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Novel Antioxidant Therapy Against Myocardial Ischemia–Reperfusion Injury During Percutaneous Coronary Angioplasty

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Abstract

Acute myocardial infarction (AMI) is the leading cause of mortality worldwide. Major advances in the treatment have included coronary interventions, such as systemic thrombolysis and percutaneous coronary angioplasty (PCA). These procedures have been aimed to recover the blood flow in the cardiac zones affected by the occlusion of a branch of the coronary artery. However, damage is generated in the heart tissue known as myocardial reperfusion injury, an event associated with increased oxidative stress. Reactive oxygen species (ROS) are able to trigger cell death pathways, and myocardial structural and functional impairment. Studies on animal models of AMI suggest that lethal reperfusion accounts for up to 50% of the final size of a myocardial infarct, a part of the damage likely to be prevented. Although a number of strategies have been aimed to ameliorate lethal reperfusion injury, up to date the beneficial effects in clinical settings remain elusive. The accumulated body of evidence suggests that redox balance is a crucial determinant of ischemia–reperfusion injury, with clear mechanistic insights into pharmacological approaches. This chapter presents the molecular basis for a novel cardioprotection of patients with AMI subjected to PCA, based on a reinforcement of the antioxidant system.

Keywords: acute myocardial infarction, ischemia–reperfusion, oxidative stress, antioxidant therapy, coronary angioplasty

1. Introduction

According to the World Health Organization, a total of 56 million deaths occurred worldwide during 2012 and 17.5 million (31.25%) were due to cardiovascular diseases, still the principal

cause of death by noncommunicable diseases. In addition, deaths due to ischemic heart disease (IHD) in 2012 were estimated as 7.4 million (13.2%), remaining as the leading cause of death over the past decade [1, 2], with a considerable social impact due to mortality, morbidity, loss of quality of life and high economic cost. In IHD, severe and prolonged myocardial ischemic events occur through thrombotic complications from atherosclerotic plaques in pericardial coronary arteries, leading to cardiomyocyte death. The latter becomes more significant when ischemia is caused by complete coronary occlusion, generating an acute myocardial infarction (AMI) where the coronary microcirculation is significantly reduced, affecting most of the left ventricular wall thickness together with structural and functional impairments, scarring and adverse remodeling [3, 4]. 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 flow using either thrombolytic therapy or percutaneous coronary angioplasty (PCA), but this process can itself induce further viable cardiomyocyte death and increased infarct size, a phenomenon known as myocardial reperfusion injury (MRI), thus reducing the beneficial effects. The MRI causes four types of cardiac dysfunction, the first two being reversible and the others irreversible: (i) reperfusion-induced arrhythmias; (ii) myocardial stunning; (iii) microvascular obstruction or no-reflow phenomenon; and (iv) lethal myocardial reperfusion injury (LMRI). LMRI is the most important because may account for up to 50% of the myocardial infarct (MI) final size as shown in both experimental ischemia–reperfusion (I/R) models and patients with ST-segment elevation MI applying therapeutic interventions solely at the onset of myocardial reperfusion [5, 6]. In addition, several experimental studies have shown the important role of oxidative stress in MRI and it has been postulated as a therapeutic target for cardioprotection [7–13]. However, the clinical trials have shown mixed results with no clear confirmation of the beneficial effects of exogenous antioxidant therapy at the onset of myocardial revascularization, possibly due to differences in the design and methodology [14]. Next, we describe the pathophysiological mechanisms involved in MRI and the molecular basis for a novel cardioprotective treatment of patients with AMI subjected to PCA, based on a reinforcement of the antioxidant system.

2. Oxidative stress and the pathophysiology of myocardial ischemia–reperfusion injury

Occlusion of a coronary artery decreases blood flow to myocardial tissue causing a state of prolonged ischemia. The lack of oxygen and nutrients triggers a series of abrupt metabolic and biochemical changes within the cardiomyocyte that lead to several mechanisms of cell death, which are enhanced in the reperfusion (**Figure 1**).

During acute myocardial ischemia, the absence of oxygen in the mitochondrial electron transport chain (mETC) causes a drop in the production of adenosine triphosphate (ATP), and the glycolytic pathway generates a shift to anaerobic respiration with intracellular accumulation of lactic acid [9, 15]. In addition, the Krebs cycle stops and CO_2 cannot be eliminated from the extracellular space due to blood flow arrest. Therefore, a decrease in the intracellular pH

(<7.0) occurs, which increases the Na^+ influx through the Na^+/H^+ exchanger, while the ATP depletion stops Na^+ efflux through Na^+/K^+-ATPase. This intracellular Na^+ accumulation activates Na^+/Ca^{2+} exchangers in the reverse direction leading to cytosolic Ca^{2+} overload [16], where the sarcoplasmic reticulum is unable of uptaking Ca^{2+} from the cytosol because sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) transporter needs ATP to function [17]. High levels of intracellular Ca^{2+} induce the conversion, via limited proteolysis and sulfhydryl oxidation, of xanthine dehydrogenase to xanthine oxidase (XO) in endothelial cells mainly, an isoform that produces superoxide anion and hydrogen peroxide (H_2O_2) from oxygen [18]. The acidic conditions exert a strong inhibitory effect on the mitochondrial permeability transition pore (mPTP) [19], despite the presence of inducing factors opening such as Ca^{2+} and inorganic phosphate overload, oxidative stress and ADP. The mPTP is an inner mitochondrial membrane protein channel that, when it is open under certain conditions, mediates non-selective permeability to molecules less than 1.5 kDa, collapsing the mitochondrial membrane potential and uncoupling oxidative phosphorylation, leading to ATP depletion, mitochondrial matrix swelling and cell death through apoptosis and necrosis [20]. In addition, acidosis and low levels of ATP reduces the myocardial contractile activity [21].

The coronary revascularization postmyocardial ischemia rapidly increases the level of tissue oxygenation, which triggers a series of mechanisms producing LMRI. The most important mediators of this process are described below.

2.1. Oxidative stress

During the first minutes of the onset of myocardial reperfusion, a burst of ROS occurs, in accordance with several experiments demonstrating direct measurements of free radicals in isolated hearts and *in vivo* I/R models [8, 10–13]. The potential enzymatic sources of ROS production in cardiac tissue exposed to I/R are xanthine oxidase (XO) in endothelial cells, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) in neutrophils, mETC, uncoupled nitric oxide synthase (uncNOS), cytochrome P450, lipoxygenase/cyclooxy-genase and monoamine oxidase [22]. XO activation and ATP catabolism to hypoxanthine occur in ischemic period, generating high levels of ROS together with uric acid from oxygen and accumulated hypoxanthine (or xanthine), when blood flow is restored [18]. The important role of NOX family in the MRI has been shown in experimental studies where NOX-isoform-specific knockout mice have significantly reduced infarct sizes compared to wild-type (WT) controls, confirming these results in buffer-perfused Langendorff models [23]. Cardiolipin peroxidation and cytochrome oxidase uncoupling in ischemic period results in the inhibition of electron flux through mETC, ATP depletion and increased superoxide anion generation, a situation that persists in the reperfusion where the Krebs cycle is reactivated and high levels of tissue oxygen can lead to increased ROS production [24]. Tetrahydrobiopterin (BH_4), a NOS cofactor, suffers oxidation to dihydrobiopterin in prolonged ischemia, resulting in loss of NOS enzyme affinity by the substrate L-citrulline together with a shift in the generation of nitric oxide (NO), a potent vasodilator, to superoxide anion during reperfusion [24, 25].
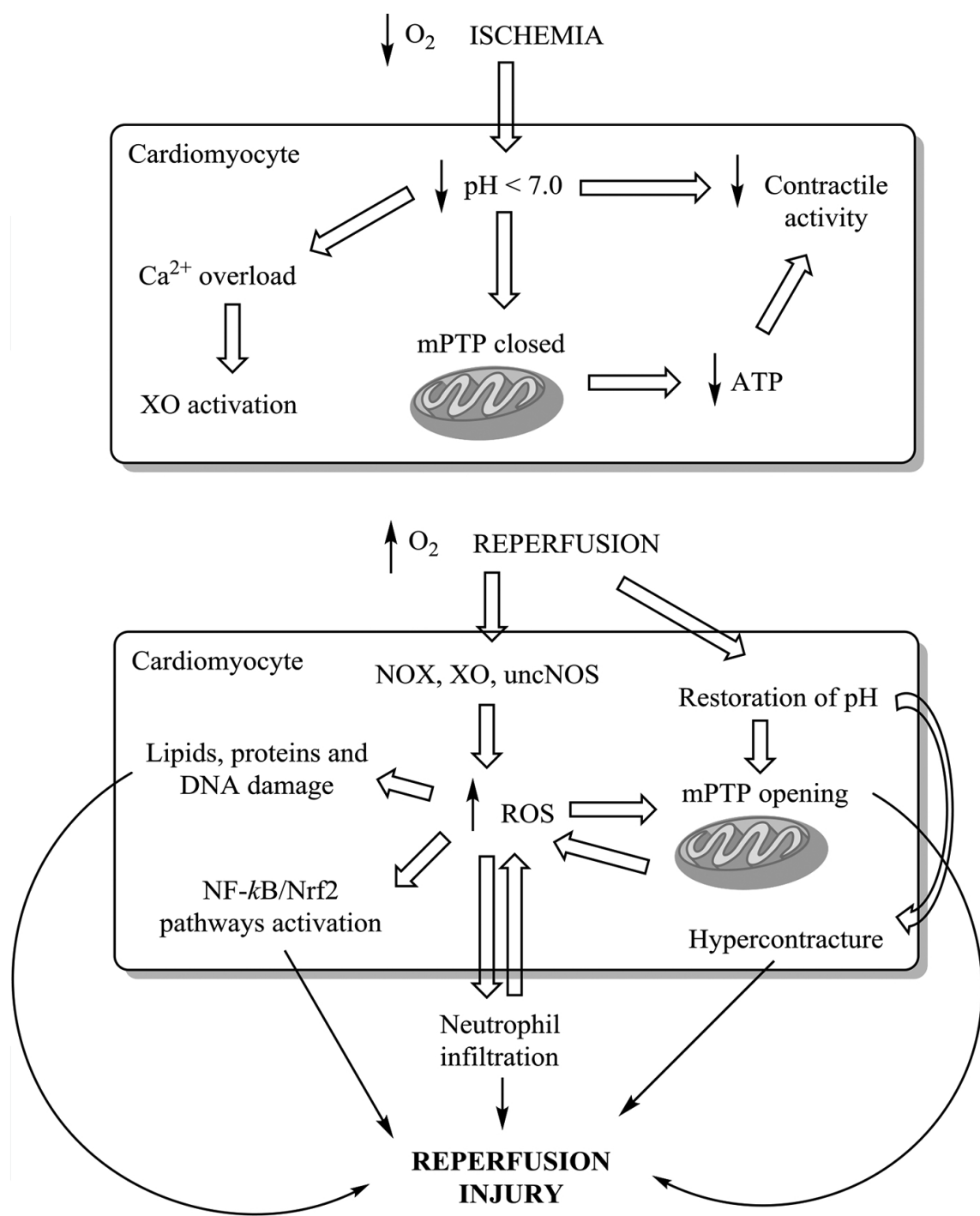
Figure 1. Schematic representation of the pathophysiological mechanism involved in myocardial damage due to I/R. ATP, adenosine triphosphate; mPTP, mitochondrial permeability transition pore; NOX, NADPH oxidase; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species; uncNOS, uncoupled nitric oxide synthase; XO, xanthine oxidase; Ca^{2+}, calcium; DNA, deoxyribonucleic acid.

Exacerbation of oxidative stress during postischemia myocardial reperfusion overwhelms the endogenous antioxidant defenses, causing free radical propagation reactions with direct damage to cellular biomolecules as lipid peroxidation, protein oxidation/nitration and DNA

damage [24, 26]. The main effecter of ROS-induced damage is the highly reactive hydroxyl radical generated from Fenton/Haber–Weiss reactions and peroxynitrite (reviewed in Section 3) [24], demonstrating its formation in a postischemia reperfused heart [11]. In addition, ROS can induce activation both of nuclear factor kappa B (NF-κB) and nuclear factor-erythroid 2-related factor 2 (Nrf2)-signaling pathways, although the ROS concentration threshold has not been experimentally determined [27]. NF-κB proteins are a family of transcription factors with a central role in regulating the expression of genes related with inflammation, immune response, cell proliferation and apoptosis [28–30], and different levels of ROS can both activate and inhibit NF-κB-signaling, depending on the context, with a high degree of complexity [31]. On the other hand, Nrf2 is a transcription factor that positively regulates the human antioxidant response element (ARE), leading to the gene expression of endogenous antioxidant defence system. Kelch-like ECH-associated protein 1 (Keap1) is a suppressor protein anchored in the cytoplasm that physically binds Nrf2, but oxidative stress facilitates the complex dissociation and Nrf2 nuclear translocation to ARE-containing promoters [32]. A study demonstrated that Nrf2 is indispensable for the regulation of both constitutive and inducible expression of antioxidants and phase-2 enzymes in mouse primary cardiomyocytes [33]. In clinical trials, the antioxidant therapy at the onset of reperfusion, in patients with AMI subjected to PCA, has mainly considered the use, alone or combined, of ROS scavengers, inhibitors of ROS sources, human recombinant antioxidant enzymes and reduced glutathione donor [14].

2.2. Intracellular pH

The intracellular acidic pH generated in ischemia returns to physiological values during myocardial reperfusion [9]. Bond et al. [34] simulated I/R conditions in cultured neonatal rat cardiac myocytes, demonstrating that when intracellular acidic pH increases to 7.4 hypercontracture and cell death occur. In addition, free Ca^{2+} increases during simulated ischemia and in simulated reperfusion. Under conditions of ischemia, it was shown in cultured cardiac myocytes and perfused papillary muscles that inhibition of Na^+/H^+ exchanger delayed the increase of intracellular pH after reperfusion and prevented reperfusion-induced cell killing, but not reduce the increase in intracellular-free Ca^{2+} [35]. By contrast, reperfusion with inhibition of Na^+/Ca^{2+} exchanger decreases intracellular free Ca^{2+} but did not reduce cell killing. These results suggest that acidotic pH is generally protective in I/R, and Na^+/H^+ exchanger contributes to reperfusion washout effect on intracellular acidic pH, leading to a Ca^{2+}-independent lethal reperfusion injury in cardiomyocytes.

2.3. The mPTP opening

Recently, it has been proposed that various potential protein components either to form the molecular structure of the mPTP or to regulate its opening [20, 36]. It was shown that the mPTP opening occurs within the first few minutes postischemia myocardial reperfusion [37], with both burst of oxidative stress and intracellular pH normalization (possibly due to the inhibitory effect of acid pH on mPTP is removed) as the main contributing factors [38, 39]. On the other hand, Ca^{2+} overload seems not to be a causative factor in I/R model. In adult rat myocytes, both

cytosolic and mitochondrial Ca^{2+} increased during ischemia but decreases to basal levels in the first minutes of reperfusion. Ca^{2+} overload occurred late in both compartments, event that was prevented by mPTP inhibitors. Besides, intramitochondrial Ca^{2+} chelation did not prevent cell death after reperfusion. Thus, Ca^{2+} overload appears to be the consequence of bioenergetic failure after mPTP opening [38]. Another study showed that, at the onset of reperfusion, there is a transient increase in cytosolic Ca^{2+} levels together with a simultaneous transient sarcoplasmic reticulum Ca^{2+} depletion [40], corroborating the latter. The mPTP is a potential pharmacological target for prevent LMRI, and experimental studies with mPTP inhibitors (such as cyplosporin A), at the onset of myocardial reperfusion, has been reported to reduce MI size by 40–50% [41–44].

2.4. Inflammation

Ischemia is associated with slow infiltration of neutrophils, but recruitment toward the necrotic zone is favored after reperfusion by increased ROS exacerbation that triggers upregulation of adhesion molecules (P-selectin, CD11/CD18, ICAM-1) in cardiomyocytes, with cytokines (TNFα, IL-1, IL-6, IL-8, NAP-1, PAF, MIP-2) and complement, which are released from ischemic-reperfused myocardium. Neutrophils adhesion to coronary vascular endothelium occurs rapidly (within minutes) after onset of reperfusion, with abundant accumulation into the infarct zone during the following 6 hours. Neutrophils release more than 20 different proteolytic enzymes (hydrolases, metalloproteinases, and proteases) and are a major ROS source by generating superoxide anions through NOX, positioning them as important contributors to MRI [45].

3. Cardioprotection by combined antioxidant therapy

After reviewing the most relevant pathophysiological processes of myocardial ischemia–reperfusion injury, the central role being played by the burst of oxidative stress in the first minutes of revascularization certainly has positioned itself as a pharmacological target of choice. In the following section, we describe the molecular basis of an innovative combined antioxidant therapy, which includes a reinforcement of endogenous antioxidant defences, aimed to prevent or at least ameliorate the MRI in patients with AMI undergoing PCA.

3.1. Ascorbate

Vitamin C (ascorbic acid or ascorbate) is a potent water-soluble antioxidant in humans, which cannot be endogenously synthesized [46] and must be incorporated through vegetables and fruits [47]. Vitamin C is an electron donor and is oxidized to dehydroascorbate when acting as a reducing agent, returning to reduced form when is used by the cell [48] (**Figure 2**). A study in a group of apparently healthy adult nonsmoking population showed an inverse correlation between plasma vitamin C and products of oxidative damage to DNA, proteins and lipids [49]. Another study evaluated oxidant and antioxidant parameters in the blood of the patients with MI before and after thrombolysis and showed that the activity of superoxide dismutase (SOD),

an antioxidant enzyme, was significantly reduced, whereas the activity of XO, an oxidant enzyme, together with the levels of malondialdehyde (MDA), a lipid peroxidation biomarker, significantly increased after reperfusion. These parameters improved to normal or near-normal levels when patients were supplemented with oral vitamin C postreperfusion [50], confirming the *in vivo* antioxidant capacity of vitamin C. Other properties of this compound are described in the following experiments. Ascorbic acid, together vitamin E, reverse endothelial dysfunction through a modulator effect by upregulating endothelial NOS (eNOS) and downregulating NOX on the vascular wall [51]. In essential hypertensive patients, impaired endothelium-dependent vasodilation improved with vitamin C supplementation, an effect that can be reversed by a NOS inhibitor, suggesting a restoration of NO availability and oxidative stress-mediated endothelium impairment in this pathology [52]. Pretreatment with vitamin C prevents vascular function damage and release of IL-6 induced by endothelin-1 in humans [53]. Intracellular vitamin C in human cell lines and primary endothelial cells, together with cell cultures in medium with dehydroascorbic acid, showed significantly decreased TNFα-induced NF-κB activation [54].
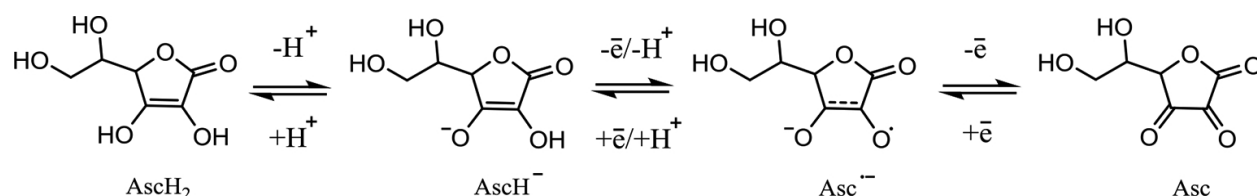

Figure 2. Oxidation of ascorbate (AscH$^-$) to dehydroascorbic acid (Asc) for the loss of two electrons in succession, through the formation of ascorbyl radical intermediate (Asc$^{\bullet-}$). Importantly, ascorbate is the ionized form of ascorbic acid (AscH$_2$) at physiological pH (7.4).

Vitamin C has been used in I/R models. Gao et al. [55] demonstrated that in isolated rat hearts subjected to I/R, glutathione monoethyl ester (GSHme), but no ascorbic acid, administered at the onset of reperfusion exerted protective effects against MRI. Furthermore, GSHme coadministered together with ascorbic acid had enhanced protective effects, suggesting a synergistic effect between the two compounds. Another *in vivo* experimental study showed that intravenous (IV) administration of vitamin C or vitamin C plus vitamin E, prior to coronary occlusion following reperfusion, had not significant differences in infarct size compared to the control group. Besides, vitamin C alone tends to increase infarct size, whereas the vitamin combination tends to decrease [56]. The clinical trials in which vitamin C is orally administered, in combination with other vitamins, in different doses to patients with cardiovascular history showed no beneficial cardioprotective long-term effects [27]. In patients with AMI subjected to PCA, administration of vitamin C orally (2.0 g) followed by a constant infusion (20 mg/min), before reperfusion, did not suppress the rapid and transient increase in levels of urinary 8-epi-prostaglandin F2α (8-epi-PGF2α), a biomarker of oxidative stress *in vivo*, after PCA [57]. A review [14] of the cardioprotective strategies using vitamin C in combination with other vitamins in AMI followed by restoration of coronary blood flow showed variable results when administered orally, but when an infusion of vitamin C (1000 mg/12 h) was followed by oral doses (1200 mg/24 h) and vitamin E (600 mg/24 h), positive clinical outcomes were obtained

within a composite endpoint. According to the latter, a study in patients undergoing elective percutaneous coronary intervention for stable angina showed that 1 g vitamin C administered by infusion (16.6 mg/min), 1 hour before of intervention, improved the impaired microcirculatory reperfusion and had significantly reduced plasma levels of oxidative stress biomarkers [58].

These results could be explained by the fact that oral administration of vitamin C shows a plasma concentration–time profile, in a dose range of 200–2500 mg/day, producing a steady-state plasma concentration approximately by 80 μmol/l (0.08 mmol/l), due to apparent saturation of tissue uptake and in less degree by function of oral bioavailability and renal excretion [59]. At these physiological concentrations, superoxide anion reacts with NO at a rate 10^4-fold greater than that at which it reacts with ascorbic acid (**Table 1A** and **C**), situation that is favored during the ROS burst in the first minutes of postischemia myocardial reperfusion because superoxide anion levels are exacerbated.

	Reaction	Rate constant ($M^{-1} s^{-1}$)
(A)	$O_2^{\cdot -} + \cdot NO \rightarrow ONOO^-$	7×10^9
(B)	$O_2^{\cdot -} + SOD \rightarrow O_2 + H_2O_2$	2×10^9
(C)	$O_2^{\cdot -} + AscH_2 \rightarrow Products$	$2.7–3.3 \times 10^5$

$ONOO^-$, peroxynitrite; H_2O_2, hydrogen peroxide; O_2, molecular oxygen [60–62].

Table 1. Comparison of the reaction rate constants of superoxide anion ($O_2^{\cdot -}$) with nitric oxide ($^\cdot NO$), superoxide dismutase (SOD) and ascorbic acid ($AscH_2$).

Superoxide anion reacts avidly with NO to form peroxynitrite ($ONOO^-$) (**Table 1A**), an agent very reactive and toxic to biomolecules, with a constant rate higher than that of the reaction between superoxide anion and SOD (**Table 1B**). Furthermore, peroxynitrite (pKa at 37°C = 6.8) can be protonated in medium with acidotic pH during ischemia, resulting in ONOOH that is inherently unstable. Reperfusion restored the intracellular pH to physiologic levels, favoring decomposition of ONOOH to hydroxyl radical and nitrogen dioxide radical (**Figure 3**) [24], both responsible for generating oxidative stress and nitrosative stress damage to cardiomyocytes. Thus, peroxynitrite contributes to MRI generating lipid peroxidation and protein nitration in tyrosine residues, affecting the function and structure of the latter; oxidation of thiol groups related to cell antioxidant capacity; rupture of double-stranded DNA; and BH_4 NOS cofactor oxidation, which reduces the formation of NO [63].

Jackson et al. [64] demonstrated *in vitro* that supraphysiologic vitamin C concentration of 10 mmol/l is needed to overcome the competition with NO for superoxide. Consequently, only IV infusion can achieve these plasma ascorbate levels. It has been documented that the use of high doses of IV vitamin C over 10 mmol/l in patients appears to have a positive safety profile, but it should be avoided in patients with renal function impairment or glucose-6-phosphate

dehydrogenase deficiency [65]. Human plasma vitamin C concentration–time profile following short-term IV infusion shows peak concentrations higher than 20 mmol/l, remaining over 10 mmol/l for 3 hours [66]. It is important to note that vitamin C needs to be incorporated into the cell to exert its effects. Specific Na^+-dependent transporters for ascorbic acid (reduced form) are called SVCT1 and SVCT2, while transporters for dehydroascorbic acid (oxidized form) are members of GLUT family (GLUT1/3/4) that facilitate transport of glucose [67]. These transporters are expressed in the human myocardium [68–70].
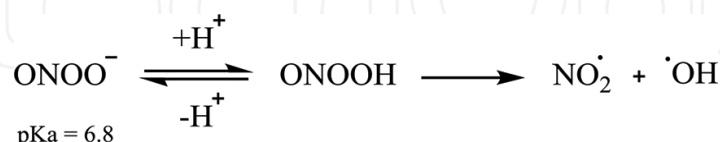
$$ONOO^- \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} ONOOH \longrightarrow NO_2^{\bullet} + {}^{\bullet}OH$$

$$pKa = 6.8$$

Figure 3. Formation of hydroxyl radical ($^{\bullet}OH$) and nitrogen dioxide radical ($NO_2^{\bullet}$) from peroxynitrite ($ONOO^-$) through an intermediary peroxynitrous acid (ONOOH).

3.2. N-Acetyl cysteine

When ascorbate is oxidized to dehydroascorbate, it can return to its reduced form through a reduced glutathione (GSH)-dependent recycling mechanism inside the cell, which may be direct [71] or enzyme mediated [72] (**Figure 4**) and can lead to a dehydroascorbate concentration-dependent decrease in intracellular GSH levels [71, 73, 74]. This process has been described in human erythrocytes [73], bovine aortic endothelial cells [74], among others and fulfills a function of blood antioxidant reserve [75]. Glutathione (γ-glutamyl-cysteinyl-glycine) is an endogenous agent playing a primary role of nonenzymatic antioxidant defence together with participating in metabolic processes and cellular regulation. It has a reduced form (GSH) and an oxidized form (GSSG) which are interconvertible. It is synthesized in all cell types of the organism, being mostly in the cytosol and to a lesser extent in the extracellular plasma [76].
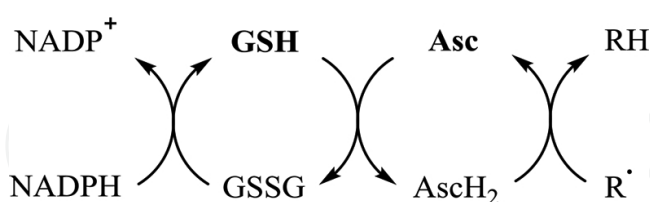
$$NADP^+ \quad \textbf{GSH} \quad \textbf{Asc} \quad RH$$

$$NADPH \quad GSSG \quad AscH_2 \quad R^{\bullet}$$

Figure 4. Reduced glutathione (GSH)-dependent reduction of dehydroascorbic acid (Asc), which may be mediated directly or by enzyme. NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidized form); GSSG, oxidized glutathione; $AscH_2$, ascorbic acid; RH, unsaturated fatty acid chain; $R^{\bullet}$, lipid alkyl radical.

If we consider an IV administration of high doses of vitamin C before, during and after PCA in patients with AMI, the burst of ROS will be counteracted by ascorbic acid, which generates large amounts of dehydroascorbic acid. We hypothesized that the latter interact with endogenous GSH and will cause a drop in their levels, limiting the cardioprotective effect. For this reason, we believe essential to reinforcement the endogenous antioxidant defence system with

a GSH donor, such as *N*-acetyl cysteine, to optimize antioxidant therapy. Furthermore, as above-mentioned, coadministration of a GSH donor and vitamin C tend to have a synergistic protective effect on infarct size in an I/R model of isolated rat heart [56].

N-Acetyl cysteine (NAC) is a drug currently used in clinic that has demonstrated a good safety profile when administered orally, although adverse effects will be more noticeable at high doses (>3 g/day) or IV administration (e.g., for the treatment of paracetamol overdose) [77]. In experimental I/R models, high NAC dose administrated intracoronary with a radiographic contrast in pigs, at the onset of reperfusion, was safe, reduced platelet reactivity and there was a trend toward a better cardiac function at 24 h, but there was no significant difference in the myocardial infarct size and did not provide significant renal protection compared to control group [78]. On the other hand, in a rat I/R model, NAC administration by continuous infusion before, during and after reperfusion produced a significant limitation of the infarct size compared to control group, but injection of NAC bolus with the same total dose, before and at onset of reperfusion, failed to reduce it [79]. In clinical trials, group of patients undergoing coronary artery bypass and/or valve surgery treated with IV infusion of NAC, before and after of surgery, decreases the incidence of postoperative atrial fibrillation compared to control group [80]. LIPSIA-N-ACC trial [81], a randomized, single-blind, controlled trial, was designed to measure the effects of high doses of NAC on contrast-induced nephropathy (CIN) and reperfusion injury in ST-segment elevation MI patients undergoing primary angioplasty intervention (PCI) with moderate contrast volumes. CIN is the acute deterioration of renal function occurring after intravascular administration of contrast media that is not attributable to other causes, defined as increase in serum creatinine > 0.3 mg/dL or 25% above baseline levels within 48 hours after contrast administration, which is associated with increased rates of morbidity and mortality. Of the 251 patients enrolled, 126 were randomized to the NAC treated group and 125 to the placebo group. NAC was administered as an IV bolus (1200 mg) before angioplasty and twice daily for 48 hours after angioplasty. In addition, all patients and controls were hydrated with IV infusion of NaCl (0.9%), at a rate of 1 ml/kg of body weight per hour for 12 h, after PCI. Iopromide was used as a nonionic low-osmolality contrast agent for PCI. In the primary outcomes, CIN (>25% increase in serum creatinine level <72 h after randomization) occurred in 14% in the NAC-treated group and 20% in the placebo group, with no significant differences; the MRI (measured as myocardial salvage index by MRI) was also not statistically significant difference in both groups, so it is concluded that NAC not provide an additional clinical benefit to placebo with respect to CIN and MRI. However, activated oxygen protein products and oxidized low-density lipoprotein were evaluated as oxidative stress markers in blood plasma (in venous blood samples collected before, immediately after PCI, and subsequently for up to 3 days) and found that the NAC-treated group had a significant reduction (20%) of these markers, while the placebo group had no significant differences. In another study, patients with AMI that received NAC infusion (a total dose of 15 g/24 h), combined with IV nitroglycerin and streptokinase, were well tolerated together with having significantly lesser oxidative stress, a trend toward more rapid reperfusion and better preservation of left ventricular function compared to control group [82].

3.3. Deferoxamine

It has been shown in I/R models of isolated perfused heart that during a period of ischemia the amount of tissue available iron (Fe) increases in a time-dependent manner. Fe is rapidly mobilized through the perfusion fluid leading to very high Fe levels (up to 50-fold compared to pre-ischemic values) in the first small volumes of coronary flow fractions (CFF), returning to baseline over time. In addition, the levels of Fe in the CFFs correlated well with the loss of cardiac function following global ischemia of varying duration [83]. Similarly, the Fe levels increase up to 30-fold in cardiac tissue during ischemia, in a time-dependent manner, due to acidification in ischemia because this effect contributes tremendously to the mobilization of Fe from intracellular ferritin storage. After reperfusion, tissue Fe levels decrease, although it is known that the superoxide anion contributes to the mobilization of Fe from ferritin [84–86]. Langendorff models with myocardial iron overload develop different functional, biochemical and ultrastructural alterations as compared to control groups of myocardial I/R, which are prevented by deferoxamine (DFO), an iron chelator [87], realizing the harmful tissue effect of Fe high levels. The role of Fe in the postischemia MRI has been demonstrated in experimental models by the use of iron chelators at the onset of reperfusion, improving cardiac function relative to control group [88, 89]. Furthermore, a long-term study conducted in randomly selected men aged 42, 48, 54 and 60, who had no symptoms of coronary heart disease at entry, showed that elevated levels of serum ferritin (stored Fe) was a strong risk factor for developing AMI [90].

Physiologically, transition metals, such as iron, are mainly stored or complexed. However, under certain pathological conditions, the nonchelated state iron levels are increased, thus generating oxidative stress. Reduced iron (Fe^{2+}) can react with hydrogen peroxide to generate hydroxyl radical ($^\bullet OH$), a process known as Fenton reaction (**Table 2A**). At the same time, oxidized iron (Fe^{3+}) can react with superoxide anion to form again Fe^{2+} and oxygen (**Table 2B**). The sum of the Fenton reaction and the superoxide-mediated reduction of Fe^{3+} originates the Haber–Weiss reaction (**Table 2C**), where hydroxyl radical is generated from superoxide anion and hydrogen peroxide [24]. Thus, during myocardial I/R increase, the Fe^{2+} availability and ROS levels that favor the formation of highly harmful and reactive hydroxyl radical through these redox reactions, can significantly contribute to MRI. This allows considering the iron overload during I/R as a pharmacological target for cardioprotection.

In addition, our interest is focused on the interaction between vitamin C and iron. Ascorbate has pro-oxidant effect because of reduction of Fe^{3+} to Fe^{2+} (**Table 2D**), which is substrate to Fenton reaction leading to ROS production [92]. Under physiological conditions, *in vivo* studies with vitamin C supplementation showed predominantly reduced levels in markers of oxidative damage in DNA, lipids and proteins, even in the presence of iron. These results were correlated with *in vitro* systems, such as isolated or cultured cells and biological fluids, where the antioxidant role, or no effect of vitamin C, predominated over a pro-oxidant role [91]. Considering that iron overload occurs in postischemia myocardial reperfusion, IV infusion of high doses of vitamin C at the onset of reperfusion could generate a strong interaction with iron, which not only decrease the concentration of ascorbate available in blood to counteract the burst of oxidative stress, reducing its antioxidant effect, but also favor pro-oxidant effects

and ROS production. Thus, use of an iron chelator, as DFO, as adjuvant to antioxidant therapy with vitamin C should be considered to reduce deleterious effects and maximize cardioprotection in patients with AMI being subjected to PCA. A experimental study in pigs showed that the combined use of vitamin C (100 mg/kg) and DFO (60 mg/kg), administered as IV infusion at the beginning, during and after reperfusion postischemia, had no difference in the measured cardiac parameters compared to the control group, although it was observed a trend toward reducing infarct size [94]. However, no markers of oxidative stress, apoptosis or other biochemical parameter related to myocardial damage were measured. Another study in sheep demonstrated that administration by IV infusion of vitamin C (1.5 g) and DFO (1 g) (in combination but not separately), before reperfusion, significantly protected against the development of ventricular arrhythmias induced by I/R, compared to control group [95].

Reaction	Rate constant ($M^{-1} s^{-1}$)
(A) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$	76
(B) $O_2^{\cdot -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$	$1.9 \times 10^6 - 10^4$
(C) $O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + \cdot OH + OH^-$	<2.3
(D) $AscH^- + Fe^{3+} \rightarrow Asc^{\cdot -} + Fe^{2+} + H^+$	10^2

H_2O_2, hydrogen peroxide; Fe^{2+}, reduced iron; Fe^{3+}, oxidized iron; $\cdot OH$, Hydroxyl radical; OH^-, Hydroxyl anion; $O_2^{\cdot -}$, Superoxide anion; O_2, Molecular oxygen; $AscH^-$, Ascorbate; $Asc^{\cdot -}$, Ascorbyl radical [91–93].

Table 2. Fenton (A), Haber–Weiss (C) and others redox reactions.

DFO has shown beneficial effects in experimental models of I/R. Isolated, perfused rabbit hearts treated with DFO, during ischemia and reflow, demonstrated improved functional and metabolic recovery of myocardium together a reduction in reperfusion-induced oxygen free-radical generation, compared to control group [96]. In a canine model of I/R, DFO pretreatment before ischemia, but not at the beginning of reperfusion, reduced significantly infarct size and release of GSSG into the coronary sinus during early reflow, compared to control group [97]. Decrease in infarct size by early treatment with DFO was corroborated by another independent study in canine model of I/R [98]. Regarding clinical trials, patients undergoing coronary artery bypass grafting, that received an IV infusion of DFO for 8 hours, prevented the increase in oxidative stress markers and improving ventricular functional parameters after surgery, compared to control group [99]. Other clinical trial in patients with ST-elevation MI subjected to primary percutaneous coronary intervention (PPCI) showed that administration of IV bolus of DFO (500 mg) immediately before surgery, followed by a 12-h infusion (50 mg/kg), significantly reduced in plasma F2-isoprostane levels, with no difference in infarct size, after PPCI compared to placebo group [100].

4. Conclusion and perspectives

In this chapter, we have reviewed the molecular processes involved in the pathophysiology of myocardial damage by postischemia reperfusion, emphasizing the central role of oxidative stress as the key mediator of this damage. Accordingly, increased ROS production can give rise to the occurrence of events ranging from inflammation, damage to biomolecules and metabolic cell impairment to even cell death. From this paradigm, a novel antioxidant therapy is proposed as cardioprotective action in patients with AMI subject to PCA. This treatment considers the use of vitamin C (sodium ascorbate) in high doses administered intravenously, combined with NAC and DFO prior to surgery so as to optimize and enhance the beneficial effects and reduce the harmful effects on myocardium occurring in this setting (**Figure 5**). However, the results from different experimental models are controversial and more studies are still lacking. On this line, it is important to note that MRI is an unsolved problem in the clinical practice. Different strategies to prevent this damage during surgery for revascularization in patients with AMI have been tried without conclusive results, and we expect that our proposal can contribute as an effective, low risk and economic alternative in the near future.
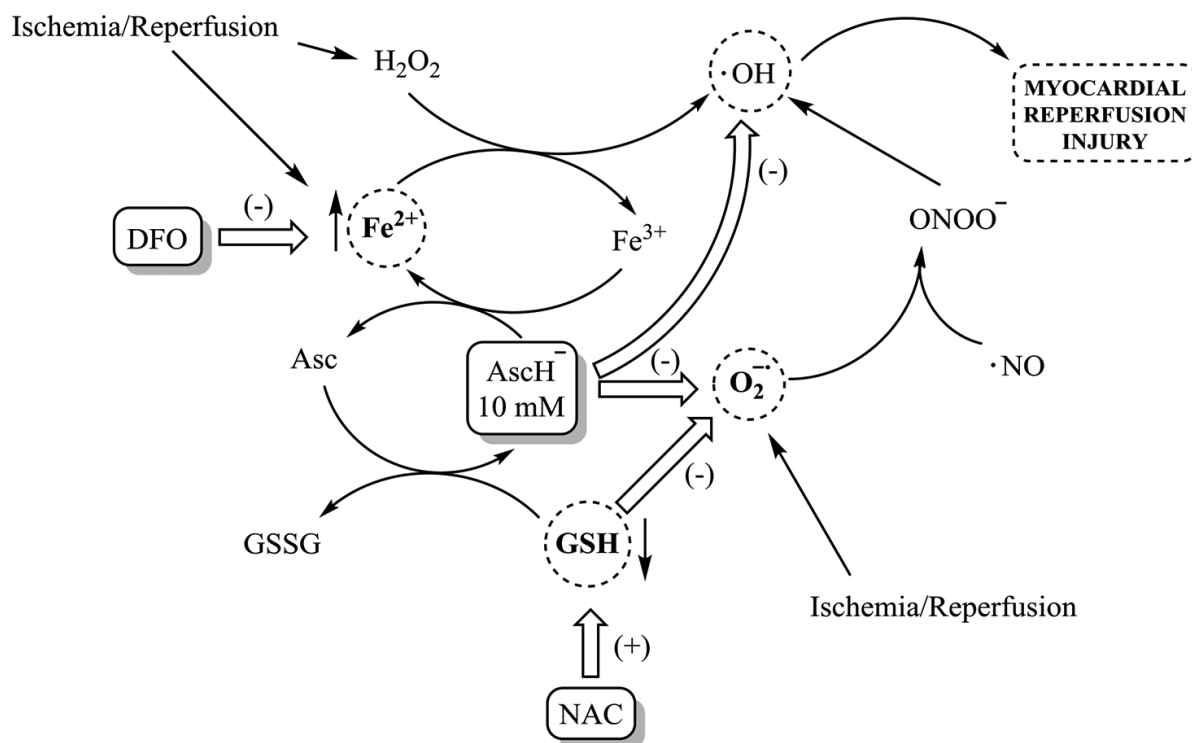

Figure 5. Diagram of the proposed combined antioxidant therapy for patients with AMI subject to PCA, which considers the use of: (i) ascorbate ($AscH^-$) in high doses to compete with nitric oxide (•NO) by superoxide anion ($O_2^{•-}$); (ii) deferoxamine (DFO) to counteract reduced iron (Fe^{2+}) overload and thus prevent the Fenton reaction and interaction with $AscH^-$; (iii) *N*-acetyl cysteine (NAC) as reduced glutathione (GSH) donor to reinforce the antioxidant defense system and mitigate its interaction with dehydroascorbic acid (Asc). In this way, it counteracts directly and indirectly the hydroxyl radical (•OH), which is the main mediator of myocardial damage by oxidative stress during reperfusion. H_2O_2, hydrogen peroxide; GSSG, oxidized glutathione; $ONOO^-$, Peroxynitrite; Fe^{3+}, oxidized iron.

Acknowledgements

Nomenclature

Asc	dehydroascorbic acid
AscH$_2$	ascorbic acid
AMI	acute myocardial infarction
ARE	antioxidant response elements
ATP	adenosine triphosphate
CFF	coronary flow fraction
DFO	deferoxamine
eNOS	endothelial nitric oxide synthase
8-epi-PGF2α	8-epi-prostaglandin F2α
GSH	reduced glutathione
GSSG	oxidized glutathione
IHD	ischemic heart disease
IL-6	interleukin 6
I/R	ischemia–reperfusion
IV	intravenous
kDA	kilo dalton
LMRI	lethal myocardial reperfusion injury
PCA	percutaneous coronary angioplasty
MDA	malondialdehyde
mETC	mitochondrial electron transport chain
MI	myocardial infarction
MRI	myocardial reperfusion injury
mPTP	mitochondrial permeability transition pore
NAC	N-acetyl cysteine
NF-κB	nuclear factor kappa B
NO	nitric oxide
NOX	NADPH oxidase
Nrf2	nuclear factor-erythroid 2-related factor 2

·OH	hydroxyl radical
ONOO⁻	peroxynitrite
PPCI	primary percutaneous coronary intervention
ROS	reactive oxygen species
SOD	superoxide dismutase
TNFα	tumor necrosis factor alpha
XO	xanthine oxidase

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