REVIEW

Reperfusion injury in acute myocardial infarction. From bench to cath lab. Part I: Basic considerations

Lésions myocardiques de reperfusion au cours de l’infarctus myocardique aigu. Du laboratoire au cath lab. Première partie : données fondamentales

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Summary Early reperfusion during evolving myocardial infarction is essential for saving myocardium and patients’ lives. Nevertheless, lethal reperfusion injury can occur, limiting myocardial salvage. Numerous experimental studies have proved the deleterious effects of reoxygenating endothelial cells and cardiomyocytes. The major breakthrough was the proof that the success of myocardial reperfusion can be modified by preconditioning and, more recently, by postconditioning, a form of progressive and interrupted reperfusion. Three theories have been put forward to explain reperfusion injury: (1) oxidative stress resulting in a burst of oxygen-radical formation, which can cause membrane damage; (2) the energy paradox, which suggests that restarting energetic mitochondrial machinery results in myofibrillar hypercontracture, cytoskeleton fragility and membrane rupture; and (3) the role of inflammation, which addresses the effects of leucocyte accumulation and activation. Fortunately, reperfusion injury salvage kinases can be up-regulated and in some circumstances may block, in a manner similar to pre- or postconditioning, the diabolical cycle leading to necrosis and/or apoptosis of viable cells. The end effectors of the survival system are two mitochondrial channels – the mK-ATP channel and the mitochondrial permeability transition pore. Better understanding of these salutary molecular mechanisms and their triggers may result in a new era of reperfusion techniques.

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Background

Acute coronary thrombosis results in acute myocardial infarction with ST-segment elevation except when collateral flow is rapidly available. The prerequisite for saving myocardium and patients’ lives is to restore coronary blood flow as early as possible using thrombolytic therapy and/or angioplasty [1,2]. Nevertheless, interventional cardiologists and clinicians have reported many paradoxical features, known as “myocardial reperfusion syndrome” [3] and “no-reflow” phenomenon [4], which occur immediately after relief of the epicardial lesion.

Specific histological aspects of canine-reperfused myocardium, which are clearly different from permanent ischaemia, were first noticed by Jennings et al. as early as 1960 [5]. Over the past 30 years, the debate between those in favour of and those against the theory of deleterious effects resulting from myocardial reperfusion has been the source of many controversies, including the suggestion that reperfusion could be a double-edged sword [6] or is only a laboratory artefact [7]. The first proof that modifying reperfusion conditions can reduce infarct size was provided by Forman et al. [8] using perfluorochemical during acute percutaneous coronary intervention (PCI). After numerous hopeful but ultimately unsuccessful pharmacological attempts [9], the demonstration in 2003 of the postconditioning phenomenon [10] mimicking preconditioning [11] clearly proved that abrupt reperfusion can kill severely ischaemic but viable myocardial cells. The positive results of the first randomized study of PCI and postconditioning in humans [12] proved definitively the concept of reperfusion injury and its clinical relevance.

The aim of this review is to combine biological data with clinical features to demonstrate that to reperfuse is necessary, but that “off–on” reperfusion is now overcome. The outcome of this novel reperfusion strategy may result in the saving of 30 to 50% more myocardium [13].

Reperfusion injury: definition

The widely accepted definition of reperfusion injury [14] includes metabolic and structural events that occur as a direct result of the reopening of the epicardial segment of the infarct-related artery, and which can be reversed by modifying reperfusion conditions. Reperfusion injury affects viable endothelial and myocardial cells and can result in their death or stunning. What is accepted as the true definition of reperfusion injury must be differentiated from the accelerated death of non-viable cells even if the mechanisms are identical.

A new paradigm

The previous paradigm underlying much of the scepticism about lethal reperfusion injury was that the main mechanism leading to cell death during reperfusion was energy deficiency due to the length and intensity of ischaemia. According to this theory, injury occurring at the time of reperfusion is the consequence of having reached a point of no return in cellular metabolism. The final size of the infarct was regarded only as the consequence of the delay between sudden artery occlusion and reopening. Discovery of the phenomenon of postconditioning [10], in which “staccato” reperfusion results in a dramatic reduction in infarct size, opened the door to a new paradigm: the final success of reperfusion for myocardial ischaemia depends not only on the ischaemic insult but also on preventable reperfusion...
Molecular basis of permanent ischaemic-myocardium death

The two basic mechanisms of acute myocardial oxygen deprivation are inhibition of adenosine triphosphate (ATP) synthesis and accumulation of metabolic products due to lack of washout. The heart is a muscle that constantly adapts its uptake of oxygen and substrates to match its activity level. ATP and creatine phosphate (CrP) are energy products, 85% of which are devoted to cardiac contraction and the remaining 15% to membrane ion transporters and protein synthesis [15]. During total regional myocardial ischaemia, as is the case in ST-segment elevation myocardial infarction, mitochondrial metabolism is depressed and ATP production falls.

From a schematic perspective, the metabolic scenario can be summarized as follows: the first part, parallel to (but not because of) reducing levels of ATP and CrP, quickly results in reduction and arrest of cardiac contraction, which could be considered a self-protective mechanism [15,16]. The second part is an attempt to open the salvage energy-producing pathways. Anaerobic glycolysis, despite its low rate of ATP production, takes over from aerobic glycolysis and may maintain sufficient energy to allow the membrane pumps to function. Unfortunately it cannot enter the tricarboxylic cycle and thus produces lactic acid, protons and CO₂, which in turn inhibit glycolysis. Favoured by endogenous catecholamine release, lipid degradation (free fatty acids and triglycerides) and beta oxidation start in order to produce ATP but these processes are much more energy consuming (oxygen wastage phenomenon) and also lead to the production of H⁺. Finally, mitochondrial damage and uncoupling of energy production stop ATP synthesis.

Then starts the third and final episode: membrane ion pumps stop and may even reverse (e.g. Ca²⁺/Na⁺ exchange), leading to intracellular accumulation of H⁺ resulting in deep intracellular acidosis, Na⁺ and Ca²⁺ influx and a large efflux of K⁺ [19–21]. Ca²⁺ enters the mitochondria, provoking a deleterious overload and inducing myofibrillar permanent contraction (rigor contraction), even at low levels of ATP [22]. Na⁺ overload favours cell swelling.

Acidosis initiates lysosomal leakage, with the release of enzymes that damage membrane phospholipids and long-chain fatty acids. Deterioration of the cytoskeleton by myofibrillar contracture also plays a key role. All of these events are reversible with the exception of any damage that has occurred to the membranes and cytoskeleton.

Historically, myocardial infarction was described by pathologists as "coagulation necrosis" in which cells are transformed into eosinophilic hyaline masses [5,23,24]. Membrane (e.g. sarcolemma, sarcoplasmic reticulum, mitochondria) fragmentation and cell swelling are observed. Myofibril contracture bands are usually considered characteristic of reperfused infarctions but can also be seen in non-reperfused tissue [13,14,22]. The progression of irreversible cell damage begins in the subendocardium and spreads out towards the epicardium as a wave-front phenomenon [17]. The speed at which irreversibility occurs depends on the degree of collateral flow and the catecholergic response [15].

Necrosis is not the only aspect associated with irreversible lesions. Both necrosis and apoptosis are present in hearts subjected to permanent total ischaemia [18]. Apoptotic cells (detected by immunohistochemical methods) retain their membrane integrity and do not release intracellular enzymes or induce acute inflammation. It is erroneous to think of myocardial infarction only in terms of necrosis. Moreover, apoptosis may start earlier than necrosis because this feature requires energy that cannot be sustained at low levels of ATP.

Molecular basis of lethal reperfusion injury

Lethal reperfusion injury means the presence of viable myocardial cells at the time of reflow. The presence of viable myocardial cells implies that the above-mentioned mechanisms have not yet resulted in irreversible lesions, at least in certain parts of the myocardium. One elegant example of the existence of viable cells after a long period of ischaemia is given in a study by Matsumara et al. involving dogs [24]. The investigators used isotopic viability markers, and showed that at the end of the ischaemic period a large proportion of infarcted myocardium is still viable but loses its viability and becomes necrotic during the first hours of reperfusion.

A very large volume of experimental work has been dedicated to the mystery surrounding reperfusion injury. It is possible to summarize the results, which can be classified into four theories, each of which has its supporters. We, however, propose to demonstrate that these four theories do not conflict with each other.

The major initial suggestion is that reperfusion injury must be viewed as two different facets, one concerning endothelial reperfusion injury and the other myocyte reperfusion injury. Reperfusion injury mechanisms are complex and involve numerous molecular features that occur rapidly within the first few minutes after reopening of the vessel. Trying to describe such a complex scenario as
clearly as possible tempts us to separate artificially the three main harmful culprit phenomena leading to cellular reperfusion death. They are, in fact, closely interconnected.

**Oxidative stress**

Oxygen free radicals or reactive oxygen species (ROS) are molecules with an odd, unpaired electron that renders the molecule unstable and highly reactive. ROS (O$_2^-$, H$_2$O$_2$, OH$^-$, NO) are normal products of endothelial and myocardial cellular metabolism and are even triggers of many physiological molecular reactions (e.g., cellular homeostasis, mitosis, differentiation and signalling). But ischaemia-reperfusion injury is the prototype example of a situation in which ROS are produced in amounts far exceeding those that cells and tissues can manage without being damaged.

In 1984, Burton et al. [25] commented on the role of ROS in inducing myocardial damage. The following year, McCord [26] claimed "It is now clear that oxygen-derived free radicals play an important part in several models of experimentally induced reperfusion injury". Increasing experimental evidence has now accumulated [27], suggesting that ROS are important mediators of postischaemic reperfusion damage explaining the "oxygen paradox" (death of reoxygenated ischaemic cells) described by Hearse et al. [28]. Numerous techniques, including electron paramagnetic resonance, have measured the explosive burst of ROS synthesis immediately after reperfusion but also later [27], with the first increase occurring 15 min after reperfusion followed by a second increase between 18 and 24 h later. The physiological antioxidative defences (e.g., catalase, glutathione peroxidase and superoxide dismutase) are overwhelmed after reperfusion [27]. ROS are formed in the cytosol of cardiomyocytes, in endothelial cells, leukocytes and mitochondria [27,29], with connections between these different sites (Fig. 2). The ROS hypothesis can potentially explain each of the mechanisms involved in reperfusion injury [27,29]:

- inhibition of the Na$^+$/K$^+$ ATPase and sarcoplasmic reticulum Ca$^{2+}$ ATPase, both leading to cellular calcium overload;
- membrane lipid peroxidation and breakdown causing cell swelling;
- chemotaxis of neutrophils resulting in white cell plugging of capillaries and microvascular obstruction.

In addition, white cells when activated are a potent source of further ROS. Cell membranes are particularly susceptible to oxidation by ROS. Rearrangement of the double bonds gives rise to the formation of lipid peroxides, which in the presence of oxygen participate in an autocatalytic chain reaction. Generation of ROS leads to functional injury and impairment of contractile function.

Reactive nitrogen species have been implicated recently in reperfusion injury [29]. NO is a well-known signalling molecule, leading to vascular smooth muscle relaxation and vasodilatation, and is also implicated in the excitation—contraction coupling. During reperfusion, due to a disturbance of the redox state of the cells, it can be combined with superoxide resulting in a very reactive radical ONOO$^-$, the peroxynitrite. It results not only in processes...
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such as membrane lipid peroxidation but also in the interruption of the normal signalling pathways. NO metabolism at reperfusion appears complex and is not fully understood. It most likely involves dysregulation of NO synthesis due to an alteration of the expression and localization of NO synthases translocated from sarcoplasmic reticulum to the sarclemma [29]. ROS and reactive nitrogen species are generated into the endothelium, the cardiomyocytes (mitochondria and cytoplasma), and by activated neutrophils.

Thus, at low concentrations, both ROS and reactive nitrogen species, which are closely linked, are physiological second messengers implicated in the normal cell function. But at high levels, such as during reperfusion, they take a pathophysiological role and tend to interrupt normal signalling pathways. They may directly injure cellular compounds, especially lipid membranes [27].

The oxidative stress theory has long been considered the primary deleterious effect of reperfusion, all the more so when ROS scavengers were proven experimentally to prevent reperfusion injury. For example, as early as 1989, Bolli et al. [30] showed that a potent and cell-permeable antioxidant administered as an intracoronary infusion in a myocardial infarction model in dogs, before and during reperfusion, resulted in a significant improvement of contractile function compared with control animals.

The energy paradox

The energy paradox hypothesis suggests that restarting mitochondrial ATP synthesis — the aim of revascularization — leads to a cascade of paradoxical events resulting in an increase in cellular damage [14] (Fig. 3). By definition, this means that the mitochondrial ATP-production machinery is not excessively damaged by ischaemia and is able, due to the return of substrates and oxygen, to start synthesizing ATP, even at only a low level. In several studies, a direct blocker of the myofibrils (2,3 butane dionemonoxide-BMD) inhibited infarct size by one half [31]. The reperfusion energy scenario results in cardiomyocyte death by two mechanisms, one leading to broken membranes, either directly and/or due to ischaemic cytoskeleton fragility; the second resulting in new damage to the energy-cell factory [14,32]. The reason behind the latter is the opening of the mitochondrial permeability transition pore (mPTP) [33], a recently discovered mitochondrial pore, which remains closed during ischaemia and opens early during reperfusion (Fig. 4).

The first act in the sequence of events is due directly to the restarting of ionic pumps, which try to correct the ionic ischaemic disequilibrium [14,19–21,32]. At the end of a prolonged period of ischaemia, the cytosol is overloaded with Na⁺, Ca²⁺ and H⁺. Acidosis is corrected rapidly by the efflux of H⁺ through the Na⁺/H⁺ exchanger and the Na⁺/HCO₃⁻ symporter located in the sarclemma. This results in intracellular Na⁺ overload, which in turn creates Na⁺ efflux in exchange for Ca²⁺, leading in a large overload of Ca²⁺. Several authors have even proposed a reverse function of the Na⁺/Ca²⁺ exchanger [19,20] and as a consequence a supplementary increase of intracellular Na⁺ parallel to that of Ca²⁺. Finally, if the Na⁺/K⁺ ATP-dependent channel is able to function again, Na⁺ will leave the cytosol in return for K⁺.

Irrespective of the precise molecular mechanism involved in the complex ionic features occurring at reperfusion, the main result is a reperfusion-linked calcium overload added to the ischaemic calcium overload. Reperfusion with a low calcium buffer abolishes the increase in Ca²⁺ uptake and results in an improvement in ventricular function recovery [34]. Activation of the Ca²⁺ sarcoplasmic reticulum ATP-dependent pump, or SERCA, leads to a temporary sequestration of excess Ca²⁺ within this intracellular storage organelle [13,14,20,22,32,35]. If the capacity of this organelle is too small for the amount of Ca²⁺ accumulated in the cytosol, a cycle of continuous release and reuptake of Ca²⁺ from and into the sarcoplasmic reticulum is initiated. These spontaneous oscillations end only if the major mechanism for Ca²⁺ extrusion from the cytosol is sufficiently activated (Na⁺/Ca²⁺ exchange). If not, Ca²⁺ promotes myofibrillar permanent contraction, or hypercontracture, due to rapid crossbridge cycling [32], which in turn increases cytoskeleton fragility and membrane rupture. Such myofibrillar permanent contraction can occur even at low levels of ATP due to a slow turnover of crossbridges. In this situation, myofibrillar bridges remain in a long-lasting state of contraction called rigor contracture, and may also damage the cytoskeleton and membranes [22].

The second part occurs within the mitochondrial membrane. A new door between the cytoplasm and mitochondria has been established recently [33,35,36]: mPTPs are non-specific pores located in the inner mitochondrial membrane and open easily under conditions of Ca²⁺ overload as well as in presence of ROS excess, ADP depletion, and inorganic phosphate (Pi) increase [35–37]. mPTP opening results in increases in mitochondrial Ca²⁺ and H⁺ as well as reactive oxygen species (ROS) synthesis. The direct role of Ca²⁺ overload in mPTP opening is nevertheless challenged [37] and ROS may have a predominant role. When mPTP opening lasts too long, it starts to destroy the inner mitochondrial protection, leading to collapse of the mitochondrial membrane potential and uncoupling of the respiratory chain [33,35]. Finally, ATP production, which had just restarted, may cease. Swelling of the mitochondrion causes a change in the mitochondrial membranes leading to an efflux of cytochrome c. In turn, this induces the caspase cascade and promotes apoptosis [38]. Numerous publications consider that reflow is the trigger of mPTP opening and that...
this is a crucial event in inducing reperfusion injury. During ischaemia, mPTPs remain closed due to acidosis, and thus when the pH returns to normal at the beginning of reperfusion, the mPTPs may open.

The consequence of pH normalization, which is the trigger of energetic deleterious mechanisms, is called the pH paradox [38]. Thus, recovery of the “energetic machinery” does not always result in the recovery of the cells' preischaemic state. On the contrary, it may cause a number of unwanted effects. One might call these events the "energy paradox", meaning that resumption of ATP synthesis could result in a cardiomyocyte dysfunction due to the speed of recovery. Alternatively, mitochondria may need time to return to their preischaemic physiological activities, whereas reperfusion acts as a booster, leading to an abrupt change in state.

To summarize, the main components of this complex interaction are calcium overload, ROS, and rapid normalization of the intracellular pH. Adding to these is the washout of molecules accumulated during the ischaemic phase, which decreases the intracellular osmotic pressure and consequently provokes water overload and swelling that may lead to rupture of the mitochondria and cell membranes.

When cells remain viable despite experiencing severe ischaemia, they need a ‘warm-up’ period to restart their metabolic activity more slowly. Mitochondrial swelling and uncoupling and myofibrillar hypercontracture are the result of abrupt reoxygenation.

Hypercontracture can progress from cell to cell through gap junctions [39]. The “energetic” reperfusion injury mechanisms lead to a peculiar histological appearance. This is characterized by hypercontracted sarcomeres (contraction bands) and frequent sarcolemmal ruptures that gave rise to the term contraction band necrosis [22]. The uncontrolled activation of the contractile machinery depends also on the intensity and duration of the ischaemic period. Its intensity is a consequence of different parameters:

- endogenous catecholamine release;
- arterial systolic pressure;
- extent of collateral vessels.

Role of inflammation

In the intact organism, ischaemic myocardial injury initiates an acute inflammatory response in which polymorphonuclear leukocytes are the major participants [39]. Evidence indicates that the interacting inflammatory reactions are augmented by reperfusion and can contribute to myocardial damage [40,41]. But the direct involvement of neutrophils in lethal reperfusion injury remains controversial. Experimental inhibition or depletion of neutrophils [42], just before or immediately after reperfusion, has provided contradictory data [42,43]. Nevertheless, the participation of neutrophils at least as co-participants and/or amplifiers of reperfusion injury seems now to be recognized [42—44].

Myocardial reperfusion induces an intense accumulation of neutrophils into the area of previously ischaemic
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Myocardium, which can be two- to sixfold greater in the ischaemic than in the non-ischaemic tissue [40]. Neutrophil accumulation begins immediately after reperfusion, increasing during the following 6 h [40,41]. A second phase of increase starts 24h later [41]. Numerous adhesion molecules and inflammation mediators are expressed and/or up-regulated and lead to the fixation of leucocytes to the endothelium and the cardiomyocytes [42,43]. Interactions between neutrophils and endothe-lium are mediated by the selectin family, particularly P-selectin expressed on the surface of endothelial cells within the first few minutes of reperfusion [42]. P-selectin expression reaches a maximum 10—20 min after reperfusion. Integrins (membrane glycoproteins) and specifically the CD11/CD18 family are also expressed on the leucocyte surface and result in firm adherence to endothelial surface [42]. The immunoglobulin ICAM-1, expressed on the endothelial surface and up-regulated by proinflammatory mediators (ROS, IL-1, TNF-alpha), is the endothelial counterligand of the leucocyte surface-adhesion molecule [42]. These immunoglobulins reach a maximum expression around 4 h after reperfusion.

Neutrophil—myocyte interactions are promoted by the up-regulation of ICAM-1 expressed on the myocyte membrane, permitting adhesion of activated leucocytes [42,44]. It must be kept in mind that leucocyte accumulation and activation, and endothelial and myocyte adhesion start immediately after reperfusion but last for only a few hours [40—42]. The three pathways of the complement cascade are activated, increasing the interaction between endothelium and leucocytes [45].

The final issue of the early but also long-lasting inflammatory response to reperfusion can be summarized as follows:

- endothelium dysfunction with the loss of vasodilatory properties, the release of vasoconstrictive molecules (thromboxane A2 and endothelin), platelet activation, and distal plugging leading to the no-reflow phenomenon [42], at least in part;
- neutrophils, considered one of the major sources of ORS [46], are involved in damage and rupture of cardiomyocyte membranes.

The role of leukocytes during ischaemia and reperfusion is the result of a complex molecular biological scenario that has not yet been fully explained. Despite experimental evidence, their role remains controversial [42,43]. Conflicting results from experimental pharmacological and immunological prevention do not rule out the role of early inflammation processes in reperfusion injury; inflammation is probably only one of the deleterious features of reperfusion. Inflammation mechanisms are present not only early but also during the next 24 h and even later.

**Molecular basis of endothelial and myocardial salvage**

The concept of preconditioning, in which pretreatment with brief episodes of ischaemia reperfusion preceding a long period of total ischaemia resulted in a dramatic reduction in infarct size, was introduced by Murry et al. [11] in 1986. Their data clearly demonstrated that cell death cannot be seen as a mere consequence of energy deficiency. Preconditioning has been reported to:

- reduce infarct size;
- preserve vascular endothelial function;
- decrease accumulation and activation of polymorphonuclear neutrophils;
- decrease calcium overload;
- delay the restoration of neutral pH;
- reduce apoptosis [38].

Postconditioning achieves the same result. Moreover, it inhibits ROS production and decreases cellular oedema [38]. Furthermore, while some authors consider that pre- and postconditioning have additive effects [47,48], others deny this suggestion [49]. The precise mechanisms explaining postconditioning efficacy have been proposed from molecular biology studies. A report by Hausenloy and Yellon [50] provides insights, showing that it is possible to protect reperfused myocardium by activating prosurvival kinase signalling pathways, now called the reperfusion injury salvage kinase pathway (RISK). To understand such a complex and still evolving mechanism, it appears necessary to go back from the end effectors of the RISK pathway, to describe the RISK cascade, and finally to identify the triggers. Whether or not the survival mechanisms are identical for pre- and postconditioning is still debated [50—52].

The end effectors of these salvage kinases are mPTP [13,33—36] and K-ATP channels [34,48,49]. When in the open setting (promoted by calcium overload, oxidative stress, and ATP depletion) mPTPs lose their mitochondrial protective role. Thus mitochondria undergo massive swelling and become uncoupled, are unable to maintain the pH gradient and membrane potential, and instead of producing ATP they start to degrade the remaining ATP [33]. A second consequence of mitochondrial swelling is that the architecture of the mitochondrial inner membrane changes, resulting in release of cytochrome c, which in turn activates proapoptotic caspases [38]. The first postconditioning mechanism appears to prevent mPTP opening and preserves mitochondrial function [33]. Opening the mitochondrial K-ATP channels could be the second end effector of postconditioning [38]. This opening provokes a partial mitochondrial membrane depolarization preventing calcium overload. Opening of K-ATP channels might also occur in the sarcoplasmic reticulum membrane and could decrease cytosolic calcium overload; as a consequence, it prevents myofibrillar contracture [52]. Murata et al. [53] consider that K-ATP channels are already acting during ischaemia, limiting Ca2+ overload, whereas mPTPs are involved only during the early reperfusion period. A ribosomal protein (p7056K) may be a third intramitochondrial RISK end effector involved in an antiapoptotic role [48]. Finally, glycogen synthase (GSK-3B) has been proposed as the pivotal kinase that serves as a RISK point of convergence, receiving inputs of these pathways and mediating both K-ATP channels and mPTPs [54].

The full mechanism of the RISK pathway is not completely known, but the current paradigm implicates one or more G protein-coupled receptors followed by a cascade of protein kinases, including protein kinase C and mitogen-activated protein kinases (MAPKs), which open the K-ATP channels and close the mPTP [50]. The MAPK pathway could be divided into two parallel circuits, one including phosphatidylinosol 3
Figure 5. Passive and active salvage pathways. Akt: serin-threonin kinase; ERK 1/2: extracellular signal-regulated kinase; GSK 3B: glyco-
gen synthase kinase-3 beta; K-ATP: potassium channels ATP-dependent; MEK 1/2: mitogen-activated protein kinase; mPTP: mitochondrial permeability transition pores; PI3K: phosphatidylinositol 3 kinase.

There are many candidate trigger molecules for initiating survival pathways [38]: adenosine is the best known of these triggers, such as in preconditioning. Bradykinin, which is synthesized during ischaemia, and paradoxically ROS could also induce salvage mechanisms. Other drugs (postconditioning-mimicking drugs) such as endogenous or exogenous opioids, nicorandil, insulin, cyclosporin A, glucagon-like peptide-1, erythropoietin, statins, cardiotrophin-1, protein kinase C, transforming growth factor-beta 1 might also activate this path and/or inhibit directly apoptosis by blocking caspases.

After preconditioning, these survival pathways have been up-regulated and can work immediately after reperfusion. In the case of postconditioning, they need time to start. This time is achieved by maintaining sufficient acidosis, which blocks the opening of mPTPs [36]. At the same time, if adenosine is not drained too quickly, it has time to trigger the RISK pathway. This is the reason why triggering the survival mechanisms is closely linked to the duration of the initial artery opening. This must be as short as possible and probably not more than 1 min. Nevertheless, the data we currently have suggest that these delays could differ from one animal species to another and especially for humans [38,51,52].

These active survival mechanisms can be associated with passive ones [51] (Fig. 5). Tsang et al. [51] proposed a dual action of postconditioning, with one being passive (not involving the RISK pathway) and the other, active one, inducing the RISK pathway. It is, however, difficult to differentiate these two mechanisms, especially because low pressure reperfusion is known to activate the RISK pathway and attenuates mPTP opening. Controlled and gradual reperfusion could limit ROS burst and neutrophil accumulation. At the same time, low-pressure reperfusion has been shown to decrease mPTP opening [55].

Thus, having tried to summarize the now accepted concept of preventing mechanisms for lethal reperfusion injury, it must be said not everything is fully understood. Nevertheless, sufficient research evidence is available to start trials in humans.

Remote preconditioning: the last good new?

Remote preconditioning occurs when an organ is subjected to sublethal transient ischaemia and confers protection to another organ [57]. This phenomenon was described recently using mesenteric ischaemia or aortic clamping and resulted in reduction in the size of the myocardial infarct. In humans this technique may be achieved by intermittent inflation of the blood pressure cuff [57,58]. Whether remote preconditioning depends on the same survival pathways remains to be demonstrated, but it at least includes protein kinase C as does postconditioning [58].

Apoptosis: the secret killer?

This genetically programmed form of cell death is very difficult to detect by conventional histological methods. Apoptotic cells are phagocytosed by adjacent cells without the occurrence of inflammatory reactions. Their membranes remain intact and only nuclear DNA fragmentation, which
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