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Pathophysiology of Ischemia-Reperfusion Injury: New Therapeutic Options for Acute Myocardial Infarction

Marisol Ruiz-Meana and David García-Dorado

Servicio de Cardiología, Hospital Universitari Vall d'Hebron, Barcelona, Spain

The impact of coronary artery disease on survival and quality of life is mainly due to cardiomyocyte death. Massive cardiomyocyte death occurs during acute myocardial infarction but emergency coronary recanalization is usually not able to prevent it. Laboratory research has demonstrated that a significant part of that cell death takes place during the first few minutes of reperfusion and that treatment aimed at disrupting the mechanisms responsible can reduce the size of the infarct. Those mechanisms include Ca^{2+} overload, mitochondrial permeabilization and cytoskeletal and membrane fragility (induced by the activation of proteases), all of which play critical roles. Moreover, cell death can propagate to adjacent cardiomyocytes via gap junctions. In addition, other myocardial and blood cells also contribute to both immediate and delayed cardiomyocyte death during reperfusion. Most forms of treatment developed to protect against reperfusion injury are still at the experimental stage, though some have been successfully tested in patients, such as atrial natriuretic peptide, inhibition of mitochondrial permeabilization and ischemic postconditioning. The possibility that myocardial salvage can be achieved by administering adjuvant treatment during coronary recanalization presents acute myocardial infarction patients with a new therapeutic option.

Key words: *Necrosis. Calcium. Hypercontracture. Mitochondria.*

Fisiopatología del daño miocárdico por isquemia-reperfusión: nuevas oportunidades terapéuticas en el infarto agudo de miocardio

La muerte de los cardiomiocitos es la principal causa del impacto de la cardiopatía isquémica en la supervivencia y la calidad de vida. Se produce masivamente durante el infarto agudo de miocardio, y la recanalización coronaria no suele ser capaz de prevenirla. Los estudios experimentales han demostrado que una parte significativa de esta muerte celular se produce en los primeros minutos de reperfusión, y que los tratamientos dirigidos a interferir con los mecanismos de su desarrollo reducen el tamaño de infarto. Entre estos mecanismos, la sobrecarga de Ca^{2+}, la permeabilización mitocondrial y la fragilidad del citoesqueleto/sarcolema (producida por la activación de proteasas) desempeñan un papel crítico. Además, la muerte celular se puede propagar a los cardiomiocitos adyacentes a través de *gap junctions*. Otras células miocárdicas y sanguíneas contribuyen a la muerte precoz y tardía de los cardiomiocitos durante la reperfusión. La mayoría de los tratamientos desarrollados contra el daño por reperfusión están circunscritos al ámbito experimental, pero algunos se han probado con éxito en pacientes, como el péptido natriurético auricular, la inhibición de la permeabilización mitocondrial y el poscondicionamiento. La posibilidad de salvar miocardio mediante tratamientos coadyuvantes aplicados durante la recanalización coronaria representa una nueva oportunidad terapéutica para los pacientes con infarto agudo de miocardio.

Palabras clave: *Necrosis. Calcio. Hipercontractura. Mitocondrias*

Coronary disease is one of the leading causes of death and disability, and epidemiological predictions indicate that its morbidity and mortality rates will exceed those of cancer and infectious disease in the near future in all countries throughout the world.[1] The devastating effects of this disease on world health are mainly caused by just one mechanism, cell death, that directly or indirectly—through contractile failure, ventricular remodeling and arrhythmias—causes the development of heart failure, disability and death. The extent of cell death (necrosis) caused by an acute coronary occlusion not only depends on

Correspondence: Dr. D. García-Dorado.
Servicio de Cardiología. Hospital Universitari Vall d'Hebron.
Pg. Vall d'Hebron, 119-129. 08035 Barcelona. España.
E-mail: dgdorado@vhebron.net

the size of the area at risk, but also on the severity and duration of ischemia. In recent years, there has been genuine medical progress that has significantly helped to improve survival and quality of life among patients with coronary disease. This has consisted in the development of treatment capable of restoring blood flow (reperfusion therapy) in patients with acute myocardial infarction.

It is well known that the survival of ischemic cells depends on various factors, and that the period cells undergo ischemia until the restoration of blood flow is the main factor determining the success of reperfusion therapy. This knowledge has led to great effort being invested in reducing the time from the moment a patient feels thoracic pain to the beginning of reperfusion therapy. However, costs continue to increase and further reductions in this period remain problematic. Despite improvements in the procedures used to reopen the coronary artery—that enable more effective, rapid, complete, and permanent recanalization of the acutely occluded coronary arteries—the vast majority of patients with ST-segment elevation acute coronary syndrome continue to present myocardial necrosis to a greater or lesser degree.

Reperfusion therapy, whether by thrombolysis or invasive procedures, does not guarantee the survival of ischemic cells, and numerous research studies conducted in the last 2 decades have unquestionably established that, although revascularization is the only possible alternative to salvage the ischemic cells from certain death, cell death is partly precipitated, paradoxically, by restoration of the flow itself.[2] This event, known as reperfusion injury, has been exhaustively explored in various experimental models, but its relevance has only recently been recognized in the context of clinical reperfusion. Thus, the possibility of improving the efficacy of thrombolysis and percutaneous coronary intervention through administering adjunctive cardioprotection therapy during revascularization provides a new therapeutic option capable of improving the clinical outcome when it is no longer possible to modify ischemia time. The development of cardioprotective strategies is based on knowledge of the physiopathological mechanisms of acute cell death during myocardial reperfusion. Although some of these mechanisms have been classically known by cardiovascular surgeons (and prevented through cardioprotective strategies used in the context of heart surgery), many others remain under investigation. In recent years, there have been major scientific advances in understanding and identifying various factors involved in cell death due to reperfusion and, based on this research, new and potentially useful therapeutic targets have been identified for its prevention.

MYOCARDIAL NECROSIS IN THE FIRST MINUTES OF REPERFUSION

When reperfusion is performed sufficiently promptly to save the ischemic myocardium at risk—that is reversibly damaged and would have developed infarction if the artery had not been opened—the cells that can recover control of ion homeostasis survive; however, in a variable proportion of cardiomyocytes ion imbalance not only is not corrected, but worsens, finally triggering immediate necrotic cell death. This type of death is characterized by occurring during the first minutes of restoring oxygen and blood flow, and is accompanied by cell membrane rupture and the release of cell content (mainly cytosolic enzymes) to the extracellular matrix, which leads to the characteristic histological features of contraction band necrosis,[3] where individual cardiomyocytes are extremely shortened and their sarcomeric structure is completely disorganized. Ultrastructural images of these cells obtained using electron microscopy show sarcolemmal rupture, mitochondrial swelling, and massive deposits of Ca^{2+} in the mitochondrial matrix, in addition to shortening and disorganization of the sarcomeric myofibrils (Figure 1).

The characteristics of this necrosis can be reproduced in a classic laboratory experiment that consists in submitting an isolated rat heart that has been retrogradely perfused using a Langendorff preparation to transient ischemia (normally between 40 min and 60 min) and later reperfusion. This experimental maneuver releases large amounts of intracellular enzymes (creatine kinase, lacatate dehydrogenase) in the first minutes of reperfusion, whose magnitude correlates with the size of the areas of contraction band necrosis seen in histological sections. This type of response experimentally demonstrates that myocardial cell death occurs early when flow is restored.[4] As will be seen, this type of cell death can be prevented by interventions administered at the time of reperfusion.

Studies conducted in isolated cardiomyocyte models have demonstrated that reoxygenation after a simulated ischemia period leads to abrupt shortening of cell length in the first minutes of reoxygenation, accompanied by cytoarchitectural disorganization, whose ultrastructural characteristics coincide with those of the necrotic contraction bands seen in histological sections.[5] This type of response at the cellular scale has been called hypercontracture and the likelihood of it occurring depends on the amount of time between severe depletion of the intracellular ATP content during ischemia and the moment of reenergization.[6] Analysis of myocardial segment length using piezoelectric ultrasonic probes has aided in determining the degree of myocardial shortening that occurs during reperfusion, detected

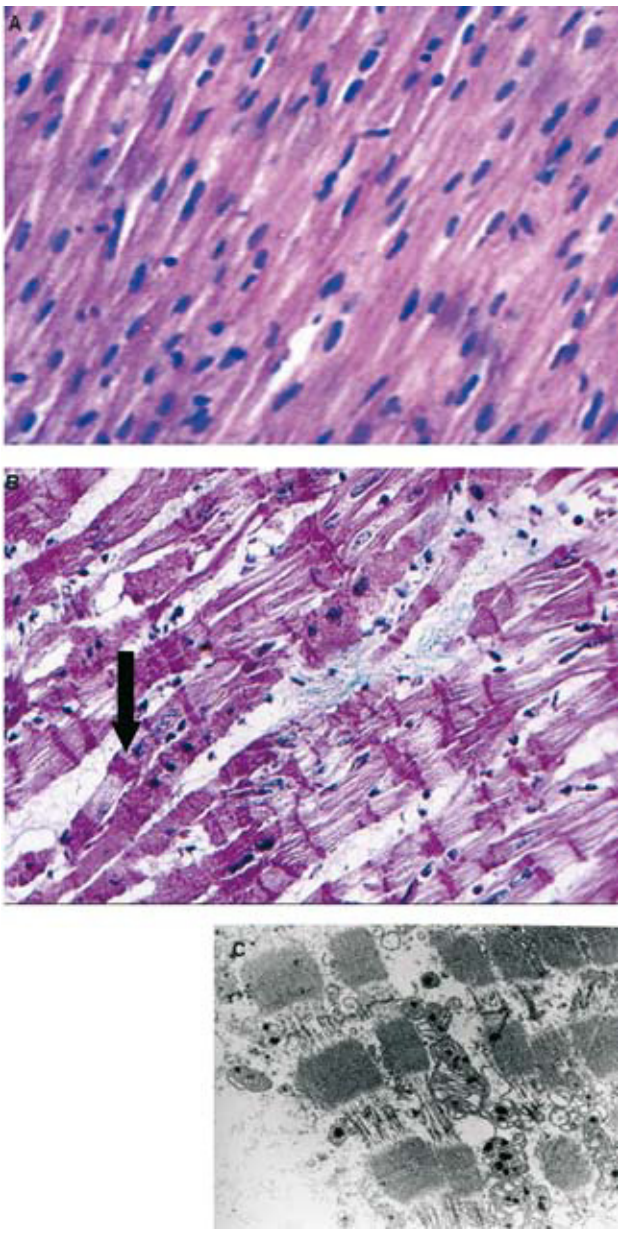

Figure 1. Histological image of pig myocardium from the control group (A) and reperfused pig myocardium after 50 min of ischemia (B), in which tissue disruption can be observed, characterized by severe cell shortening and the development of contraction bands (indicated by the arrow). C: ultrastructural detail of the contraction band, with clumps of sarcomeric protein and mitochondrial swelling and disruption.

as a reduction in end-diastolic length below the baseline level, and whose magnitude correlates with the area of necrotic contraction bands.[7] This variable is one of the many experimental determinations that indicate that necrotic contraction bands in reperfused infarcted tissue reflect the hypercontracture observed in isolated cardiomyocytes.

Loss of Ion Homeostasis During Ischemia-Reperfusion

The mechanisms that lead to extreme cell shortening through hypercontracture have been intensely investigated using isolated myocyte models, where morphological changes and ion imbalances can be simultaneously analyzed in a controlled environment. These types of studies have demonstrated that hypercontracture is caused by reoxygenation (that reactivates the contractile activity of the ATP-dependent myofibrils) coinciding with abnormally high concentrations of intracellular Ca^{2+} (that, in presence of ATP, generates uncontrolled and excessive contractile force) (Figure 2).[8] The loss of Ca^{2+} homeostasis begins during the previous ischemia period, when important changes occur in cytosolic composition.[6]

One of the first ion imbalances that is established during ischemia is the sustained increase of intracellular Na^+ concentrations due to Na^+/K^+ pump inhibition of the sarcolemma—which cannot operate in the absence of energy—and progressive cytosolic acidification by anaerobic glycolysis. The cell tries to correct the Na^+ overload through membrane Na^+/Ca^{2+} exchange in reverse mode—that does not require energy to function—and that extrudes intracellular Na^+ while introducing Ca^{2+} into the cells, and thus begins a process of progressive loss of control of Ca^{2+}, which, in physiological conditions, is one of the most strictly regulated cations in the intracellular matrix.

The reoxygenation of cells at risk can precipitate an abrupt worsening of cation control, mainly by intracellular acidosis correction mechanisms that further worsen cytosolic Na^+ overload. The return of blood flow rapidly washes out the catabolites (basically H^+) from the extracellular matrix, which leads to a pH gradient between the cells and their environment, thereby activating the mechanisms of correction of intracellular acidosis (mainly through the plasma membrane Na^+/H^+ exchanger and the $Na^+/HCO3^-$ cotransporter). This corrective response to intracellular acidosis further worsens the cytosolic Na^+ overload that, once again, is managed by the cell by activating reverse Na^+/Ca^{2+} exchange which causes[9] an additional influx of Ca^{2+}. The Ca^{2+} influx through reverse mode Na^+/Ca^{2+} exchange is of little relevance in physiological conditions in human myocytes, but can be detrimental when the cell is overloaded with Na^+. As a result of this chain of correction mechanisms, a great amount of Ca^{2+} accumulates in the reoxygenated cell, thus seriously compromising its own survival. In fact, pharmacological inhibition of Na^+/Ca^{2+} exchange using KB-R7943 has been shown to reduce cytosolic Ca^{2+} overload in isolated cardiomyocytes and

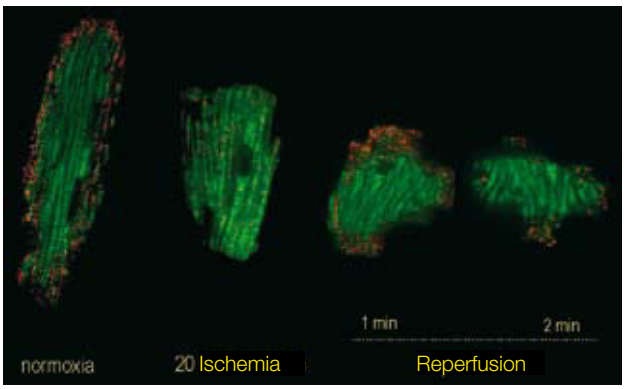

Figure 2. Temporal sequence obtained by confocal microscopy (3400) of a rat cardiac myocyte under controlled conditions (normoxia), after 20 min of simulated ischemia, and during the first 2 min of reenergization. The cell is stained with a specific fluorochrome that reflects changes in mitochondrial membrane potential (JC-1) used as an index of oxidative phosphorylation. The loss of the color red compared to green indicates mitochondrial depolarization. After 20 min of ischemia, most mitochondria were depolarized due to severe energy depletion accompanied by cell shortening or ischemic rigor contracture. During the first minutes of reperfusion rapid mitochondrial repolarization occurred with the simultaneous development of hypercontracture.

infarct size in an in situ transient coronary occlusion model.[9]

Cell Swelling, Structural Fragility, and Intercellular Junctions

In addition to the ion exchanges that occur in the cytosol previously mentioned, reactivation of the energy metabolism has direct consequences on the integrity and operation of vital cellular structures (cytoskeleton, intracellular organelles, and sarcolemma). First, the appearance of cell swelling due to washing out the metabolites accumulated in the extra-cellular space creates a trans-sarcolemmal osmotic gradient and favors the entry of water into cells. The increase in the volume of cells that have a weakened cytoskeleton and cell membrane promotes the loss of cellular integrity. Experimental studies have demonstrated that hyperosmotic reperfusion can have favorable effects on infarct size by reducing the degree of swelling and cell death.[10]

Second, the development of mechanical fragility during ischemia can significantly reduce the resistance of cells to the actual mechanical stress caused by reperfusion. The mechanisms that lead to cellular structures becoming fragile are not well understood, but it has been reported that calpain activation can cause proteolysis of structures of the subsarcolemmal cytoskeleton.[11,12] Calpains are Ca^{2+}-activated proteases that are inhibited by acidosis, but play a key role in cardiomyocyte death during reperfusion due to the combination of Ca^{2+} overload and pH normalization. The calpain-mediated degradation of ankyrin—a protein that participates in anchoring the Na^+/K^+ATPase membrane pump to the subsarcolemmal cytoskeleton—is a major cause of Na^+ pump impairment during initial reperfusion.[13] Such proteolytic degradation can lead to a vicious circle, where Na^+ overload favors additional Ca^{2+} influx and perpetuates calpain activation that, in turn, worsens intracellular Na^+ overload, which triggers hypercontracture and cell death.

Even when mechanical fragility is present, hypercontracture does not usually induce sarcolemmal rupture in isolated myocytes, but does induce the rupture of intact cells that are part of the myocardial tissue. This difference in response to mechanical stress caused by contractile hyperactivation may have several explanations. One is that the absence of osmotic edema in reoxygenated isolated myocytes (in which washout does not occur during reperfusion) reduces mechanical tension of the sarcolemma secondary to the development of hypercontracture.[14,15]

Another possible explanation is that the presence of intercellular junctions in intact tissue create tension and tear forces during cell shortening that facilitate rupture of the cell membrane. Studies conducted in our laboratory have demonstrated that hypercontracture of a myocyte can be transmitted to adjacent myocytes through gap junctions and that cell-to-cell propagation of hypercontracture contributes to the final size of the reperfused infarcts.[16,17] The use of pharmacological intercellular communication blockade has been proven effective in reducing cell death during the first minutes of reperfusion in various experimental models.[18,19] Our group has demonstrated that the mechanism of such cell injury propagation is based on the passage of Na^+ through gap junctions from the hypercontracted myocyte to the adjacent myocytes and the subsequent activation of the Na^+/Ca^{2+} exchanger in the adjacent cell, which in turn induces Ca^{2+} overload and the development of hypercontracture in that cell.[20]

Sarcoplasmic Reticulum and Mitochondria in the Genesis of Hypercontracture and Cell Death

In physiological conditions cardiomyocytes consume a great quantity of chemical energy to produce the mechanical work. Myofibril contraction is a highly controlled synchronized event, that is mediated by transient increases in cytosolic Ca^{2+} most of which come from the sarcoplasmic reticulum. It has been known for some time that the sarcoplasmic reticulum

is involved in some diseases and, in particular, in the genesis of certain types of arrhythmias, the progression of heart failure, and diabetic cardiomyopathy. However, much less is known about the contribution of the sarcoplasmic reticulum to the development of lethal reperfusion injury, even though there is solid experimental evidence demonstrating that it may play a decisive role in cardiomyocyte necrosis. During the first minutes of myocardial reperfusion, the sarcoplasmic reticulum is under severe Ca^{2+} overload, and this mitochondrial reactivation of ATP synthesis triggers Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA), which is responsible for cytosolic Ca^{2+} uptake, despite the persistence of increased Ca^{2+} flow from the extracellular matrix. As a result of this, there is a great accumulation of Ca^{2+} within the sarcoplasmic reticulum that exceeds its storage capacity, due to which Ca^{2+} is finally extruded through the ryanodine receptors (RyR) and again undergoes uptake, which leads to a rapid Ca^{2+} oscillation pattern that propagates through the cell and creates a mechanical force that can exceed the elastic capacity of the sarcomeres.[21] Various experimental studies have demonstrated that pharmacological blockage of Ca^{2+} oscillations by the sarcoplasmic reticulum reduces the incidence of hypercontracture in reoxygenized myocytes[22] and that the activation of intracellular signaling pathways that interfere with the movements of sarcoplasmic reticulum-dependent Ca^{2+}—for example, with natriuretic peptide agonists—confers protection during myocardial reperfusion, both in animals and humans.[23,24]

Although hypercontracture by itself can be a determining factor in sarcolemmal rupture, other mechanisms have been recently proposed that can also contribute to reperfusion injury and cell death. One which is currently the object of intense research is the loss of mitochondrial integrity in the presence of certain pathological conditions (Ca^{2+} overload, energy deficit, oxidative injury) due to an abrupt change in membrane permeability, an event known as mitochondrial permeability transition.[25] This abrupt change in mitochondrial permeability causes the uncoupling of cell respiration and an energetic collapse incompatible with cell survival.[26] Mitochondrial permeability transition has been documented during myocardial reperfusion, mainly as a consequence of oxidative injury and Ca^{2+} overload and by the rapid correction of intracellular acidosis, one of the most effective inhibitors of this change of mitochondrial permeability (Figure 3).[27-29] Although it seems clear that mitochondrial permeability transition causes cytochrome C release and implements the signaling chain that leads to cell apoptosis, the mechanism by which it causes necrosis in reperfused myocardium is not well understood.[30] In fact, the energetic collapse secondary to mitochondrial permeabilization is difficult to reconcile with the development of hypercontracture, since the initial studies on lethal reperfusion injury,[31] as well as recent data obtained using magnetic resonance spectroscopic imaging (unpublished data), demonstrate that hypercontracture is an energy-dependent event. Nor is it clear by which mechanism the energy depletion induced by the change of mitochondrial permeability can cause sarcolemmal rupture in the first minutes of reperfusion. Recent studies in isolated cardiomyocytes, where mitochondrial permeability transition was induced by pulsed-laser stimulation, indicate that permeability transition in a small number of mitochondria within the cell can worsen Ca^{2+} management in cells, since this is accompanied by mitochondrial Ca^{2+} release into the cytosolic space, which favors the development of hypercontracture whenever the cell preserves a sufficient number of intact mitochondria able to maintain energy demand.[32] Figure 4 shows a schematic representation of the physiopathological mechanism of reperfusion injury.

The Contribution of Other Cell Types to Ischemia-Reperfusion Injury

Finally, it should be borne in mind that although most of the characteristics of necrosis secondary to transient myocardial ischemia can be experimentally reproduced in isolated cardiomyocyte preparations and in isolated perfused hearts, other cell types, such as blood platelets, neutrophils and fibroblasts, among others, can contribute to reperfusion injury and finally to cell death. In particular, blood platelets activated during ischemia-reperfusion adhere to the microvascular endothelium of the reperfused myocardium through L-selectin and release factors that contribute to the loss of Ca^{2+} homeostasis and myocyte death.[33-35]

FROM LABORATORY RESEARCH TO PATIENTS: THE CHALLENGES OF TRANSLATIONAL MEDICINE

Lethal reperfusion injury can be a therapeutic target in patients with acute myocardial infarction. The possibility of administering adjunctive treatment during angioplasty should be based on knowledge of the molecular and cellular mechanisms that lead to acute necrotic death during myocardial reperfusion. However, basic research often encounters numerous obstacles regarding its transfer to the clinical context, and although certain drugs and interventions have proven effective in reducing cell death in cellular and animal models, they are rarely developed to the point of being marketed for use in humans. The reasons for failure in biomedical research transfer

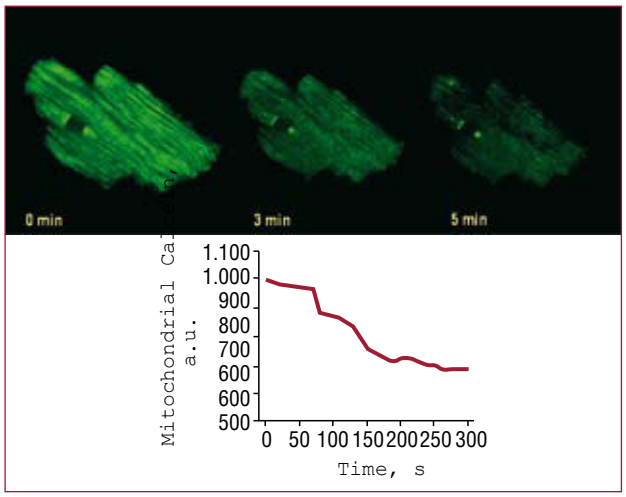

Figure 3. Temporal sequence obtained by confocal microscopy (×400) of a rat cardiac myocyte during the first minutes of reperfusion, where the mitochondria have been stained with calcein, a fluorochrome that becomes trapped in its interior unless mitochondrial permeability transition occurs. In this specific example, the contractile apparatus of the cell was blocked with BDM to prevent the development of hypercontracture and avoid artifacts in the intensity of the fluorescent signal. During the first minutes of reperfusion, there was a strong decrease in the mitochondrial fluorescent signal which is a sign of mitochondrial permeabilization.

tend to be attributed to the differences between species in disease pathogenesis and in response to treatment, and that experimental models lack the capacity to suitably reproduce the complexity of the physiopathological processes that take place in clinical contexts, but many other reasons exist which may explain this failure.

The Breakdown in Knowledge Transfer

One of the experimental approaches that has had a clear protective effect both in animal models and in perfused organs or cells in culture is Na^+/H^+ exchanger inhibition using cariporide.[36,37] The progressive establishment of intracellular acidosis during ischemia contributes to pathological Na^+ overload by cell membrane Na^+/H^+ exchanger activity. This mechanism does not operate in normal cells, so that exchange inhibition could be a good therapeutic target with specific action during the course of the disease. Experiments conducted by our research group and others have demonstrated that Na^+/H^+ exchanger inhibition using cariporide before coronary occlusion is in fact capable of significantly reducing the extent of necrosis during reperfusion, but its mechanism of action is not based on prolonging acidosis, as had been predicted, but on delaying the depletion of cell energy reserves and the onset of ischemic rigor contracture.[38,39] Its administration at the time of reperfusion has no effect on infarct size because the cardiomyocytes are able to correct intracellular acidosis despite the Na^+/H^+ exchanger being inhibited through other parallel mechanisms (basically the Na^+/HCO_3 cotransporter)

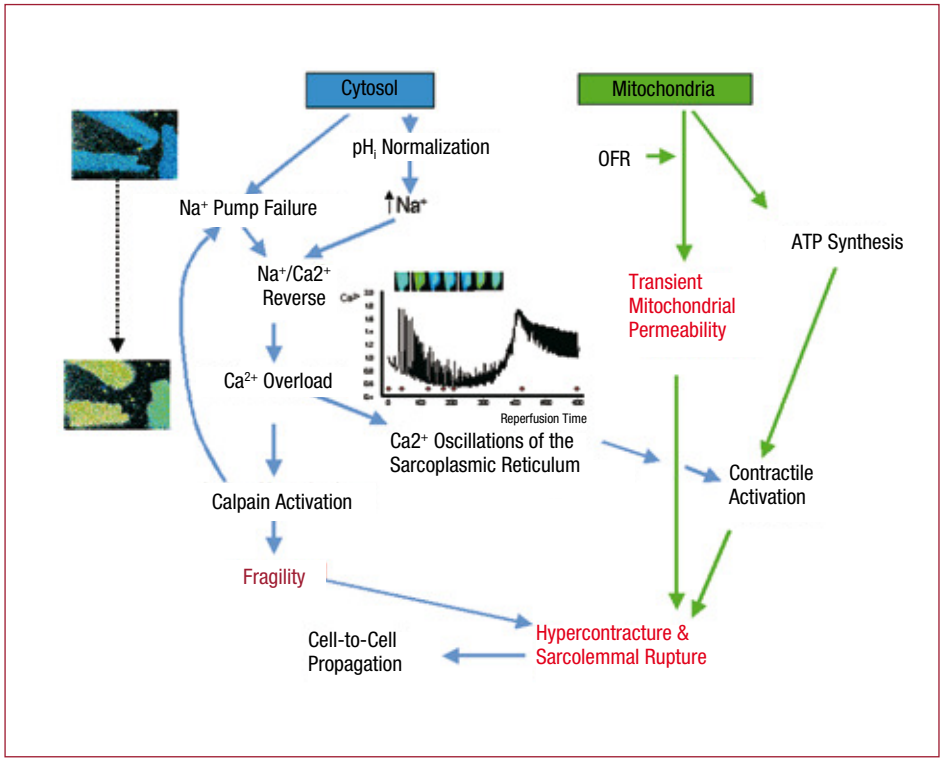

Figure 4. Schematic representation of the mechanisms that lead to cell death during the first minutes of reperfusion. OFR: oxygen free radicals.

that contribute to Na^+ and Ca^{2+} overload and counteract the protective effect of cariporide.[40]

Despite this evidence, various clinical trials have been conducted with the aim of investigating the protective effect of Na^+/H^+ exchanger inhibition in patients with acute myocardial infarction: the Guard During Ischemia Against Necrosis (GUARDIAN) study,[41] the Evaluation of the Safety And Cardioprotective Effects of Eniporide in Acute Myocardial Infarction (ESCAMI) study[42] and the Sodium-Hydrogen Exchange Inhibition to Prevent Coronary Events in Acute Cardiac Conditions (EXPEDITION) study.[43] The GUARDIAN study included more than 11 000 patients with acute coronary syndrome who underwent angioplasty or surgery and who received cariporide at different doses (20 mg, 80 mg, or 120 mg). The administration of high-dose cariporide offered protection when patients received the treatment before ischemia (a requirement that was only fulfilled in the subgroup of patients who underwent bypass surgery). The drug offered no protective effect on the other study variables (reinfarction rate, evolution of patients in whom ischemia was not severe), as predicted by experimental studies. The ESCAMI study specifically selected patients with coronary occlusion with ST-segment elevation who underwent reperfusion (959 patients) and were administered eniporide (up to 200 mg) at the time of reperfusion. The results of this study were negative and the pharmacological treatment was safe but ineffective. In view of the positive results among the subgroup of patients who underwent surgery in the GUARDIAN study, the EXPEDITION study was designed, a large clinical trial that included more than 5700 patients who underwent aortocoronary bypass surgery, and who received a very high dose of cariporide (more than 200 mg) over a very long period. The results of this study also demonstrated a reduction in myocardial injury, as described in the GUARDIAN study, but there was a significant increase in the incidence of treatment-associated stroke. Overall, this represents a group of instances where translational research was unsuccessful due to failure to match the design of the clinical trial (regarding both population selection and the study variables, doses and the time of administration of pharmacological treatment) to the experimental results.

Another problem that often occurs when transferring experimental results to clinical trials is that they test therapeutic targets whose physiopathological role is questionable or for which there is a lack of sufficiently robust scientific evidence. Thus, for example, numerous clinical trials have been designed to test the cardioprotective effect of drugs that inhibit oxygen free radical formation, with negative or controversial results,[44] and that are based on the hypothesis that oxygen free radicals are detrimental when applied exogenously. However, a serious limitation to this hypothesis is that there are no mechanistic experimental data establishing a cause-and-effect relationship between oxygen free radical production during reperfusion and the development of hypercontracture and cell death.

The Difficulty of Selecting Therapeutic Targets of Interest for Drug Development

The cell membrane Na^+/Ca^{2+} exchanger is the final cause of Ca^{2+} overload during reperfusion.[9,20,45] The activation of this exchanger in its reverse mode allows the cell to eliminate the excess Na^+ that has accumulated during ischemia at the expense of deleterious Ca^{2+} influx from the extracellular matrix. This exchange is irrelevant in healthy cells with good control over intracellular Na^+ concentrations, but is crucial for myocytes that have suffered previous ischemia. Experimental studies have contributed solid evidence demonstrating that the inhibition of this exchanger at the time of reperfusion using a specific noncommercial drug, KB-R7943, is able to significantly reduce cytosolic Ca^{2+} overload and hypercontracture in isolated reperfused cardiac myocytes, leading to a reduction in infarct size in isolated rat heart and in pig heart submitted to in situ transient coronary occlusion.[9,20] Other studies, using genetically modified animals, have confirmed the mechanism by which this drug offers protection in reperfusion injury.[46] Nevertheless, despite the robustness of the experimental evidence, no pharmaceutical company has invested the effort necessary to develop a drug able to specifically and safely inhibit the Na^+/Ca^{2+} exchanger. As a consequence of this, no clinical trial has been conducted in humans in the context of myocardial reperfusion.

Cyclic guanosine monophosphate (cGMP) is a second messenger that has numerous functions in cells and various studies have demonstrated that it decreases in cardiomyocytes submitted to ischemia-reperfusion.[47] The initial experiments designed to explore the potential cardioprotective effect of cGMP during reperfusion were conducted in isolated cardiac myocytes,[48] and demonstrated that stimulation of cGMP synthesis (or exposure to soluble cGMP analogues) was able to reduce hypercontracture and cell death during reperfusion. Later studies have proven that exogenous administration of L-arginine (which can increase NO availability and, as a result, cGMP availability) also protects isolated rat heart and intact pig heart against reperfusion injury.[49] However, the increase in NO availability by L-arginine can have many

other effects in addition to increasing cGMP synthesis, and is an intervention that should be applied before reperfusion, which significantly limits its therapeutic potential. To mitigate this limitation, other experimental approaches have been designed with the aim of increasing myocardial cGMP concentrations through stimulation of particulate guanylate cyclase with urodilatin. This intervention, applied in the first minutes of reperfusion, can attenuate hypercontracture and significantly reduce the extent of necrosis in isolated rat heart submitted to ischemia-reperfusion[50] and in intact pig heart submitted to transient coronary occlusion, in the absence of hemodynamic effects.[23] Other laboratories have consistently documented a protective effect of interventions aimed at increasing cGMP concentrations in the reperfused myocardium. Furthermore, various mechanisms have been proposed by which cGMP can have beneficial effects during myocardial reperfusion, such as the reduction of Ca^{2+} oscillations from the sarcoplasmic reticulum and of arrhythmias,[51] inhibition of intercellular communication,[52] and platelet activation inhibition. Therefore, this represents a therapeutic target which can reduce the results of cell injury associated with Ca^{2+} overload once this has already been established.

Unlike other experimental interventions, the concept of cardioprotection through cGMP stimulation has in fact been tested in patients. Intravenous administration of atrial natriuretic peptide at the time of reperfusion in patients with acute myocardial infarction has been associated with decreased left ventricular remodeling, after 1 month of follow-up, compared to controls.[53] More recently, the protective effect of recombinant human ANP has been demonstrated in a double-blind clinical trial.[54] Despite this evidence, an insufficient number of clinical trials have been conducted in patients on the potential cardioprotective effect of these drugs, as it has not been considered a high-priority subject.

A pilot study has recently been conducted in patients to test the protective effect of mitochondrial permeability transition inhibition by administering cyclosporin A immediately before percutaneous coronary intervention.[55] Mitochondrial permeability transition has been identified in numerous experimental studies as a mechanism that induces necrotic cell death during reperfusion. In the pilot study mentioned, 58 patients with ST-segment elevation acute myocardial infarction were randomized to receive an intravenous bolus of cyclosporin A (2.5 mg/kg) or placebo. Although the previous duration of ischemia and the area at risk were similar in both groups, administration of cyclosporin A significantly reduced infarct size (CK, troponin I and MRI) in the group receiving treatment. It can be argued that cyclosporin A is a pleiotropic drug, with immunosuppressive effects but with little specificity, and that the mechanism of action remains uncertain regarding how it achieves its cardioprotective effect, although this study is of value in that it successfully tested an experimental hypothesis in patients.

Similarly, the concept of cardioprotection by ischemic postconditioning has been transferred to patients with acute myocardial infarction. Ischemic postconditioning is the event by which brief episodes of occlusion and reopening of the coronary artery at the time of reperfusion reduces cell death, a strategy that has been tested in various animal models and in perfused organs, although the molecular mechanisms involved remain a matter of debate. In the study conducted by Staat et al,[56] 30 patients undergoing coronary angioplasty were randomized to a control group or postconditioning group, in which an angioplasty balloon was alternately inflated and deinflated for 4 episodes of 1 min each. Infarct size, determined by CK release over 72 h, was significantly smaller in the postconditioning group.

THE FUTURE OF MYOCARDIAL PROTECTION AGAINST CELL DEATH SECONDARY TO ISCHEMIA-REPERFUSION

Despite the enormous progress that has occurred during the last two decades regarding knowledge of the physiopathological mechanisms that lead to lethal reperfusion injury, some results have been challenged, and many of the factors involved remain unknown. The focus of cardiovascular research in the future should be less compartmentalized and include the following: *a)* systems biology; *b)* integrated physiological studies; and *c)* a multidisciplinary approach.

Systems biology can help to unravel the complexity of biological models that, in general, function as networks made up of an infinite number of nodes, through the integral study of complex systems based on high-throughput quantitative techniques and using computer-simulation models.[57] This approach increases predictive accuracy (predictive biology) and more reliably establishes the association between a specific biological effect and the stimulus that triggered it (integrative biology). Studies on systems biology networks require high-throughput technology and the development of sophisticated mathematical models which have just begun to be available. An example of this is magnetic resonance spectroscopic imaging, a tool by which complex metabonomic studies can be conducted in which the general patterns of many metabolites can be simultaneously compared.[58] On the other

hand, integrated physiological studies can directly investigate the relationship between the metabolic pathways involved in a specific process and the real effect of this process on cell morphology or function. This approach contributes relevant information on the integrated response of a cell or organism to a specific stimulus, although its main limitation continues to be its strong dependency on the experimental conditions. Finally, a multidisciplinary approach should help to address biological problems based on a broader perspective, from both technical and knowledge standpoints, thus facilitating the development of new cardioprotection strategies and the correct design of clinical studies.

CONCLUSIONS

The treatment of reperfusion injury provides a real opportunity to reduce cell death and improve the prognosis of patients with myocardial infarction undergoing reperfusion. The positive results obtained in several recent clinical trials support this idea. However, intense efforts will be needed in translational research as well as a strong commitment from industry to transfer cardioprotective treatment to clinical practice in patients with acute myocardial infarction.

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