain a large electrochemical proton gradient across the inner membrane, so that the electron transport chain can generate ATP via F₁Fₒ ATPase (46). High Ca²⁺ loads and ROS, which occur during postischemic reperfusion, can make the inner mitochondrial membrane more permeable to ions, termed as MPT, by forming large nonselective pores. When MPT allows permeation of relative small solutes and ions <300 Da, it is termed as low conductance (32). High-conductance MPT results in permeation of higher solutes up to 1,500 Da, which results in oncotic pressure and water influx, with mitochondrial matrix swelling and collapse of the mitochondrial membrane potential (ΔΨm) and reversal of ATP synthase, effectively converting mitochondria from ATP producers to ATP consumers. Eventually, immense matrix swelling will result in rupture of the outer mitochondrial membrane and release of proteins from the intermembrane space. As aforementioned, one of the important factors that is released is cytochrome c, which after binding Apaf-1 in the cytosol causes activation of caspase-9, triggering the apoptotic cascade (17).

An important additional concept is the notion that ROS by itself can also induce ROS release by a positive feedback loop, which is associated with mitochondrial depolarization along with MPT induction (77). It seems that mitochondrial ROS can be generated and can trigger MPT when the components of the mitochondrial respiratory chain are going from the reduced to the oxidized state, as occurs during I/R. Most likely, the source of ROS is a result of the diversion of electrons from the electron transport chain in the inner mitochondrial membrane (78). In addition, it has been demonstrated that the induction of MPT results in a massive ROS release in isolated mitochondria that are supplemented with NADP (8). This concept was further supported by the observation that the MPT inhibitor bongkrekic acid prevented a large burst of ROS release, despite the presence of triggering ROS. Thus triggering by ROS alone may not be sufficient for a large burst of ROS and may require additional involvement of MPT. Nevertheless, the mechanism of this ROS-induced ROS release is still not clear (78).

Crompton et al. were the first who reported that MPT pore opening plays a key role in myocardial I/R injury (15). Although it has been widely accepted that MPT pore opening is associated with postischemic reperfusion (23), there are still studies suggesting that MPT pore opening might be caused by ischemia in the absence of reperfusion (11). During reperfusion, reoxygenation and resumption of the electron transport chain cause a burst of ROS. At the same time, intracellular Ca²⁺ and phosphate rise and intracellular Mg²⁺ decreases, while acidosis is resolving. These conditions during reperfusion indeed promote MPT pore opening (71). Subsequently it became clear that preventing MPT pore opening at reperfusion is protective against myocardial I/R injury. The potent MPT pore inhibitors cyclosporine-A and sanglifehrin-A have been found highly protective even when administered only during reperfusion (11, 26).

In addition, it was soon revealed that cardioprotection against I/R injury by IPC and pharmacological preconditioning ultimately involves prevention of MPT pore opening. For instance, the mitochondrial potassium channel opener diazoxide, as well as PKCe stimulation by PMA, both showed protection against Ca²⁺ MPT in isolated and in situ mitochondria (39). Moreover, one of the targets of PKCe showed a strong association and interaction with a variety of mitochondrial proteins including the MPT pore (7), and pretreatment of isolated cardiac mitochondria with recombinant PKCe clearly protected Ca²⁺-induced MPT pore opening (6). Furthermore, the infarct size-limiting effects of late preconditioning with the NO donor diethylenetriamine/NO could be abrogated by MPT pore opening with atractyloside (68). Finally, ischemic postconditioning has also been reported to eventually converge into inhibition of the MPT pore (2).

The MPT pore is hypothesized as a multiprotein complex that spans the mitochondrial inner and outer membrane, although its exact molecular identity is still in dispute. Whether the MPT pore is a “single” pore complex or a population of complexes with distinct properties is currently unknown. There is significant evidence suggesting that the outer membrane voltage-dependent anion channel (VDAC) participates in MPT.
Three isoforms, VDAC1, VDAC2, and VDAC3, have been identified, but more recently a role for VDAC1 has been suggested in mitochondria-induced cell death. As the adenine nucleotide translocase (ANT) ligands bongkrekate and atractylside can respectively inhibit and induce MPT, most models of the MPT pore also include ANT on the inner mitochondrial membrane as a structural pore component, which interacts with and is regulated by cyclophilin D (CyP-D). In mice, ANT1 and ANT2 would be important, whereas in humans it is ANT1, ANT2, and ANT3. Recently, it has been convincingly demonstrated in CyP-D-deficient mice that CyP-D plays a crucial role in MPT and I/R injury. Nevertheless, recently it was shown that liver mitochondria from mice lacking both ANT1 and ANT2 still underwent MPT, which makes the involvement of ANT in MPT a matter of discussion. In addition, the role of VDAC as a pore component has also been questioned, since VDAC-deficient mitochondria can still exhibit equal cytochrome c release followed by caspase cleavage. It is however not clear how both ANT- and VDAC-deficient mice can survive, and whether other compensatory mechanisms may be responsible for MPT. Other suggested regulatory MPT pore components are the benzodiazepine receptor, hexokinase, and creatine kinase. Most likely, the use of proteomic technologies will provide us with more insight into the architecture of the protein complexes that form the MPT pore.

The antiapoptotic members of the Bcl-2 protein family, Bcl-2 itself and Bcl-XL, have been reported to abrogate Bax/Bak-induced MPT and apoptosis by either interacting with Bax/Bak and/or directly binding to the pore components and inhibiting MPT. Despite many efforts, the exact mechanism by which Bcl-2 inhibits cell death is not well understood. The proapoptotic Bcl-2 family member Bax may form channels in the mitochondrial outer membrane, either alone or with mitochondrial proteins, that result in the release of cytochrome c and induction of apoptosis. Binding of Bcl-2 to Bax can mediate the antiapoptotic effect by preventing Bax from forming channels in the mitochondrial outer membrane. Bcl-2 may also bind to VDAC to inhibit apoptosis. In addition to limiting myocardial I/R injury, it was also found that Bcl-2 overexpression can reduce ischemic acidification and the rate decline of ATP, suggesting that Bcl-2 has other significant effects on mitochondrial function than cytochrome c release.

As summarized in Fig. 1, activation of the RISK pathway can be induced by both IPC and by IPO. In addition, pharmacological agents that can act as preconditioning mimetics have also been suggested to activate the RISK pathway. Inhibition of the MPT pore and activation of the RISK pathway during posts ischemic reperfusion have both been implicated to protect the heart from cell damage. In this regard, activation of the RISK pathway upstream from the MPT pore may initiate a protective effect through the inhibition of MPT.

CONCLUSIONS AND FUTURE DIRECTIONS

Despite many advances in basic science and clinical studies, ischemic heart disease is still the number one cause of mortality and morbidity in western society. In the 20th century, classical physiology studies have provided valuable insight into the pathophysiology of myocardial ischemia and reperfusion. Next to reperfusion therapy, preconditioning of the heart with nonlethal ischemic episodes may be the most promising means to limit damage by myocardial infarction. It became clear that the cardioprotective effect of preconditioning is...
mediated by signal transduction molecules. Elucidating these intracellular signal transduction molecules mediating preconditioning allowed for the identification of pharmacological agents that can mimic cardioprotection. Consequently, it was observed that pharmacological activation of the RISK pathway and inhibition of the MPT pore during postischemic reperfusion strongly protects the heart from I/R injury. The focus of research in cardioprotection against myocardial infarction has shifted from physiology and biochemical studies at whole organ level into molecular studies at organelle and intracellular level. Over the past decade, the use of biochemistry and cellular and molecular biology, genomics, and proteomics has become more important to identify the signaling complexes mediating cardioprotection against I/R injury. In this regard, many classical methods have been used, including protein expression and protein assay studies. Another very important tool has been the use of pharmacological inhibition of certain intracellular signaling molecules. Nevertheless, many of the intracellular signal transduction molecules that are involved in the underlying mechanisms of cardioprotection are still elusive. It is still not clear how certain protective signaling molecules of the RISK pathway can modulate the MPT pore, and if so, which interacting molecular complexes are involved.

To further study the biological function of signal transduction molecules beyond the level of expression, it is important to also consider other factors that can have an important effect. One important fact that can determine the biological function of signal transduction molecules is spatiotemporal location within a cell or organelle. Which proteins are part of the MPT pore, their precise structure, and how their structure can modify the MPT core’s biological function are still matters of dispute. Another important determinant of the biological function of signal transduction molecules is posttranslational modification, such as phosphorylation, methylation, and acetylation. To study the structure and posttranslational modifications of signal transduction molecules and MPT pore components in greater detail, the best tool would be the use of functional proteomic studies. Therefore, an ideal combination of physiology, biochemistry, molecular biology, and more recently the use of functional proteomics may be the best approach to understanding myocardial I/R injury and cardioprotection at whole organ and intracellular level (53).

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REFERENCES

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54. Reimer KA, Jennings RB. The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest* 40: 633–644, 1979.


