

Can We Improve Myocardial Protection during Ischaemic Injury?

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Key Words

Myocardial ischaemia · Cardiac reperfusion · Cardioprotection

Introduction

In recent years medicine has evolved markedly in the understanding of pathophysiology and the development of responsive therapies. Cardiology has contributed significantly to this evolution. Perhaps one of the most relevant contributions was that, after the demonstration that coronary thrombosis is the cause instead of the result of STEMI, the timely restoration of flow to the ischaemic myocardium became the standard treatment for the majority of patients. This has resulted in a very significant reduction in acute cardiovascular mortality: risk-adjusted in-hospital mortality for STEMI has decreased from 20% in the 1980s to less than 5% nowadays [1–3]. Without any question, timely reperfusion either by thrombolytic therapy or primary percutaneous coronary angioplasty is the most effective therapy for limiting infarct

size, reducing the healing pattern of the infarcted zone, preserving left-ventricular systolic function and delaying the onset of remodelling and heart failure [1, 2].

The reason for such success is the very strict dependence of the myocardium on a continuous delivery of oxygen through the coronary arteries to support mitochondrial oxidation and, ultimately, the adenosine triphosphate (ATP) production necessary for cardiac function and the maintenance of the complex myocardial structure. Successful revascularization means restoration of the continuous delivery of oxygen, which is particularly relevant for the heart because myocytes have, at most, a very limited reserve of oxygen. Thus, the abrupt, thrombotic occlusion of an epicardial coronary artery causes ischaemia of the myocardium distal to the thrombosis, a condition which, if it persists, results in the death of myocytes with destruction of the myocardium previously supplied by the same coronary artery. This type of death, which usually involves millions of myocytes at a time and causes an immunological reaction leading to fibrotic repair and scar formation, is called ‘necrosis’ [3]. Recently, however, other forms of death have been identified with-

in the ischaemic area, termed apoptosis, autophagy and necroptosis, etc. [3]. Although relevant from a scientific point of view, the clinical community is questioning, particularly in this situation, the importance of a myocyte's mode of death as, in any case, death is death. It has been suggested that such a distinction does not matter in the context of the acute death of myocytes immediately after the coronary occlusion. It might, however, matter in the sequel of acute myocardial infarction, i.e. post-ischaemic remodelling and heart failure [3].

A large body of experimental evidence suggests that the final myocardial damage during acute myocardial infarction is actually the result not only of acute flow and therefore oxygen deprivation (ischaemia) but, paradoxically, also of the subsequent restitution of flow and oxygen supply (reperfusion). This latter form of injury is called ischaemia/reperfusion injury. In the present review, we will analyse the pathophysiology of ischaemia/reperfusion injury, the different attempts made to limit it, and examine whether the entity truly exists.

Reperfusion Injury: A Multifaceted Phenomenon

So-called reperfusion injury can manifest in several ways. Four different types of reperfusion damage have been proposed, as outlined below.

Reperfusion Arrhythmias

Arrhythmias during or immediately after reperfusion are seen both in different experimental models and in humans [4]. Among the animal studies, those of Manning and Hearse [5] using isolated and perfused hearts provided a very clear description of reperfusion-induced arrhythmias and the possibility to reduce their occurrence with different antiarrhythmic drugs [5]. In humans, arrhythmias are often present in reperfused-STEMI patients, particularly after thrombolysis. Actually, early reperfusion arrhythmias together with the early blood increase of intracellular biomarkers are considered evidence of successful reperfusion. The most common arrhythmias are idioventricular rhythm, ventricular tachycardia and ventricular fibrillation, all of which can generally be resolved without great difficulty [6]. It follows that this form of reperfusion damage is treatable and can also have a benign/positive implication.

Myocardial Stunning

This term refers to reversible post-ischaemic contractile dysfunction that can occur both in large animal mod-

els and in humans [7]. It is common knowledge that ischaemic myocardium does not necessarily recover its function immediately after reperfusion. On the contrary, this can happen at a later stage, even weeks later in humans. Like arrhythmias, stunning is considered a benign form of reperfusion damage. Normally it does not need any specific treatment because it is unpredictable and a specific anti-stunning treatment is not available. Nonetheless, in the unfortunate cases when reperfusion results in severe stunning, causing low cardiac output, positive inotropes and vasodilators are used in the hope that severe stunning and not irreversible damage is the ultimate cause. Stunning or severe ventricular dysfunction is sometimes encountered after surgical reperfusion, in the setting of coronary artery bypass grafting, especially after prolonged clamping. Despite several experimental efforts the mechanisms responsible for stunning have not been clearly identified. The most likely and popular explanation is linked to the effects of oxidative stress and calcium overload on the contractile apparatus, but even this hypothesis has never been confirmed [8, 9].

Microvascular No-Reflow

Aside from arrhythmias and stunning, the so-called no-reflow phenomenon has been another suggested form of reperfusion damage. The coronary microcirculation plays a fundamental role in the success of reperfusion. If blood flow is restored to the epicardial artery but there is an obstruction of the microcirculation, the myocardium will remain ischaemic and will never recover. Thus, microvascular obstruction is considered to be an irreversible form of damage which results in both myocyte and endothelial death. The phenomenon was first described in a feline heart [10] and confirmed in dogs [11] as the presence of severe capillary damage, swollen, ruptured endothelial cells, intraluminal thrombosis and irreversibly injured cardiomyocytes [12]. Thus, the no-reflow phenomenon, when it occurs, greatly contributes to myocardial ischaemia/reperfusion damage despite the successful reopening of large epicardial coronary arteries [10]. However, whether the phenomenon itself causes further ischaemic damage is doubtful: if reperfusion does not occur, there will inevitably be no reflow both at the epicardial and microvascular level, in any case resulting in further damage. In the clinic the no-reflow phenomenon is detected angiographically (in the case of PCA) from no or slow flow of contrast media, despite successful recanalization of the epicardial coronary artery. It normally develops within minutes after reperfusion and it might persist for a week [13]. As the ischaemic zone and the infarct area

are not reperfused, the washout of intracellular biomarkers is also reduced or abolished, contributing to underestimation of the final infarct size. This is particularly relevant in the case of thrombolysis, in the absence of angiographic data, and might explain the often detected discrepancy between the estimated (from the biomarkers curve) and actual area of necrosis. It follows that it is difficult to establish the frequency of the no-reflow phenomenon in the clinic, although it has been estimated to occur in 10–30% of patients with reperfused STEMI [14, 15].

The underlying cause of the microvascular obstruction is unclear. A number of mechanisms have been proposed, including: (a) embolization of particulate debris from the spontaneous or induced rupture of the culprit atherosclerotic plaque, (b) vasoconstriction caused by a release of vasoconstrictor substances, again from the culprit lesion, (c) microvascular compression due to oedema of the ischaemic myocytes, and (d) platelet and leukocyte aggregates from the culprit lesion or from blood flow as part of the general inflammation associated with STEMI. The role of leukocyte infiltration, however, is more important in the later phase of the infarct, such as healing and remodelling. Obviously these different mechanisms are not mutually exclusive and, probably, they are all involved [16, 17].

Currently, there is no possibility of predicting the no-reflow phenomenon or an effective specific therapy. Therefore, the slow- or no-reflow phenomenon unfortunately remains a neglected therapeutic target.

Lethal Reperfusion Injury

Although reperfusion is essential for the functional recovery of ischaemic myocardium, it has been indicated that, especially when it occurs late, it can contribute to further myocyte damage and death [17]. The first description was reported from histological features of reperfusion causing explosive oedema and swelling of the cytosol, massive hypercontraction bands and sarcolemma damage, as well as mitochondrial calcium accumulation in the form of phosphate granules [18, 19]. Figures 1–4 show a sequence of electron microscopic changes occurring in isolated and perfused rabbit hearts during different periods of ischaemia and reperfusion, while figures 5 and 6 report the sequence of events in terms of mechanical activity, lactate, creatine phosphokinase (CPK), Mg^{2+} , high-energy phosphate, mitochondrial function and calcium homeostasis in the same preparation. Reperfusion seems to suddenly and massively potentiate myocardial damage, characterized by intense oedema and cytosolic and mitochondrial calcium overload, causing an increase of myofilament contracture and a further reduction of

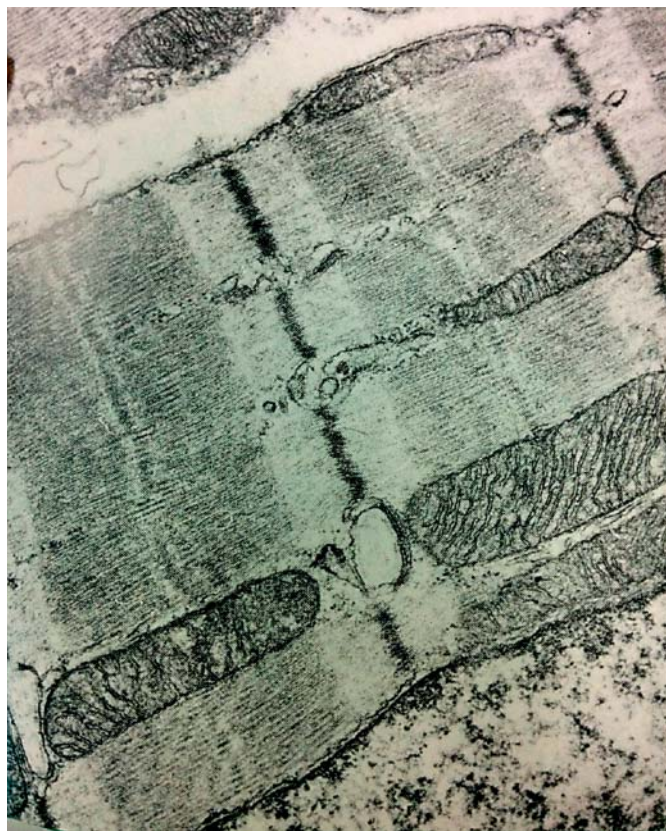


Fig. 1. Typical ultrastructure of the normal well-oxygenated rabbit myocardium after 90 min of aerobic reperfusion with Krebs-Henseleit buffer.

ATP production. Figure 7 shows that this type of reperfusion injury is evident in an isolated and perfused heart preparation when readmission of flow occurs rather late, after several (90) min of ischaemia, which roughly correspond to 6–12 h in humans [20].

However, in the 1960s a controversy arose about the existence of such specific reperfusion-induced myocardial damage. It was argued that, at best, the damage detected immediately after reperfusion represents just an acceleration of damage that in any case would have occurred if the myocardium had never been reperfused. The only way to support the concept of reperfusion damage was to show that the damage could be reduced by an intervention provided at the time of reperfusion. This, however, proved to be difficult, at least in the isolated and perfused heart, as all the putative cardioprotective agents (which at that time, were mainly calcium antagonists, beta-blockers and antioxidants) needed to be adminis-

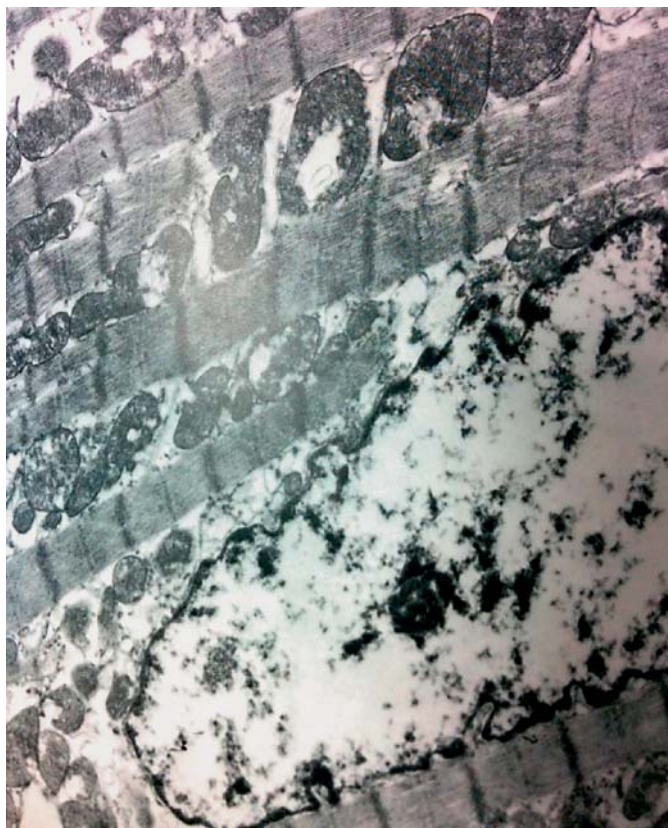


Fig. 2. Ultrastructural changes after 30 min of ischaemia in isolated and perfused rabbit heart. Within 30 min of severe ischaemia the typical electron microscope view of a dense and orderly appearance of the myocardium disappears. The nucleus shows margination of the chromatin material. Intracellular oedema, inflammatory cell infiltration and total loss of stainable glycogen are also present. The sarcolemma and mitochondria are relatively unchanged. Injury at this stage of severe ischaemia may still be reversible. The extent of cell damage observed morphologically following prolonged ischaemia depends in part on the site from which the sample is taken. Irreversibly injured cells near the centre of an infarct die without marked microscopic change, whereas those closest to normally perfused areas, at the periphery of the infarct, develop marked morphologic abnormalities.



Fig. 3. Ultrastructural changes after 90 min of ischaemia in isolated and perfused rabbit heart. After 90 min of severe ischaemia, when irreversible damage has occurred, the electron microscopic view clearly indicates a severe disarrangement of ultrastructure. Disorganization of myofibrils is the most prominent finding, accompanied by the appearance of relatively dense contraction bands. Margination of nuclear chromatin is prominent and profound mitochondrial changes are evident, including oedema and loss of the matrix density, fragmentation of the cristae and alteration of the external membrane. Extramitochondrial accumulation of 'lipid droplets' is always present. The sarcolemma is not necessarily damaged.

tered before the onset or at the time of ischaemia if they were to be effective [21, 22].

More favourable evidence of possible reperfusion damage was provided in large animal preparations, especially in dogs, in which it is possible to precisely measure the infarct size, the reduction of which has become the goal of any protective intervention. The concept of cardioprotection then became fashionable and the object of several experimental studies. In large animals, cardiac protection was linked to the extension of infarct size,

while in small animals and in isolated heart preparations it was linked to the recovery of cardiac function during reperfusion.

The Concept of Infarct Size and Cardioprotection

Cardiac protection is a broad term that refers to different strategies aimed at the reduction of myocardial damage, generally induced by ischaemia and reperfusion but,

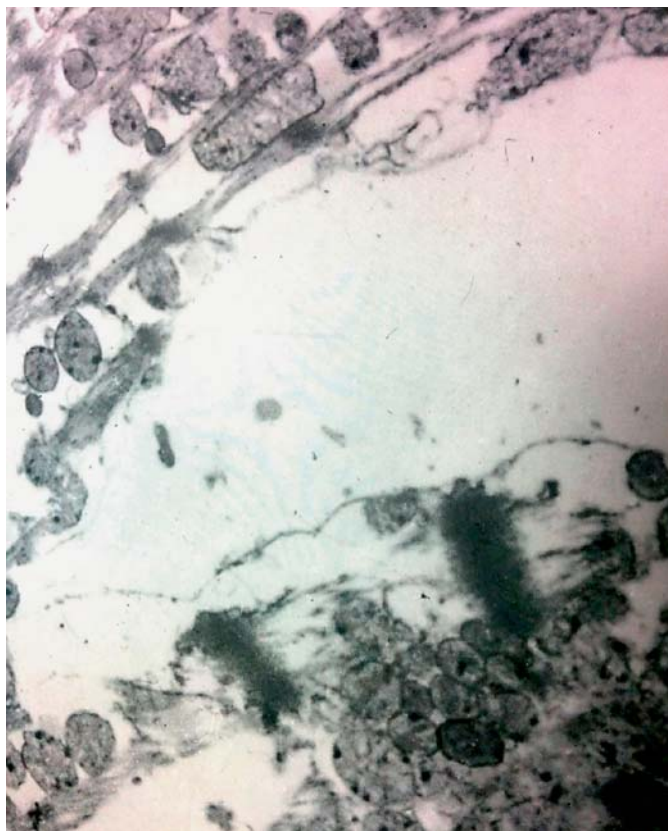


Fig. 4. Ultrastructural changes, immediately after reperfusion, following 90 min of severe ischaemia in isolated and perfused rabbit hearts. In the cytosol there are stretched and separated fibres with a disrupted myofibrillar pattern and formation of contraction bands, massive accumulation of lipid droplets and oedema. The sarcolemma and sarcoplasmic reticulum show signs of loss of integrity and continuity. The mitochondrial alterations are of a type that appears in all dying cells. The matrix is relatively clear, with loosely packed cristae. Prominent dense bodies, suggesting the accumulation of calcium, are always present.

occasionally, also by other insults. Cardioprotection can be achieved by drugs, chemical substances or other interventions, acting directly or through the recruitment of endogenous substances. The size of the infarct is the major determinant of final damage in large animals and of prognosis in humans. Therefore, it is the unambiguous end point for cardioprotection studies. Infarct size can be measured mainly in experimental settings, the standard method being triphenyltetrazolium chloride staining. In humans it can be measured by the area under the curve subtended from released intracellular biomarkers, such as CPK or troponin, and by magnetic resonance imaging, which is the current standard. The major determinants of

infarct size are the extent of the perfusion territory distal to the occluded coronary artery and the duration of ischaemia. Duration is important because, even after complete coronary occlusion, there is a period in which the injury is still reversible. The area not yet irreversibly damaged constitutes the so-called 'area at risk'. The duration of ischaemia necessary to cause irreversible damage varies from seconds in isolated myocytes, a few minutes in small rodents, to about 30–40 min in large mammals and even longer in humans (perhaps as long as 6–8 h), although in humans it is difficult to establish with precision the onset of ischaemia [16, 19, 20]. It follows that the area at risk does not necessarily correspond to the actual infarct size, which normally begins in the inner myocardial layers and spreads transmurally and laterally. Since the area at risk is the primary determinant of infarct size, infarct size is normalized for the area at risk, which is determined by microspheres which measure the hypoperfused myocardium or, post-mortem, with the injection of dye that stains all non-risk myocardium [23]. Another major determinant of infarct size is the amount of collateral flow which, in turn, depends on the level of microcirculation and, eventually, on the degree of angiogenesis. Obviously, all these factors contribute to the spatial and temporal development of necrosis and infarction. Optimally, reperfusion of the ischaemic myocardium is sufficiently early to avoid further expansion of the area at risk and also to reduce it by providing flow and oxygen to the ischaemic but not yet necrotic myocytes. Late reperfusion, on the other hand, could have the opposite effect, further extending infarct size. To prove the existence of late reperfusion injury in humans, however, is, at best, extremely difficult, mainly because infarct size before and after reperfusion cannot be measured. Normally it is possible to measure periprocedural myocardial injury during percutaneous coronary intervention (PCI) or coronary artery bypass grafting in terms of the increased release of intracellular biomarkers, but it is not clear whether the damage has occurred during ischaemia or has been brought about by reperfusion. Paradoxically, if there is no reperfusion and, therefore, massive damage, there is no washout of biomarkers! Imaging techniques such as myocardial perfusion scintigraphy with thallium or sestamibi single-photon emission CT and gadolinium contrast-enhanced MRI are better measurements of infarct size in humans, but it is impractical to apply any of these techniques just before and immediately after reperfusion. It follows that the definitive proof of reperfusion injury relies on the reduction of infarct size by interventions given immediately prior to or at the time of reperfusion versus placebo. This has been

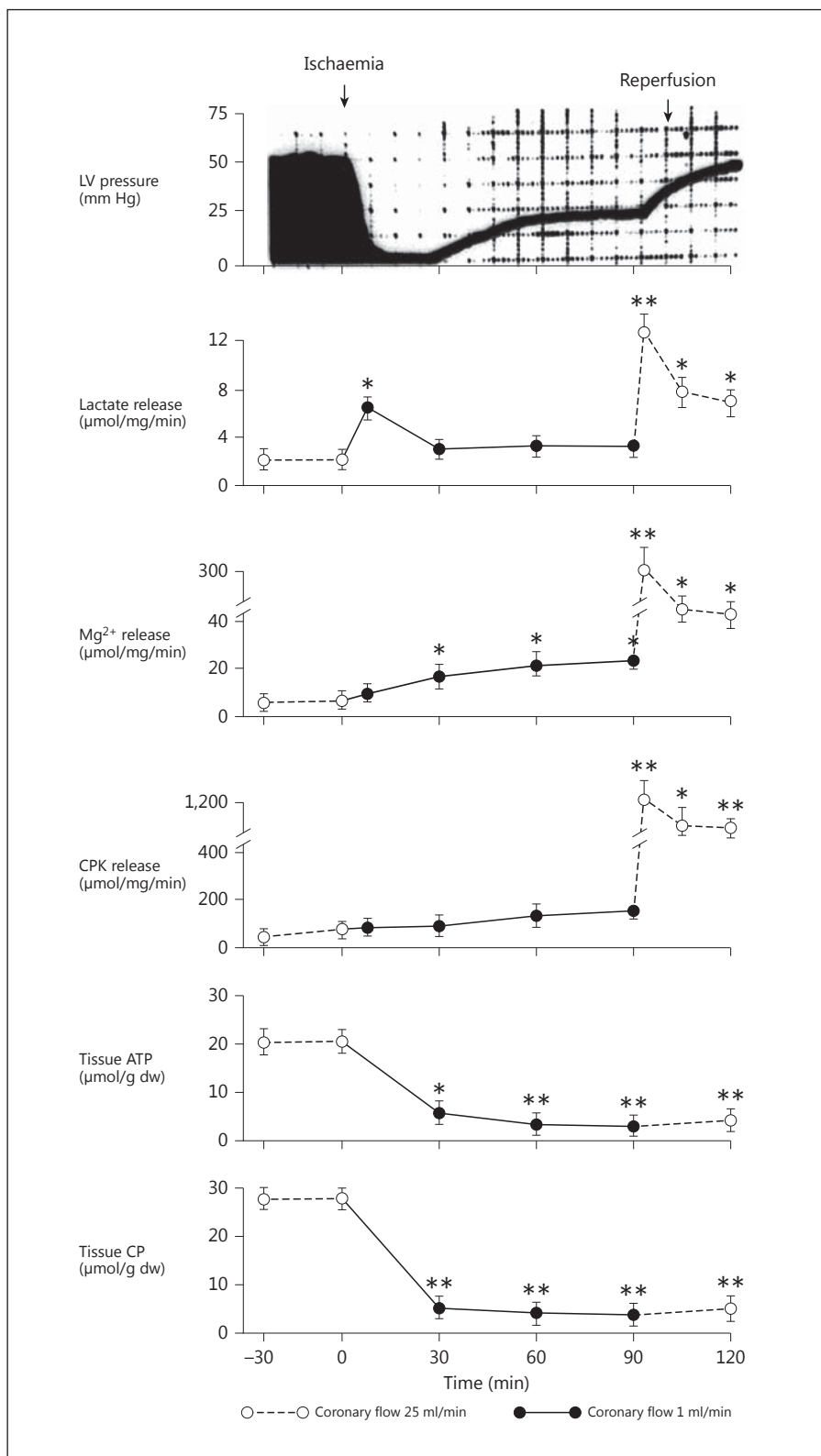
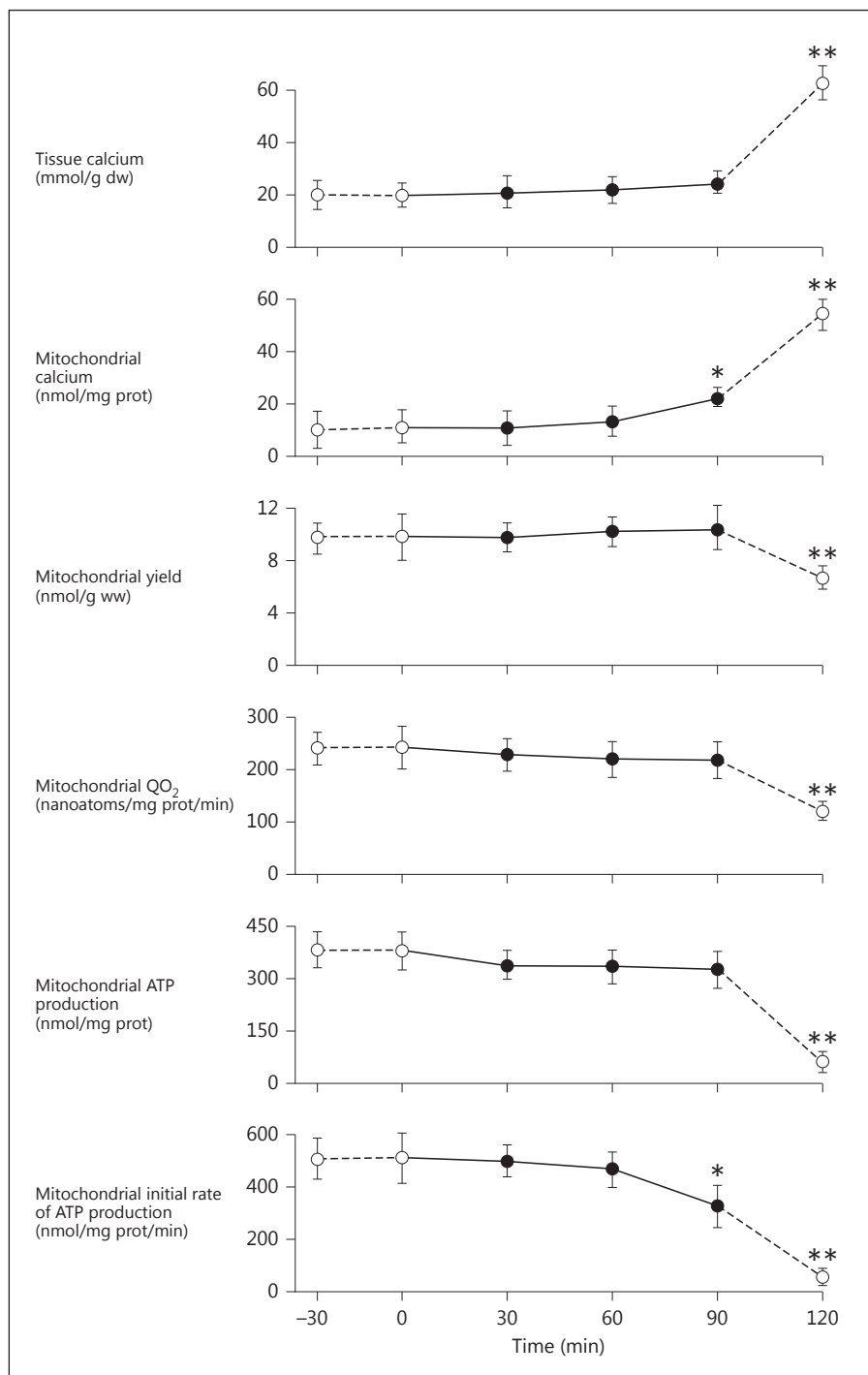


Fig. 5. Time course of mechanical function, lactate magnesium (Mg^{2+}) and CPK release, as well as the tissue content of high-energy phosphate (ATP and creatine phosphate, CP) of rabbit hearts isolated and perfused for 30 min under aerobic conditions followed by 90 min of ischaemia (coronary flow reduced from 22 ml/min under aerobic conditions to 1 ml/min) and then reperfused for a further 30 min. dw = Dry weight.

Fig. 6. Time course of calcium homeostasis (measured in terms of tissue content of total and mitochondrial calcium) and mitochondrial function (measured in terms of isolated mitochondrial oxygen consumption, QO_2 , initial rate and total ATP production) of rabbit hearts isolated and perfused for 30 min under aerobic conditions followed by 90 min of ischaemia (coronary flow reduced from 22 ml/mm under aerobic conditions to 1 ml/mm) and then reperfused for a further 30 min. dw = Dry weight; ww = wet weight; prot = protein.



done in large animals with different therapeutic agents or strategies targeting different possible mediators of reperfusion injury, often reporting positive results. However, the attempts to translate this success into the clinic have consistently failed.

Possible Causes of Lethal Reperfusion Injury

Several hypotheses have been proposed to explain why reperfusion should result in further injury. Interestingly, this research has followed several lines of enquiry, de-

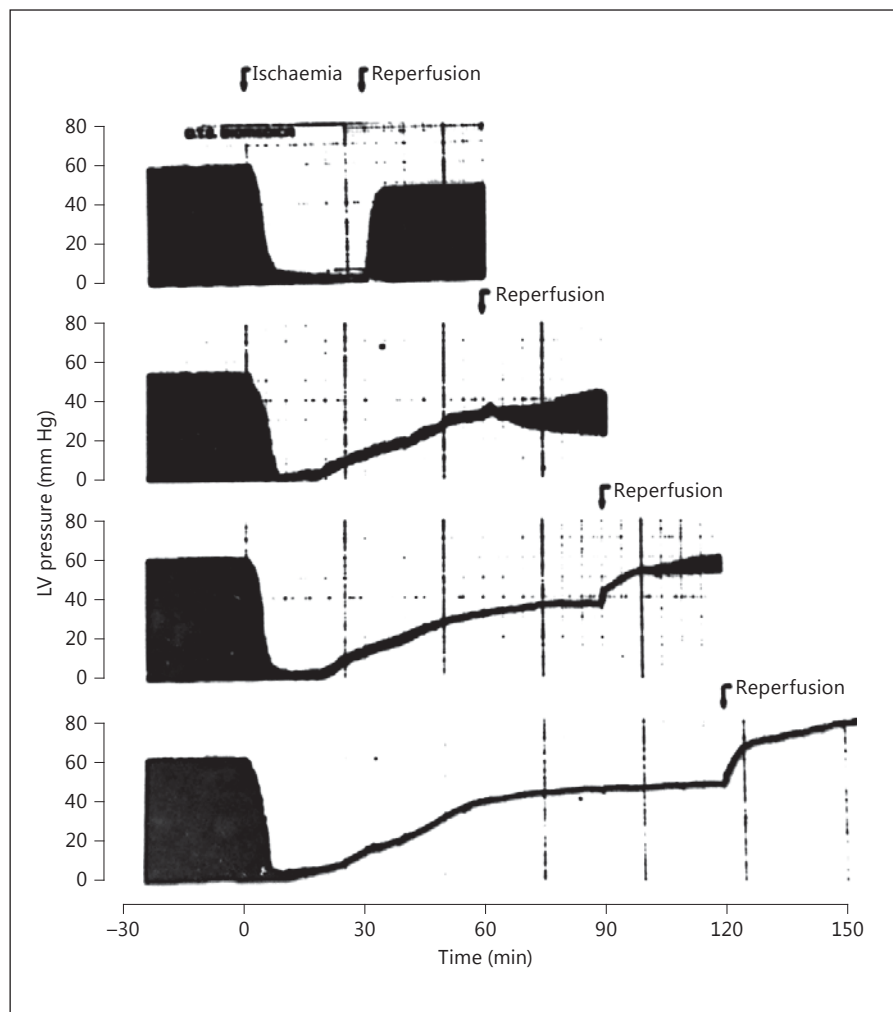


Fig. 7. Mechanical function of isolated and perfused rabbit hearts subjected to increasing periods (from 30 to 90 min) of ischaemia followed by 30 min of reperfusion.

pending on the specific time and on the trend of the moment.

Calcium Paradox

One of the first ideas was linked to the so-called 'calcium paradox', i.e. the readmission of calcium after a short period of calcium-free or low-flow (ischaemic) perfusion causes massive immediate damage to the myocyte similar to that seen after late reperfusion. As in the calcium paradox, after late reperfusion important damage to the sarcolemma and the sarcoplasmic reticulum has been described with consequent intracellular and mitochondrial calcium overload (fig. 6) [19]. This later event is particularly detrimental as the mitochondria, during reperfusion, may use the restored oxygen for calcium transport instead of ATP production, with the two processes competing for the same energy source, i.e. the $\Delta\Psi$ generated

across the inner mitochondrial membrane [20]. As a consequence, it was logical to consider calcium antagonists to attenuate calcium overload. These agents, however, despite being highly protective when used before or at the time of ischaemia, failed to reduce late reperfusion damage in isolated and perfused heart preparations [21]. This is not surprising, considering that the entity of sarcolemma damage (see fig. 4) precludes even the existence of the calcium channel, the ultimate site of action of calcium antagonists. However, when tested in larger animal preparations, these agents decreased infarct size [23, 24]. The results of the corresponding clinical trials, however, have been unequivocally negative irrespective of whether the calcium antagonists were administered before, during or after ischaemia [25]. Another possibility to reduce calcium overload is to inhibit the sodium-hydrogen exchange. Again, the literature reports promising experimental re-

sults when the inhibitors were used during ischaemia [26], but failure when provided during PCI [27]. Ranolazine, an inhibitor of late sodium influx, has also been suggested to be cardioprotective by reducing the calcium overload. However, when tested in two large clinical trials, it failed to improve prognosis or reduce the occurrence of myocardial infarction [28, 29].

Oxygen Paradox and Oxidative Stress

The concept of calcium overload as a possible cause of reperfusion injury has generated considerable attention as a potential reason for the abrupt damage of the sarcolemma and its sequelae. After reperfusion, it has been suggested that the readmission of oxygen causes oxidative stress with consequent membrane peroxidation and immediate damage. This process was considered likely to occur as ischaemia causes a time-dependent reduction of the natural myocardial defence mechanism against oxygen free radicals, i.e. mitochondria superoxide dismutase activity and the cytosolic glutathione-peroxidase and reductase system, as well as sarcolemmal α -tocopherol [30–32]. The readmission of oxygen in the absence of all existing natural scavengers could cause rapid damage to the sarcolemma, which would then become permeable to Ca^{2+} . Unfortunately, despite strong logic, both animal and clinical studies examining the cardioprotective effect of different antioxidants have always been inconclusive [33, 34].

As oxidative stress reduces the availability of nitric oxide (NO), it was also thought that the administration of NO donors could be cardioprotective [35]. When tested in the clinic and administered to reperfused-STEMI patients, nicorandil, an NO donor, and trimetazidine, a metabolic modulator with antioxidant properties, failed to limit the infarct size [35, 36]. Today, the damaging effect of oxygen free radicals is recognized by the scientific community in various diseases but, unfortunately, it is not a target for reperfusion therapy.

pH Paradox

As for the Ca^{2+} and/or oxygen paradoxes, even the abrupt pH restoration during reperfusion has been considered deleterious and a possible cause of reperfusion damage. Myocardial ischaemia causes a rapid drop of intracellular pH as a consequence of H^+ and lactate accumulation. The ischaemia-induced acidosis is, in turn, responsible for freezing all calcium movements and, thus, for the rapid cessation of contraction in the ischaemic zone. Reperfusion results in a rapid washout of lactate (see fig. 5) and activation of the sodium-hydrogen exchanger and the sodium-bicarbonate symporter, with

rapid restoration of physiologic pH. Experiments in rat cardiomyocytes have shown that reperfusion with acidic buffer is cardioprotective, an effect that has been linked to inhibition of the mitochondrial permeability transition pore (mPTP) [37]. However, in the clinic, sodium-hydrogen exchange inhibitors administered to delay the restoration of physiologic pH during reperfusion failed to protect the heart or to improve functional recovery [38].

Inflammation

Acute ischaemia in humans and in large animals triggers a process of inflammation with neutrophil migration in the ischaemic tissue, and the consequent vascular plugging and release of degenerative enzymes. All this is facilitated by reperfusion, which activates cell-adhesion molecules. Several interventions, including leukocyte-depleted blood, pharmacologic inhibitors of complement activation, CD11 and CD18, antibodies against the cell-adhesion molecules, and adenosine, have been proposed and tested experimentally with differing results that were often inconclusive [39–43]. The corresponding clinical studies failed to show any cardioprotective effects of these interventions.

Mitochondrial Permeability Transition Pore

All of the above mechanisms, when explored alone, failed to play a clinical role. It is likely that they are not mutually exclusive and most probably they all play a synergistic role. Recently, a possible common pathway has been identified – the opening of mPTP. This is a non-selective channel of the inner mitochondrial membrane which, in physiological conditions, is closed. As described, during ischaemia, cardiomyocytes are subjected to calcium overload, reactive oxygen species production, inflammation and the accumulation of long fatty acids. All these factors increase the susceptibility but do not cause mPTP opening and, until a very critical value is reached, the pH remains low. During reperfusion, the respiratory chain is suddenly re-exposed to oxygen with the restoration of the mitochondrial membrane potential of the still viable and not yet necrotic myocytes. This leads to neutralization of the acidic pH which induces the mPTP opening, allowing passage through the inner mitochondrial membrane of all molecules <1.5 KDa. This, of course, is a major problem for the cell, as the inner mitochondrial membrane becomes freely permeable to protons, effectively uncoupling oxidative phosphorylation, and disrupting ATP production (see fig. 6). Thus, progressively, osmotic swelling, damage and irreversible disruption of mitochondria occur. The mPTP and the con-

sequent abrupt increase of calcium trigger the different caspases, which, in turn, cause apoptosis, leading to cell death. Thus, the cells that survive the ischaemic insult might die from the damage generated by coronary reperfusion [44]. Griffiths and Halestrap [44] have demonstrated that mPTP opening occurs only upon reperfusion of the ischaemic heart, when the intracellular milieu is suited for its opening. Given the relevance of mPTP as the final, common pathway of all the possible players of reperfusion injury, it is not surprising that a considerable amount of still ongoing work has focused on the identification of the molecular structure of the mitochondrial pore. The most plausible model for the mPTP is a supra-molecular structure, assembled in the inner mitochondrial membrane by proteins composing mitochondrial contact sites between the outer and inner mitochondrial membranes. The list of possible proteins is long, but none have been proven to be the mPTP [45, 46]. The C subunit of mitochondrial ATP synthase has been proposed to play a critical role in mPTP opening phenomena [47]. Many factors support this hypothesis. For example, pH influences mPTP opening and also regulates ATP synthesis by modulation of the ATP synthase. The ATP synthase is sensitive to oxidative stress by the oxidation of specific cysteine residues. More specifically, the C subunit binds calcium and has pore-forming properties. In addition, dephosphorylation of the C subunit prevents mPTP opening while overexpression promotes its opening [48]. Several pharmacological agents have been suggested to limit reperfusion injury by inhibiting mPTP, including cyclosporine, TRO40303, adenosine, FX06, carperitide, atorvastatin, exenatide, glucose-insulin-potassium, bendavia and NO. Once again, as for all other attempts, the clinical results are not encouraging. The compounds that have been most extensively tested are cyclosporine and TRO40303. Cyclosporine-A inhibits mPTP opening by binding to mitochondrial targeted cyclophilin-D. Piot et al. [49] randomized 58 patients to receive single boluses of cyclosporine-A or placebo immediately before PCI. The infarct size measured by the area under the curve of CPK was significantly smaller in the cyclosporine group. This encouraging result generated a larger multi-centred trial in 970 patients with clearer outcomes [50]. Unfortunately, the results did not show any differences between the placebo and treated groups at 1 year. In contrast to cyclosporine-A, TRO40303 binds mitochondrial translocation proteins at the cholesterol site. A recent study in vitro has shown that the translocation protein does not play a role in mPTP opening. Accordingly, the clinical trials testing the role of TRO40303 as an adjunct therapy to

PCA (MITOCARE) showed no cardioprotective effects [51]. All these studies emphasize the need to clearly identify the composition of the mPTP and the trigger for its opening before moving to the clinic. In this context, the C subunit of ATP synthase could be an interesting new target.

Pre-, Post- and Remote Conditioning

Ischaemic preconditioning is the prototype of non-pharmacological cardiac protection since the magnitude of protection achieved by this protocol in the experimental setting is larger and more consistent than that from any other intervention. The phenomenon was identified by chance by Murry et al. [52], who showed that four cycles of 5 min of coronary occlusion and 5 min of reperfusion before a sustained coronary occlusion for 40 min remarkably reduces the infarct size in dogs. Importantly, the protection was independent from collateral blood flow. The protection is time-limited, i.e. if ischaemia is prolonged to 2 or 3 h, ischaemic preconditioning has no effect [53]. The beneficial effects of preconditioning were linked to a large extent to a reduction of reperfusion injury, even though it is likely that preconditioning reduces ischaemic damage as well. The mechanisms that underlie the cardiac protection induced by preconditioning relies on the activation of existing endogenous signalling molecules [54]. These molecules trigger intracellular mediator cascades and the activation of different effectors that stabilize myocytes and prevent their death. An abundance of signalling pathways have been discovered in many species and preparations which will not be discussed in detail here, but NO, protein kinase activation and mitochondria with the opening of mPTP are considered indispensable elements of preconditioning in the signal pathways [55].

The unpredictability of STEMI makes the application of preconditioning impossible in this clinical setting, although it could have a role in planned cardiac surgery [56, 57]. Zhao et al. [58] showed (in dogs) that brief episodes of ischaemia and reperfusion, performed immediately after reperfusion following prolonged ischaemia, reduce the final infarct size and named this phenomenon 'post-conditioning'. Staat et al. [59] applied a similar protocol in men with STEMI, within 1 min after primary angioplasty revascularization, by inflating/deflating the balloon in a 1-min cycle. This resulted in a 34% reduction of the area under the curve for CPK. However, the largest randomized clinical trial of post-conditioning in STEMI was neutral [60].

Another very attractive form of myocardial conditioning that does not involve manipulation of the culprit cor-

onary lesion is the so-called remote conditioning: a conditioning performed in a distant organ [61]. The mechanism of transfer of the cardioprotective signal from distance is unclear. Both neuronal and humoral hypotheses have been suggested [53]. Interestingly, a few groups have routinely applied remote preconditioning protocols in the ambulance during transfer to the PCI centre, using 4- to 5-min brachial cuff inflation with encouraging results [62, 63].

Conclusions

Despite improvements in its prevention and acute therapy, myocardial infarction remains a major cause of death and its incidence is rising. Reperfusion, by means of thrombolytic therapy or primary PCI, is the most effective strategy for reducing infarct size and improving clinical outcome. The process of myocardial reperfusion itself, however, can induce injury to the myocardium, thereby reducing the beneficial effects of myocardial reperfusion. Nonetheless, the existence of reperfusion injury has been and still is a matter of debate. Despite the convincing experimental evidence outlined in this review, definitive clinical demonstration is lacking. This is partly due to the gap between clearly defined and controlled experimental models and the difficulty of properly designing phase III clinical trials in humans subjected to revascularization for STEMI with either thrombolysis or PCI, and to the lack of a clearly defined target for the therapy. Because of the consistent lack of clinical success, reperfusion injury has been considered a laboratory issue, or even a fantasy, by most cardiologists. However, recent conditioning strategies have provided new enthusiasm for attenuating reperfusion injury and for it to be considered a reality. Preconditioning is not clinically feasible. Post- and remote conditioning are more feasible but not routinely used due to a lack of definitive evidence of any benefit. Even the recent intracoronary delivery of cell therapy to repair infarcted myocardium (which is not within the scope of this review) seems to have failed, at least in the clinic.

So, today, the only realistic strategy to reduce reperfusion injury in STEMI patients is to reperfuse as early as possible. This can be achieved through early diagnosis and immediate intervention using an effective network allowing ambulance diagnosis with direct access to intervention facilities. Clearly, the implementation of these networks has resulted in remarkably good results but still to the benefit of too few patients. For the others, who are

reperfused after a longer time of ischaemia, reduction of the injury remains a neglected therapeutic target, even though highly needed. The real risk is that, after such a long series of failures, the drug industry and the academia will withdraw their support and investments, which is to be regretted. Any such withdrawals may be self-defeating in the long term. On the contrary, we should aim at providing a clear understanding of ischaemic and reperfusion injury which will eventually allow the development of specific drugs or interventions to counteract the damage of acute myocardial infarction, which today is the most relevant killer. However, it would be unfair to conclude that more than 40 years of research on ischaemic reperfusion has failed to provide clinical results that have changed the natural history of the disease. This research has led to the discovery and wide application of cardiac protection with cardioplegia and hypothermia during surgical coronary artery bypass grafting, thus allowing complex cardiac interventions that were unimaginable in the absence of cardioprotection. Why such a consolidated success in comparison to the failure in the other clinical settings? Very simply, the surgeons apply cardiac protection before (and not after) ischaemia and during the surgically induced ischaemia to a quiescent heart that does not need to contract. Two important issues that make the difference!

Acknowledgements

This work was supported by a grant from Fondazione Anna Maria Sechi per il Cuore (FASC), Italy. The funders had no role in the preparation of or decision to publish the paper.

Conflict of Interest

Roberto Ferrari reports that he received a honorarium from Servier for steering committee membership, consulting and speaking, as well as support from Servier for travel to study meetings. In addition, he received personal fees from Amgen, Boehringer-Ingelheim, Novartis, Merck Serono and Irbtech.

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