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Role of Oxidative Stress as a Novel Therapeutic Target in Myocardial Injury Due to Ischemia/Reperfusion in Patients with Acute Myocardial Infarction

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Abstract

The most effective therapeutic intervention for reducing infarct size and improving outcomes in patients with acute myocardial infarction is the thrombolytic therapy or percutaneous coronary angioplasty. However, this process itself can generate ischemia-reperfusion injury that can be responsible for up to 50% of the final infarct size. Considering oxidative stress as the main damaging agent in this pathology, it has been postulated that reinforcing antioxidant defenses could improve cardiac function. However, up to date clinical trials based on monotherapies have been consistent in the favorable results. In this review the pathophysiological mechanisms of myocardial injury due to ischemia/reperfusion in patients undergoing percutaneous coronary angioplasty are updated. In addition, new therapeutic alternatives for cardioprotection in this population, are explored, with emphasis in the combined therapy as a novel antioxidant treatment for this myocardial injury.

Keywords: Oxidative stress, Antioxidants, Cardioprotection, Acute myocardial infarction, Vitamin C, Vitamin E, N-acetylcysteine, Polyphenols, Ischemia-reperfusion injury.

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Contents

Contribution of this paper to the literature

This review contributes as an updated of the main pathophysiological mechanisms of myocardial injury due to ischemia/reperfusion in patients with acute myocardial infarction, with emphasis in the role of oxidative stress as a therapeutic target that could reinforce the antioxidant defense for cardioprotection.

1. Introduction

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. It was reported that 17.9 million people died from CVDs in 2019, representing 32% of all global deaths [1], among which coronary heart disease (CHD) is the leading cause of death, being responsible for 16% of total deaths worldwide [2]. This represents an estimated total cost of 196,000 million euros per year in CVDs in Europe, approximately 54% of the total investment in health [3]. Moreover, CHD was one of the 10 most expensive conditions treated in US hospitals in 2013 ($9.0 billion) [4].

In ischemic heart disease, cardiomyocyte death almost always occurs in the context of severe and prolonged myocardial ischemic events, which are a consequence of thrombotic complications from atherosclerotic plaques [5]. In most cases, disruption of a vulnerable atherosclerotic plaque or erosion of the coronary artery endothelium generates arterial lumen occlusion and produces a series of biochemical and metabolic changes that eventually lead to the death of cardiomyocytes. This cell death is further exacerbated when the occlusion of the coronary arteries is complete, generating an acute myocardial infarction (AMI) [6].

The most effective therapeutic intervention for reducing the size of a myocardial infarct and improving the clinical outcome is timely and effective restoring of coronary blood flow using either thrombolytic therapy or percutaneous coronary angioplasty, but this process itself can induce further cardiomyocyte death and increased infarct size, a phenomenon known as ischemia-reperfusion injury (IRI), thus reducing the beneficial effects [7, 8]. In fact, IRI can be responsible for up to 50% of the final infarct size [7].

Although the molecular mechanisms underlying myocardial IRI are not well defined [9], several experimental studies have shown the important role of oxidative stress in this complication, and it has been postulated as a therapeutic target for cardioprotection [10-16]. Therefore, reinforcement of the antioxidant defense system should be expected to protect the myocardium against IRI, however, up to date the results of this proposal has not been successful and needs more studies.

Although multiple therapies appeared to be effective in attenuating reperfusion injury in the experimental setting, translation into clinical practice has not been demonstrated to be consistent [17]. Therefore, the present work describes the pathophysiological mechanisms of myocardial IRI, with emphasis on the role of oxidative stress as a target for novel therapeutic strategies for cardioprotection.

2. Pathophysiology of Myocardial Reperfusion Injury

2.1. Alterations in Myocardial Function

The impairment of cardiac function during myocardial reperfusion can generate four types of cardiac dysfunctions [7]:

a) *Myocardial stunning*, it is defined as mechanical dysfunction that persists after reperfusion, despite the absence of irreversible damage and the restoration of normal or almost normal coronary flow [18]. It is a result from the detrimental effects of oxidative stress [19-21] and intracellular calcium overload on the myocardial contractile apparatus [19, 20, 22, 23]. This phenomenon occurs in a wide variety of pathophysiological conditions [20] and the affected zone usually recovers after several days or weeks [7].

b) *Reperfusion arrhythmias*, is a disturbance of cardiac rhythm that arises as a consequence of a total or partial restoration of flow in tissue which has been previously globally or regionally ischemic [24]. Arrhythmias during or immediately after reperfusion are seen in experimental animal models and in humans [25]. In fact, they are often present in reperfused acute ST-segment elevation myocardial infarction (STEMI) patients, particularly after thrombolysis. In this population, the most commonly encountered reperfusion arrhythmias are idioventricular rhythm, ventricular tachycardia, and fibrillation [26], which usually self-terminate or are easily treated [7, 26, 27]. Early reperfusion arrhythmias are considered evidence of successful reperfusion and vitality of the cardiomyocytes [26].

c) *Microvascular "No-reflow"*, is a phenomenon that describes when the previously occluded epicardial artery restores the blood flow, but it remains the inability to reperfuse the infarct zone [28, 29]. The microvascular obstruction (MVO) is considered the main responsible mechanism, and it causes an irreversible form of damage that results in both myocyte and endothelial cells death [30]. The underlying etiology of MVO is unclear [25], but some factors have been associated, including capillary damage with diminished vasodilation, capillary compression by inflammation of endothelium and cardiomyocyte, micro embolization of particles released from the atherosclerotic plaque, release of vasomotor and thrombogenic substances, and platelet micro-thrombi [31-34].

d) *Lethal reperfusion injury* (LRI), occurs when, as a consequence of the reperfusion of the infarct area, the damage to the previously affected tissue is enhanced [35]. The role of lethal reperfusion injury as a mediator of cardiomyocyte death is currently controversial. In this regard, they have suggested that reperfusion exacerbates the cellular injury suffered during the ischemic period [36].

2.2. Cellular Metabolic Alterations

Adult cardiomyocytes are terminal cells without replicative capacity [37] and with high demand for adenosine triphosphate (ATP). This ATP is provided by the high amounts of mitochondria that cardiomyocytes possess. The aerobic/anaerobic glycolysis and β-oxidation of free fatty acids generates Acetyl-CoA, which is metabolized through the tricarboxylic acid cycle to supply ATP. Therefore, this cell type works primarily with aerobic metabolism [38].

The metabolic and cellular changes associated with ischemia and subsequent reperfusion are described below:

2.2.1. Ischemia

When the myocardium is exposed to ischemia and AMI, the reduced oxygen supply to the mitochondrial electron transport chain (mETC) causes a drop in the production of ATP. Thus, the glycolytic pathway activates the anaerobic respiration with accumulation of lactic acid [9, 13, 39]. The decrease in intracellular pH forces the cardiomyocyte to excrete H^+ through the Na^+/H^+ exchanger, with the subsequent increase in intracellular Na^+. Meanwhile, intracellular ATP depletion deactivates ATPases such Na^+/K^+ ATPase, which leads to intracellular Na^+ accumulation [40-42]. Consequently, Na^+ accumulates within the cell, activating Na^+/Ca^{2+} exchangers in the reverse direction and increasing cytosolic Ca^{2+} [41, 43, 44]. Due to ATP depletion, the sarcoplasmic reticulum is unable to uptake Ca^{2+} from the cytosol because sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) transporter needs ATP to function, resulting in Ca^{2+} overload [38, 45]. Furthermore, rapid increases in intracellular Ca^{2+} leads to a non-physiologic opening of the mitochondrial permeability transition pore (MPT) However, the low intracellular pH is inhibitory [46]. Additionally, activation of intracellular proteases, such as calpain, causes a fragile cellular structure or hypercontracture, leading to contraction band necrosis [38, 44]. Without appropriate restoration of blood supply after ischemia, the lack of ATP content and high Ca^{2+} levels activate myocyte atrophy, and finally apoptosis and necrosis [47].

2.2.2. Reperfusion

Although reperfusion is essential to restore oxygen and nutrients to support cell metabolism and remove byproducts of cellular metabolism, paradoxically, it can by itself inflict further damage. The mechanisms involved in reperfusion injury are complex and multifactorial. This review includes the effects of generation of reactive oxygen species (ROS), cytosolic calcium accumulation, opening of the MPT, and pronounced inflammatory responses Figure 1 [7-9].

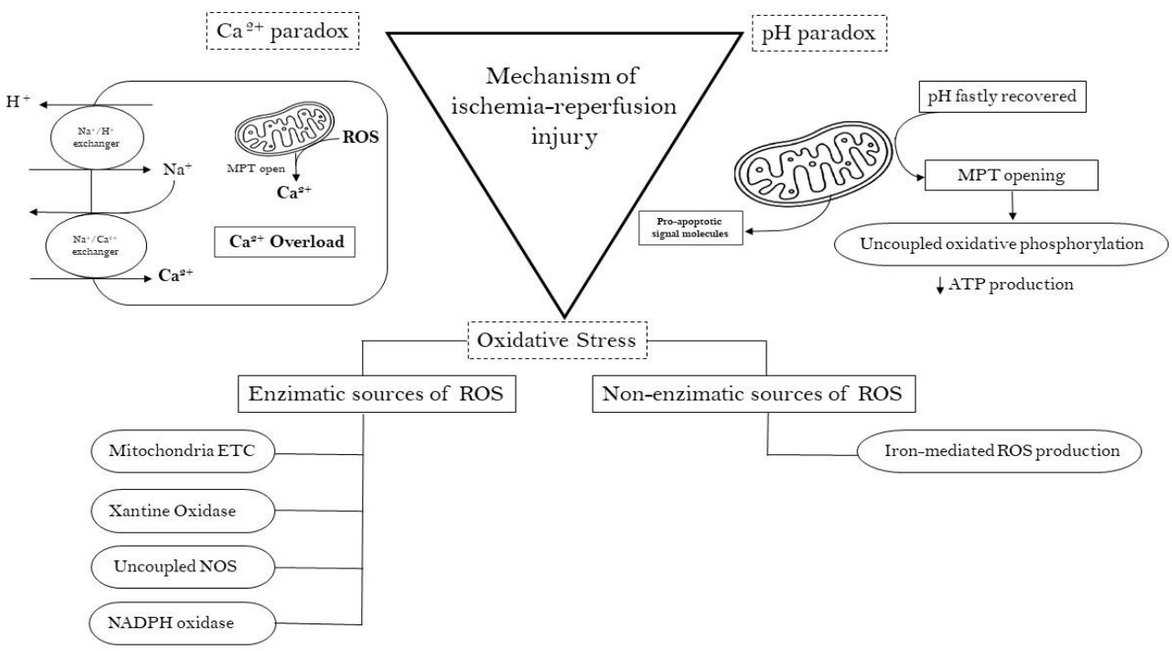

Figure 1. Pathophysiology of Myocardial Ischemia-Reperfusion. The mechanism of ischemia-reperfusion injury includes: (1) Ca^{2+} paradox due Ca^{2+} overload with MTP opening and activation of Na^+/H^+ and Na^+/Ca^{2+} exchangers; (2) the rapidly recovered pH from acidosis opens the MPT, uncoupling oxidative phosphorylation and ATP production; (3) oxidative stress due to enzymatic and non-enzymatic ROS production.

Note: ROS: reactive oxygen species; MPT: mitochondrial permeability transition pore; ATP: adenosine triphosphate; Mitochondria ETC: mitochondrial electron transport chain; Uncoupled NOS: Nitric oxide synthase.

2.3. Oxidative Stress

In the first few minutes following the onset of myocardial reperfusion, a ROS burst is produced by different sources [11, 48]. To control this increases in oxidative stress, the myocardial cells have endogenous free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and thioredoxin peroxidase, among others [49]. In addition, nitric oxide (NO) derived from endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) are thought to protect against myocardial IRI by their cardioprotective effect [50]. Further, there are non-enzymatic antioxidant defenses, such as ascorbic acid, α-tocopherol, reduced glutathione (GSH), coenzyme Q10, cysteine, carotenoids, flavonoids, polyphenols, and other various exogenous antioxidants that are currently taken in the diet or as supplements. There are many mechanisms whereby antioxidants may act such as (1) scavenging ROS or their precursors, (2) inhibiting the formation of ROS, (3) attenuating the catalysis of ROS generation via binding to metal ions, (4) enhancing endogenous antioxidant generation, and (5) reducing apoptotic cell death by up-regulating the antiapoptotic gene Bcl-2 [35, 51].

The ROS production sources can be enzymatic or non-enzymatic, and their role in the pathophysiology of IRI will be addressed.

2.3.1. Enzymatic Sources of ROS Production

2.3.1.1. Mitochondria

There are different sites in the mETC in mammalian mitochondria generating superoxide anion ($O_2^{\cdot-}$) and/or hydrogen peroxide (H_2O_2). Electrons derived from NADH or some other donor can directly react with oxygen and

generate $O_2^{\cdot-}$, primarily at complexes I and III, causing partial reduction of molecular oxygen to $O_2^{\cdot-}$ instead of reduction to H_2O [52, 53] Particularly, reverse electron transport at complex I is the main source of $O_2^{\cdot-}$ upon reperfusion of ischemic tissue [54, 55].

2.3.1.2. Xanthine Oxidoreductase

It can be found in two interconvertible forms: xanthine dehydrogenase (XDH) preferably using NAD^+ as the electron acceptor; and xanthine oxidase (XO) using O2 as the terminal electron acceptor. Xanthine oxidoreductase catalyzes the transformation of hypoxanthine and xanthine to uric acid, with $O_2^{\cdot-}$ or H_2O_2 generation as by-products. Moreover, under acidic conditions (pH~6.5), XDH may oxidize NADH instead of xanthine, thus promoting $O_2^{\cdot-}$ production [56-58].

2.3.1.3. Uncoupled Nitric Oxide Synthase

While NO derived from constitutive eNOS and nNOS protect against IRI, the inducible nitric oxide synthase (iNOS) derived ones aggravate the damage, causing cardiac hypertrophy and oxidative stress [50]. Tetrahydrobiopterin (BH_4) is an essential NOS co-factor, however BH_4 is oxidized in the presence of ROS. Consequently, in the absence of L-arginine, BH_4, or both, eNOS changes to its uncoupled condition and become a source of $O_2^{\cdot-}$ rather than NO, and contribute to oxidative stress [59-61]. Additionally, it has been demonstrated that the iNOS is increased after the reperfusion, producing large amounts of NO and leading to direct cytotoxic effects, or reacting with $O_2^{\cdot-}$ to form the highly oxidizing agent peroxynitrite ($ONOO^-$) that causes further cell damage [62-65].

2.3.1.4. Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase

NADPH oxidase isoforms (NOXs) are members of a transmembrane proteins family that transport electrons donated by NADPH across biological membranes, leading to reduce O_2 to $O_2^{\cdot-}$. Some studies have shown that NOX can also produce H_2O_2 [66-68]. Seven isoforms have been described previously: NOX1 to NOX5, dual oxidase- (DUOX-) 1 and DUOX-2 [66, 67, 69, 70]. DUOX-1, DOUX-2 and NOX4 predominantly produce H_2O_2. On the other hand, the remaining NOX isoforms produce mostly $O_2^{\cdot-}$ [53, 66, 71]. NOX1, NOX2, NOX4 and NOX5 are highly expressed in cardiovascular system; and specifically up-regulation of NOX2 and NOX4 are related to oxidative stress via production of $O_2^{\cdot-}$ and H_2O_2 in hearts subjected to IRI [53, 72, 73].

2.3.2. Non-Enzymatic Sources of ROS Production

The non-enzymatic generation of ROS occurs in the presence of metal ions, such as iron ones [74]. To understand this mechanism, it is necessary to describe iron homeostasis:

This essential element is necessary for cell survival, in fact cardiomyocytes are sensitive to iron deficiency because they require large amounts of mitochondria with its enzymes that contain hemoproteins. However, they also are poorly protected against iron overload [75]. Therefore, the role of ferritin (FT) as the main ion storage in a non-toxic and readily available manner is essential. The small percentage of total intracellular iron (less than 5%) that does not bind to FT is defined as a labile iron pool (LIP), and exists in the cytosol, in the mitochondrial matrix, and lysosomes as a redox-active iron pool [76, 77]. If FT becomes saturated, the LIP will be increased and it will be incorporated into the Fenton reaction Equation 1 and Haber-Weiss reaction Equation 2, producing ROS. The Fenton reaction is a chemical reaction between ferrous iron (Fe^{2+}) and H_2O_2, which produces hydroxyl radical ($^{\cdot}OH$). On the other hand, in the Haber–Weiss reaction, ferric iron (Fe^{3+}) is reduced back to Fe^{2+} in the presence of $O_2^{\cdot-}$ radicals [77, 78].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + -OH + {}^{\cdot}OH \quad \textit{Fenton Reaction} \tag{1}$$

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + -OH + {}^{\cdot}OH \quad \textit{Haber-Weiss reaction} \tag{2}$$

During IRI, iron-mediated non-enzymatic ROS production can trigger cell death processes such as apoptosis, necroptosis, pyroptosis, and ferroptosis [77]. Among these, ferroptosis highlights as a regulated form of necrosis caused by the accumulation of lipid peroxidation products and ROS production derived from iron metabolism, mainly when GSH levels in the cell are depleted or when the enzyme GSH-Px4 is inhibited [79]. GSH-Px4 can inhibit ferroptosis by converting phospholipid hydroperoxides to lipid alcohols using GSH [80].

Both GSH-Px4 and acyl-CoA synthetase long-chain family member 4 (ACSL4) are recognized biomarkers of ferroptosis, and it has been reported that when an ischemic rat heart is reperfused there is greater expression of ACSL4 and lower levels of GSH-Px4. And this response is not replicated in the ischemic phase [81]. In addition, a previous study showed that ferroptosis was more active 30 min after reperfusion and not during other moments of this phase [82].

In summary, the iron overload can induce a non-enzymatic production of ROS via Fenton and Haber-Weiss reactions. The product of oxidative stress can potentially lead to cellular death mediated by ferroptosis, which is produced in myocardial IRI and may be related to the extent of the infarct size [74]. The latest results related to IRI-ferroptosis are important to take into consideration for the development of cardioprotective therapies based on ferroptosis inhibition to reduce heart IRI.

2.4. Ca^{2+} Paradox

During reperfusion, there is a rapid restoration of essential substrates for the generation of ATP, such as glucose or free fatty acids, an instantaneous increase in oxygen supply and a prompt normalization of the extracellular pH. This creates an extreme H^+ gradient across the plasma membrane that triggers the Na^+/H^+ exchanger, leading to a massive Na^+ influx with H^+ outflow to the extracellular [38]. This gradient can trigger the inverted action of the surface Na^+/Ca^{2+} exchanger, which excretes accumulated Na^+ but leads to intracellular Ca^{2+} overload [83]. Therefore, excess of Ca^{2+} induces cardiomyocyte death by causing hypercontracture of the heart cells and MPT opening [7].

In parallel, upon re-oxygenation the xanthine oxidase is activated by Ca^{2+}-sensitive proteases, increasing ROS production [84]. Also, under oxidative stress conditions, Ca^{2+} is induced by ROS to influx into the cytoplasm and then influx into the mitochondria via mitochondrial Ca^{2+} uniporter, resulting in the opening of MPT, the collapse of mitochondrial membrane potential, and release of apoptotic signaling molecules such as cytochrome c and apoptosis-inducing factor (AIF) from the intermembrane space [85-88].

After 30–60 min of reperfusion, a gradual recovery of Ca^{2+} excretion and ATP-dependent Ca^{2+} reuptake in sarcoplasmic reticulum (SR) takes place, and the cells return to normal homeostasis. This ischemia-reperfusion process makes the intracellular Ca^{2+} concentration dual peaked [89], with one peak occurring at 15–60 min after the onset of index ischemia and the other peak occurring within 30 min of reperfusion [38].

Experimental studies have shown that pharmacologic antagonists of the sarcolemmal Ca^{2+} channel [90] or the mitochondrial Ca^{2+} uniporter [91], administered at the onset of myocardial reperfusion, reduce MI size by up to 50%. However, clinical studies of calcium channel blockers administered at the onset of myocardial reperfusion have not shown beneficial results [92].

2.5. pH Paradox and MTP Opening

Once the reperfusion has started, the previously lowered pH is rapidly restored by the washout of lactate, the activation of the Na^+/H^+ exchanger and the Na^+-HCO_3^- symporter. This can contribute to lethal reperfusion injury, and is termed the pH paradox [93]. This effect may be mediated by the opening of the MPT, which is a non-selective channel of the inner mitochondrial membrane that is closed under physiological conditions [94]. During ischemia, the susceptibility of the MPT is increased, but the pore remains closed when the pH is low. However, when pH is rapidly recovered, the neutralization of the acid media triggers the actual MPT opening, thus allowing passage through the inner mitochondrial membrane of molecules >1.5 kDa, leading to uncoupled oxidative phosphorylation and disrupting ATP production [95]. To date, the molecular nature of the MPT is still unclear [96]. Moreover, preventing MPT opening at the time of reperfusion by administering MPT inhibitors (such as the immunosuppressant cyclosporin A) at the onset of myocardial reperfusion has been reported in experimental studies to reduce infarct size by 40%–50% in small and large animal models [97-100]. However, in clinical studies, delaying the restoration of physiologic pH during myocardial reperfusion using Na^+/H^+ exchange inhibition did not protect the heart [41, 101].

2.6. Inflammation

It is unclear whether the inflammatory response that accompanies an AMI contributes to the pathogenesis of myocardial LRI or whether it is a reaction to the acute myocardial injury [102]. Nevertheless, it has been reported the release of chemoattractant draws neutrophils into the infarct zone during the first 6 hours of myocardial reperfusion, and during the next 24 hours they migrate into the myocardial tissue. These neutrophils cause vascular plugging and release degradative enzymes, along with ROS [7, 102].

The association between high ROS production and inflammation is mediated by the pro-inflammatory transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is sensitive to the redox state. High oxidative stress induces the separation of NF-κB from the NF-κB-IκB complex. Thus, NF-κB alone translocates into the nucleus, where it interacts with the genome and stimulates the expression of genes for inflammatory cytokines, leading to the initiation of the inflammatory response [103].

Finally, through the ischemia and reperfusion models of multiple organs, Toll-like receptors have been demonstrated to play an important bridging role in the interaction between oxidative stress and inflammatory response [104]. It is thought that ROS up-regulates IL-1β through the NLRP3 inflammasome activation and caspase-1 expression [105]. It was demonstrated that thioredoxin-interacting protein mediated NLRP3 inflammasome activation in cardiac microvascular endothelial cells was a novel mechanism of MI/RI [106].

3. Therapeutic Focus for Myocardial Reperfusion Injury

In patients with AMI, the opportunity to intervene is limited between the onset of myocardial ischemia and the time of myocardial reperfusion. However, therapeutic targeting of the individual components of myocardial LRI, including oxidative stress, calcium overload, pH correction, and, more recently, inflammation have produced disappointing results [107, 108]. Nevertheless, there have been emerging therapeutic strategies for preventing myocardial LRI in the last years.

The concept of "ischemic conditioning" includes several endogenous cardioprotective strategies, applied either directly to the heart (ischemic preconditioning or post-conditioning) or to another region of the body, for example a limb (remote ischemic preconditioning, preconditioning or post-conditioning [109, 110]. In this regard, ischemic preconditioning (IPC) is one of the most potent cardioprotective strategies against IRI, originally described by Murry, et al. [111]. Sub-lethal amounts of ROS can serve as a trigger for IPC by functioning as signaling messengers to protect against LRI. In the early phase of IPC, post-translational modification of redox-sensitive proteins provides cardioprotective signal transduction pathways [53]. On the other hand, the late phase of IPC is mediated by cardioprotective gene expression, like hypoxia-inducible factor 1 (HIF-1), which generates low amounts of mitochondrial ROS that is going to act as intracellular signals [112]. Although the molecular mechanism triggered by HIF that leads to sub-lethal ROS accumulation remains unknown [53], it has been hypothesized that HIF-induced pathways seem to converge on Akt activation and inhibition of MTP opening [113-116].

In parallel to ischemic conditioning, there are many drugs that can reduce myocardial IRI, mainly based on their antioxidant capacity.

3.1. Ascorbic Acid

Vitamin C (Vit C), ascorbic acid or ascorbate is a water-soluble antioxidant agent that acts as a ROS scavenger. Oral ingestion of fruits, vegetables or supplements is the primary route of administration for Vit C [117]. Although low plasma concentrations are achieved, between 100 μmol/L or 150 μmol/L with food intake and

supplementation, intravenous injection of ascorbate has been reported to lead to concentrations reaching 25 to 30 mmol/ L in plasma [118]. However, its intracellular levels and plasma levels do not always have a correlation in cells other than red blood cells, since ascorbate tends to accumulate within the cell [35].

Due to its properties, Vit C can be found in two states: the reduced form, ascorbic acid, and the oxidized form, dehydroascorbic acid (DHA) [119]. SVCT1 and SVCT2 are the Na^+-dependent Vit C transporters, responsible for entering ascorbic acid into the cell [120], while the glucose transporters GLUT1, GLUT3, and GLUT4 are responsible for the DHA entry [119]. All three types of glucose transporters are expressed in the myocardium [35]. Vit C antioxidant potential is explained by its ability to donate electrons, being oxidized to DHA. Later it returns to its reduced form after being used by the cell [121].

By acting as an electron donor, ascorbic acid generates ascorbyl radical, and it is capable of being oxidized by acting as an antioxidant or enzymatic cofactor [121]. So it is now accepted that Vit C together with glutathione constitute the primary cellular defenses against ROS production [122]. In this regard, it has been shown an inverse correlation between Vit C plasma concentration and products of oxidative damage to DNA, proteins and lipids in healthy adult nonsmoking population [123]. In another study where oxidative and antioxidant parameters were evaluated in patients with AMI before and after reperfusion, it was found that the activity of the antioxidant enzyme SOD decreased, while the activity of the oxidant enzyme XO increased in conjunction with the marker of lipid peroxidation malondialdehyde (MDA) after thrombolysis. Patients who were supplemented with post-reperfusion oral Vit C showed improvements in these parameters to almost normal levels [124]. Also, in another IRI model, it has been reported that Vit C administered after the reperfusion decreases lactate dehydrogenase (LDH) levels in blood, as a marker of oxidative stress [125]. In addition, a significant decrease in hydroperoxides concentration at 48 hours post reperfusion [126] and a decrease in 8-isoprostanes after 6–8-hour post reperfusion [127], has been described following the administration of Vit C. The results of several studies [128-130] have shown a significant decrease in the levels of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative damage to DNA, post reperfusion and administration of Vit C. In the same way, the treatment with Vit C caused a significant decrease in 8-iso-prostaglandin F2 alpha at 6-8 hours after reperfusion [128], that is, it decreased the peroxidation of arachidonic acid in lipid membranes.

Vitamin C must reach a plasma concentration of 10 mml/L to displace the reaction of the O_2^--radical with NO, which acts at a rate 105 times faster than the reaction between ascorbic acid and the superoxide anion radical [35, 131]. But when it has been administered at higher concentrations prior to reperfusion, no significant improvements have been observed in reducing the infarct size [132]. However, there are few studies of Vit C in human IRI models, so the results are not conclusive. Other limitations of the current evidence are the lack of consideration of basic aspects such as the mechanistic approach of the drug and its pharmacokinetic properties [133, 134].

Although the administration of Vit C improves the total antioxidant capacity at 48 hours after reperfusion, it has been observed that the GSH/GSSG ratio decreased significantly in the groups supplemented with Vit C [127, 132]. Likewise, in a setting of myocardial IRI, Vit C at high doses could activate the Fenton reaction due the iron overload and acting as a pro-oxidant species [74].

In addition to these results, it has been described multiples antioxidant effects of Vit C:

i. Synergistic effects with vitamin E (Vit E), ascorbic acid recycles α-tocopherol (α-TOH) into lipid bilayers and erythrocytes [35]. Also, in conjunction with Vit E, Vit C is able to up-regulate eNOS activity [135].
ii. Decrease in ROS production by the down-regulation of NOX enzymes activity [136].
iii. Suppression in NF-κB activation induced by tumor necrosis factor α (TNF-α) [137].
iv. It prevents the oxidation of BH_4, cofactor of NOS, thus avoiding uncoupled eNOS function and $O2^{\cdot-}$ overproduction [35].

3.2. Vitamin E

Vit E is a group of fat soluble molecules, known as one of the most potent antioxidants, among which α-TOH stands out as one of the most active forms [74]. α-TOH is the major peroxyl radical scavenger in biological lipid membranes [138, 139], because it acts like a lipid based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation through its own conversion into an oxidized product, α-tocopheroxyl radical [35]. To restore the α-TOH, the α-tocopheroxyl radical is reduced by redox-active molecules such as Vit C or ubiquinol [140]. If the α-tocopheroxyl radical is not reduced, it can react with lipids and generates lipid radical compounds, producing damage to the lipid membranes. Therefore, to have a beneficial therapeutic effect, the α-TOH requires co-antioxidants such as Vit C [141].

The cardioprotective effect of Vit E has been described previously in a study, showing that higher α-TOH baseline serum concentration is associated with a decreased risk overall and causes specific mortality for cardiovascular and heart diseases [142]. In IRI animal models, the α-TOH reduces infarct size and preserved cardiac function, in association with lower neutrophil infiltration locally in the ischemic myocardium and increases in anti-inflammatory monocyte function [143]. Furthermore, it has been reported positive effects in clinical studies of revascularization surgeries of the lower extremities [144], kidney transplantation [145], liver surgery [146], and aortic aneurysm repair [147]. In parallel, preoperative administration of Vit E has proven to exert beneficial effects on liver surgery by reducing the impact of IRI [148].

Besides its role as ROS scavenger, Vit E has been associated with increased GSH-Px activity [35] and decreased ROS production via down-regulation of NOX enzymes [136]. In addition, Vit E also has anti-inflammatory effects by inhibiting the transcriptional activity of NF-κB, that contributes to diminish the proinflammatory gene expression [149].

3.3. N-Acetylcysteine

N-acetylcysteine (NAC) is an acetylated cysteine compound that acts as a blood antioxidant reserve. It could prevent the reduction of GSH/GSSG ratio during exposure to ascorbic acid by behaving as a GSH donor when it is oxidized to DHA [74].

In ischemia-reperfusion rat models it has been demonstrated that the administration of NAC by continuous infusion before, during and after reperfusion produced a smaller infarct size compared to the control group. However, this effect did not occur when NAC was administered as an intravenous bolus at the same dose [150]. Also, the results of a clinical trial showed a decrease in the incidence of postoperative atrial fibrillation in patients undergoing coronary artery bypass and valve surgery treated with intravenous infusion of NAC, before and after surgery [151].

Since co-administration of a GSH donor with Vit C shows a synergistic protective effect on infarct size in an isolated rat heart IRI model [152], it has been postulated that reinforcement antioxidant defenses with a GSH donor such as NAC could prevent the decrease in GSH and the reduction of the GSH/GSSG ratio during the administration of high doses of ascorbic acid to control burst of ROS in the reperfusion phase of AMI treatment [135].

3.4. Deferoxamine

Deferoxamine (DFO) is a Food and Drug Administration (FDA) approved drug to treat either acute or chronic iron overload and it has a well-defined role as an iron chelator [153]. His effects in myocardial IRI are based on the increase in free iron during ischemic phase due media acidification, which promotes the mobilization of iron from intracellular ferritin. In the same way, once the reperfusion is onset, even if the iron levels decrease, the O2·- contributes to more mobilization of iron from ferritin [135]. In fact, elevated serum FT levels is an important risk factor for developing AMI in middle-aged men without prior coronary artery disease [154]. Therefore, under conditions of oxidative stress, the LIP can react to produce ·OH in Fenton and Haber-Weiss reactions [74], as previously described.

The use of iron chelators at the onset of reperfusion has been proved to improve cardiac function relative to the control group [155]. Furthermore, when isolated and perfused rabbit cardiomyocytes are treated with DFO during ischemia and reperfusion, it has been associated with greater functional and metabolic recovery of the myocardium, as well as a reduction in the generation of ROS induced by perfusion, compared to the control group [156]. Likewise, in IRI canine model, pretreatment with DFO before ischemia, but not at the beginning of reperfusion, significantly reduced infarct size and GSSG release in the coronary sinus during reperfusion [157]. In addition, a clinical trial of patients with STEMI submitted to coronary angioplasty shows that intravenous bolus administration of 500 mg of DFO immediately before surgery, followed by a 12-hour infusion to 50 mg/kg, significantly reduced plasma levels of F2-isoprostane after angioplasty compared to control group [158].

DFO could have a synergistic role with ascorbate, since the latter reduces Fe^{3+} to Fe^{2+}, which is the substrate of the Fenton reaction that leads to higher ROS production. Therefore, besides the iron overload during reperfusion, a high dose of intravenous Vit C infusion could interact with Fe^{2+}, enhancing the pro-oxidant effects of Vit C [135]. In this scenario, the use of iron chelators such as DFO could be considered in conjunction with Vit C antioxidant therapy in patients with myocardial IRI.

In this regard, in an experimental sheep model it was shown that the combined use of ascorbic acid (1.5 g) and DFO (1 g) administered by intravenous infusion was protective against the development of ventricular arrhythmias induced by myocardial IRI, compared to the control group [159].

3.5. Polyphenols

This group of bioactive molecules currently occurring in some foods have antioxidant and anti-inflammatory effects. In addition, they also exhibit anti-cardiac hypertrophy, anti-atherosclerosis, anti-diabetic and anti-apoptotic effects through different signaling pathways [160].

According to the number of phenol rings and the elements that are attached to them, polyphenols can be classified into flavonoids and non-flavonoids. Flavonoids are present in plants, including vegetables and fruits, representing 2/3 of the total polyphenols ingested in the diet. The best known flavonoids are quercetin, catechin, and myricetin [160]. In this regard, a myocardial IRI model study in rats described a cardioprotective role of quercetin by preventing a decrease in the XDH to XO ratio [161].

On the other hand, the non-flavonoids are found in different citrus fruits and berries, coffee, olive and sesame. The most important non-flavonoids are phenolic acids, like resveratrol [160]. They have shown antioxidant and anti-inflammatory effects by reducing inflammatory markers, such as IL-1β, IL-8, monocyte chemoattractant protein (MCP-1), COX-2, and iNOS [162]. Particularly, resveratrol increases the expression and activity of eNOS due their capacity to enhance the serine 1177 residue phosphorylation, diminishing the endogenous eNOS inhibitor asymmetric dimethylarginine (ADMA) [163], and/or activating the AMP-activated protein kinase pathway in the cardiomyocyte [164].

Finally, the polyphenol treatment can stimulate the cytochrome P450 system, enhancing the functional recovery in a reperfused heart after ischemia, due the diminishes in the production of free radicals [165]. Also, the polyphenols administration has been shown to have antiarrhythmic effects and minimize mitochondrial IRI [166]. In addition, it can preserve the integrity of endothelial cells by stimulating the endothelium-derived hyperpolarizing factor [167, 168] and increase coronary flow via endothelium-dependent relaxation [169] and directly promote NO production.

4. Novel Perspectives and Conclusions

In the light of the results of the available studies up to date, myocardial IRI appears as a pathophysiological entity without therapeutic solution in the clinical practice. However, oxidative stress plays a leading role in the process of cell death and inflammation after reperfusion. Therefore, it has been postulated that reinforcing antioxidant defenses prior to reperfusion could improve the clinical outcomes, as assessed by markers of oxidative stress, infarct size, and myocardial function.

In this regard, the administration of Vit C at doses greater than 10 mml/L has proven to be safe, reducing markers of DNA damage and lipid peroxidation, as well as increasing the activity of the antioxidant enzyme SOD. Despite this, Vit C can act as a pro-oxidant species by reducing Fe^{3+} to Fe^{2+} and activating the Fenton reaction,

leading to a greater amount of ROS production. Furthermore, during ischemia and reperfusion of the cardiomyocyte, there is a greater mobilization of $Fe^{2+}$from ferritin, which increases the LIP and ROS production.

For these reasons, a novel antioxidant therapy has been proposed as a cardioprotective treatment in patients with AMI subjected to reperfusion. It consists in a continuous intravenous infusion of high doses of Vit C combined with NAC and DFO, administered before and during reperfusion. This would allow to optimize the positive antioxidant effects that have been described with each drug separately in IRI models. On the one hand, NAC will act as a GSH donor, preventing the reduction of the GSH/GSSH ratio as a result of the oxidation of ascorbate to DHA. In turn, DFO will decrease Fe^{2+} levels as LIP, decreasing the ROS production rate due to the Fenton reaction. Thus, the co-administration of these drugs is expected to have a synergistic behavior in avoiding the deleterious effects of Vit C.

To date, more studies of combination therapy are needed in subjects with AMI who underwent reperfusion via thrombolysis or percutaneous angioplasty, since the favorable results of experimental studies have not been able to be transferred to the clinical setting. However, the novel treatment described would allow a safe therapy with low doses of each drug, being a potential alternative in the short or medium term for the management of ROS burst during reperfusion in patients with AMI.

Abbreviations:

ACSL4, acyl-CoA synthetase long-chain family member 4; AIF, apoptosis-inducing factor; AMI, acute myocardial infarction; ATP, adenosine triphosphate; CAT, catalase; CHD, coronary heart disease; CVDs, cardiovascular diseases; DHA, dehydroascorbic acid; DUOX-x, dual oxidase-; eNOS, endothelial nitric oxide synthase; FT, ferritin; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; HIF-1, hypoxia-inducible factor 1; H_2O_2: hydrogen peroxide. IMI, ischemia-reperfusion injury; iNOS, inducible nitric oxide synthase; IPC, ischemic preconditioning; LDH, lactate dehydrogenase; LIP, labile iron pool; LRI, lethal reperfusion injury; MCP-1, monocyte chemoattractant protein; MDA, malondialdehyde; mETC, mitochondrial electron transport chain; MPT, mitochondrial permeability transition pore; MVO, microvascular obstruction; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; NF-κβ, nuclear factor kappa-light-chain-enhancer of activated B cells; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOX, NADPH oxidases; $ONOO^-$, peroxynitrite; OH, hydroxyl radical; $O2^{\cdot-}$, superoxide anion; ROS, reactive oxygen species; SERCA, sarcoendoplasmic reticulum Ca^{2+}-ATPase; SOD, superoxide dismutase; STEMI, ST-segment elevation myocardial infarction; SR, sarcoplasmic reticulum; Vit E, vitamin E.; Vit C, vitamin C; XDH, xanthine dehydrogenase; XO, xanthine oxidase; α-TOH, α-tocopherol.

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