ARTÍCULO DE REVISIÓN

Myocardial Ischaemia-Reperfusion Syndrome

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Abstract

Reperfusion is the definitive treatment to salvage ischemic myocardium from infarction. A primary determinant of infarct size is the duration of ischemia. Early reestablishment of blood flow to ischaemic myocardium limits infarct size and reduces mortality. In a paradoxical manner, the return of blood flow to ischemic myocardium may result in additional injury in the area at risk. This condition is known as reperfusion injury, and the damage is more likely when reperfusion therapy is delayed. Even when the majority of the clinical trials designed to evaluate agents for preventing reperfusion injury have been disappointing, therapies to limit reperfusion injury remain an active area of investigation.

Key words: Myocardial reperfusion therapies, ischaemic myocardium, reperfusion injury.

Título: Síndrome de lesión por reperfusión miocárdica

Resumen

La reperfusión es el tratamiento definitivo para salvar miocardio isquémico del infarto. Un determinante principal del tamaño del infarto es la duración de la isquemia. El restablecimiento temprano de flujo sanguíneo al miocardio isquémico reduce los límites del tamaño del infarto y la mortalidad. De manera paradójica, el retorno del flujo sanguíneo al miocardio isquémico puede resultar en lesiones adicionales en el área de riesgo. Esta condición se conoce como lesión por reperfusión, y el daño es más probable que suceda cuando la terapia de reperfusión se retrasa. Incluso cuando la mayoría de los ensayos clínicos diseñados para evaluar agentes para prevenir la lesión por reperfusión han sido decepcionantes, terapias para limitar la lesión por reperfusión siguen siendo un área de investigación activa.

Palabras clave: terapias de reperfusión miocárdica, isquemia miocárdica, lesión por reperfusión.

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Received: 09/02/2014 Reviewed: 18/02/2014: Accepted: 04/03/2014

Introduction

Cardiovascular diseases occupy the first place in worldwide mortality. Much of this proportion is due to heart disease caused by ischaemia, which is usually caused by the obstruction of a coronary artery secondary to the rupture of an atherosclerotic plaque. Prolonged ischaemia can lead to irreversible myocardial damage and cell death, and it has been demonstrated that if the ischaemia is quickly reversed, myocardial tissue recovery occurs. Therapeutic efforts are aimed to achieve the restoration of blood flow to ischaemic myocardial tissue for this reason, a strategy that has been termed 'reperfusion' and is thought to stop the main mechanism of cell damage, i.e. hypoxia. However, there is a phenomenon in which despite having achieved early restoration of blood flow and consequently the supply of oxygen and nutrients, cell damage remains irreversible and results in cell death. This article will explain the mechanisms by which ischaemia causes cellular damage, the phenomenon of reperfusion injury and what treatment options are available.

Definition

Reperfusion injury is defined as myocardial, vascular or electrophysiological dysfunction caused by restoring blood flow to a previously ischemic tissue [1].

Mechanisms of Ischaemic Myocardial Injury

Myocardial reperfusion injury was first reported in 1960 by Jennings et al. It has been shown that only those ischaemic cardiomyocytes able to recover their ionic homeostasis will survive. However, a variable number of ischaemic cardiomyocytes fail to recover the ionic balance, thus triggering cell death [2]. Interruption of myocardial blood flow causes a series of events that lead to alterations in the myocyte's physiology and architecture including mitochondrial and sarcolemma injury and alterations in intracellular calcium handling, culminating in injury and cell death [3]. Those processes include the decrease of intracellular ATP levels, the change in energy usage from aerobic to anaerobic metabolism and a reduction in intracellular pH.

Initially, this damage is reversible and the restoration of blood flow during this period could recover the normal structure and function of the cells. But if the ischaemia persists for a long period of time, the damage becomes irreversible. During this period of time the ischaemic myocytes are viable, but paradoxically, the restoration of blood flow and the reintroduction of oxygen and energy into an abnormal cellular environment will lead to additional cells' injury and death. The factors contributing to reperfusion injury include damage to cellular membranes and organelles, free-radical formation,

leukocyte aggregation, increased mediators of inflammation, platelet and complement activation, endothelial damage, activation of the pro-apoptotic signalling cascade and vasoconstriction [4-7].

From a physiological point of view, four types of injury have been described: The first, it´s the stunned myocardium, which is the mechanical myocardial dysfunction that persists despite the absence of irreversible damage and despite restoration of coronary blood flow. The second is the no-reflow phenomenon that is the inability to reperfuse a previously ischaemic region. The third, it´s the cardiac dysfunction accompanied by arrhythmia. The last type is the lethal reperfusion injury that consists of death of the cardiomyocytes after reperfusion [1].

Factors Contributing to this Phenomenon

Calcium

Calcium is an essential element for cell function, including the regulation of cell death. Evidence shows that calcium is one of the main elements involved in myocardial reperfusion injury, since the cytosolic increase of this element gives rise to various processes that terminates in cardiomyocyte injury or death [1,7,8]. The intracellular calcium overload was first demonstrated by Zimmerman et al., in 1967 [9]. Ischaemia induces intracellular accumulation of ions of sodium, hydrogen and calcium culminating in tissue acidosis. Reperfusion produces alterations in the ionic fluxes and evidence suggests that rapid normalization of pH after the ischaemic tissue is reperfused leads to an increase in cytotoxicity. Sodium-dependent buffering mechanisms, including pumps Na+/H+ and Na+/HCO_3- are activated leading to the intracellular accumulation of Na+. Consequently, this high concentration of Na+ increases the exchange of Na+ by the sarcoplasmic Ca_2+. The increased Ca_2+ entry through L-type calcium channels and the decrease in its reuptake by the sarcoplasmic reticulum promotes the overload of this ion in the myocardial cell. This ionic disbalance results in myocardial stunning, myofibrillar hypercontractility, mitochondrial ultrastructural damage and ATP depletion [10].

Oxygen and Free Radicals

Cardiomyocytes are cells with high energy requirements, which is why they have a high density of mitochondria. It is not strange that these organelles loaded with reactive-oxygen species and pro-apoptotic materials are directly related to myocardial reperfusion injury. The mitochondrial inner membrane, which is responsible for the potential of the membrane, in normal conditions is impermeable to ions and proteins. The

alteration of the inner membrane potential is the process called 'transitional permeability', a process that is believed to be performed by the mitochondrial permeability transition pore (mPTP). mPTP formation creates a non-selective channel between the inner mitochondrial membrane and the sarcoplasm.

The result is an electrochemical gradient where the mitochondrial matrix elements are released into the sarcoplasm. This triggers the biochemical phenomena experienced in reperfusion injury such as oxidative stress, Ca_2+ overload and rapid normalization of pH [10]. This is why there has been so much interest in the mPTP as a therapeutic target. It also explains the generation of free radicals through the incomplete reduction of oxygen during the reperfusion injury. Although the cardiomyocytes express endogenous enzymes for scavenging free radicals such as superoxide dismutase, catalase and glutathione peroxidase, its capacity is exceeded after ischaemia and reperfusion.

It has been demonstrated that exogenous free radical entrance also increases the Ca_2+ ion load in the cell thereby causing more damage. The leukocytes reaching the site of the injury are also a source of free radicals. Free radicals cause lipid peroxidation leading to rupture of the cell membrane, resulting in cellular oedema and necrosis, denaturation of proteins including ion channels and enzymes, and rupture of the DNA strands, playing an important role as mediators of cell apoptosis [11].

Platelet Activating Factor (PAF)

PAF is a phospholipid with diverse and potent physiological effects. Under normal physiological conditions, PAF is minimally expressed. Its expression has been reported in various cells including cardiomyocytes. In myocardial reperfusion injury, in addition to its inflammatory role, PAF is believed to be released during oxidative stress mediated by free radicals and there is evidence that this alters the tone of the coronary arteries by inducing prolonged vasoconstriction [12].

Nitric Oxide (NO)

NO is a gas that is synthesized by the enzyme nitric oxide synthase primarily by the endothelial cells, with multiple —mainly vasoactive— actions in the human body. During reperfusion injury, the NO can preserve coronary blood flow, decrease platelet aggregation and reduce the interaction between neutrophils and the endothelium. At low concentrations, NO can improve cardiomyocyte function. In contrast, at high concentrations, NO can depress its function, alter mitochondrial respiratory chain and may even induce apoptosis [13,14].

Cell Apoptosis

Reperfusion induces apoptosis. It requires the activation of caspases, which in turn activate endonucleases responsible for DNA degradation and subsequent cell death. Certain target proteins mark the apoptotic process (In situ end labelling - TUNEL, Annexin V). In essence, viable cells that are reperfused and are provided of oxygen and glucose remain alive. However, reperfusion also provides the energy necessary to complete the apoptotic process and even accelerate it. During reperfusion, it is believed that the failure of ion channels, nitric oxide and some growth factors can trigger apoptosis. Several studies have shown that during myocardial infarction, most of the cardiomyocytes die through apoptosis. However, these studies have been questioned because of their methodology for identifying and measuring apoptosis in infarct tissue. In turn, apoptosis has been a great source of research to reduce the size of the infarct zone [14]. Also, recent work has implicated autophagy in the pathogenesis of ischaemia reperfusion injury. Over time, we will know the real mechanism and implications [15].

Neutrophils

During myocardial reperfusion injury, the endothelium and myocardial cells generate inflammatory signals to which neutrophils respond directly. The evidence shows that neutrophils are directly implicated during the reperfusion injury. Several studies have shown that neutrophils are related to the lethal reperfusion injury type, in which cardiomyocytes that were completely viable die at the end of ischaemia. Neutrophils produce various derivatives that are mediators of reperfusion injury, and produce enzymes such as proteases, gelatinases and collagenases, which damage the extracellular matrix. Neutrophils are a primary source of free radicals. The neutrophils are activated and respond to a large number of mediators.

Some of these mediators are produced in endothelial cells, cardiomyocytes and mast cells. Other mediators are the complement C5a, and various cytokines such as TNFa, IL-1, IL-6, IL-8 and PAF. It is known that the intracoronary infusion of complement C5a stimulate neutrophil adhesion to the vascular endothelium and is associated with decreased blood flow. Arachidonic acid is released in reperfused myocardium by neutrophils and this is a potent neutrophil chemoattractant. The interaction between neutrophils and vascular endothelium is a key step in the inflammatory process, which usually takes place in postcapillary venules. This step is dependent on selectins. In terms of time, during the insult, neutrophil infiltration is a slow process that can begin from 12 hours to 4 days post event. During reperfusion, this time is

accelerated and in turn infiltration is increased. The results of all these events are cell necrosis, endothelial damage and microvascular damage [16].

Clinical Manifestations

Reperfusion Arrhythmias

Reperfusion arrhythmias may be mediated by mitochondrial dysfunction and are common among patients treated with thrombolytic therapy, primary percutaneous coronary intervention and cardiac surgery. The most common arrhythmia during reperfusion is accelerated idioventricular rhythm. Ventricular tachycardia and fibrillation can also occur post-thrombolytic therapy, but these arrhythmias are more likely to be seen in persistent vessel's occlusion and infarction. Subsequent to the period of ischaemia, mitochondria may not be able to restore or maintain their internal membrane potential, thereby destabilising the action potential and increasing the susceptibility to the development of arrhythmias [17].

Microvascular Dysfunction

Final recovery of myocardial function requires the preservation of the coronary microvasculature [18]. Microvascular dysfunction, or the phenomenon of "no-reflow" previously mentioned, refers to the insufficient blood flow within the post-ischemic vasculature in rest, and it has been associated with worse cardiovascular outcomes [19]. Platelet activation and activation of complement are associated with microvascular dysfunction, so the aggressive antiplatelet therapy could have beneficial effects in its prevention, including the use of inhibitors of glycoprotein IIb/IIIa [20-23].

Myocardial Stunning

It's defined as the transient myocardial dysfunction occurring after reperfusion and is believed to be due to persistent anaerobic metabolism even after reperfusion. It has also been associated with microvascular injury [24-26]. Myocardial stunning may recover over time, so inotropic agents can be used for a short time to improve cardiac function and perfusion of organs. Other therapies that may prevent its occurrence are still under investigation.

Myocyte Death

The myocytes' death is the worst consequence of reperfusion injury and data from diverse studies suggest that more than 50% of the size of the infarction can be attributed to this kind of lesion [27,28]. Whereas reperfusion may accelerate or cause further damage to the injury that occurred during the previous ischemia, several studies have focused on the modification of the conditions of reperfusion to evaluate if there is any effect on the extent of damage [29,30].

Treatment of Myocardial Ischaemia-Reperfusion Syndrome

Based on the pathophysiology of reperfusion injury, several possible therapeutic strategies could be considered. However, only few interventions have proved useful in clinical practice, which could be explained by the multiple mechanisms related to myocardial infarction and reperfusion injury. Besides, some mechanisms may play a dual role, as mediators of injury as in the healing process. Targeted therapies against these mechanisms can be unpredictable [16]. Other conditions that may be associated with this potential therapeutic failure include the time of initiation of therapy (some agents might work better if patients are being treated with them previously) and the presence of comorbidities such as diabetes and hypercholesterolaemia. The following are some of the strategies that have been studied with this purpose:

Inhibitors of Glycoprotein IIb/IIIa

Platelet activation plays a role in the myocardial microvascular and reperfusion injuries. GP IIb/IIIa inhibitors are potent inhibitors of platelet activity, but there is not enough evidence for routine use in the management of patients with myocardial ischaemia — reperfusion syndrome [22,23].

Adenosine

It's a substrate for ATP reestablishment, induces vasodilation and inhibits platelet and neutrophils function. Adenosine has been evaluated in different trials, with variable results. Acute Myocardial Infarction Study of Adenosine (AMISTAD) was the first large-scale clinical trial testing adenosine as an adjunct to reperfusion therapy. This trial showed that adenosine infusion (70 µg/kg/min) was efficacious with regard to reducing infarct size in patients with anterior AMI without causing serious adverse clinical events (33% relative reduction in infarct size) [31].

AMISTAD II was designed to examine the effect of adenosine as an adjunct therapy for patients with acute anterior STEMI undergoing either thrombolysis or PCI. In this trial, 2.118 patients were randomized to receive a 3-h intravenous infusion of low-dose adenosine (50 µg/kg/min), high-dose adenosine (70 µg/kg/min), or placebo before PCI or within 15 min of the initiation of fibrinolysis. Although the high-dose adenosine group showed a significant reduction in infarct size compared with the control group, no difference in the primary clinical composite endpoint of death, new-onset congestive heart failure, or rehospitalization for congestive heart failure within 6 months was observed [32]. Intracoronary administration of adenosine and a syn-

thetic agonist of adenosine have been studied in smaller clinical trials, with promising result, but further studies are needed [33,34].

Vasodilators

Papaverine has proven useful in improving TIMI flow of the epicardial arteries, but its use has been limited by its association with the occurrence of ventricular arrhythmias, primarily with intracoronary administration [35]. Inhibitors of angiotensin converting enzyme (ACE) can have several beneficial actions on reperfusion, including free-radical scavenging, coronary vasodilation and elevation of bradykinin and prostacyclins levels [36]. In animal models, ACE inhibitors increased coronary blood flow, but have failed to produce improvement in regional ventricular function [37]. Several studies have investigated the effects of inhibiting the synthesis of endothelin-1 or its receptor blockade. Although the results are conflicting, larger studies are ongoing with these drugs [38-41].

Modulation of Ion Channels

Considering that changes in intra- and extra-cellular ion concentrations play a role in some of the processes involved with reperfusion injury, ion channels constitute an attractive therapeutic target for the management of this type of injury. Sodium/hydrogen ion exchange (NHE) is an important regulator of intracellular pH and calcium concentration. Blocking this exchange could reduce calcium uptake and preserve cellular architecture. Experimental studies have demonstrated marked limitation of infarct size when NHE-inhibitors are administered prior to ischaemia [42-45]. However, in two randomized studies the sodium/hydrogen exchange inhibitor eniporide had no impact in terms of reduction in infarct size or clinical outcomes [46,47]. The efficacy of NHE inhibitors administered just prior to reperfusion remains controversial.

Ischaemia of myocardium is associated with increases in the late sodium current (I_{Na}), intracellular sodium and calcium concentrations, calcium overload, and impairment of contractile relaxation. Ranolazine reduces the late I_{Na} and, is expected to decrease sodium entry into ischaemic myocardial cells. As a consequence, ranolazine is proposed to reduce calcium uptake indirectly via the sodium/calcium exchanger and to preserve ionic homeostasis and reverse ischaemia-reperfusion induced contractile dysfunction. It is effective in the treatment of chronic angina and may be effective in reperfusion injury and in limiting infarct size in AMI patients [48-50]. Finally, within this group, it is important to mention the ATP-sensitive potassium (K-ATP) channel activators. These channels play a role

in ischaemic preconditioning and microvascular vasodilation. In small studies, the activator of K-ATP channel nicorandil, was associated with better perfusion and left ventricular wall motion and reduction of adverse events [51,52]. Furthermore, magnetic resonance imaging suggests that the use of nicorandil may improve microvascular obstruction [53]. None of these trials showed satisfactory proof of infarct size reduction in adequately sized trials. Consequently this agent is not currently being used in AMI, and awaits evaluation in larger trials.

GIK Solution

The concept of glucose–insulin-K+-therapy (GIK) was introduced by Sodi-Pallares and thought to be protective by stabilization of the membrane [54]. Combination therapy of glucose-insulin-potassium has been evaluated as a potential way to stimulate anaerobic glycolysis, increase ATP levels and decrease the release of free fatty acids. Large and well-designed studies including the CREATE-ECLA and DIGAMI 2 studies did not support its use in clinical practice. In the CREATE-ECLA trial (n=20,000; reperfusion therapy in 83%), GIK treatment failed to improve 30-days clinical outcome although there was a trend towards improved clinical outcome in patients undergoing reperfusion therapy by PCI (9% of all patients) [55].

Anti-Neutrophilic and Anti-complement Therapy

Inhibition of accumulation and activation of neutrophil has been associated with controversial results, and has been correlated with decreased infarct size in some studies but not in others [56-58]. Unfortunately, the observed delay between the onset of AMI and the administration of anti-neutrophilic therapy (anti-CD-18) may have been related to the decrease in the ability of these agents in mitigating neutrophil-mediated cell injury [56]. Similarly, the results reported with the complement inhibitor pexelizumab were disappointing. This can be explained by the complexity and the multiple steps involved in immune activation reperfusion injury [59-61]. Moreover leukocyte inhibition may have negative effects on the healing process associated with AMI as previously mentioned.

Antioxidant Therapy

The prominent role of oxygen free-radicals in the pathophysiology of reperfusion injury has stimulated several studies to assess the effectiveness of antioxidants in reducing the damages associated [62-70]. The results have been contradictory. Currently, investigation with different molecules (erythropoietin, oestrogens, heme-oxygenase 1, hypoxia-inducible factor 1) is ongoing.

Magnesium

The role of magnesium to minimise reperfusion injury was evaluated for the first time in the ISIS-4 (Fourth International Study of Infarct Survival) study, but no benefit was demonstrated. Controversy persisted about the moment of initiation of magnesium infusion, so the MAGIC study was conducted, which showed that magnesium infusion has no effect on mortality even when administered at the time of reperfusion [71,72].

Cyclosporine

In addition to its immunosuppressive capabilities, the administration of cyclosporine at the time of percutaneous coronary intervention has been found to reduce in infarct size. Calcium overload and excessive production of reactive oxygen species in the first few minutes after reperfusion set off a cascade of biochemical changes that result in the opening of the mitochondrial permeability transition pore (MPTP) in the cardiac cells. The opening of the MPTP leads to mitochondrial dysfunction. The cessation of energy production results in cellular death. Protecting mitochondria is a viable cardioprotective strategy. A pilot study of 58 patients showed that a single dose of cyclosporine administered prior to primary angioplasty in patients with AMI resulted in reduction of the 20% reduction in infarct size assessed by MRI [73,74].

Ischaemic Post-Conditioning

Postconditioning, defined as brief intermittent cycles of ischemia alternating with reperfusion applied after the ischemic event, has been shown to limits infarct size, reducing the number of necrotic, apoptotic and autophagic cells [75-77]. When applied to patients with ST-segment elevation myocardial infarction, the technique includes interventional angioplasty with an inflated balloon for a short period of time and at low pressure, temporarily occluding the infarct-related artery after it achieved an adequate permeability. In a study evaluating the potential of post-ischaemic conditioning after 20 min of sustained ischaemia, post-ischaemic conditioning improved endothelial function. No protection was observed if the applied protocol lasted for more than a minute, suggesting that this strategy should be applied during the first minute of full reflow [78,79]. Some studies evaluating patients with ST-segment elevation myocardial infarction taken to successful percutaneous coronary intervention submitted to ischemic post-conditioning protocols showed that these ones had higher attenuation of ST-segment elevation and improvement in the degree of distal coronary perfusion [80]. Mechanical postconditioning reduces infarct size in patients with ST-segment-elevation myocardial infarction treated with PCI. The impact of mechanical postconditioning seems to be indepen-

dent of the size of myocardium at risk. More studies are needed with a large number of patients to determine the optimal protocol, to assess treatment efficacy using more accepted measurements of infarct size post-reperfusion and to determine long-term prognosis [81].

Endovascular Cooling

Since myocardial metabolism decreases at lower temperatures, endovascular cooling could achieve some degree of cardioprotection. Endovascular coils and external cooling blankets to achieve temperatures below 33 °C during PCI for AMI have been used with this purpose. The COOL-MI study and the ICE-IT study, showed no effect on infarct size in the total population. However, in patients with anterior myocardial infarction cooled to <35 °C at the time of reperfusion, infarct sizes were roughly halved [82-84].

Conclusion

Currently, the treatment for AMI is early reperfusion; however, this itself may contribute to the final myocardial infarct size, condition known as reperfusion injury. Reperfusion injury contributes to up to 50% of the total myocardial damage. Despite of many successful results in animals, the translation into the clinical setting has been disappointing for many years. Over the last few decades, the discovery of the phenomena of ischemic preconditioning and postconditioning, along with significant advances in our understanding of the cardioprotective pathways underlying these phenomena, have provided the possibility of successful mechanical and pharmacological interventions against reperfusion injury. Further investigation is required into this field.

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