Oxidative Stress in Cardiac Remodeling Post-Ischemia/Reperfusion: Friend or Foe?

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Abbreviations

WHO	World Health Organization
CVD	Cardiovascular disease
Ca^{2+}	Calcium
ROS	Reactive Oxygen Species
I/R	Ischemia Reperfusion
I/RI	Ischemia-Reperfusion Injury
MI	Myocardial Infarction
CHD	Coronary Heart Disease
PCI	Percutaneous Coronary Intervention
CABG	Coronary Artery Bypass Grafting
ATP	Adenosine triphosphate
Na^{+}	Sodium
mPTP	Mitochondrial Permeability Transition Pore
H^{+}	Hydrogen ion
NCX	Na^{+}/Ca^{2+}-exchanger
PKC-δ	Protein Kinase C delta
PKC-ε	Protein Kinase C epsilon
PARP	poly (ADP-ribose) polymerase
O_2^{-}	Superoxide anion
XO	Xanthine oxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate

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NOS	Oxidase Synthase
MPO	Myeloperoxidase
nNOS	neuronal NOS
eNOS	endothelial NOS
iNOS	inducible NOS
NO	Nitric Oxide
$ONOO^-$	peroxynitrite
BH4	tetrahydrobiopterin
H_2O_2	hydrogen peroxide
ETC	Electron Transport Chain
XDH	Xanthine dehydrogenase
IL-1	Interleukine 1
IL-6	Interleukine 6
TNF-α	Tumor Necrosis Factor alpha
PMNs	Polymorphonuclear Lukocytes
HIF-1α	Hypoxia-inducible factor 1-alpha
MIM	Mitochondrial Inner Membrane
MnSOD	Manganese Superoxide Dismutase
H_2O_2	Hydrogen peroxide
MAPKs	Mitogen-activated Protein Kinases
RAF-MEK	Rapidly Accelerated Fibrosarcoma- Mitogen-activated protein kinase kinase pathway
PI3K	PI-3 kinase
HMGB1	High-mobility box 1
TLRs	Toll-Like Receptors
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PKA	Protein Kinase A
Akt/PKB	Protein kinase B
Bcl-2	B-cell lymphoma 2
MMPs	Matrix metalloproteinases
BK	Big Potassium channels
mitoKATP	Mitochondrial ATP-sensitive K+ channel

12.1 Introduction

Given the heart's high energy demand and function, along with its vital physiological role to the body, a prolonged and non-managed ischemia is detrimental with high risk of morbidity and mortality [1, 2]. Half a century ago, myocardial reperfusion following coronary blood flow obstruction emerged as a promising therapy to rescue the heart from ischemic damage. However, challenging reports emerged since 1970s contradicting the beneficial role of reperfusion on myocardial tissue recovery following ischemia, and highlighting the myocardial ischemia-reperfusion injury (I/RI) concept [3]. Multiple studies, thereafter, exposed the underlying

mechanisms behind those findings. Hearse et al [4] were among the first group to report that sudden resumption of metabolic activity to energy-(and oxygen-) starved tissue resulted in a reoxygenation-dependent injury response independent of the hypoxic stress, commonly called "reperfusion injury". I/RI development is multifactorial involving alterations in both mitochondrial and cellular homeostasis, including a shortage in ATP production, alterations in ion gradient homeostasis, excessive inflammation, Ca^{2+} handling dysregulation, and excessive ROS production. In fact, myocardial ROS surge following reperfusion was for long proposed to be the mediator of I/RI [5, 6]. Consistently, a large number of studies have intensively addressed the role of excessive ROS formation during I/R [7]. Of note, ROS is a well-known potent mediator of metabolic disruption, inflammation, necrosis, and cell death in multiple diseases including myocardial injuries [8]. In this chapter we emphasize the importance of ROS-mediated reperfusion injury, and highlight the promising mito-targeted antioxidant therapy. We also examine the paradoxal evidence supporting the beneficial effects of ROS bursts in pre- and postconditioning mechanisms.

12.2 From Permanent Occlusion to Reperfusion: The Bad, the Good, and the Ugly

12.2.1 The Bad: Myocardial Infarction

Coronary blood flow obstruction, commonly termed MI, is characterized by an inadequate blood flow and subsequent nutrient and oxygen deprivation to the affected area. The severity of MI is strongly dependent on the size of the area at risk, the duration of ischemia, and the presence or absence of comorbidities [9]. The onset of MI itself is characterized by multiple life-threatening pathologies, including ventricular fibrillation, atrio-ventricular block [10] and cardiogenic shock [11]. Following hospitalization and stabilization of potentially existing arrhythmias, non-reperfused MI patients undergo adverse remodeling of the myocardium with very poor prognosis and high risk of heart failure development and death. Based on the American Heart Association statistical report, an approximate number of 720,000 Americans are hospitalized either for a first time MI or coronary heart disease (CHD) events with a projection of a median survival of 8.4, 5.6, 7, and 5.5 years for ≥45 year old white males, white females, black males, and black females respectively. Additionally, sudden cardiac death accounts for 13.5% of death certificates with a relatively high lifetime risk for cardiac arrest survivors [12].

12.2.2 The Good: Reperfusion

Given the well-established positive correlation between the duration of ischemia and the extent of myocardial damage, coronary blood flow restoration was an inevitable solution. In the last two decades, researchers have conducted a multitude of

studies and reported that the salvage of ischemic cells from inevitable death is only possible by revascularization. Thus, multiple interventions such as percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), and pharmacological (thrombolysis) approaches to remove the occluding clot were developed and adopted [13]. Reperfusion has proven to limit the ischemic injury and subsequently the infarct size area. The importance of reperfusion therapy in MI patients was surveyed over the past 20 years and reported a continuous decline of 6-month mortality, along with a further 22% reduction in standardized mortality, from 2010 to 2015 following reperfusion therapy [14] .

12.2.3 The Ugly: Reperfusion Injury

Despite the perpetual improvement of multiple procedures to ensure a rapid, complete, effective, and permanent reopening of the acutely occluded coronary artery, numerous studies revealed that myocardium salvage following blood flow restoration is highly predisposed to another form of injury, known as reperfusion injury [11]. Aside from the reperfusion impact on cardiac remodeling, multiple pathological conditions are known to occur at the onset of blood flow restoration, including arrhythmias, myocardial stunning, and potential microvascular occlusion that could be life-threatening [15].

12.3 Mechanisms of Cellular Cardiac Injury Following I/R

12.3.1 At the Onset of Ischemia

Following coronary artery clotting, cessation of cellular oxygen supply halts mitochondrial membrane polarization, reducing therefore adenosine triphosphate (ATP) formation and increasing mitochondrial ROS production [16]. Subsequently, reduced ATP-dependent Na^+/K^+ pump activity, leads to Na^+ accumulation in the myocyte and lowered mitochondrial resting membrane potential. Na^+ overload within the cell is counter-regulated by the reverse activity of Na^+/Ca^{2+}-exchanger (NCX) that pumps Na^+ out in exchange for Ca^{2+}, resulting eventually in intracellular and intra-mitochondrial Ca^{2+} overload. Concurrently with Ca^{2+} and Na^+ overload, the absence of oxygen supply switches cellular metabolism to anaerobic glycolysis promoting lactate accumulation and cellular acidosis [17]. In summary, ischemia-induced accumulation of intracellular sodium, ROS, and calcium ions, favors, if sustained, the opening of the mitochondrial permeability transition pore (mPTP) [18]. This, together with ATP shortage, determines a loss of contractility, structural disorganization, and apoptotic, necroptotic, and necrotic cell death [19, 20]. However, the acidic conditions during ischemia prevent opening of the mPTP and subsequent cardiomyocyte death (Fig. 12.1a).

12.3.2 At the Onset of Reperfusion

Reperfusion is intended to restore ATP production and reactivate the Na^+/K^+ ATPase to slowly re-establish the sodium gradient, leading to normal cation fluxes and eventually extruding the excess cytosolic and mitochondrial Ca^{2+}. However a massive mitochondrial ROS burst follows reoxygenation during reperfusion, which is further fueled by inflammation, increasing the risk of mPTP opening and cell death [18]. Additionally, persistent high intracellular Ca^{2+} levels observed during the early phase of reperfusion, increase the risk of a damaging myocardial hypercontracture that was otherwise inhibited during acidic ischemia (Fig. 12.1b). Besides, in the setting of ischemic–reperfusion injury, ROS burst is also responsible of the activation of protein kinase C delta (PKC-δ) stimulating its translocation to the mitochondria where it results in cytochrome c release, caspase 3 activation, and a decrease in the activity of pro-survival Akt, as well as poly (ADP-ribose) polymerase (PARP) cleavage in the nucleus. Pharmacological inhibition of PKC-δ is exploited in many therapeutic strategies like preconditioning [21]. In summary, reperfusion-induced cellular damage is largely dependent on ROS burst, Ca^{2+} overload, and mPTP opening [20].

12.4 Myocardial ROS in I/R: Types and Sources

Compelling evidence pointing to the causal interconnection between oxidative stress and I/RI is well established [22]. Oxidative stress is a consequence of the imbalance between ROS production and antioxidant capacity, either because of heightened ROS release and/or an ineffective antioxidant system [23]. Under ischemic conditions, mitochondrial complexes I and III are primarily responsible of the conversion of molecular oxygen to unstable/reactive superoxide (O_2^-) [24]. Cardiomyocytes, containing the highest number of mitochondria, consume a higher level of oxygen than any other cell and subsequently become major ROS producers [25]. As a result, heightened cellular ROS levels ultimately alter cellular homeostasis primarily by damaging proteins, lipids, and nucleic acids [26, 27]. In addition to local ROS production, immune cell infiltration into the myocardium following I/RI contributes substantially to increase ROS levels [28]. Upon reperfusion of the ischemic myocardium, inflammatory reaction is noticeably accelerated. Although inflammation is crucial for myocardial tissue healing, the re-establishment of blood flow to ischemic tissue accelerates and prolongs inflammatory response detrimentally. Among multiple immune cell infiltrations, neutrophils are considered the earliest and the most potent releaser of ROS, followed by macrophages [29]. Interestingly, clinical anti-neutrophil therapies did not succeed in slowing or preventing adverse myocardial remodeling post-MI [30]. These findings imply that local free radical outburst following reperfusion is potentially the main source of ROS-mediated injury during I/R. Xanthine oxidase (OX), nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, mitochondrial electron transport damage and uncoupling, uncoupled nitric oxidase synthase (NOS), and

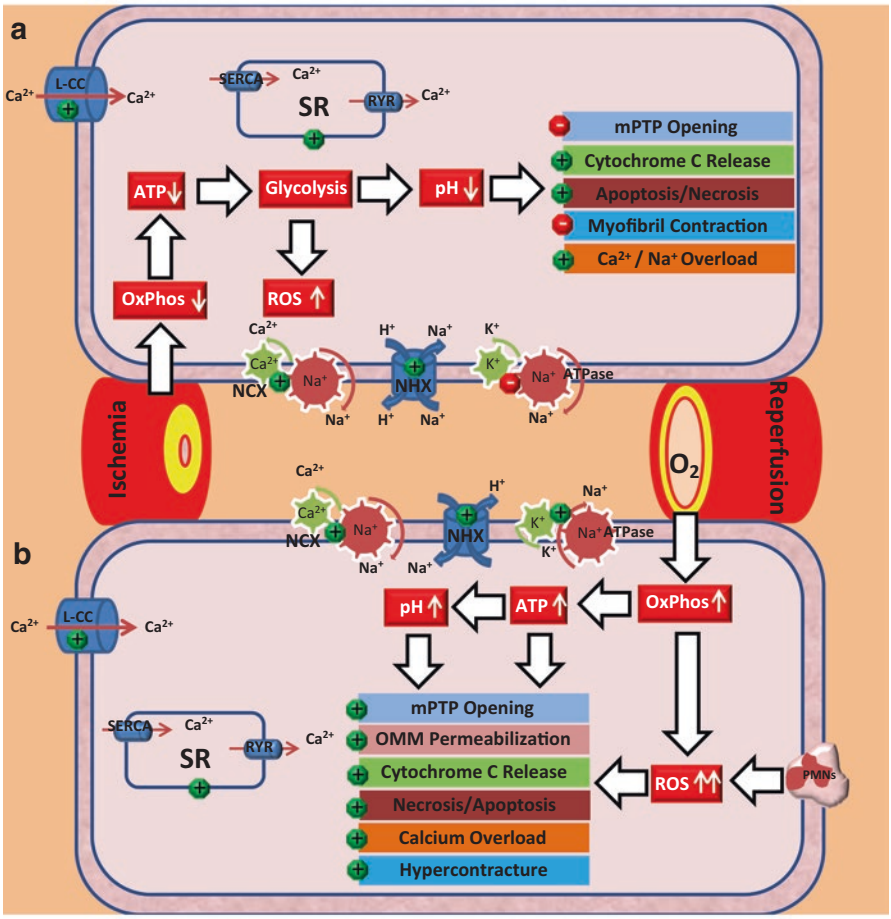


Fig. 12.1 Schematization of the key components of acute myocardial ischemia reperfusion injury: (**a**) Loss of oxygen supply in ischemia leads to a loss of ATP production and a switch to anaerobic respiration, resulting in a drop in intracellular pH, accompanied with an increased intracellular and mitochondrial-derived ROS. The ATP consuming Na^+-K^+-pump ceases to function, leading to Na^+ accumulation in the myocyte and the resting membrane potential is lowered. With the development of acidosis, the NHX further increases intracellular Na^+ exacerbating Ca^{2+}overload by forcing the NCX to manage, in a reverse mode the extrusion of Na^+ and the influx of Ca^{2+} into the cell. The sarcolemmal L type voltage-gated Ca^{2+} (L-CC) are activated allowing more Ca^{2+}entry as the resting membrane potential is low. Ca^{2+}pump SERCA2 is now taken up the excess of Ca^{2+}into the SR that releases it subsequently via RYR, leading to contraction and contracture. The acidic conditions during ischemia however prevent the opening of the mPTP and cardiomyocyte hypercontracture. (**b**) During reperfusion ATP production increases leading to the Na^+-K^+-pump reactivation, a slow restoration of both sodium gradient and NCX normal activity extruding the excess of cytosolic Ca^{2+}. An excessive production of ROS accompanies reoxygenation, electron transport chain activation, and immune cells infiltration. ROS burst mediates myocardial reperfusion injury by inducing the opening of the mPTP, causing outer mitochondrial membrane permeabilization, apoptosis, necrosis, acting as a neutrophil and cytokines chemoattractant, mediating dysfunction of the SR and causing myofibril hypercontacture. Restoration of physiological pH

myeloperoxidase (MPO) are the major producers of ROS in reperfused ischemic myocardium [31] and will be discussed in this chapter.

12.4.1 Nitric Oxide Synthases (NOS)

One of the most studied sources of physiological and pathophysiological ROS are the three well-recognized isoforms of NOS enzymes known as neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) that normally produce NO during the oxidation of L-arginine to L-citrulline [32]. While eNOS and nNOS are known to be constitutively expressed in the myocardium, iNOS, although primarily induced in immune cells, is expressed in cardiomyocytes under ischemic conditions [33, 34]. Constitutive myocardial nitric oxide (NO) generation under physiological conditions is essential for physiologic cell signaling [35]. Blood flow and oxygen restoration following reperfusion significantly increase NOS activity and subsequent NO production [36]. Although NO has been reported to be protective against I/R-induced injury in different organs of experimental animals [37] and humans [38], the beneficial effects of NO activity are negated by increased O_2^--mediated peroxynitrite ($ONOO^-$) generation following reperfusion (Table 12.1).

$$NO + O_2^- = ONOO^-$$

Contrarily to NO, $ONOO^-$ is very detrimental to proteins and lipids. $ONOO^-$ can negatively and irreversibly alter the structure and function of NOS by damaging its heme domain and oxidizing the tetrahydrobiopterin (BH4) cofactor [32, 39, 40] ultimately leading to NOS uncoupling an important source of I/R-induced ROS generation [32]. NOS function during I/R depends as well on its structural form; two cellular forms of constitutive NOS exist, the monomer and the homodimer forms. The monomer form is responsible of O_2^- generation in small amounts; the shift towards excessive O_2^- production depends on the homodimer/monomer ratio, intracellular L-arginine supply, and on BH4 oxidation [1].

A decrease in the local BH4/NOS ratio makes the balance, of a stoichiometric relationship between BH4 and eNOS, fall towards increased O_2^- instead of NO [41, 42]. Uncoupled NOS, furthermore, produces more O_2^- that acts as a positive feedback loop leading to further BH4 to BH2 oxidation and the propagation of NOS uncoupling. Besides the described loop, XO [43] and/or NADPH oxidase [44] play an important role in the I/R-induced reduction in BH4 levels by promoting O_2^- generation.Also, an essential factor required for the synthesis of NO by eNOS is

Fig. 12.1 (continued) following reperfusion along with Ca^{2+} overload accentuates mPTP opening leading to an increased infract size, cellular dysfunction, and cell death. *Ca^{2+}* calcium, *Na^+* sodium, *K^+* potassium, *H^+* hydrogen, *O_2* oxygen, *SR* sarcoplasmic reticulum, *SERCA* sarco/endoplasmic reticulum Ca^{2+}ATPase, *ATP* adenosine triphosphate, *OxPHos* oxidative phosphorylation, *ROS* reactive oxygen species, *mPTP* mitochondrial permeability transition pore, *NCX* $3Na^+/1Ca^{2+}$ exchanger, *NHX* Na^+-H^+-exchanger, *PMNs* polymorphonuclear lukocytes, (+) stimulation, (−) inhibition, ↑ increase, ↓ decrease

Table 12.1 Potential sources of reactive oxygen species in the cardiac tissue exposed to ischemia and reperfusion

Evidence of ROS involvement in I/RI		References
↓Superoxide dismutase activity		[5]
↓Endogenous cellular antioxidant systems		
↓Cellular glutathione-to-glutathione disulfide ratio		
↑ Lipid peroxidation,		
↑O_2^- production at reperfusion		
Oxygen-derived radicals act like mediators of reperfusion injury in isolated heart models in the presence or absence of superoxide		[116–118]
Oxygen-derived free radicals are directly implicated in I/RI		[119–121]
O_2^- was identified as the parent radical that serves as a precursor to the formation of both OH^- and the carbon-centered radical		
Free-radical are highly generated in an intact dog model of I/R		
O_2^- is the parent radical at reperfusion		
Oxygen, nitrogen, and carbon-centered free radicals are generated during I/R in an isolated rabbit and rat heart models		[122–124]
Exogenous administered ROS at the same levels as those observed during reperfusion induced similar calcium overloading, functional depression, and metabolic changes		
Major sources and outcomes of ROS generation in I/RI		
Xanthine oxidase	The time-course of ROS production elicited by I/R in isolated rat hearts is closely correlated with the kinetics of XO substrate accumulation.	[125, 126]
	Increased tissue xanthine and hypoxanthine levels determine the severity of the I/RI	
	Pharmacologic blockade of xanthine oxidase (XO) substrate formation:	
	(−) XO-dependent ROS production	
	(−) Contractile dysfunction that accompanies reperfusion	
	Exogenous administration of hypoxanthine and xanthine :	
	(−) The protective effects of blockade of xanthine oxidase substrate formation	
	Inhibition of XO:	
	↑ Levels of XOR antigen in vascular endothelium of myocardial ischemic tissues	
NADPH oxidase	↑ROS generation	[127–131]
	↑Tissue injury following reperfusion	
	Blunted reperfusion-induced neutrophil accumulation:	
	↓ Tissue injury and/or ROS production	
	The application of a simulated I/R on purified cardiomyocytes in culture:	
	(+) Tissue injury-related responses that are dependent on Nox activity	
	NOX inhibition:	

(continued)

Table 12.1 (continued)

Evidence of ROS involvement in I/RI		References
	↓ ROS production	
	↓ Myocardial infarct size	
	↓Cell death	
	↑Protective effect in isolated buffer (cell free) perfused hearts exposed to I/R	
	Mutant mice deficient in either Nox-1 or Nox-2/ Nox-1, Nox-2 and Nox-1/Nox-2 double knockout mice :	
	↑Protective effect in buffer-perfused Langendorff preparations with I/R hearts models	
	Myocytes release of Nox isoforms:	
	↑ROS generation during I/R	
Mitochondria	**I/RI*:***	[125, 132, 133]
	↑Mitochondrial H_2O_2 generation	
	↓Cytochrome c release from the MIM	
	↑Reduction state of cytochrome c	
	Ischemic damage to complex I and III:	
	↑Capacity to generate O_2^- at reperfusion	
	Both associated and/or separated complex I and III isolated from mitochondria obtained from reperfused hearts can generate O_2^-	
Nitric oxide synthase	***I/R:***	[127–131]
	↑Uncoupled NOS	
	↑Myocardial $ONOO^-$ generation by NOS	
	↓Endothelium-dependent vasodilation in porcine coronary arteries	
	In vitro and in vivo models of I/R:	
	BH4 supplementation replenish NOS activity in isolated rat hearts	
	↑↑ uncoupled NOS-derived O_2^- production	
	↑ I/R-induced cardiac inflammation and tissue damage	
	After reperfusion of ischemic heart:	
	↑ Arginase activity	
	↑ O_2^- generation increases	
	↓Arginine levels and NO production decrease	
	I/R+ treatment with a combination of arginine and BH4:	
	↓Infarct size	

I/RI ischemia reperfusion injury, *I/R* ischemia reperfusion, O_2^- superoxide, *ROS* reactive oxygen species, *XO* xantine oxidase, *XOR* xantine oxidase receptor, *Nox* NADPH oxidase, H_2O_2 hydrogen peroxide, *MIM* mitochondrial inner membrane, *NOS* nitric oxide synthase, $ONOO^-$ Peroxynitrite, *BH4* tetrahydrobiopterin, *NO* nitric oxide, (+) stimulation, (−) inhibition, ↑ increase, ↓ decrease

arginine, the nitrogen donor, and substrate for arginase I and II [45]. Increased arginase activity leads as well to increased production of O_2^- by NOS, a mechanism called "arginine steal". Finally, it was very early reported that myocardial eNOS actively produces NO during ischemia and reperfusion; however, parallel observations have shown that the enzyme is affected during ischemia. In fact, a prolonged ischemia is accompanied by intracellular acidosis that reversibly or irreversibly inhibits eNOS activity independently of the duration of acidosis [46] (Table 12.1).

12.4.2 Monoamine Oxidase and p66shc

During I/R, mitochondria are responsible of the generation of hydrogen peroxide (H_2O_2) through serotonin oxidization via monoamine oxidase [47]. Serotonin accumulation, as well as increased monoamine oxidase activity, is noted during ischemia and substantially increased following reperfusion [48]. Moreover, mitochondria are also capable of H_2O_2 production using a novel pathway that involves the 66-kDa isoform of the growth factor adaptor protein, p66shc. Ischemic conditions are responsible of translocation of p66shc from the cytosol to the mitochondrial intermembrane space, allowing it to use reducing equivalents from the electron transport chain (ETC) via the oxidation of cytochrome c to make H_2O_2. This reaction acts in a vicious cycle to provide p66shc with increased substrate in the intermembrane space during ischemia [49].

12.4.3 Cellular Xanthine Dehydrogenase vs. Xanthine oxidase

During normoxic physiological conditions, xanthine dehydrogenase (XDH) catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid by coupling the reaction with NAD^+ reduction to yield NADH.

$$\text{hypoxanthine} + \text{NAD}^+ \overset{\text{XDH}}{\rightarrow} \text{xanthine} + \text{NADH}$$

$$\text{xanthine} + \text{NAD}^+ \overset{\text{XDH}}{\rightarrow} \text{uric acid} + \text{NADH}$$

However, ischemic context enhances the conversion of XDH to XO by the modification of a sulfhydryl group or by proteolytic cleavage. XO is a molybdoflavoenzyme complex that controls, in addition to uric acid production, ROS generation through catalyzing the oxidation of hypoxanthine to xanthine.

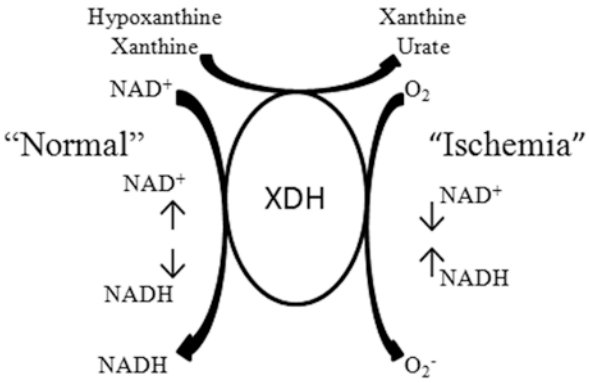
$$\text{hypoxanthine} + \text{O}_2 \overset{\text{XO}}{\rightarrow} \text{xanthine} + \text{O}_2^-$$

$$\text{xanthine} + \text{O}_2 \overset{\text{XO}}{\rightarrow} \text{uric acid} + \text{O}_2^-$$

XO-derived ROS contribute to multiple pathologic conditions including I/RI (Table 12.1). The accumulation of XO following ischemia will increase O_2^- formation. Besides, the oxygen burst at the onset of reperfusion drastically increases O_2^- formation [50] .

12.4.4 Cellular Xanthine Dehydrogenase

Nevertheless, other mechanisms can explain the enhanced superoxide release, independently of XDH to XO conversion. In fact, XDH has an NADH oxidase activity in the presence of acidic conditions (pH 6.5) wherein NADH is oxidized rather than xanthine [33]. XDH is capable of generating superoxide at 4-times the rate of XO. Besides, XDH is the dominant isoform in the early reperfusion period and is most likely a more important source of superoxide than the XO isoform at the onset of the reperfusion.



Post-transcriptional regulation of XDH expression is reported during I/R, wherein the hypoxic and inflammatory environments are stimuli associated with increased XDH transcription [51]. On the other hand, XO activity is also regulated at the post-translational level. These modifications have been attributed to O_2 tension that results in phosphorylation of the enzyme by p38 kinase [52]. In addition, along with hypoxic environment, the inflammatory context (mast cell degranulation and macrophage activation), which accompanies I/R, participates via multiple cytokines such as IL-1, IFN-γ, IL-6 and TNF-α to increase XDH/XO mRNA. Another feature of the XO capacity to produce ROS under ischemic conditions is its capacity to act as a nitrate/nitrite reductase (Table 12.1). This enzymatic reaction catalyzes the production of NO by one electron reduction of nitrite, and is optimal under anoxic/hypoxic and acidic conditions [53]. The generated NO, an important substrate for peroxynitrite generation, enhances the oxidative burst in the presence of an ischemia/inflammation loop in the ischemic heart [54] (Table 12.1). Finally, XO participates in leucocyte recruitment upon I/R, followed by neutrophil recruitment and XO-derived ROS secretion [55].

12.4.5 Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase

The Noxs are a family of 7 isoforms expressed in multiple types of cells, including vascular endothelial cells, smooth muscle cells, fibroblasts, cardiomyocytes, and polymorphonuclear leukocytes (PMNs). Nox isoforms are known as Nox-1 to Nox-5 and dual oxidases (Duox)-1 and -2 [56, 57]. Nox/Duox are considered the major source of ROS in multiple pathological conditions including cardiac remodeling post-MI [58–60] (Table 12.1). The contribution of Nox enzymes to reperfusion injury is documented by multiple studies reporting both an increased expression and/or activity of Nox in ischemic tissue and attenuation of I/R induced injury following Nox inhibition [61, 62]. A large amount of data reported the involvement of multiple factors in the activation of Noxs in I/RI. For example, studies confirmed that hypoxia inhibitory factor-1α (HIF-1α) activation, evoked by the hypoxic state that accompanies ischemia, promote production and activation of Noxs [63]. Among all Nox isoforms, Nox2 is one of the most widely expressed in cardiac cells and, therefore, a prominent ROS producer in myocardial I/R (Table 12.1). The activation of XO and the resulting increase in ROS and intracellular Ca^{2+} levels have been reported to be indispensable for Nox2 activation under ischemic conditions. The stimulation of PKC by XO-generated ROS also contributes to ischemic-evoked Nox2 activation. Furthermore, the inhibition of XO halts ischemic-induced upregulation of HIF-1α proving that Nox2 activation by XO is essential for HIF-1α activation under ischemic conditions [64]. Of note, both the activation of the complement system and increased generation of angiotensin II are also associated with an increase in Nox activity in cardiac post-ischemic tissue [61, 65].

12.4.6 Mitochondrial ETC ROS Production

Mitochondria constitute 33% of the total cardiac myocyte cell volume, highlighting their fundamental role in cardiac function and the high energy demand of the myocardium. The mitochondrial ETC complex is comprised of a series of multi-subunit complexes (complexes I–IV) located in the inner mitochondrial membrane (IMM) and coupled to mobile carriers such as coenzyme Q and cytochrome c. The complexes and cytochrome c contain redox groups (Fe-S clusters and/or heme) that allow for the transfer of electrons along the components of the ETC, generating a proton electrochemical gradient, ultimately promoting ATP production via ATPase [25, 66]. Mitochondria are considered a normal source of ROS that play a crucial role as cell signaling intermediates in order to maintain cellular homeostasis. Under normal physiological conditions, ETC reduces oxygen to water using more than 97% of the entire electron flux through mitochondria. The remaining 2–3% of electrons consistently leak from ETC to form O_2^-. In addition to its important role in signaling, physiological production of O_2^- plays a critical role in multiple crucial cell functions such as metabolism, proliferation, and apoptosis [67].

Following ischemia, the decrease in mitochondrial respiration as well as ATP production, along with complex I/III alterations, increases NADH:NAD^+ ratio and reduces flavin mononucleotide prosthetic group within the NADH dehydrogenase component of complex I. These changes increase the leakage of electrons that form O_2^- via univalent reduction of O_2 and subsequent ROS production beyond physiological levels [24, 25, 68, 69]. Although reduced cytochrome c controls mitochondrial ROS levels by scavenging O_2^-, persistent ischemia increases the oxidized state of cytochrome c contributing further to mitochondrial damage and the accumulation of O_2^- (Table 12.1).

Upon reperfusion, oxygen burst into an already stunned mitochondria drastically increase ROS production to a much higher extent than during ischemia. Additional sites within complex I may contribute to ROS generation. Mitochondrial increase in superoxide production is normally accompanied by an increase in H_2O_2 formation through MnSOD activity within the mitochondrial matrix [70]. Superoxide dismutase enzymes contain either copper, manganese, or nickel metal centers that are reduced or oxidized to convert cellular O_2^- into H_2O_2 (Table 12.1) [71, 72]. H_2O_2 interaction with NO also increases formation of $ONOO^-$. Of note, ROS is able to freely spread within the mitochondrial network mainly through the mPTP and inner membrane ion channels, centralizing therefore cellular damage [25, 73].

12.5 ROS Mediated Adverse Effects in I/R

ROS production during the ischemic, reperfusion, and remodeling phases contribute to cardiac injury post-MI. The extent of injury, however, varies based on the size of the affected myocardium, the magnitude of ROS reactions, and the severity of cardiomyocyte damage. Uncontrolled sustained ROS burst causes modification and denaturation of a multitude of structural and functional molecules leading to irreversible tissue damage. The effect of each ROS, however, depends on its type. OH^- for instance acts instantly right after generation. O_2^- and NO^- radicals on the other hand, are of much lesser reactivity, more specific, and can mediate radical reactions on sites that are distant form their site of production. In the absence of appropriate ROS scavengers, sustained ROS production triggers oxidative vicious cycles that could permanently damage the cells. Of the well-known ROS-mediated cellular damage, lipid peroxidation, protein denaturation, mitochondrial, and DNA damage constitute the basis behind those effects.

12.5.1 DNA Oxidation

OH-mediated hydrogen extraction interferes with cellular DNA, causing purine and/or pyrimidine direct modification and/or fragmentation producing a plethora of DNA lethal lesions [74]. These lesions can induce mutagenesis, crosslinks between DNA strands and proteins, stand breaks, which affect thereafter DNA replication

and transcription [75] and ultimately promote a pro-apoptotic and pro-necrotic effect (Table 12.2).

12.5.2 Lipid Peroxidation

Lipid peroxidation is a typical 3 phase oxidative reaction that occurs abundantly during I/R. The alkenes, unsaturated fatty acids and major component of biological membrane's phospholipid bilayers, are very susceptible to hydrogen extraction by ROS. The generated carbon-centered and peroxyl radicals constitute the initial phase of ROS attack followed by an amplification phase also known as the propagation phase [76]. Lipid peroxidation continues with additional similar abstractions until two radical species combine in a termination phase. Reactive aldehydes such as malondialdehyde (MDA), 4-hydroxynonenal, and isoprostane are major end products of this classical oxidation cascade and are known to increase during I/R [77]. Lipid peroxidation byproducts are bioactive and well-involved in the adverse remodeling of I/R. 15-F2t-isoprostane, for example, is reported to induce a dose-dependent vasoconstriction in coronary arteries, promoting therefore cardiac dysfunction following I/R [78] (Table 12.2).

12.5.3 Protein Oxidation

ROS-mediated activation of necrotic and pro-apoptotic proteins determines the severity and the extent of infarct size [79]. For instance, ROS can modify cellular proteins via oxidation and nitration, impairing subsequent myocardial contraction and promoting myocardial stunning following I/R [80]. Similarly to what is observed with lipids, hydrogen extraction by $OH^{\cdot}$ is a key player in the initiation phase of the oxidative attack on proteins by affecting amino-acid functional groups [81]. Denaturation of proteins by ROS oxidation reactions is due to the cleavage of peptide bonds, functional group cross-linking, and by hydrophobicity alterations of amino acids on protein surfaces [81] (Table 12.2).

Proteins with signaling roles, such as kinases and phosphatases, can also be oxidized by $ONOO^{-}$, affecting therefore their signaling capacities and impact [82]. By regulating mitogen-activated protein kinases (MAPKs), ROS contribute to cellular responses to mitogens, inflammatory cytokines, and (un)physiological stimuli [83]. Activation of p38 can have either pro- or anti-apoptotic effects, and is exaggerated during I/R. Also, p38 has been reported to play a role in regulating mitochondrial ROS levels and intracellular signaling pathways, as well as controlling mitochondrial events associated with development of I/R-associated damage (Table 12.2). Other signaling pathways that have also been shown to be involved in this regulation include: the RAF-MEK pathway that, in contrast, prevents mitochondrial accumulation of ROS/Ca^{2+} and cell death [83], and the PI-3 kinase (PI3K)/protein kinase C (PKC/AKT) pathway that has a protective role against cellular I/R-induced cell death.

Table 12.2 ROS targets following I/RI

Oxidized target	Effects	References
Lipids	↑Lipid peroxidation.	[77, 134]
	↑Alkene hydrogen abstraction	
	↑Generation of carbon-centered and peroxyl radicals	
	↑Peroxyl and lipid isoforms generation	
	↑Production of MDA ,4-hydroxynonenal, and isoprostane	
	↑Cardiolipin depletion	
Proteins	↑ Physical and chemical modification of myocardial proteins	[79–81, 99, 135–140]
	↑Non-enzymatic modification of cellular proteins	
	↑ Amino acid oxidation and nitration	
	↑ Formation of nitrotyrosine residues on proteins	
	↑ Products of tyrosine oxidation: myocardial 3-nitrotyrosine and dityrosine	
	↑ Particular risk of tyrosine nitration in mitochondrial proteins	
	↑ $OH^{\cdot}$ hydrogen abstraction in functional groups and backbone α-carbons of all amino acids	
	↑ Cleavage of peptide bonds and cross-linking of functional groups	
	↑Alteration of the hydrophobicity of amino acids on protein surfaces	
	↑Cellular and mitochondrial sulfhydryl groups	
	↑Tyrosine kinases and protein tyrosine phosphatases oxidation	
	↑↑Mitochondrial tyrosine and cysteine residues nitration and oxidation	
	↑↑Reactive aldehydes-mediated electrophilic attack towards nucleophilic amino acids	
	↑4-hydroxynonenal provoked modification and inhibition of the cytochrome oxidase	
	↑ Oxidative activation of necrotic and pro-apoptotic protein	
	↑ MMP-9 cleavage and activation	
DNA	↑ $OH^{\cdot}$-mediated hydrogen abstraction	[74, 75, 78, 141–143]
	↑ Purine or pyrimidine direct modification and/or fragmentation	
	↑DNA lesions	
	↑DNA bases modification	
	(+) Inter and intra-strand crosslinks	
	↑ DNA–protein crosslinks	
	(+) strand break formation	
	↑Adducts with MDA, and ROS-mediated lipid-peroxidation products	
	↓DNA replication and transcription	

(continued)

Table 12.2 (continued)

Oxidized target	Effects	References
Mitochondria homeostasis	↑Perturbation of the mitochondrial energy production	[93, 96, 99, 144]
	↑Overexuberant liberation of mitochondrial ROS	
	↑Mitochondrial DNA rearrangement and fragmentation	
	↓Mitochondrial enzymes activities	
	↑The susceptibility of mitochondrial DNA to oxidative modification in circulating leukocytes	
	↑Oxidative inactivation of mitochondrial aconitase	
	↑Mitochondrial production of hydroxyl radicals	
	↓Mitochondrial structure and function	
	↑Mitochondrial Ca^{2+} levels	
	↑mPTP opening	
	↑Perforation and lysis	
	↑Mitochondrial depolarization and cell death	

MDA malondialdehyde, *OH^-* hydroxyl radical, *MMP-9* matrix metalloproteinase 9, *DNA* deoxyribonucleic acid, *ROS* reactive oxygen species, *Ca^{2+}* calcium, *mPTP* mitochondrial permeability transition pore, (+) stimulation, (−) inhibition, ↑ increase, ↓ decrease

ROS are implicated as well in inflammatory signaling, not only by fueling the pro-inflammatory response in a self-perpetuating manner, but also by regulating the process of high-mobility box 1 (HMGB1) protein release that occurs especially in response to cellular damage. HMGB1 is an agonist for Toll-like Receptors (TLRs). Accordingly, TLR4-mediated NFκB activation is recruited for oxidative stress-activated intracellular signaling pathways [84].

Two additional developmental pathways also figure among the most important pathways in this context: the Wnt/s-catenin signaling that is activated by ROS [84] and NOTCH signaling that suppresses ROS production [85]. Nevertheless, this sort of crosslink between intracellular signaling and regulation of mitochondrial ROS production has been demonstrated for p53 [86], protein kinase A (PKA) [87], rapidly accelerated fibrosarcoma (RAF) kinase, protein kinase B (Akt/PKB), and B-cell lymphoma 2 (Bcl-2) [83]. The tyrosine kinase pathway plays a role via p66shc, which acts as a redox enzyme that generates mitochondrial ROS through oxidation of cytochrome c [49]. In addition, oxidative stress leads to alterations in the activation state of different PKCs. This activation provides a protective role in the context of preconditioning by activating the specific PKC-ε isoform [88]. However, activation of PKCδ isoform increases, in a positive-loop manner, ROS generation. Activation of PKCδ by ROS regulates the expression and function of apoptosis-related proteins, and represents a target for caspases leading to cellular death [88].

Activation of enzymes including MMPs and caplains is also pronounced following increased ROS production and pH restoration, and is capable subsequently of degrading crucial functional proteins, such as myosin light chain [89], α-actinin [90], and cardiac troponin [91, 92] (Table 12.2). During ischemia, oxidative stress is

also broadly responsible for Na^+/H^+ exchanger (NHX) activation, a mechanism that attempts to restore intracellular pH by increasing cellular Na^+ levels. The Na^+/Ca^{2+} exchanger (NCX) is thus activated, leading to intracellular accumulation of Ca^{2+} and a state of mitochondrial Ca^{2+} overload and depolarization. This phenomenon is exacerbated upon reperfusion with mitochondrial calcium uniporter (CaU) exaggerated opening. ROS-mediated alterations of anion exchanger function leads to pH recovery. Excessive ROS, however, favor opening of the mPTP (Fig. 12.1b) [93], leading to mitochondrial matrix swelling and loss of MOM. This result is fatal due to the pro-apoptotic molecules that are released from the mitochondrial intermembrane space (IMS). Additionally, another type of Ca^{2+} permeable cationic channel is affected by increased ROS production during I/R. The transient receptor potential melastatin 2 (TRPM2) is in fact a ROS sensor [94]. Oxidative stress-mediated activation of TRPM2 results in mitochondrial Na^+ and Ca^{2+} overload, which leads to a disrupted mitochondrial membrane, cytochrome c release, PARP-1 cleavage via induction of caspase-8 activation, and finally apoptotic cell death [95].

It is worthwhile to point out that radical reactions are of a semi-random nature, so they do not necessarily yield irreversible cell damage. For example, the magnitude of the oxidative attack on membranes, proteins, or DNA may not be enough to have an adverse effect on their functions. Besides, if the damaged protein is not of critical functional relevance, normal cell processes, such as phospholipid and protein turnover, can remove the altered biomolecule and the cell will survive. Understanding the contribution of ROS to the development of I/RI may identify additional targets for therapeutic interference. That being said, the understanding of aberrant signaling in this particular pathological condition holds the promise for novel therapeutic approaches that specifically target the regulation of mitochondrial function (Table 12.3).

12.6 General and Mitochondria Targeted Antioxidants Reduce I /R Injury

Mitochondrial Ca^{2+} overload and overexuberant mitochondrial ROS burst, constitute the hallmark of I/R-mediated cardiac cell injury [96, 97]. A massive burst of ROS following reperfusion localizes in mitochondrial regions that progress to swelling and eventually stimulates opening of the mPTP [98, 99]. mPTP opening is

Table 12.3 Summary of selected clinical trials using general ROS scavengers therapeutics for MI

Antioxidant	Effect	Outcome	References
β-carotene	*Harmful*	↑ CAD risk	[145]
Edaravone	*Effective*	↓ Infarct size and reperfusion arrhythmia	[145]
L-carnitine	*Effective*	↓ Level of cardiac MI markers	[146]
Vitamin E	*Effective*	↓ CVD risk	[145]
	Harmful	↑ Increased HF risk	

CAD coronary artery disease, *MI* myocardial infarction, *CVD* cardiovascular disease, *HF* heart failure, ↑ increase, ↓ decrease

directly linked to mitochondrial DNA rearrangement and fragmentation, a complete disrupted mitochondrial structure and function (Table 12.2), followed by mitochondrial perforation and lysis [18]. Mitochondria-targeted antioxidant therapy has the ability to salvage I/R-assaulted cardiomyocytes more so than general antioxidants (Table 12.4) at different levels including: (1) preventing excessive detrimental cellular ROS production that is largely and mainly produced by mitochondria with I/R, (2) promoting low and beneficial ROS signaling through protein kinase Cε and its downstream substrates, and (3) preventing harmful ROS signaling through protein kinase Cδ and its downstream effectors. Examples of protective therapies targeting mitochondrial ROS are detailed in Table 12.4.

12.7 The Paradoxal Cardioprotective Effects of ROS

Pre- and postconditioning are manipulations during which short periods or bouts of ischemia are applied by occluding and opening the coronary artery, prior or subsequent to, permanent occlusion [100]. Pharmacological and interventional ischemia pre- and postconditioning has gained immense attention due to its protective effects on cardiac remodeling prior to or following reperfusion [101, 102]. This protection is, however, impeded with application of antioxidants. In fact, unlike excessive and sustained ROS burst that is now proven to be detrimental, low levels of ROS are protective (Table 12.4). A growing body of recent evidence has established that generation of ROS at low levels can serve as a signal mediating physiologic responses. The protective role of preconditioning on the myocardium was first described in 1986 by Murry et al., as a slower ATP depletion rate and smaller infarct size in the heart treated with brief episodes of I/R before prolonged occlusion, followed by reperfusion [101, 102]. Mitochondrial pathways play an important role in promoting the activation of cell survival programs following preconditioning via ROS signaling-dependent mechanisms [103]. A good example of cardioprotective roles of reliable amounts of ROS is the metabolic vasodilator effect of H_2O_2, produced by myocardial mitochondria. H_2O_2 serves as a mediator that couples oxygen consumption to coronary blood flow by acting as an activator of redox- and 4-aminopyridine-sensitive voltage-dependent potassium (Kv) channels in smooth muscle cells [100]. In addition, H_2O_2 that derives from complexes I and III in the endothelial mitochondria's electron transport chain is capable of triggering calcium activated potassium (BKCa) channels in order to enhance acetylcholine- and flow-induced coronary vasodilation [104, 105]. More recently, several methods of preconditioning have been developed including ischemic preconditioning (IPC), exercise preconditioning, and pharmacological preconditioning [106–108]. The opening of mitochondrial ATP-sensitive K^+ (mitoKATP) channel is one of the most important mechanisms activated by preconditioning stimuli (Table 12.4). This activation allows potassium to flow into mitochondria leading to depolarization and matrix alkalization. Subsequently, an increase in ROS production activates downstream survival signaling events through PKC, preventing mPTP opening [106, 109]. Additionally, the generation

Table 12.4 Different mitochondrial ROS targeting strategies

Mitochondrial Target	Antioxidant/ Target name	Description	Effect/outcome	Study model	References
Targeting Defective Mitochondria to Prevent Production of Excessive ROS	**MitoQ**	An ubiquinol with a lipophilic TPP+ modification, which enables mitochondrial delivery of the compound	***Effect:*** Antioxidant activity ↓LDH release ***Outcome:*** (−) Tissue damage (−) Mitochondrial swelling ↓Cytochrome c release (−) Caspase 3 activation ↓Tissue and mitochondrial damage ↓Cardiac disfunction	Langendorff model of rat heart I/R damage	[147]
	SkQ	TPP+ modified ROS scavenger	***Effect:*** (−) Oxidative modification of cytochrom c (−) Peroxidase activity of cytochrom c ***Outcome:*** (−) Cytochrom c/ H_2O_2 induced liposomes permeabilization (−) Apoptosis ↑Protection from oxidation-caused I/RI	Langendorff model of rat heart I/R damage	[148]

(continued)

Table 12.4 (continued)

Mitochondrial Target	Antioxidant/ Target name	Description	Effect/outcome	Study model	References
	SS31 (Bendavia)	A four amino acid synthetic peptide(phenylalanine-darginine-phenylalanine-lysine)	***Effect:*** ↓ ROS levels in the MIM ↓ Mitochondrial ROS production ↓Pathological ROS production in aged mitochondria ***Outcome:*** (−) mPTP opening (−) Hydrophobic cytochrome *c* interactions with cardiolipin in the IMM ↑ Cytochrome *c* to function as an electron carrier ↑Cardioprotection	Multiple animal models of MI and heart failure (mice, rats, guinea pigs, rabbits, and sheep)	[149–151]
	Mitochondrial catalase (mCat)	Mitochondrial enzymatic anti-oxidants	***Effect:*** (−)ROS production ***Outcome:*** ↓Mitochondrial oxidative damage (-)ROS-induced cardiac hypertrophy, fibrosis, and heart failure ↑ Lifespan of mice	Different models of angiotensin II-induced cardiac hypertrophy	[152]

	P110	Slective Drp1 inhibitor	***Effect:*** ↓ROS production ↓ATP production (−) Drp1 enzyme activity (−) Drp1/Fis1 ***Outcome:*** ↓Excessive mitochondrial fission ↓Pathological mitochondrial fragmentation without interfering with normal Drp1 functions ↑ Mitochondrial function ↓Infarct size	Primary cardiomyocytes, *ex vivo*, and an *in vivo* MI models	[153–155]
	iGAPDH	Metabolically inactive or oxidized GAPDH: results from high levels of ROS released by mitochondria	***Effect:*** (−) Nearby GAPDH oxidative post-translational modifications Molecular sensor for detecting and tagging damaged mitochondria ***Outcome:*** ↑ Mitophagy ↑Direct uptake of damaged mitochondria into a lysosomal-like structure	HL1 murine cardiomyocyte cell culture model subjected to simulated I/R	[156]

(continued)

Table 12.4 (continued)

Mitochondrial Target	Antioxidant/ Target name	Description	Effect/outcome	Study model	References
Preventing Harmful ROS Signaling Through Protein Kinase Cδ and its Downstream Effectors	**δV1-1**	Specific PKCδ translocation peptide inhibitor	***Effects:*** (−) PKCδ activation (−) PKCδ translocation to the endoplasmic reticulum ↓ROS generation ***Outcomes:*** ↓Endothelial vascular dysfunction ↑Survival of coronary endothelial cells ↓Cell death	I/R model in cardiac myocytes	[21]
Promote Beneficial ROS Signaling Through Protein Kinase C ε and its Downstream Substrates	**Antimycin A**	Secondary metabolites produced by Streptomyces bacteria	***Effect:*** ↑Physiological ROS production ↑PKCε translocation to the mitochondria ***Outcome:*** ↓Cardiac infarct size	I/R Langendorff model of mice heart	[157]

	ψεRAC	PKCε agonist	***Effect :*** ↑PKCε–HSP90 protein–protein interaction ↑PKCε translocation to the mitochondria ↑Phosphorylation and activity of an intra-mitochondrial PKCε substrate ↑ ALDH2 ↑Physiological ROS generation ***Outcome:*** ↑Cardioprotection following I/RI	I/R model of isolated cardiomyocytes	[158]
Promote Beneficial ROS Signaling through preconditioning mimetic	**Diazoxide**	Pharmacological preconditioning mimetic: mitoKATP channel opener	***Effects:*** ↑ROS generation in a connexin 43-dependent manner ↓Cytosolic LDH release ***Outcome:*** ↑Vasodilation ↑ Hyperglycemic effects	Isolated rat heart model of MI	[159]

(continued)

Table 12.4 (continued)

Mitochondrial Target	Antioxidant/ Target name	Description	Effect/outcome	Study model	References
	Diosgenin and atorvastatin	Pharmacological preconditioning mimetic: mitoKATP channel activators/openers	***Diosgenin effect:*** ↓ROS ↑LDH release ***Diosgenin Outcome:*** Induces a cardioprotective preconditioning effect: ↓Infarct area ***Atorvastatin effect:*** ↓OGD/R-induced ROS levels ↑LDH activity in serum ↓OGD/R-induced mitochondrial Ca^{2+} overload (−) mPTP opening (−) $\Delta\Psi m$ depolarisation (+) mitoKATP channels ***Atorvastatin Outcome:*** ↓ Infarct area (−) NAD^+ release Improves the ultrastructure of myocardium Improves hemodynamic variables	Isolated rat heart model of MI	[160, 161]

	Alda-1	A selective Aldehyde dehydrogenase activator	***Effect :*** ↓H_2O_2 release ↑ALDH2 enzymatic activity ↓Aldehydic load in cardiomyocytes	Oxidative stress-related cardiovascular conditions or models	[162–165]
			Outcomes: (−) down-regulation of cardiac mitochondrial ETC(I and V) (+) Mitochondrial bioenergetic status ↓Ventricular diastolic diameter ↓Posterior wall thickness ↑Cardiac function preservation after I/R (−) MI-induced heart failure ↑↑Mitochondrial function ↑Left ventricular function ↑Viability of iPSC-CMs after ischemia	MI rats models Human ischemic hearts	

TPP+ triphenylphosphonium cation, *I/R* ischemia reperfusion, *LDH* cytosolic enzyme lactate dehydrogenase, *ROS* reactive oxygen species, H_2O_2 hydrogen peroxide, *I/RI* ischemia reperfusion injury, *MIM* mitochondrial inner membrane, *ATP* adenosine triphosphate, *MI* myocardial infarction, *mPTP* mitochondrial permeability transition pore , *Drp1* dynamin related protein 1, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *iGAPDH* inactive or oxidized GAPDH, *PKCδ* protein kinase C-delta, *PKCε* protein linase C-epsilon, *HSP90* heat shock protein 90, *OGD/R* oxygen–glucose deprivation/recovery, $\Delta\Psi m$ mitochondrial membrane potential, NAD^+ nicotinamide adenine dinucleotide, *ETC* electron transport chain, *iPSC-CMs* induced human pluripotent stem cell-derived cardiomyocytes, (+) stimulation, (−) inhibition, ↑ increase, ↓ decrease

of mild matrix swelling improves ATP synthesis and fatty acid oxidation, conditioning the cells to any potential ischemic injury [109].

Ischemic postconditioning on the other hand was first introduced by Zhao et al. in 2003. This term refers to brief periods of ischemia alternating with brief periods of reflow applied at the onset of reperfusion following sustained ischemia. The timing of post-conditioning interference is crucial given that reperfusion injuries occurs only within several minutes following blood reflow [110]. The basics of preconditioning- and postconditioning-mediated protection are very similar [110, 111]. In fact, similar to preconditioning, the mitoKATP/ROS/PKC axis pathway constitutes the basis of postconditioning protective therapy [110]. However, the degree of protection largely depends on the timing of axes activation following reperfusion [112]. Of note, both pre- and postconditioning share an important effect that underlines their cardioprotective efficacy. In fact, the associated prolongation of cellular acidosis that takes place initially during early reperfusion after ischemia favors inhibition of mPTP opening for a few minutes following reperfusion. Pre- and postconditioning released ROS take advantage of delayed protective pH normalization to induce activation of cell survival programs. Therefore, following pH normalization, an arsenal of downstream effectors that prevent mPTP opening is boosted, to preserve mitochondrial and cellular integrity [113].

Several other signaling pathways are implicated in the infarct-sparing effect of pre- or postconditioning [114]. The Reperfusion Injury Salvage Kinases (RISK) pathway involves the activation of two signaling pathways consisting of pro-survival kinases ERK1/2 and Akt that converge on mitochondria to decrease mPTP opening [115]. The Survivor Activating Factor Enhancement (SAFE) pathway involves the induction of JAK-STAT3 signaling. The relative contribution of the RISK and SAFE pathways to cardiac protection varies with the experimental ischemic protocol, as well as species. Some studies have linked SAFE signaling to the initiation of the RISK pathway, although the mechanism is not defined [115]. Both the RISK and SAFE pathways are activated by ROS.

12.8 Conclusion and Future Direction

Reperfusion of the coronary circulation is necessary to prevent irreversible loss of the myocardium. Yet reperfusion causes further harm to the heart via the generation of ROS, which invariably leads to heart failure and shortened lifespan. These ROS target phospholipids of the cell membrane, various structural, transport, and signaling proteins, and DNA, which may then act synergistically to further ROS generation and damage the heart. MMPs, caspases, and calpains are activated as well, further exacerbating structural damage. Much progress has been made in identifying the sources of ROS, which include NOXs, MAO, uncoupled NOSs, p66shc, xanthine dehydrogenase/oxidase, and mitochondria. Paradoxically, lower levels of ROS may activate a number of signaling mechanisms that tamp down excessive ROS generation by mitochondria as initially revealed in preconditioning experiments. The targets of this manipulation include both direct effects on mitochondria,

as well as the upregulation of protective proteins at later time points. For practical reasons, direct preconditioning strategies have little if any translational potential. However, complementary approaches, such as exercise-induced preconditioning and ischemic postconditioning, offer clinical promise. Pharmacological manipulations that specifically target mitochondrial complexes that generate ROS during reperfusion are gaining interest as therapies. Although much progress has been realized in the last decade in understanding the source and implications of ROS as foe in I/R-mediated injury to the heart, the upcoming decade should result in the practical application of therapeutic strategies that are based on the revelation of mechanisms defined by the protective actions of ROS in the myocardium.

References

1. Raedschelders K, Ansley DM, Chen DD (2012) The cellular and molecular origin of reactive oxygen species generation during myocardial ischemia and reperfusion. Pharmacol Ther 133(2):230–255
2. Frank A, Bonney M, Bonney S, Weitzel L, Koeppen M, Eckle T (eds) (2012) Myocardial ischemia reperfusion injury: from basic science to clinical bedside. Seminars in cardiothoracic and vascular anesthesia. SAGE, Los Angeles
3. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB (1977) The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation 56(5):786–794
4. Hearse D, Humphrey S, Chain E (1973) Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release. J Mol Cell Cardiol 5(4):395–407
5. Guarnieri C, Flamigni F, Caldarera C (1980) Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. J Mol Cell Cardiol 12(8):797–808
6. Granger DN, Rutili G, McCord JM (1981) Superoxide radicals in feline intestinal ischemia. Gastroenterology 81(1):22–29
7. Granger DN, Kvietys PR (2015) Reperfusion injury and reactive oxygen species: the evolution of a concept. Redox Biol 6:524–551
8. Davidson SM, Yellon DM, Murphy MP, Duchen MR (2011) Slow calcium waves and redox changes precede mitochondrial permeability transition pore opening in the intact heart during hypoxia and reoxygenation. Cardiovasc Res 93(3):445–453
9. Frangogiannis NG (2015) Pathophysiology of myocardial infarction. Compr Physiol 5(4):1841–1875
10. Spann JF Jr, Moellering RC Jr, Haber E, Wheeler EO (1964) Arrhythmias in acute myocardial infarction: a study utilizing an electrocardiographic monitor for automatic detection and recording of arrhythmias. N Engl J Med 271(9):427–431
11. Bajaj A, Sethi A, Rathor P, Suppogu N, Sethi A (2015) Acute complications of myocardial infarction in the current era: diagnosis and management. J Investig Med 63(7):844–855
12. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S et al (2018) Heart disease and stroke statistics-2018 Update: a report from the American Heart Association. Circulation 137(12):e67–e492
13. Yellon DM, Hausenloy DJ (2007) Myocardial reperfusion injury. N Engl J Med 357(11):1121–1135
14. Puymirat E, Simon T, Cayla G, Cottin Y, Elbaz M, Coste P et al (2017) Acute myocardial infarction: changes in patient characteristics, management, and 6-month outcomes over a period of 20 years in the FAST-MI program (French Registry of Acute ST-Elevation or Non-ST-Elevation Myocardial Infarction) 1995 to 2015. Circulation 136(20):1908–1919

15. Ferdinandy P, Schulz R, Baxter GF (2007) Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev 59:418–458
16. Ashraf M, Enthammer M, Haller M, Koziel K, Hermann M, Troppmair J (2012) Intracellular signaling in ischemia/reperfusion injury (IRI): from mechanistic insights to therapeutic options. J Transplant Technol Res 3:002
17. Liu T, O'Rourke B (2013) Regulation of Na^+/Ca^{2+} exchanger by pyridine nucleotide redox potential in ventricular myocytes. J Biol Chem M113:496588
18. Seidlmayer LK, Juettner VV, Kettlewell S, Pavlov EV, Blatter LA, Dedkova EN (2015) Distinct mPTP activation mechanisms in ischaemia–reperfusion: contributions of Ca^{2+}, ROS, pH, and inorganic polyphosphate. Cardiovasc Res 106(2):237–248
19. Avkiran M, Marber MS (2002) Na+/H+ exchange inhibitors for cardioprotective therapy: progress, problems and prospects. J Am Coll Cardiol 39(5):747–753
20. Zhou T, Chuang CC, Zuo L (2015) Molecular characterization of reactive oxygen species in myocardial ischemia-reperfusion injury. Biomed Res Int 2015:864946
21. Monti M, Donnini S, Giachetti A, Mochly-Rosen D, Ziche M (2010) δPKC inhibition or εPKC activation repairs endothelial vascular dysfunction by regulating eNOS post-translational modification. J Mol Cell Cardiol 48(4):746–756
22. Vina J, Borras C, Abdelaziz KM, Garcia-Valles R, Gomez-Cabrera MC (2013) The free radical theory of aging revisited: the cell signaling disruption theory of aging. Antioxid Redox Signal 19(8):779–787
23. Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24(5):981–990
24. Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417(1):1–13
25. Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 94(3):909–950
26. Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL et al (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 515(7527):431
27. Chouchani ET, Pell VR, James AM, Work LM, Saeb-Parsy K, Frezza C et al (2016) A unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury. Cell Metab 23(2):254–263
28. VanTeeffelen JW (2008) How to prevent leaky vessels during reperfusion? Just keep that glycocalyx sealant in place! Critical care (London, England) 12(4):167
29. Slegtenhorst BR, Dor FJ, Rodriguez H, Voskuil FJ, Tullius SG (2014) Ischemia/reperfusion injury and its consequences on immunity and inflammation. Curr Transplant Rep 1(3):147–154
30. Vinten-Johansen J (2004) Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. Cardiovasc Res 61(3):481–497
31. Perkins K-AA, Pershad S, Chen Q, McGraw S, Adams JS, Zambrano C et al (2012) The effects of modulating eNOS activity and coupling in ischemia/reperfusion (I/R). Naunyn Schmiedeberg's Arch Pharmacol 385(1):27–38
32. Forstermann U, Munzel T (2006) Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113(13):1708–1714
33. Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA et al (2002) Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature 416(6878):337–339
34. Oess S, Icking A, Fulton D, Govers R, Muller-Esterl W (2006) Subcellular targeting and trafficking of nitric oxide synthases. Biochem J 396(3):401–409
35. Paulus WJ, Bronzwaer JG (2002) Myocardial contractile effects of nitric oxide. Heart Fail Rev 7(4):371–383
36. Fleming I (2010) Molecular mechanisms underlying the activation of eNOS. Pflugers Arch – Eur J Physiol 459(6):793–806

37. Godinez-Rubi M, Rojas-Mayorquin AE, Ortuno-Sahagun D (2013) Nitric oxide donors as neuroprotective agents after an ischemic stroke-related inflammatory reaction. Oxidative Med Cell Longev 2013:297357
38. Roberts BW, Mitchell J, Kilgannon JH, Chansky ME, Trzeciak S (2013) Nitric oxide donor agents for the treatment of ischemia/reperfusion injury in human subjects: a systematic review. Shock (Augusta, Ga) 39(3):229–239
39. Chen W, Druhan LJ, Chen CA, Hemann C, Chen YR, Berka V et al (2010) Peroxynitrite induces destruction of the tetrahydrobiopterin and heme in endothelial nitric oxide synthase: transition from reversible to irreversible enzyme inhibition. Biochemistry 49(14):3129–3137
40. Zhang Y, Tocchetti CG, Krieg T, Moens AL (2012) Oxidative and nitrosative stress in the maintenance of myocardial function. Free Radic Biol Med 53(8):1531–1540
41. Alkaitis MS, Crabtree MJ (2012) Recoupling the cardiac nitric oxide synthases: tetrahydrobiopterin synthesis and recycling. Curr Heart Failure Rep 9(3):200–210
42. Crabtree MJ, Tatham AL, Al-Wakeel Y, Warrick N, Hale AB, Cai S et al (2009) Quantitative regulation of intracellular endothelial nitric-oxide synthase (eNOS) coupling by both tetrahydrobiopterin-eNOS stoichiometry and biopterin redox status: insights from cells with tet-regulated GTP cyclohydrolase I expression. J Biol Chem 284(2):1136–1144
43. De Pascali F, Hemann C, Samons K, Chen C-A, Zweier JL (2014) Hypoxia and reoxygenation induce endothelial nitric oxide synthase uncoupling in endothelial cells through tetrahydrobiopterin depletion and S-glutathionylation. Biochemistry 53(22):3679–3688
44. Siu KL, Lotz C, Ping P, Cai H (2015) Netrin-1 abrogates ischemia/reperfusion-induced cardiac mitochondrial dysfunction via nitric oxide-dependent attenuation of NOX4 activation and recoupling of NOS. J Mol Cell Cardiol 78:174–185
45. Pernow J, Jung C (2013) Arginase as a potential target in the treatment of cardiovascular disease: reversal of arginine steal? Cardiovasc Res 98(3):334–343
46. Giraldez RR, Panda A, Xia Y, Sanders SP, Zweier JL (1997) Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart. J Biol Chem 272(34):21420–21426
47. Mialet-Perez J, Bianchi P, Kunduzova O, Parini A (2007) New insights on receptor-dependent and monoamine oxidase-dependent effects of serotonin in the heart. J Neural Transm (Vienna, Austria: 1996) 114(6):823–827
48. Manni ME, Rigacci S, Borchi E, Bargelli V, Miceli C, Giordano C et al (2016) Monoamine oxidase is overactivated in left and right ventricles from ischemic hearts: an intriguing therapeutic target. Oxidative Med Cell Longev 2016:4375418
49. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C et al (2005) Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell 122(2):221–233
50. George J, Struthers AD (2009) Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress. Vasc Health Risk Manag 5(1):265–272
51. Wang G, Qian P, Jackson FR, Qian G, Wu G (2008) Sequential activation of JAKs, STATs and xanthine dehydrogenase/oxidase by hypoxia in lung microvascular endothelial cells. Int J Biochem Cell Biol 40(3):461–470
52. Berry CE, Hare JM (2004) Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol 555(Pt 3):589–606
53. Cantu-Medellin N, Kelley EE (2013) Xanthine oxidoreductase-catalyzed reactive species generation: a process in critical need of reevaluation. Redox Biol 1:353–358
54. Maia LB, Pereira V, Mira L, Moura JJ (2015) Nitrite reductase activity of rat and human xanthine oxidase, xanthine dehydrogenase, and aldehyde oxidase: evaluation of their contribution to NO formation in vivo. Biochemistry 54(3):685–710
55. Shafik AN (2013) Febuxostat improves the local and remote organ changes induced by intestinal ischemia/reperfusion in rats. Dig Dis Sci 58(3):650–659
56. Kahles T, Brandes RP (2013) Which NADPH oxidase isoform is relevant for ischemic stroke? The case for nox 2. Antioxid Redox Signal 18(12):1400–1417

57. Brandes RP, Weissmann N, Schroder K (2010) NADPH oxidases in cardiovascular disease. Free Radic Biol Med 49(5):687–706
58. Doerries C, Grote K, Hilfiker-Kleiner D, Luchtefeld M, Schaefer A, Holland SM et al (2007) Critical role of the NAD(P)H oxidase subunit p47phox for left ventricular remodeling/dysfunction and survival after myocardial infarction. Circ Res 100(6):894–903
59. Lambeth JD, Krause KH, Clark RA (2008) NOX enzymes as novel targets for drug development. Semin Immunopathol 30(3):339–363
60. Kleikers PW, Wingler K, Hermans JJ, Diebold I, Altenhofer S, Radermacher KA et al (2012) NADPH oxidases as a source of oxidative stress and molecular target in ischemia/reperfusion injury. J Mol Med (Berlin, Germany) 90(12):1391–1406
61. Simone S, Rascio F, Castellano G, Divella C, Chieti A, Ditonno P et al (2014) Complement-dependent NADPH oxidase enzyme activation in renal ischemia/reperfusion injury. Free Radic Biol Med 74:263–273
62. Gan X, Su G, Zhao W, Huang P, Luo G, Hei Z (2013) The mechanism of sevoflurane preconditioning-induced protections against small intestinal ischemia reperfusion injury is independent of mast cell in rats. Mediat Inflamm 2013:378703
63. Mittal M, Roth M, Konig P, Hofmann S, Dony E, Goyal P et al (2007) Hypoxia-dependent regulation of nonphagocytic NADPH oxidase subunit NOX4 in the pulmonary vasculature. Circ Res 101(3):258–267
64. Nanduri J, Vaddi DR, Khan SA, Wang N, Makarenko V, Semenza GL et al (2015) HIF-1alpha activation by intermittent hypoxia requires NADPH oxidase stimulation by xanthine oxidase. PLoS One 10(3):e0119762
65. Nguyen Dinh Cat A, Montezano AC, Burger D, Touyz RM (2013) Angiotensin II, NADPH oxidase, and redox signaling in the vasculature. Antioxid Redox Signal 19(10):1110–1120
66. Sanderson TH, Reynolds CA, Kumar R, Przyklenk K, Huttemann M (2013) Molecular mechanisms of ischemia-reperfusion injury in brain: pivotal role of the mitochondrial membrane potential in reactive oxygen species generation. Mol Neurobiol 47(1):9–23
67. Cadenas E (2004) Mitochondrial free radical production and cell signaling. Mol Asp Med 25(1-2):17–26
68. Drose S, Brandt U (2012) Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol 748:145–169
69. Pasdois P, Parker JE, Griffiths EJ, Halestrap AP (2011) The role of oxidized cytochrome c in regulating mitochondrial reactive oxygen species production and its perturbation in ischaemia. Biochem J 436(2):493–505
70. Chen Q, Lesnefsky EJ (2006) Depletion of cardiolipin and cytochrome c during ischemia increases hydrogen peroxide production from the electron transport chain. Free Radic Biol Med 40(6):976–982
71. Bleier L, Wittig I, Heide H, Steger M, Brandt U, Drose S (2015) Generator-specific targets of mitochondrial reactive oxygen species. Free Radic Biol Med 78:1–10
72. Holzerova E, Prokisch H (2015) Mitochondria: much ado about nothing? How dangerous is reactive oxygen species production? Int J Biochem Cell Biol 63:16–20
73. Aon MA, Cortassa S, Akar FG, Brown DA, Zhou L, O'Rourke B (2009) From mitochondrial dynamics to arrhythmias. Int J Biochem Cell Biol 41(10):1940–1948
74. Jena N, Mishra P (2012) Formation of ring-opened and rearranged products of guanine: mechanisms and biological significance. Free Radic Biol Med 53(1):81–94
75. Niles JC, Wishnok JS, Tannenbaum SR (2006) Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: structures and mechanisms of product formation. Nitric Oxide 14(2):109–121
76. Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Med Cell Longev 2014:360438
77. Musiek ES, Yin H, Milne GL, Morrow JD (2005) Recent advances in the biochemistry and clinical relevance of the isoprostane pathway. Lipids 40(10):987–994

78. Xu X, Muller JG, Ye Y, Burrows CJ (2008) DNA− protein cross-links between guanine and lysine depend on the mechanism of oxidation for formation of C5 vs C8 guanosine adducts. J Am Chem Soc 130(2):703–709
79. Krijnen PA, Nijmeijer R, Meijer CJ, Visser CA, Hack CE, Niessen HW (2002) Apoptosis in myocardial ischaemia and infarction. J Clin Pathol 55(11):801–811
80. Kloner RA, Jennings RB (2001) Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 2. Circulation 104(25):3158–3167
81. Stadtman E, Levine R (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25(3-4):207–218
82. Peluffo G, Radi R (2007) Biochemistry of protein tyrosine nitration in cardiovascular pathology. Cardiovasc Res 75(2):291–302
83. Kuznetsov AV, Smigelskaite J, Doblander C, Janakiraman M, Hermann M, Wurm M et al (2008) Survival signaling by C-RAF: mitochondrial reactive oxygen species and Ca^{2+} are critical targets. Mol Cell Biol 28(7):2304–2313
84. Lehwald N, Tao GZ, Jang KY, Sorkin M, Knoefel WT, Sylvester KG (2011) Wnt–β-catenin signaling protects against hepatic ischemia and reperfusion injury in mice. Gastroenterology 141(2):707-18. e5
85. Yu HC, Qin HY, He F, Wang L, Fu W, Liu D et al (2011) Canonical notch pathway protects hepatocytes from ischemia/reperfusion injury in mice by repressing reactive oxygen species production through JAK2/STAT3 signaling. Hepatology 54(3):979–988
86. Zhu Y, Prives C (2009) P53 and metabolism: the GAMT connection. Mol Cell 36(3):351–352
87. Acin-Perez R, Salazar E, Kamenetsky M, Buck J, Levin LR, Manfredi G (2009) Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. Cell Metab 9(3):265–276
88. Brodie C, Blumberg P (2003) Regulation of cell apoptosis by protein kinase c δ. Apoptosis 8(1):19–27
89. Cohen MV, Yang X-M, Downey JM (2007) The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. Circulation 115(14):1895–1903
90. Zhang Z, Feng H-Z, Jin J-P (2011) Structure of the NH2-terminal variable region of cardiac troponin T determines its sensitivity to restrictive cleavage in pathophysiological adaptation. Arch Biochem Biophys 515(1-2):37–45
91. Sung MM, Schulz CG, Wang W, Sawicki G, Bautista-López NL, Schulz R (2007) Matrix metalloproteinase-2 degrades the cytoskeletal protein α-actinin in peroxynitrite mediated myocardial injury. J Mol Cell Cardiol 43(4):429–436
92. Hernando V, Inserte J, Sartório CL, Parra VM, Poncelas-Nozal M, Garcia-Dorado D (2010) Calpain translocation and activation as pharmacological targets during myocardial ischemia/reperfusion. J Mol Cell Cardiol 49(2):271–279
93. Perrelli M-G, Pagliaro P, Penna C (2011) Ischemia/reperfusion injury and cardioprotective mechanisms: role of mitochondria and reactive oxygen species. World J Cardiol 3(6):186–200
94. Perraud A-L, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C et al (2001) ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature 411(6837):595
95. Yang K, Chang W, Yang P, Chien C-L, Lai M, Su M et al (2006) Activation of the transient receptor potential M2 channel and poly (ADP-ribose) polymerase is involved in oxidative stress-induced cardiomyocyte death. Cell Death Differ 13(10):1815
96. Camara AK, Bienengraeber M, Stowe DF (2011) Mitochondrial approaches to protect against cardiac ischemia and reperfusion injury. Front Physiol 2:13
97. Ong S-B, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ (2010) Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation 121(18):2012–2022
98. Zorov DB, Juhaszova M, Sollott SJ (2006) Mitochondrial ROS-induced ROS release: an update and review. Biochim Biophys Acta (BBA)-Bioenergetics 1757(5–6):509–517
99. Kalogeris T, Bao Y, Korthuis RJ (2014) Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox Biol 2:702–714

100. Rogers PA, Chilian WM, Bratz IN, Bryan RM Jr, Dick GM (2007) H_2O_2 activates redox- and 4-aminopyridine-sensitive Kv channels in coronary vascular smooth muscle. Am J Physiol Heart Circ Physiol 292(3):H1404–H1411
101. Ytrehus K, Liu Y, Downey JM (1994) Preconditioning protects ischemic rabbit heart by protein kinase C activation. Am J Phys 266(3 Pt 2):H1145–H1152
102. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74(5):1124–1136
103. Wang Q, Sun AY, Simonyi A, Kalogeris TJ, Miller DK, Sun GY et al (2007) Ethanol preconditioning protects against ischemia/reperfusion-induced brain damage: role of NADPH oxidase-derived ROS. Free Radic Biol Med 43(7):1048–1060
104. Zhang DX, Gutterman DD (2007) Mitochondrial reactive oxygen species-mediated signaling in endothelial cells. Am J Physiol Heart Circ Physiol 292(5):H2023–H2031
105. Xi Q, Cheranov SY, Jaggar JH (2005) Mitochondria-derived reactive oxygen species dilate cerebral arteries by activating Ca^{2+} sparks. Circ Res 97(4):354–362
106. Quindry JC, Hamilton KL (2013) Exercise and cardiac preconditioning against ischemia reperfusion injury. Curr Cardiol Rev 9(3):220–229
107. Ascensao A, Ferreira R, Magalhaes J (2007) Exercise-induced cardioprotection – biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. Int J Cardiol 117(1):16–30
108. Frasier CR, Moore RL, Brown DA (2011) Exercise-induced cardiac preconditioning: how exercise protects your achy-breaky heart. J Appl Physiol (1985) 111(3):905–915
109. Boengler K, Heusch G, Schulz R (2011) Mitochondria in postconditioning. Antioxid Redox Signal 14(5):863–880
110. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ et al (2010) Postconditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res 87(3):406–423
111. Skyschally A, van Caster P, Iliodromitis EK, Schulz R, Kremastinos DT, Heusch G (2009) Ischemic postconditioning: experimental models and protocol algorithms. Basic Res Cardiol 104(5):469–483
112. Hausenloy DJ, Wynne AM, Yellon DM (2007) Ischemic preconditioning targets the reperfusion phase. Basic Res Cardiol 102(5):445–452
113. Cohen MV, Yang XM, Downey JM (2008) Acidosis, oxygen, and interference with mitochondrial permeability transition pore formation in the early minutes of reperfusion are critical to postconditioning's success. Basic Res Cardiol 103(5):464–471
114. Rossello X, Yellon DM (2017) The RISK pathway and beyond. Basic Res Cardiol 113(1):2
115. Zouein FA, Altara R, Chen Q, Lesnefsky EJ, Kurdi M, Booz GW (2015) Pivotal importance of STAT3 in protecting the heart from acute and chronic stress: new advancement and unresolved issues. Front Cardiovasc Med 2:36
116. Shlafer M, Kane PF, Kirsh MM (1982) Superoxide dismutase plus catalase enhances the efficacy of hypothermic cardioplegia to protect the globally ischemic, reperfused heart. J Thorac Cardiovasc Surg 83(6):830–839
117. Sies H (1993) Strategies of antioxidant defense. Eur J Biochem 215(2):213–219
118. Ambrosio G, Zweier JL, Jacobus WE, Weisfeldt ML, Flaherty JT (1987) Improvement of postischemic myocardial function and metabolism induced by administration of deferoxamine at the time of reflow: the role of iron in the pathogenesis of reperfusion injury. Circulation 76(4):906–915
119. Arroyo CM, Kramer JH, Dickens BF, Weglicki WB (1987) Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitrone DMPO. FEBS Lett 221(1):101–104
120. Arroyo CM, Kramer JH, Leiboff RH, Mergner GW, Dickens BF, Weglicki WB (1987) Spin trapping of oxygen and carbon-centered free radicals in ischemic canine myocardium. Free Radic Biol Med 3(5):313–316

121. Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB (1988) Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest 82(2):476–485
122. Garlick PB, Davies MJ, Hearse DJ, Slater TF (1987) Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. Circ Res 61(5):757–760
123. Corretti MC, Koretsune Y, Kusuoka H, Chacko VP, Zweier JL, Marban E (1991) Glycolytic inhibition and calcium overload as consequences of exogenously generated free radicals in rabbit hearts. J Clin Invest 88(3):1014–1025
124. Josephson RA, Silverman HS, Lakatta EG, Stern MD, Zweier JL (1991) Study of the mechanisms of hydrogen peroxide and hydroxyl free radical-induced cellular injury and calcium overload in cardiac myocytes. J Biol Chem 266(4):2354–2361
125. Xia Y, Zweier JL (1995) Substrate control of free radical generation from xanthine oxidase in the postischemic heart. J Biol Chem 270(32):18797–18803
126. Webb A, Bond R, McLean P, Uppal R, Benjamin N, Ahluwalia A (2004) Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. Proc Natl Acad Sci U S A 101(37):13683–13688
127. Victorino GP, Ramirez RM, Chong TJ, Curran B, Sadjadi J (2008) Ischemia-reperfusion injury in rats affects hydraulic conductivity in two phases that are temporally and mechanistically separate. Am J Phys Heart Circ Phys 295(5):H2164–H2H71
128. Borchi E, Parri M, Papucci L, Becatti M, Nassi N, Nassi P et al (2009) Role of NADPH oxidase in H9c2 cardiac muscle cells exposed to simulated ischaemia-reperfusion. J Cell Mol Med 13(8b):2724–2735
129. Donoso P, Finkelstein JP, Montecinos L, Said M, Sánchez G, Vittone L et al (2014) Stimulation of NOX2 in isolated hearts reversibly sensitizes RyR2 channels to activation by cytoplasmic calcium. J Mol Cell Cardiol 68:38–46
130. Braunersreuther V, Montecucco F, Ashri M, Pelli G, Galan K, Frias M et al (2013) Role of NADPH oxidase isoforms NOX1, NOX2 and NOX4 in myocardial ischemia/reperfusion injury. J Mol Cell Cardiol 64:99–107
131. Dworakowski R, Anilkumar N, Zhang M, Shah A (2006) Redox signalling involving NADPH oxidase-derived reactive oxygen species. Portland Press Limited, London
132. Chambers DE, Parks DA, Patterson G, Roy R, McCord JM, Yoshida S et al (1985) Xanthine oxidase as a source of free radical damage in myocardial ischemia. J Mol Cell Cardiol 17(2):145–152
133. Webb A, Bond R, McLean P, Uppal R, Benjamin N, Ahluwalia A (2004) Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia–reperfusion damage. Proc Natl Acad Sci 101(37):13683–13688
134. Lesnefsky EJ, Chen Q, Moghaddas S, Hassan MO, Tandler B, Hoppel CL (2004) Blockade of electron transport during ischemia protects cardiac mitochondria. J Biol Chem 279(46):47961–47967
135. White MY, Tchen AS, McCarron HC, Hambly BD, Jeremy RW, Cordwell SJ (2006) Proteomics of ischemia and reperfusion injuries in rabbit myocardium with and without intervention by an oxygen-free radical scavenger. Proteomics 6(23):6221–6233
136. Liu B, Tewari AK, Zhang L, Green-Church KB, Zweier JL, Chen YR et al (2009) Proteomic analysis of protein tyrosine nitration after ischemia reperfusion injury: mitochondria as the major target. Biochim Biophys Acta 1794(3):476–485
137. Zhang L, Chen C-L, Kang PT, Garg V, Hu K, Green-Church KB et al (2010) Peroxynitrite-mediated oxidative modifications of complex II: relevance in myocardial infarction. Biochemistry 49(11):2529–2539
138. Viappiani S, Nicolescu AC, Holt A, Sawicki G, Crawford BD, León H et al (2009) Activation and modulation of 72 kDa matrix metalloproteinase-2 by peroxynitrite and glutathione. Biochem Pharmacol 77(5):826–834
139. Fert-Bober J, Leon H, Sawicka J, Basran RS, Devon RM, Schulz R et al (2008) Inhibiting matrix metalloproteinase-2 reduces protein release into coronary effluent from isolated rat hearts during ischemia-reperfusion. Basic Res Cardiol 103(5):431–443

140. Leon H, Baczko I, Sawicki G, Light PE, Schulz R (2008) Inhibition of matrix metalloproteinases prevents peroxynitrite-induced contractile dysfunction in the isolated cardiac myocyte. Br J Pharmacol 153(4):676–683
141. Jena N, Kushwaha P, Mishra P (2008) Reaction of hypochlorous acid with imidazole: formation of 2-chloro-and 2-oxoimidazoles. J Comput Chem 29(1):98–107
142. Shukla P, Jena N, Mishra P (2011) Quantum theoretical study of molecular mechanisms of mutation and cancer-a review. Proc Natl Acad Sci India Sect A-Phys Sci 81:79–98
143. Minko IG, Harbut MB, Kozekov ID, Kozekova A, Jakobs PM, Olson SB et al (2008) Role for DNA polymerase kappa in the processing of N2-N2-guanine interstrand crosslinks. J Biol Chem
144. Loor G, Kondapalli J, Iwase H, Chandel NS, Waypa GB, Guzy RD et al (2011) Mitochondrial oxidant stress triggers cell death in simulated ischemia-reperfusion. Biochim Biophys Acta 1813(7):1382–1394
145. Sugamura K, Keaney JF Jr (2011) Reactive oxygen species in cardiovascular disease. Free Radic Biol Med 51(5):978–992
146. Xue Y-Z, Wang L-X, Liu H-Z, Qi X-W, Wang X-H, Ren H-Z (2007) L-carnitine as an adjunct therapy to percutaneous coronary intervention for non-ST elevation myocardial infarction. Cardiovasc Drugs Ther 21(6):445–448
147. Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP et al (2005) Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. FASEB J 19(9):1088–1095
148. Anisimov VN, Egorov MV, Krasilshchikova MS, Lyamzaev KG, Manskikh VN, Moshkin MP et al (2011) Effects of the mitochondria-targeted antioxidant SkQ1 on lifespan of rodents. Aging (Albany NY) 3(11):1110
149. Birk A, Chao W, Bracken C, Warren J, Szeto H (2014) Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. Br J Pharmacol 171(8):2017–2028
150. Kloner RA, Hale SL, Dai W, Gorman RC, Shuto T, Koomalsingh KJ et al (2012) Reduction of ischemia/reperfusion injury with bendavia, a mitochondria-targeting cytoprotective peptide. J Am Heart Assoc 1(3):e001644
151. Chakrabarti AK, Feeney K, Abueg C, Brown DA, Czyz E, Tendera M et al (2013) Rationale and design of the EMBRACE STEMI study: a phase 2a, randomized, double-blind, placebo-controlled trial to evaluate the safety, tolerability and efficacy of intravenous Bendavia on reperfusion injury in patients treated with standard therapy including primary percutaneous coronary intervention and stenting for ST-segment elevation myocardial infarction. Am Heart J 165(4):509-14. e7
152. Dai D-F, Johnson SC, Villarin JJ, Chin MT, Nieves-Cintrón M, Chen T et al (2011) Mitochondrial oxidative stress mediates angiotensin II–induced cardiac hypertrophy and Gαq overexpression–induced heart failure. Circ Res 108(7):837–846
153. Yeon J-Y, Min S-H, Park H-J, Kim J-W, Lee Y-H, Park S-Y et al (2015) Mdivi-1, mitochondrial fission inhibitor, impairs developmental competence and mitochondrial function of embryos and cells in pigs. J Reprod Dev 61(2):81–89
154. Qi X, Qvit N, Su Y-C, Mochly-Rosen D (2012) Novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. J Cell Sci 126:789
155. Disatnik MH, Ferreira JC, Campos JC, Gomes KS, Dourado PM, Qi X et al (2013) Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. J Am Heart Assoc 2(5):e000461
156. Yogalingam G, Hwang S, Ferreira JC, Mochly-Rosen D (2013) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) phosphorylation by protein kinase C delta (δPKC) inhibits mitochondrial elimination by lysosomal-like structures following ischemia and reoxygenation-induced injury. J Biol Chem M113:466870
157. Kabir AM, Clark JE, Tanno M, Cao X, Hothersall JS, Dashnyam S et al (2006) Cardioprotection initiated by reactive oxygen species is dependent on activation of PKCε. Am J Phys Heart Circ Phys 291(4):H1893–H18H9

158. Budas GR, Churchill EN, Disatnik M-H, Sun L, Mochly-Rosen D (2010) Mitochondrial import of PKCε is mediated by HSP90: a role in cardioprotection from ischaemia and reperfusion injury. Cardiovasc Res 88(1):83–92
159. Heinzel FR, Lisa F (2005) Impairment of diazoxide-induced formation of reactive oxygen species and loss of cardioprotection in connexin 43 deficient mice. Circ Res 97:583–586
160. Badalzadeh R, Yousefi B, Tajaddini A, Ahmadian N (2015) Diosgenin-induced protection against myocardial ischaemia-reperfusion injury is mediated by mitochondrial KATP channels in a rat model. Perfusion 30(7):565–571
161. Zhao Z, Cui W, Zhang H, Gao H, Li X, Wang Y et al (2015) Pre-treatment of a single high-dose of atorvastatin provided cardioprotection in different ischaemia/reperfusion models via activating mitochondrial KATP channel. Eur J Pharmacol 751:89–98
162. Beretta M, Gorren AC, Wenzl MV, Weis R, Russwurm M, Koesling D et al (2009) Characterization of the East Asian variant of aldehyde dehydrogenase-2: bioactivation of nitroglycerin and effects of Alda-1. J Biol Chem M109:014548
163. Gong D, Zhang Y, Zhang H, Gu H, Jiang Q, Hu S (2012) Aldehyde dehydrogenase-2 activation during cardioplegic arrest enhances the cardioprotection against myocardial ischemia–reperfusion injury. Cardiovasc Toxicol 12(4):350–358
164. Gomes KM, Bechara LR, Lima VM, Ribeiro MA, Campos JC, Dourado PM et al (2015) Aldehydic load and aldehyde dehydrogenase 2 profile during the progression of post-myocardial infarction cardiomyopathy: benefits of Alda-1. Int J Cardiol 179:129–138
165. Ebert AD, Kodo K, Liang P, Wu H, Huber BC, Riegler J et al (2014) Characterization of the molecular mechanisms underlying increased ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism using a human induced pluripotent stem cell model system. Sci Transl Med 6(255):255ra130–255ra130