The role of redox dysregulation in the inflammatory response to acute myocardial ischaemia-reperfusion injury - adding fuel to the fire

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Abstract

The inflammatory response to acute myocardial ischaemia/reperfusion injury (IRI) plays a critical role in determining myocardial infarct (MI) size and subsequent post-MI left ventricular (LV) remodeling, making it a potential therapeutic target for treating patients presenting with an acute myocardial infarction (AMI). Recent experimental studies using advanced imaging and molecular techniques have yielded new insights into the mechanisms through which reactive oxygen species (ROS) contribute to the inflammatory response during acute myocardial IRI - "*adding fuel to the fire"*. The infiltration of inflammatory cells into the MI zone, leads to elevated myocardial concentrations of ROS, cytokine release, and activation of apoptotic and necrotic death pathways. Anti-oxidant and anti-inflammatory therapies have failed to protect the heart against acute myocardial IRI. This may be, in part, to a lack of understanding of the time course, nature and mechanisms of the inflammation and redox dysregulation which occur in the setting of acute myocardial IRI. In this article, we will examine the inflammatory response and redox dysregulation induced by acute myocardial IRI, and highlight potential therapeutic options for targeting redox dysregulation in order to attenuate the detrimental effects of the inflammatory response following an AMI so as to reduce MI size and prevent heart failure.

Keywords

Myocardial ischaemia/reperfusion injury, Redox dysregulation, Inflammation, Reactive Oxygen Species, Oxidative stress, Neutrophils.

Contents

1. Introduction

Acute myocardial infarction (AMI) and the heart failure which often ensues are one of the leading causes of death and disability worldwide. For patients presenting with an AMI, the treatment of choice is to restore coronary blood flow in the infarct-related artery, to salvage viable myocardium. However, despite optimal therapy the morbidity and mortality of AMI patients remain significant with 7% death and 25% heart failure at one year [1]. The reason for this, is in part, due to the presence of 'myocardial reperfusion injury' which refers to the myocardial injury and cardiomyocyte death, that is paradoxically induced by myocardial reperfusion, and which can contribute up to 50% of the final myocardial infarct (MI) size, and for which, there is currently no effective therapy [2] [3]. As such, novel therapies are required to protect the heart against acute myocardial ischaemia/reperfusion injury (IRI), in order to reduce MI size and prevent heart failure.

The inflammatory response to acute myocardial IRI plays a critical role in determining MI size and subsequent post-MI left ventricular (LV) remodelling, making it a potential therapeutic target for preventing heart failure following AMI. Experimental studies using molecular techniques and advanced biomedical imaging have yielded new insights into the mechanisms through which reactive oxygen species (ROS) contribute to the inflammatory response during acute myocardial IRI, "*adding fuel to the fire"*. The infiltration of inflammatory cells into the MI zone, leads to elevated concentrations of ROS in the myocardium, cytokine release, and the activation of apoptotic and necrotic death pathways. The complex interplay between ROS and inflammation can amplify the effects of ROS as mediators of myocardial injury and determinants of cell death. As such, ROS represent important therapeutic targets for reducing MI size and preventing adverse LV remodelling in AMI patients. In this review article, we highlight the complex interplay between ROS and inflammation in the setting of acute myocardial IRI, and explore emerging therapeutic targets for attenuating ROS and modulating the inflammatory response in patients presenting with AMI.

2. Oxygen Paradox and ROS formation

When the myocardium is re-oxygenated after a prolonged period of energy depletion, it rapidly hypercontracts. The hypercontracture and cytolysis induced by reoxygenation have become known

as the “Oxygen Paradox” [4], [5], [2]. Bresnahan et al. [6], demonstrated in a canine model that the potentiation of haemorrhage and extension of myocardial infarction is attributable to the re-administration of molecular oxygen. Using the isolated perfused rat heart, in 1973, Hearse and Chain showed that the reoxygenation kills the heart cells and exacerbates cardiac enzyme creatine phosphokinase (CPK), ATP, AMP phosphotransferase (MK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) release. The myocardial injury was not identified. However the possible responsible listed was ROS overproduction [4].

Following AMI, ROS are generated in the first minute of reperfusion, and peak 4-7 minutes later, although ROS production continues at lower sustained levels for quite some time after [7]. Oxidative stress or redox dysregulation occurs in the myocardium when ROS production is enhanced, and the anti-oxidant reserve is exhausted. This highly reactive and unstable group of compounds are formed as a result of the addition of an unpaired electron in the outer orbit of the molecule. Superoxide (O^{-}_{2}), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (•OH) are the most prominent free radicals in the pathogenesis of acute myocardial IRI. In the presence of iron, superoxide and H_2O_2 can lead to the formation of highly reactive •OH, which can damage cellular proteins, RNA, DNA and lipids. Interaction of ROS with nitrogen monoxide (NO•) [8] or fatty acids can produce peroxynitrite or peroxyl radicals, respectively. The first ROS produced in response to acute IRI is O^{-}_{2}, resulting from the univalent reduction of molecular oxygen. Dismutation of O^{-}_{2} produces H_2O_2, which, in turn, may be entirely reduced to water or partially reduced to •OH, one of the strongest pro-oxidants in nature. Also, O^{-}_{2} may react with nitric oxide in a reaction controlled by the rate of diffusion of both radicals [9] [10] [11] (fig. 1). The generation of ROS has been connected to stress responses, apoptosis, aging and death. However, the ROS are now being recognized as molecules involved in the cardiac adaptation to different types of physiological stimuli [12] [13] [14] [15] [16].

3. Biological roles of ROS in the heart

The most recognized ROS with physiological effects includes the O^{-}_{2}, NO• and the non-radical specie H_2O_2. There have been implicated in the regulation of inflammation [17] [18] [19], calcium signaling [20], hypertrophy [21], autophagy [22] and cardioprotection [23].

Cysteine (Cys) and methionine (Met) possess reactive sulfur-containing side chains that present targets for ROS [16] [24]. Oxidation of these specific and reactive residues, in turn can, lead to the reversible modification of enzymatic activity. Four oxidation states of Cys can be generated: disulfide (-S-S), sulfenic acid (-SOH) and sulfonic acid ($-SO_3H$) [16]. Sulfenic acid is readily reduced to cysteine by the cellular reducing agents, glutathione (GSH) and thioredoxin (Trx) [25] [26]. Methionine is oxidized to methionine sulfoxide (MetO) by the addition of an extra oxygen atom [27]. The Anderson laboratory found that following initial Ca^{2+}-dependent activation of CaMKII (Ca^{2+}/calmodulin-dependent kinase II), the specific oxidation of conserved Met 281/282 residues in the regulatory domain could increase CaMKII activity independent of Ca^{2+}/calmodulin [28].

Substantial evidence has revealed that H_2O_2 production has been shown to be a major component of endothelium-derived hyperpolarizing factor to control blood pressure [29] [30]. H_2O_2 also caused interprotein disulfide bond in protein kinase G (PKG) which activated the kinase independently pf the NO•-cyclic guanosine monophosphate (cGMP) pathway and coupled to vasodilation [31]. In a redox-dead Cys42Ser PKGI-α knock-in mouse, Prysyazhna et al., demonstrated that H2O2 inuce an oxidation and activation of PKG which cause vessel hyperpolarization and relaxation [32]. Also, the treatment of endothelial cells or aortic vessels with vascular endothelial growth factor (VEGF) induced growth signaling and angiogenesis dependent of protein kinase A (PKA) oxidation [33].

Modulation of the redox potential of reactive thiols may be a general control mechanism by which sarcoplasmic/endoplasmic (SR/ER) reticulum and ryanodine receptor (RyR) controls cytoplasmic Ca^{2+} concentrations in the skeletal muscle [34] and myocardium [35]. Yi X et al., demonstrated in coronary artery smooth muscle that a local NADPH oxidase system on SR/ER regulates RyR/Ca^{2+} channel activity and Ca^{2+} release from SR/ER by producing O^{-}_{2} [20]. The thiol-disulfide exchange model in cardiac muscle has been proposed to describe the mechanism by which O^{-}_{2} can directly activate the RYR/Ca^{2+}. In this model, intermolecular thiol-disulfide

interexchange reaction within RyR control open or closed states of its Ca^{2+} release channels. When the thiol groups of RyR is in a reduced status (-SOH form), the channel is closed. In contrast, the channel is open when disulfide is formed by oxidation of thiol groups of RyR (-S-S-form) [36] [37] [38] [39].

In vascular smooth muscle (VSM), angiotensin II increases NADPH oxidase-dependent ROS production, which is thought to activate signaling pathways involved in the hypertrophic response [40] [41]. The redox signaling modulation of the small G proteins Ras kinases such as ERK1/2, p38MAPK, protein kinase C (PKC) and Akt contribute to the development of GPCR agonist-induced hypertrophy [31] [42].

Hydrogen peroxide can induce kinase activation via tyrosine phosphorylation or via the induction of the released zinc from zinc-finger domains of PKC [43]. It has been proposed that O^{-}_{2} and H_2O_2 may play an important signaling role in cardioprotection. Most of the signaling pathways of cardioprotection converge at the mitochondria and the mitochondrial ROS formation mediates signal transduction through post-translational modifications of redox-sensitive proteins [44] [45] [46]. Perrelli et al., demonstrated for the first time that the cardioprotective effect of catestatine as a pharmacological postconditioning (CST-Post) depends on the activation of PI3K/Akt, PKCs and mitoKATP channels, which may include a ROS signaling [23].

It may seem paradoxical that ROS are essential for promoting normal cellular processes, as opposed to having a toxic effect on the heart. Even cell death that was previously thought to result from oxidative damage is now considered to be the result of ROS triggering a physiological pathway for cell death. Maintaining a basal level of ROS which is above a cytostatic level, but below cytotoxic, therefore enables proper redox biology reactions and the regulation of numerous processes essential for life.

4. Sources of ROS and the interplay with inflammation during acute myocardial IRI

A number of different mechanisms and sources are known to underlie ROS generation in the myocardium in the setting of acute IRI. The enzymes systems most commonly implicated in ROS production are cytochrome P-450 (CYP), xanthine oxidase, NADPH oxidase, monoamine oxidases

(MAO), uncoupled nitric oxide synthase (NOS), the unfolded-protein response (UPR)-regulated oxidative protein folding machinery in the SR/ER, and the mitochondrial electron transport chain [47] (fig. 2A).

a. Cytochrome P-450

The cytochrome P-450 (CYP) family of proteins are mono-oxygenases, which catalyse the oxidation of hydrophobic organic molecules mainly in the liver, but also in the heart [48]. The CYP system is known to be a potential source of ROS following reperfusion of the acutely ischaemic myocardium, mainly from endothelial cells [49], macrophages, and neutrophils [50]. It has already been shown that ROS can arise from the decay of oxygenated CYP intermediates produced during the catalytic mechanism of mixed-function oxidation. The contents of ROS derived from cytochrome P-450 have been shown to increase in an oxygen concentration-dependent manner as CYP generates O^{-}_{2} and H_2O_2 through an uncoupling reaction. It is conceivable that the increase in ROS produced by CYP upon reperfusion are due to an increase in uncoupling, concomitant with the increment of oxygen supply to myocardium [51].

Members of the cytochrome P-450 2-epoxygenases family (CYP 2), primarily 2C8, 2C9 and 2J2, and the hydroxylase CYP 4F are capable of metabolising endogenous arachidonic acid (AA) into vasoactive products such as epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs). Although EETs have been reported to play a cardioprotective role, CYP 2C9 can also generate O^{-}_{2}, H_2O_2, and •OH during the CYP reaction cycle [52]. Using a rat Langendorff preparation, Granville et al., showed that CYP 2C9 is a potent source of ROS during acute myocardial IRI, and contributes to the extension of MI size [53]. The O^{-}_{2} and H_2O_2 produced by CYP 2C9 can trigger NFκB activation resulting in the upregulation and secretion of pro-inflammatory cytokines and adhesion molecule expression [52]. The selective CYP 2C9 inhibition with sulfaphenazole has ben reported to result in a significant reduction in MI size after 2 hours of reperfusion in a rat acute IRI model (fig. 2B) [53].

Over-expression of endothelial CYP 2C8 has been shown to increase ROS generation and leukotoxin diols formation, thereby augmenting coronary vasoconstriction and increasing MI size

[49] [53]. During acute myocardial ischaemia, AA accumulates, leading to increased generation of 20-HETE (20-hydroxy-5,8,11,14-eicosatetraenoic acid) through CYP 4F [54]. 20-HETE acts directly on cardiomyocytes via the stimulation of NADPH oxidase-derived ROS production, and induces cardiomyocyte apoptosis. The treatment of endothelial cells with endogenous 20-HETE leads to an increase in NFκB activity and endothelial activation, characterised by the increased expression of intracellular adhesion molecules and interleukin-8 (IL-8) levels [55] [56] [57]. Inhibition of ROS production during acute IRI may be more beneficial than a free radical scavenger because such anti-oxidants must compete with cellular targets to protect tissue from ongoing ROS production. For example, the administration of cimetidine upon reperfusion has been demonstrated to reduce MI size, prevent cardiac dysfunction, and attenuate ROS production in the ischaemic region [58]. The inhibition of 20-HETE with HET0016 (N-hydroxy-N'-(4-butyl-2-methylphenyl)-formamidine) prevents the activation of inflammatory genes and the endothelial dysfunction [56] [55]. The selective hydroxylase inhibition with N-methylsulfonyl-12, 12-dibromo-11-enamide (DDMS) 10 minutes before coronary artery occlusion or 5 minutes before reperfusion was found to reduce MI size [59] [60]. These data suggest that the inhibition of CYP hydroxylases may induce cardioprotection. However, further studies are warranted to determine whether pharmacological interventions that disrupt CYP 2C and CYP 4F signalling prevent the development of inflammation associated with acute myocardial IRI.

b. Xanthine oxidase

Xanthine oxidoreductase catalyses the oxidation of hypoxanthine to xanthine and the latter to uric acid as the final steps of purine degradation [61]. Xanthine oxidoreductase has the peculiar property of existing in two interconvertible forms, xanthine oxidase (XO) and xanthine dehydrogenase (XDH). XO is formed from XDH under ischaemic conditions and upon myocardial reperfusion [62]. It can react with purine substrates (hypoxanthine or xanthine) and O_2 as the terminal electron acceptor, thereby exhibiting the ability to generate O^{-}_{2} and H_2O_2 [63]. The $\cdot OH$ and O^{-}_{2} radicals produced by the enzyme can, in turn, react with cellular proteins and membranes causing cellular injury. XO is present predominantly in the vascular endothelium in the healthy heart, and has been implicated as

a primary source of cytotoxic ROS. This is largely based on the observation that allopurinol, an inhibitor of xanthine oxidoreductase, is as effective as an oxygen radical scavenger in attenuating the tissue injury associated with acute IRI [64]. Allopurinol has been shown to decrease MI size and improved the recovery of LV function following acute IRI [65]. Oxypurinol was found to increase cardiac output and improve regional LV function after sustained coronary artery occlusion in the canine heart [66]. Pre-treatment with the XO inhibitor, allopurinol, is effective in inhibiting generation of ROS during reperfusion and improving recovery of LV function [67].

XO has also been implicated in the leukocyte recruitment that occurs during reperfusion. Leukocyte-endothelial cell adhesion in post-ischaemic models, and increased neutrophil adhesion after hypoxia have been reported to be significantly attenuated by XO inhibitors [68]. However, one problem inherent to the use of allopurinol is that its therapeutic effect is not dose dependent: at higher doses, it becomes the substrate for XO, which will in turn produce O^{-}_{2} and thus exacerbate myocardial damage. ROS generated by XO promote the formation of pro-inflammatory stimuli, modify the expression of adhesion molecules on the surface of leukocytes and endothelial cells, and reduce levels of the potent anti-adhesive agent nitric oxide. This latter effect is exacerbated by the decline in nitric oxide synthase (NOS) activity and oxidation of soluble guanylyl cyclase during reperfusion, which serves to amplify the intense inflammatory response [69]. Based on these observations it has been proposed that XO plays an important role in mediating the reperfusion injury response by promoting the recruitment and activation of leukocytes [67], [70].

c. NADPH oxidases

The NADPH oxidases (NOX) family comprises seven members, five NOX and two dual oxidases (Duox-1 and Duox-2) [71], [72]. They contain six or seven transmembrane spanning domains, respectively. NADPH oxidase catalyses electron transport from NADPH to molecular oxygen, thereby producing ROS [73]. Among these isoforms, NOX3 is highly expressed in the cochlea [74]; NOX1 is expressed in endothelial cells, VSMC and adventitial fibroblasts [75]. NOX2 and NOX 4 are abundantly expressed in cardiomyocytes [72]. NOX5 is located in vascular endothelial cells

[76], and vascular smooth muscle cells [72], [77] and Duox-1 and Duox-2 are predominantly expressed in epithelial cells [78].

The proposition that NOX enzymes contribute to acute myocardial IRI is based on two experimental strands of evidence: (1) the increased expression and activity of NOX in the post-ischaemic myocardium and (2) the attenuation of ROS following pharmacologic inhibition of NOX. Meischl et al. demonstrated that NOX2 is the predominant isoform that is expressed in cardiomyocytes, and its expression is upregulated in response to acute IRI [79]. The use of apocynin and diphenylene iodonium (DPI) (non-specific NOX inhibitors) has been found to reduce the increase in lipid peroxidation, cell death, and apoptosis after simulated IRI in cardiac cells [80].

NOX can also indirectly cause damage by enhancing the inflammatory response. Neutrophils that express NOX2 are the primary source of ROS in acute IRI [81], [82]. Some studies have shown that a phagocyte-like NADPH oxidase is the primary source of O^{-}_{2} in vascular tissue [83]. The potential involvement of neutrophils is supported by the observation that the time course of the inflammatory cell accumulation corresponds with ROS generation and the MI size following acute IRI. The activation of NOX in neutrophils is triggered via PKC-mediated phosphorylation of cytosolic p47phox (for neutrophil cytosolic factor 1), which then binds to membrane-associated gp91phox [84]. ROS generated by NADPH oxidase promote the formation of proinflammatory stimuli, modify the expression of adhesion molecules on the surface of leukocytes and endothelial cells, and reduce levels of the potent anti-adhesive agent nitric oxide. Coincident with these changes, perivascular cells become activated and release another inflammatory mediators such as tumor necrosis factor alpha (TNF-α) and cytokines [85], [86]. The regulation of different cytokines in various organs suggest a cell-specific or organ-specific effect of NOX2. However, with respect to other NOX isoforms, no solid data on their involvement in inflammation and chemotaxis after reperfusion are available.

d. Monoamine oxidases

Monoamine oxidases (MAOs) are flavoenzymes located within the mitochondrial outer membrane, responsible for the oxidative deamination of neurotransmitters and dietary amines [87], [88].

Monoamine oxidase A (MAO-A) and B (MAO-B) share 70% amino acid identity, and both contain a covalently bound FAD cofactor attached to an enzyme cysteine via the 8α-methylene of the isoalloxazine ring [89]. This flavin moiety is the only redox-dependent factor necessary for their activity. The reaction of oxidative deamination occurs in several steps, ultimately resulting in the formation of the aldehyde from the corresponding amine, ammonia and H_2O_2. MAOs catalyse oxidative deamination of several monoamines (serotonin [5-hydroxytryptamine (5-HT)], noradrenaline, dopamine), resulting in significant ROS production [88]. Recent studies suggest that MAOs contribute to increasing H_2O_2 production and catecholamine release in the early reperfusion period (5-15 minutes) [90]. MAO-A generated H_2O_2 in acute IRI induces sphingosine kinase inhibition, ceramide accumulation, and sphingosine-1-phosphate degradation in cardiomyocytes thereby leading to mitochondria-mediated apoptosis in H9c2 cells [91]. Currently, efforts are underway to investigate the mechanisms underlying the protective effect of MAO inhibitors (selegiline, D-Deprenyl), and the roles of MAO in the setting of acute IRI [92], [93].

e. Mitochondrial electron transport chain

Mitochondria have been implicated as a major source of ROS in acute myocardial IRI. The rapid movement of electrons through the electron transport chain (ETC) of the inner mitochondrial membrane can result in the leakage of electrons, which form O^{-}_2 via univalent reduction of O_2. All of the ETC complexes have been implicated as both sources and targets of the ROS generated during myocardial IRI, although most evidence supports a role for complexes I and III.

Mitochondrial complex I is viewed as a major contributor of ROS [11]. Oxidative impairment of complex I is detected in rat models of acute myocardial IRI [94]. Mitochondrial complex I has two catalytically and structurally distinct forms; one the fully competent, active A-form and the other, the deactivated, D-form. The reversible D-form of complex I predominates under ischaemic conditions, produces O^{-}_2 and H_2O_2, and may potentially increase the susceptibility of mitochondria to oxidative damage [95]. Reperfusion also induces disruption of complex II; Chen et al. found that ADP-stimulated state 3 respiration driven by succinate was 50% impaired in mitochondria from reperfused hearts, a finding which was attributed to the impairment of complex II. The

deglutathionylation of complex II predisposes the 70-kDa flavin binding subunit to oxidative stress induced by ROS during reperfusion injury [96].

Complex III is also considered an important source for mitochondrial ROS production in reperfused hearts. In the ischaemic heart, mitochondrial complex activity is reduced by 22% compared with healthy hearts. Increase unstable semiquinone radical ($\bullet Q^{-}$), is attributed to be the source of O^{-}_{2}. Mammalian complex III contains bound cardiolipin molecules that are essential for the catalytic function. The impairment of complex III activity due to the ROS-induced cardiolipin oxidative damage may increase the electron leak from the electron transport chain, generating more O^{-}_{2} and perpetuating a cycle of oxygen radical-induced damage, which ultimately leads to an increase in MI size [97]. The burst of ROS from mitochondrial complexes induce the oxidation of cholesterol and the production of oxysterols. Oxyesterols can induce interleukin-1 beta (IL-1β) secretion in vascular endothelial cells and, consequently, the expression of adhesion molecules necessary for the recruitment of immune cells [98]. Additionally, CYPs have been found in the mitochondria of diverse animal species. Mitochondrial CYPs are proteins bound to the inner membrane, and receive electrons for monooxygenation reaction from NADPH via adrenodoxin and NADPH-adrenodoxin reductase [99]. Mitochondrial CYP catalyses the conversion of cholesterol in pregnenolone and play essential roles in cholesterol homeostasis and steroid hormone biosynthesis [100]. Recently, it has been shown that myocardial reperfusion induces mitochondrial cholesterol accumulation. Peradis et al. showed that acute myocardial IRI produces high cholesterol and oxysterol concentrations in the matrix and a simultaneous decrease in mitochondrial membrane fluidity related to oxidative stress [101]. In this setting, the oxysterols 5, 6-epoxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol and 25-hydroxycholesterol exert a potent cytotoxic effect by their ability to induce inflammatory effects [102].

Liu et al. have demonstrated that the oxysterol, 25-hydroxycholesterol, enhances IL-8 production [103]. It is noteworthy to mention that IL-8 is a cytokine which might play an important role in the recruitment of T lymphocytes and monocytes into the arterial subendothelial space [104]. As summarised in figure 2C, by inhibiting cholesterol uptake into mitochondria at reperfusion with 4'-chlorodiazepam, the accumulation of oxysterols can decrease the inflammatory response and

induce cardioprotection. Further investigation is required to explore in more detail the relationship between oxysterols and inflammation in the setting of acute IRI (fig. 2C).

f. UPR-regulated oxidative protein folding machinery in the SR/ER

The cardiomyocyte sarco/endoplasmic reticulum (SR/ER) is an intracellular organelle specialising in the regulation of Ca^{2+} fluxes and different oxidative functions. There is increasing evidence that SR/ER stress plays a crucial role in IRI-induced cell dysfunction. Oxygen starvation during ischaemia and ROS and Ca^{2+} overload during reperfusion results in SR/ER stress and the activation of the pro-inflammatory pathway.

Mitochondria and SR/ER are in close apposition and the interface, commonly known as the mitochondrial-associated SR/ER membrane, is believed to act as the focal point for signaling [105]. During acute myocardial IRI, both the release and uptake of calcium from the SR/ER are dysregulated, resulting in enhanced Ca^{2+} release [46]. Much of the calcium is taken up by the mitochondria and Ca^{2+} within the mitochondria, and induces the superoxide formation. Several *in vitro* studies have demonstrated that the calcium pump on the SR/ER membrane is quite sensitive to oxidative stress and the fact that the SR/ER contains a large amount of lipids and that it produces ROS could also make this organelle very easily damaged by ROS. This vicious cycle of Ca^{2+} leakage, calcium overload and ROS generation inhibits cardiac contractility.

SR/ER stress initiates the activation of the unfolded protein response (UPR). The UPR increases the capacity of the protein folding machinery resulting in the production of more oxidative equivalents, and future deteriorating the redox state. UPR can induce TNF-α production in response to ER stress through IRE1α (inositol-requiring transmembrane kinase and endonuclease 1α) and the ER-localised protein kinase PERK pathway [106] [107]. PERK-induced translational arrest leads to the loss of IκB, thereby activating NFκB [108]. In addition, the phosphorylation of IRE1α in response to stress induces a conformational change in its cytosolic domain, which can then bind to the adaptor protein, TNF-α-receptor-associated factor 2 (TRAF2), the receptor that can activate canonical NFκB JNK MAPK signaling pathway [109]. The efflux of Ca^{2+} from the SR/ER generates ROS and NFκB activation and gene expression that drive inflammation [110] [111]. NFκB

induction by SR/ER stress is prevented by pre-incubation of cells with intracellular Ca^{2+} chelators, suggesting that Ca^{2+} release precedes ROS formation in the NFκB-mediated SR/ER-nuclear signal transduction pathway [112]. Several studies have demonstrated that hypoxia/reoxygenation is sufficient to induce SR/ER stress [113] [114]. NFκB acts as a link between SR/ER stress and inflammation after hypoxia/reoxygenation. NFκB inhibitors can protect cells against IRI by selectively inhibiting the translocation of NFκB to the nucleus. In this regard, Wu et al. have shown that SN50 can effectively can reduce damage to cardiomyocyte after reoxygenation [107].

g. Nitric oxide synthase

Neuronal nitric oxide synthase (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) generate NO• via the oxidation of L-arginine. NOS isoforms contain both an oxygenase and reductase domain. The reductase domain contains flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and binds NADPH, while the oxygenase domain contains heme and tetrahydrobiopterin (BH4), and binds arginine. Uncoupling of NOS results in the loss of NO• production, and O^{-}_{2} production. A recent study by Lin et al. demonstrated that phosphorylation of eNOS at threonine 497 mediated the switch between NO• production to superoxide generation [115].

However, the most prominent cause of NOS uncoupling is the loss of the critical NOS co-factor, BH4, either by oxidation or decreased expression of the recycling enzyme dihydrofolate reductase (DHFR) [116]. BH4 depletion is involved in both endothelial and cardiomyocyte dysfunction in hearts following acute IRI. Myocardial levels of BH4 levels have been shown to be markedly decreased after 60 minutes of reperfusion, and NOS uncoupling occurs with the increase in myocardial O^{-}_{2} formation. When electron flow is uncoupled from arginine oxidation, the reduced O^{-}_{2} is released from the heme as O^{-}_{2}.

Endothelial NOS is mostly expressed in endothelial cells, cardiomyocytes and platelets. eNOS synthesises NO• in a pulsatile manner with eNOS activity markedly increasing when intracellular Ca^{2+} is increased [117]. Recent evidence has indicated that reversing NOS uncoupling in acute myocardial IRI may be a therapeutic strategy [118]. Also, several studies have demonstrated that

low levels of heart BH4 result in myocardial inflammation. Zsuzsnna et al. demonstrated that plasma BH4, TNFα- and IL-6 levels showed an inverse correlation with the absolute values of LV function, suggesting that oxidative stress and inflammation may be responsible for LV systolic dysfunction in IRI [119]. However, further studies are warranted to determine whether NOS uncoupling induces inflammation associated with acute myocardial IRI.

5. Dysregulation of myocardial anti-oxidant pathways during acute myocardial IRI

Myocardial reperfusion increases the production of ROS and undermines the anti-oxidant defence in heart tissue, cause a redox imbalance. Myocardial anti-oxidants can be divided into the endogenous anti-oxidant system and exogenous anti-oxidants. The first line of endogenous anti-oxidants include anti-oxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase, and non-enzymatic anti-oxidants including ∝-tocopherol (vitamin E), ubiqinol or coenzyme Q_{10} (Q_{10}), ascorbic acid (vitamin C) and glutathione (GSH) amongst others [120], [121]. In the setting of acute IRI, levels of myocardial non-enzymatic anti-oxidants are suppressed. Total myocardium ascorbate, Q10, and glutathione levels decline as a function of the length of reperfusion period, and the administration of exogenous anti-oxidants can mediate cardioprotection [122].

The presence of a higher glutathione peroxidase (GPx) activity is vital for the heart to survive the attack of ROS produced in the reperfused myocardium. GPx catalyses the peroxidation of H_2O_2 in the presence of reduced glutathione (GSH) to form H_2O and oxidised glutathione (GSSG). Cardiomyocytes contain a GSH redox cycle, in which GPx reaction accepts peroxides and peroxide-derived alkoxyl and peroxyl radicals as substrates. Glutathione is a tripeptide, γ-L-glutamyl-L-cysteinylglycine, present in the heart at 1-10mM concentrations [123]. GSH reductase replenishes the loss of GSH using NADPH as a donor for reducing equivalents, however, the oxidative stress during reperfusion results in the depletion of myocardial GSH and NADPH and efflux of GSSG [124], [125]. Yoshida et, al, have demonstrated that the GPx knockout (KO) mouse hearts are more susceptible to acute IRI [126].

445 The transcription factor Nrf2 is a master regulator of a spectrum of genes related to GSH
446 metabolism via the anti-oxidant responsive element (ARE) on target genes, and also plays a role in
447 xenobiotic detoxification and proteome maintenance [127], [128]. In response to oxidative stress,
448 Nrf2 dissociates from the inhibitory regulator Keap1, and translocates to the nucleus to induce the
449 transcription of anti-oxidant genes GSH synthetase, glutathione-S-transferase, GSH peroxidase,
450 GHS reductase and NADPH quinone oxidoreductase [127], [129]. The stimulation of Nrf2 is
451 connected with activation of the PI3K/Akt kinase pathway which was shown to play a role in the
452 mechanism of increased myocardial tolerance to acute IRI and reduction of oxidative stress [129] ,
453 [130].
454 Oxidative stress depolarises mitochondria by causing lipid peroxidation, which further leads
455 to mitochondrial dysfunction. Q_{10} is a well-characterized electron carrier of the respiratory chain,
456 which is mainly localised in the inner mitochondrial membrane where it serves as a highly mobile
457 carrier of electrons and protons between the flavoproteins and the cytochrome system [131].
458 Because of its ability to transfer electrons, it acts as an anti-oxidant. Q_{10} must be reduced to
459 ubiquinol denoted quinol (QH2) to yield its maximum anti-oxidative function. In its reduced form
460 (ubiquinol), the Q_{10} molecule holds electrons loosely and will quite easily give up one or two
461 electrons to neutralise free radicals [131], [132]. The anti-oxidant properties of Q_{10} and its locatoin
462 within the mitochondria make it an potential therapeutic target for the treatment of acute IRI [133]. In
463 conditions of high oxidative stress, the rate of inactivation of NO• to peroxynitrite by superoxide
464 anions may be reduced by Q_{10}, and reduce the products of lipid peroxidation levels [134].
465 Coenzyme Q_{10} also decreases blood viscosity, improves coronary vasodilation by protecting the
466 endothelial function in patients with ischaemic heart disease [135], [136]. It decreases inflammatory
467 cytokines and prevents the hyperglycemia-induced endothelial cell damage, monocyte adhesion
468 and evolution of atherosclerotic lesions in diabetic patients [137].
469 SOD is an enzyme that converts superoxide anion to hydrogen peroxide, which is
470 subsequently converted to water by catalase [138]. It protects against oxidative stress and has
471 three isoforms: Cu-Zn SOD (SOD1), located in the cytosol; Mn-SOD (SOD2), located in the
472 mitochondrial matrix; and extracellular SOD (SOD3) [139]. Anti-oxidant enzymes have specific

targets, and they function as a sensor of specific types of ROS. For instance catalase and peroxiredoxins target H_2O_2 whereas SODs only target superoxide. Inadequate delivery of anti-oxidants to their target sites within the cell where ROS are produced may be the cause of the controversial results obtained so far because these enzymes can detoxify ROS only at the sites to which they are delivered.

Mammals have seven sirtuins (SIRT1-7) that possess NAD^+-dependent deacetylase, deacetylase, and ADP-ribosyltransferase activities. Sirtuins are found in different subcellular locations, including the nucleus (SIRT1, SIRT6, and SIRT7), cytosol (SIRT2), and mitochondria (SIRT3, SIRT4, SIRT5) [140]. SIRT3 deacetylates several lysine residues of MnSOD thereby increasing MnSOD activity and detoxification of superoxide radicals. The dependence of SIRT3 on the NAD^+/NAD ratio may determine the function of SIRT3 [141]. During myocardial reperfusion, mitochondrial NAD^+ levels decrease [142], suggesting that SIRT3 activity may be compromised during reperfusion and may contribute to the extent of acute IRI. In the Langendorff model, seven months old *SIRT3*$^{+/-}$ mice showed impaired recovery of cardiac function and larger MI size following 25 minutes of ischaemia [143]. SIRT6 reduced oxidative stress injury via an AMPK-dependent pathway. Under normal nutrients conditions, SIRT6 binds to the promoters of glycolytic genes, keeps histone H3K9 acetylation levels low, and directs glucose into the mitochondria for efficient ATP production and away for glycolysis. SIRT6 deficiency also significantly reduced both the expression and activity of SOD and catalase in ischaemic hearts. *SIRT6*$^{-/-}$ mice showed more severe acute myocardial IRI resulted from the collapse of the endogenous ROS-scavenging enzyme system, which induces ROS accumulation and stronger oxidative stress [144]. Oxidative stress activates FOXOs in cardiomyocytes mediated by AMPK and sirtuins (SIRT1 and SIRT2). SIRT1 protects the heart from acute IRI through upregulation of anti-oxidants and downregulation of proapoptotic molecules. FOXO promotes cardiomyocytes survival upon induction by oxidative stress. SIRT1 enhances transcription factor of some FOXO target genes. Cells lacking Sirt3 exhibited altered metabolism, including a significant increase in mitochondrial superoxide levels when exposed to cellular stress [140].

6. The acute-phase response: tissue damage, inflammatory response, and more ROS

Myocardial reperfusion is associated with an inflammatory response, which ultimately leads to healing and scar formation. Acute myocardial IRI involves degradation of extracellular matrix components by metalloproteinases (MMPs), oxidative stress, apoptosis and activation of complement system. The inflammatory response in the reperfused myocardium is related to the coordinated activation of a series of cytokine and adhesion molecule genes resulting in loss of barrier integrity and release of ROS into the extracellular matrix. It increases expression of adhesion molecules; acts as a chemoattractant for neutrophils, initiating their recruitment; activates the complement cascade and promotes apoptotic cell death.

The production of ROS peaks during the first 2–10 minutes of reperfusion after coronary artery occlusion and has been considered to be the first stimuli from neutrophils that invade the ischaemic region [50]. Necrotic cell death triggers release of cell contents with some of the endogenous compounds being able to activate immune cells. NF-κB is activated by various local substances including ROS [145], [146]. Upon activation, NF-κB stimulates inflammatory and immune responses. This factor also triggers gene expression of pro-inflammatory cytokines, such as TNF-α and interleukins, initiating an inflammatory response [147]. Upregulation of chemokines and cytokines results in extravasation of activated blood-derived cells into the infarcted area. Platelets are the first cells recruited to the site of infarct area, as a result of the coagulation process. Subsequently, various subsets of leukocytes infiltrate the myocardium and remove the dead cells and matrix debris [148]. During the adhesion process, the activated platelets release adhesion proteins (fibrinogen, fibronectin, P-selectin, glycoprotein IIb/IIIa), growth factors (PDGF), endothelial growth factor, fibroblast growth factor, chemokines, epithelial neutrophil-activating, cytokine-like factors (interleukins) and coagulation factors into the local environment, thereby altering chemotactic, adhesive and proteolytic properties of endothelial cells and supporting chemotaxis adhesion and transmigration of monocytes to the site of inflammation [149], [150]. Concomitantly, intercellular tight junctions are compromised, which leads to endothelial barrier dysfunction and increased vascular permeability. Also, platelets are capable of initiating complement activation and may play a role in localising the inflammatory response to the area of injury [151].

Neutrophils arrive on the scene very early after the tissue damage (4 hours after reperfusion) [152]. Their principal role appears to be mediated by adhesive interactions with activated endothelial cells of the vessels. Neutrophil infiltration into the infarcted area implies the generation of ROS and proteolytic enzymes contributing to the clearance of dead cells and debris from the infarcted area [153]. Also, they may express mediators capable of amplifying cell recruitment. Experiments have suggested that the mechanism of neutrophil-cardiomyocyte adhesion is dependent on CD18 integrin activation on neutrophils and expression of ICAM-1, one of the primary ligands for the CD18 integrins. Neutrophils block capillaries preventing reperfusion of the tissue, which leads to tissue necrosis and an exacerbated immune response. In vitro the mechanism of neutrophil–cardiomyocyte injury was shown to be strictly dependent on CD18 integrin activation and ICAM-1 expression by damaged cardiac cells. A neutrophil NADPH oxidase inhibitor and a monoclonal antibody against the neutrophil CD18 adhesion molecule markedly reduced oxygen radical levels, in addition to reducing MI size and no-reflow [81].

7. Physiological consequences of the inflammatory response

7.1. No-reflow

No-reflow (NR) or microvascular obstruction is the term used to describe the inadequate perfusion of a given coronary segment without angiographic evidence of epicardial vessel obstruction. Between 2 minutes and 8 hours of reperfusion, the area of NR increases 3-fold with most of the expansion occurring within the first 1–2 h of reperfusion [154]. The factors associated with the establishment of NR include endothelial dysfunction, compression of capillaries by swollen myocytes, alteration of the vasoregulation pathways, epicardial spasm, mechanical obstruction from embolization, extrinsic coagulation pathways, leukocyte adherence, microvascular ischaemia, oedema and vasoconstriction mediators [155], [156]. Endothelial cell injury occurs in approximately 20% of vessels after 60 minutes of reperfusion, and in 40% of vessels at 20–80 minutes of reperfusion. Indeed, initial reports showed tightly packed erythrocytes and endothelial gaps plugged by platelet and fibrin thrombi with many extravascular red blood cells in capillaries from hearts reperfused only during 20 minutes [157]. Platelet aggregates or fibrin clots could be implicated as

557 factors responsible for obstructing capillaries. Besides mechanical obstruction, leukocytes release a
558 variety of pro-inflammatory cytokines that may contribute to NR by recruiting additional inflammatory
559 cells enhancing leukocyte adhesion to the endothelium, altering coagulation or increasing
560 vasoconstriction (Figure 3).
561
562 ***7.2. Post-MI left ventricular remodeling***
563 The inflammatory reaction following AMI controbutes to cardiac structural remodelling, followed by
564 scar formation at the site of infarction as well as changes in the non-infarcted myocardium, including
565 interstitial fibrosis and vascular remodelling. The term remodelling was proposed to characterize the
566 response of remote myocardium to regional infarction and the progression from acute myocardial
567 infarction to chronic heart failure.
568 There is growing recognition and experimental evidence that oxidative stress-mediated and
569 inflammation regulate the pathogenesis of myocardial remodelling following AMI. Circulating
570 neutrophils and macrophages arrive at the infarct site after reperfusion. They contribute to the
571 proteolytic digestion and phagocytosis of the infarcted tissue, respectively. The inflammatory
572 response peaks at weeks 1 and 2 post-MI. Collagen synthesis, preferentially mediated by
573 myofibroblasts, is induced in response to different stimuli; these include mechanical stress,
574 vasoactive factors such as angiotensin II and growth factors such as transforming growth factor-β
575 (TGF-β), which can act directly or through the up-regulation of connective tissue growth factor
576 (CTGF). The fibrogenic component, which substitutes for lost parenchymal cells, follows the initial
577 phase of collagen degradation. Collagen degradation is mediated by a family of zinc-containing
578 endoproteinases- matrix metalloproteinases. These enzymes are found in the heart at low levels in
579 normal conditions but can be up-regulated after MI in response to inflammatory cytokines and TGF-
580 β [158], [159].
581
582 **7. Therapeutic targeting of ROS and inflammation**
583 It is not surprising that research over recent years has focused on anti-oxidants as a potential
584 therapy in the setting of acute myocardial IRI [160]. Although many initial studies in cells or animal

585 models have been successful, clinical trials have produced disappointing results, owing to
586 differences between animal models and human disease, the inability of the agents to reach the
587 important cellular locations, and the stage and cell-specific regulation of oxidant and anti-oxidant
588 pathways.

589 According to previously discussed data, it may be preferable to adopt a pharmacological
590 strategy that decreases mitochondrial oxidative damage to reduce acute IRI. The relatively poor
591 efficacy of conventional anti-oxidants may be the consequence of their low penetrance to the
592 mitochondrial matrix, which not only is the main site of ROS production but also suffers from
593 oxidative stress. Pretreatment with or infusion of Q_{10} soon after coronary artery ligation has been
594 shown to reduce MI size and preserve systolic function in rat models of AMI [161]. Q_{10} prevents the
595 peroxidation of the cell membrane and subcellular lipids, which occurs during acute IRI [162]. Q_{10}
596 also regulates the release of nitric oxide, and it creates endothelial regeneration and
597 immunostimulation [163], [164] and recovery of LV function after AMI [165]. However, lipophilic
598 cations have a disadvantage. Since the charge accumulation into the matrix leads to mitochondrial
599 membrane depolarisation, at the concentration greater than 10mM, toxicity has been observed, and
600 the low solubility of ubiquinone in water makes it difficult to use *in vitro*, and animals must be fed
601 Q_{10}-enriched diets for several weeks to increase levels in subsequently isolated mitochondria. More
602 hydrosoluble molecules than ubiquinone have been developed and tested in different diseases.
603 Therefore, to manipulate mitochondrial Q_{10} or ubiquinone Kelso et al. synthesised a ubiquinone
604 analogue selectively targeted to mitochondria by the addition of a lipophilic triphenylphosphonium
605 cation, mitoquinone (MitoQ) [166]. The lead compound, MitoQ, consists of a targeting lipophilic
606 triphenyl phosphonium (TPP) cation linked to a ubiquinone moiety. Using this model, it was
607 demonstrated that the administration of MitoQ before the onset of ischaemia reduced oxidative
608 damage and severity of acute IRI, thereby providing functional protection to the heart [167].
609 Myocardial treatment with MitoQ prevented the initial damage and the activation of the inflammatory
610 response. MitoQ also can reduce Ca^{2+} overload and mPTP opening [168].These findings suggest
611 that mitochondria-targeted therapies designed to minimise mitochondrial oxidative damage may
612 decrease post-reperfusion dysfunction.

A growing number of studies suggest that the compound, Szeto-Schiller-31 (SS-31) peptide, also known as Bendavia may be cardioprotective. SS-peptides were developed by Szeto and Schiller and constitute a series of 4 small, cell-permeable anti-oxidant compounds with three positive charges in homeostatic pH conditions [169]. The SS-31 peptide can scavenge H_2O_2 and ONOO• and inhibit lipid peroxidation, anti-oxidant actions attributed to a tyrosine or dimethyl tyrosine residue in their structure, the latter of the two being more efficient concerning ROS scavenging. In a recent study, Liu et al. showed that SS-31 prevented swelling of mitochondria and protected mitochondrial cristae in both endothelial and epithelial cells. It was associated with a significantly reduced loss of peritubular capillaries and cortical arterioles, interstitial inflammation, and fibrosis four weeks after ischaemia [170]. However, the EMBRACE-MI clinicla study failed to demonstrate a reduction in MI size in AMI patients administered Bendavia prior to reperfusion [171].

Another strategy to preserve redox balance and maintain mitochondrial function is the induction of endogenous anti-oxidants Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid) is a water-soluble analogue of the free radical scavenger α-tocopherol. Due to its enhanced water solubility, Trolox may function more rapidly during acute oxidative stress, while α-tocopherol requires several days of pretreatment to exhibit anti-oxidant benefits. Du *et al.* demonstrated that chitosan nanoparticles, when used as drug carriers for the delivery of Trolox, exerted a protective effect against hypoxia-mediated oxidative stress and can block the mitochondria-dependent apoptotic pathway through upregulation of Bcl-2 expression and inhibition of Bax activation and Caspase-3 expression [172].

Recently, the use of natural molecules with specific physicochemical properties has emerged. Vitamin E, C, A and other agents in complementary and alternative medicine have been studied and whereas some had protective effects in animal models, although none of them has demonstrated clear benefit for patients. Curcumin is the major active component of turmeric, a yellow compound isolated from the plant Curcuma longa, used for centuries in traditional medicine [173]. This molecule has shown therapeutic potential against a wide range of diseases, mainly due to its anti-inflammatory [174]. Curcumin exerts both direct and indirect anti-oxidant effects by scavenging ROS. Also, it has been shown that early treatment with curcumin attenuates cardiac

hypertrophy and remodelling. Curcumin might have therapeutic potential in the treatment of heart disease by attenuating oxidative stress-related events as cardiac remodelling, mitochondrial dysfunction and cell death [175].

The phosphorylated form of GSK-3 correlates with the activity of cardioprotection. Via inhibition of GSK-3, protective signalling pathways act on the end effector mitocohndrial permeability transition pore; that is, they prevent the induction of the mitochondrial permeability transition, restore mitochondrial membrane potential, and decrease ROS production. The synthetic 17β-aminoestrogen Prolame [17β-(3-hydroxy-1-propylamino)- 1,3,5(10)-estratrien-3-ol)] is an estradiol analogue in which the C17 position of the steroid nucleus is substituted by an amino-alcohol side chain-NH-(CH2)3-OH with three methylenes groups. Prolame might diminish the no-reflow phenomenon and provide cardioprotection in rats with AMI followed by reperfusion [156].

Initial success has been established in preclinical models of acute myocardial IRI for a handful of therapeutics that target neutrophils [176] [177]. A monoclonal antibody targeted against the CD11/CD18 integrin showed promising results in animal models, but clinical trials failed to show a significant reduction in MI size [178].

CONCLUSION

Following AMI, the production of ROS and the ensuing inflammatory response are critical determinants of myocardial injury, cardiomyocyte death and subsequent LV remodelling in the setting of acute IRI, providing therapeutic targets for cardioprotection. However, a number of anti-oxidants have been tested in the setting of AMI, and despite being positive in the experimental setting, most have failed in the clinical setting. The reasons for this are unclear, but may relate to the inability to achieve sufficent concentrations of anti-oxidant at the site of ROS production. Proteomic and metabolomic approaches may help in discover novel pathways underliyng the redox-mediated inflammation progression. Overcoming these issues would greatly enhance the development of successful therapies to combat oxidative stress, inflammation and cell damage, providing new treatments for AMI patients.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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Figure 1. Formation of different ROS and reactive nitrogen species from dioxygen. ischaemia/reperfusion injury. Dioxygen (O_2) is shown to undergo reduction to form superoxide (O^{-}_2). Superoxide is shown to dismutate to form hydrogen peroxide (H_2O_2), and hydrogen peroxide is shown to interact with Fe^{2+} and to form hydroxyl radical (•OH) via the Fenton reaction. Superoxide dismutase (SOD), nitrogen monoxide (NO•), peroxynitrite (ONOO•).

Figure 2. Schematic representation of (A) the sources of reactive oxygen species during acute myocardial ischaemia/reperfusion injury. (B) During acute myocardial ischaemia, AA accumulates, leading increased generation of 20-HETE through CYP 4F. 20-HETE acts directly via the stimulation of NADPH oxidase-derived ROS production and induces NFκB activation. (C) Myocardial reperfusion generates accumulation of cholesterol into mitochondria. This induces the formation of oxysterols, which can induce IL-1β and IL-8 secretion.
Cytochrome P-450 (CYP), xanthine oxidase, NADPH oxidase, monoamine oxidases (MAO), sarco/endoplasmic reticulumun (SR/ES), nitric oxide synthase (NOS), arachidonic acid (AA), 20-HETE (20-hydroxy-5,8,11,14-eicosatetraenoic acid), EETs (epoxyeicosatrienoic acids), NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), N-methylsulfonyl-12, 12-dibromo-11-enamide (DDMS), HET0016 (N-hydroxy-N'-(4-butyl-2-methylphenyl)-formamidine), cholesterol (chol), IL-1β (interleukin-1 beta), IL-8 (interleukin-8), IRI (Ischaemia/Reperfusion Injury), ROS (reactive oxygen species).

Figure 3. No-reflow following acute myocardial ischaemia/reperfusion injury. (A) Example of infact assessment using triphenyl tetrazolium chloride (TTC) staining (B) Example of no-reflow by transillumination of Microfil-perfused coronary; and (C) Schematic diagram demonstrating the multiple etiologies of no-reflow in the reperfused coroanry artery.
ROS (reactive oxygen species).

Figure 1

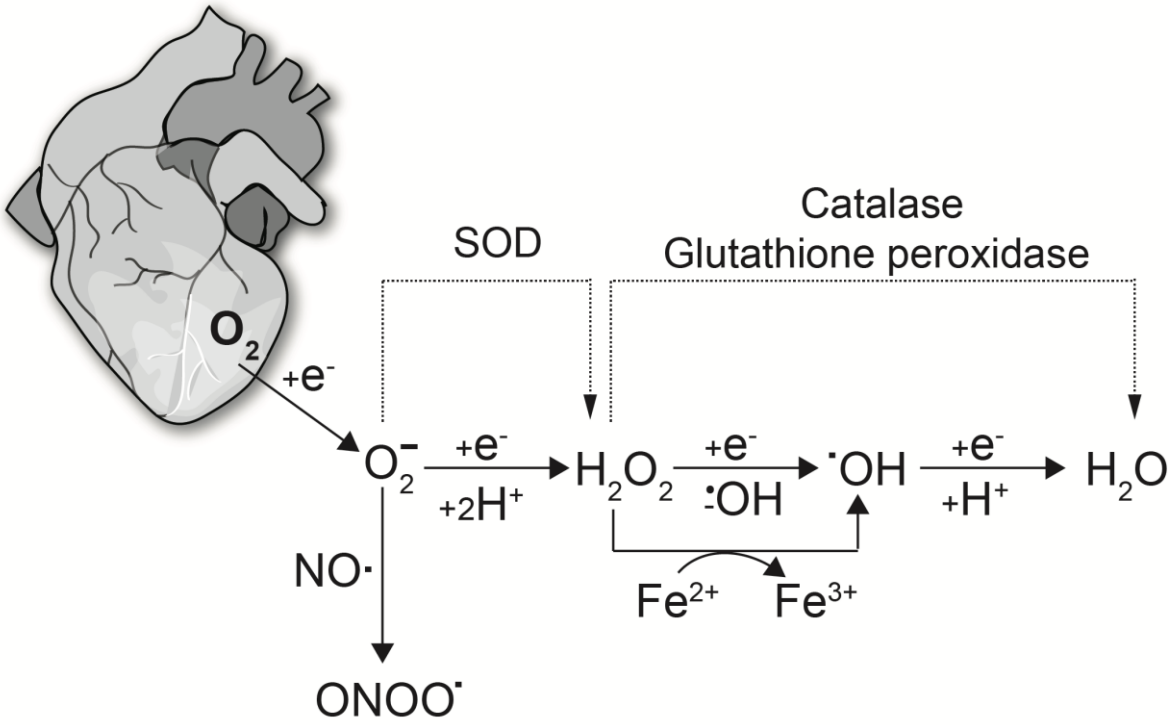

SOD
Catalase
Glutathione peroxidase
O2
+e-
O2-
+e-
+2H+
H2O2
+e-
·OH
+e-
+H+
H2O
NO·
ONOO·
Fe2+
Fe3+

Figure 2

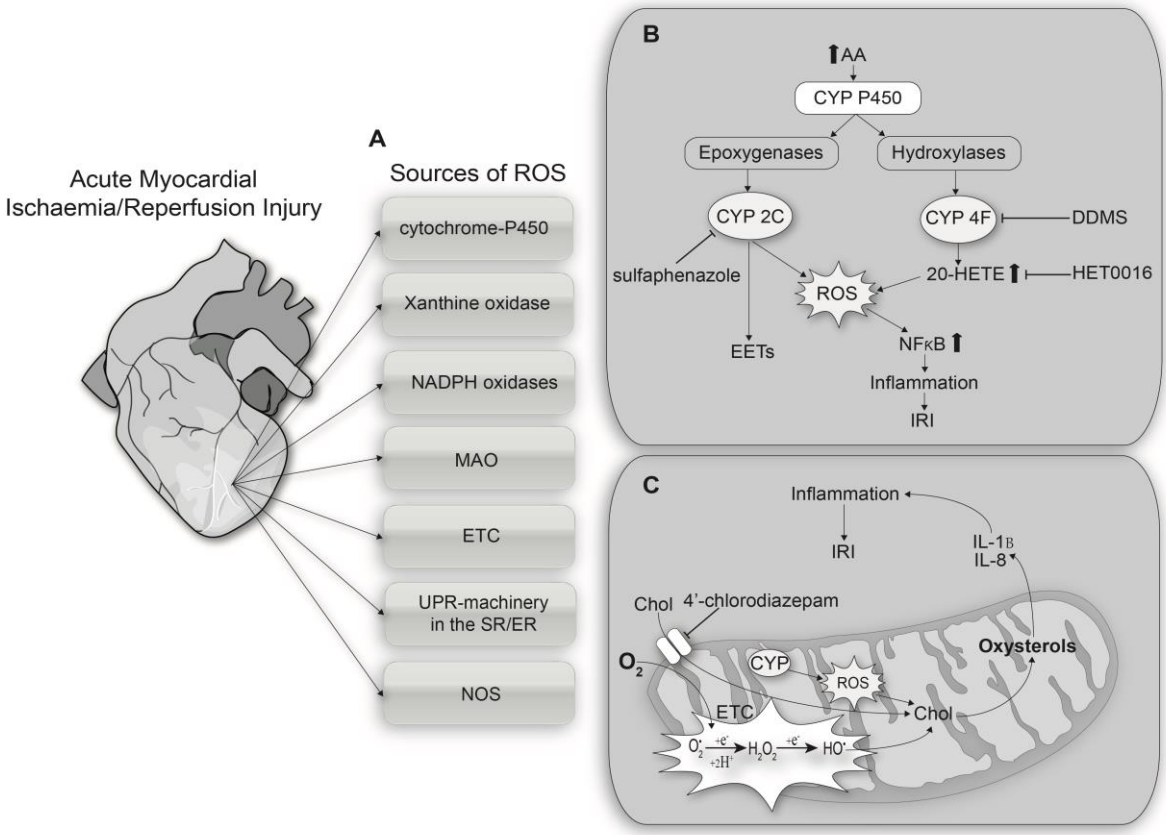
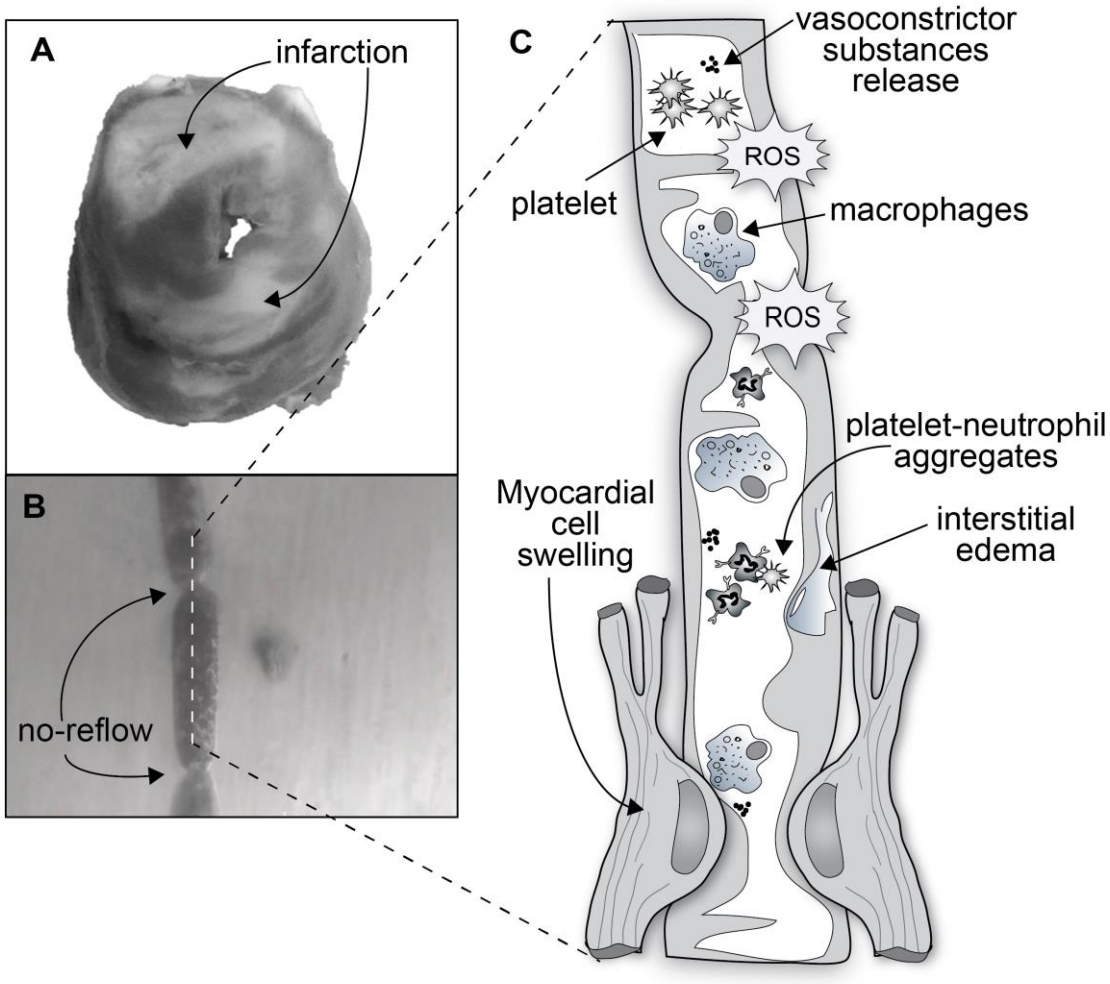

A
Acute Myocardial Ischaemia/Reperfusion Injury
Sources of ROS
cytochrome-P450
Xanthine oxidase
NADPH oxidases
MAO
ETC
UPR-machinery in the SR/ER
NOS
B
AA
CYP P450
Epoxygenases
Hydroxylases
CYP 2C
CYP 4F
DDMS
sulfaphenazole
ROS
20-HETE
HET0016
EETs
NFκB
Inflammation
IRI
C
Inflammation
IRI
IL-1B
IL-8
Chol
4'-chlorodiazepam
Oxysterols
O2
CYP
ROS
ETC
Chol

Figure 3


A
infarction
B
no-reflow
C
vasoconstrictor substances release
ROS
platelet
macrophages
ROS
platelet-neutrophil aggregates
Myocardial cell swelling
interstitial edema