REVIEW ARTICLE

Neutrophil-Mediated Cardiac Damage After Acute Myocardial Infarction: Significance of Defining a New Target Cell Type for Developing Cardioprotective Drugs

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Abstract

Significance: Acute myocardial infarction (AMI) is a leading cause of death worldwide. Post-AMI survival rates have increased with the introduction of angioplasty as a primary coronary intervention. However, reperfusion after angioplasty represents a clinical paradox, restoring blood flow to the ischemic myocardium while simultaneously inducing ion and metabolic imbalances that stimulate immune cell recruitment and activation, mitochondrial dysfunction and damaging oxidant production.

Recent Advances: Preclinical data indicate that these metabolic imbalances contribute to subsequent heart failure through sustaining local recruitment of inflammatory leukocytes and oxidative stress, cardiomyocyte death, and coronary microvascular disturbances, which enhance adverse cardiac remodeling. Both left ventricular dysfunction and heart failure are strongly linked to inflammation and immune cell recruitment to the damaged myocardium.

Critical Issues: Overall, therapeutic anti-inflammatory and antioxidant agents identified in preclinical trials have failed in clinical trials.

Future Directions: The versatile neutrophil-derived heme enzyme, myeloperoxidase (MPO), is gaining attention as an important oxidative mediator of reperfusion injury, vascular dysfunction, adverse ventricular remodeling, and atrial fibrillation. Accordingly, there is interest in therapeutically targeting neutrophils and MPO activity in the setting of heart failure. Herein, we discuss the role of post-AMI inflammation linked to myocardial damage and heart failure, describe previous trials targeting inflammation and oxidative stress post-AMI, highlight the potential adverse impact of neutrophil and MPO, and detail therapeutic options available to target MPO clinically in AMI patients. *Antioxid. Redox Signal.* 33, 689–712.

Keywords: myocardial infarction, ischemia reperfusion, myeloperoxidase, nitroxides

Introduction

CORONARY ARTERY DISEASE (CAD) is generally asymptomatic for the first four decades of life; thereafter, the risk of the onset of clinical complications caused by acute cardiac ischemia or myocardial infarction (MI) increases.

The primary cause of CAD is the accumulation of coronary artery atherosclerotic plaques that narrow the lumen, thereby decreasing blood flow and cardiac function. Myocardial ischemia induces hypoxic and oxidative responses in both cardiac cells and the vasculature, which together promote myocardial tissue damage (21, 121), often presenting as

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angina or fatigue on physical exertion, as the heart continues to work under a limited oxygen supply. Often, the first indication of ischemia is a heart attack or acute coronary syndrome (ACS) triggered by coronary artery occlusion or acute rupture of an atherosclerotic plaque. Myocardial ischemia and infarction increase the risk of subsequent heart failure by weakening or irreversibly compromising the function of the affected myocardium. Together, CAD and heart failure are leading causes of mortality and morbidity worldwide (133).

The pathogenesis of atherosclerosis is characterized by a chronic inflammatory response within the arterial intima that facilitates excessive lipid accumulation (both native and oxidized) in the arterial sub-endothelium and atheromatous plaque formation (175). A key early atherogenic event precipitated by cardiovascular risk factors such as hyperlipidemia, hyperglycemia, smoking, or hypertension is endothelial dysfunction, which manifests as impaired bioactivity of the vasoprotective signaling molecule nitric oxide (NO), as well as increased vascular permeability and endothelial-leukocyte interactions (184) (Fig. 1A). These events facilitate the infiltration of activated immune cells and low-density lipoprotein into the sub-endothelial space (175).

This initial innate immune response is likely aimed at negating the atherogenic stimuli (93). However, the continued presence of cardiovascular risk factors manifests as a chronic inflammatory/immune response that promotes cycles of vascular insult, inflammation, and remodeling (92). Thus, monocytes recruited by the inflamed endothelium into the sub-intimal space differentiate into macrophages that accumulate excessive amounts of lipid to form foam cells; the hallmark of early vascular lesions (152, 175) (Fig. 1B). Continued intimal inflammation underpins the transition of these early lesions to more complex atheromatous plaques characterized by a fibrous cap and a necrotic core, derived from dying cells and the extracellular accumulation of cholesterol crystals (Fig. 1C) (152, 175). Progressive inflammation at the shoulder regions of advanced plaques can signal for smooth muscle cell apoptosis, increased proteolytic matrix metalloproteinase (MMP) production, and fibrous cap thinning (Fig. 1C); this series of events may lead to acute plaque rupture and formation of occlusive luminal thrombi in the coronary artery to precipitate an acute myocardial infarction (AMI), resulting in ischemic cardiomyocyte death (121) (Fig. 1D).

Cardiomyocyte death in the sub-endocardium occurs within minutes of AMI onset and progresses to the epicardium with prolonged ischemia. Ischemia facilitates cardiomyocyte ATP deficiency and alterations in intracellular ion gradients (especially Ca^{2+}), thereby promoting mitochondrial dysfunction and decreased cell viability (121). These events manifest as the pathological production of reactive oxygen species (ROS) and stimulation of the innate immune response, which, in turn, further promote tissue damage including cardiomyocyte death (126).

Prolonged ischemia within the infarct region may expand after the initial event and induce cardiac dysfunction in the adjacent myocardium. Loss of cardiac contractility after AMI is a common cause of chronic heart failure (35). To circumvent this complication, clinical intervention aims at rapidly restoring blood flow and preventing further infarction by rapidly opening occluded coronary arteries, which has led to a decrease in mortality rates (72). Paradoxically, reperfu-

sion therapy that is critical to recovery of the affected myocardium also results in subsequent myocardial dysfunction that correlates with increased heart failure rates within the same patient cohort (196). It is now recognized that reperfusion after myocardial ischemia elicits inflammation that also yields oxidative host-tissue injury, which together adversely affects myocardial cells and instigates cardiac complications after AMI (38). This review discusses the implications of the post-AMI inflammatory response in driving subsequent heart failure with a focus on the adverse role of neutrophils and their potential as therapeutic targets to limit chronic myocardial dysfunction.

Myocardial Ischemia and Reperfusion Injury

The myocardium becomes significantly altered both during and after an AMI, culminating in the loss of contractility and cell death in the infarct region (21). The biochemical and cellular events that facilitate these changes are specifically related to the disparate, yet inter-linked processes of ischemia and reperfusion (Fig. 2). Ischemia deprives the myocardium of nutrients and oxygen distal to the site of coronary occlusion, rendering cardiomyocytes unable to undergo aerobic metabolism to support their energy requirements (121). The low oxygen environment is rapidly sensed by homeostatic intracellular signaling systems, such as the hypoxia inducible factor (HIF) transcription factor cascade (87), which induces the upregulation of numerous compensatory genes involved in elevating anaerobic glycolysis and promoting angiogenesis and vascularization (71, 108). However, increased anaerobic metabolism yields lactic acid that provokes acidosis and myocyte death (99) and, therefore, expansion of the primary infarct area (Fig. 2).

Considering the relatively high requirement of the myocardium for ATP, this shift in energy production to glycolysis results in the inefficient production of ATP and constitutes a pathological feature, which needs to be quickly reversed to protect against the progression toward detrimental complications such as heart failure and ischemia-induced ventricular fibrillation (11, 44). The uncoupling of glycolysis and glucose oxidation has recently been identified as an early contributing factor in the progression toward heart failure with preserved ejection fraction (44). Accordingly, restoration of coupling between glucose oxidation and glycolysis, by inhibition of fatty acid oxidation, has been reported to improve cardiac function in a rat model of AMI induced by the permanent ligation of the left anterior descending coronary artery (200). Decreased glucose oxidation and increased anaerobic glycolysis is also observed during postischemia ventricular fibrillation, with the addition of a stimulator of pyruvate dehydrogenase noted to decrease anaerobic glycolysis, induce glucose oxidation, and mitigate postischemic myocardial contractile dysfunction (11) (Fig. 2).

In addition, ischemia post-AMI decreases Na^+/K^+ pump activity that reduces ATP and alters Na^+/H^+ ion exchange *via* decreased intracellular pH, which drastically changes the intracellular ion balance by facilitating intracellular Na^+ ion accumulation (27, 137) (Fig. 2). Multiple adverse events then ensue, including myocyte swelling due to altered osmotic gradients in response to elevated Na^+ levels (21) and cellular Ca^{2+} -overload *via* increased $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity (80, 96), which manifests as contractile dysfunction (188),

FIG. 1. The pathogenesis of atherosclerosis. (A) Cardiovascular risk factors such as hyperlipidemia, hyperglycemia, smoking, or hypertension induce endothelial dysfunction that initiates the process of atherogenesis. (B) Endothelial dysfunction results in increased vascular permeability and expression of leukocyte adhesion molecules and, hence, endothelial-leukocyte interactions. Further, MPO released from incoming leukocytes binds to endothelial cells and transcytoses into the subendothelial space, where it releases oxidants such as HOCl, which covalently modify structural proteins of the subendothelial matrix such as fibronectin. This results in de-adhesion of endothelial cells. These events facilitate the transport of LDL and activated immune cells into the subendothelial space, which renders the LDL susceptible to oxidative modification. Infiltrating monocytes differentiate into macrophages, which engulf the modified LDL to become foam cells that release cytokines that are capable of further amplifying the inflammatory response. Medial SMCs undergo phenotypic modulation into synthetic SMCs that permits them to migrate into the intima and release large amounts of ECM to form the fibrous cap. These synthetic SMCs can also take up LDL to become foam cells. (C) Continued intimal inflammation results in the formation of a necrotic core of dying cells and extracellular cholesterol crystals that is encapsulated by the overlying fibrous cap. (D) Progressive inflammation at the shoulder regions of advanced plaques causes fibrous cap thinning, which could eventually lead to acute plaque rupture and formation of occlusive luminal thrombi in the blood vessel lumen. ECM, extracellular matrix; HOCl, hypochlorous acid; LDL, low-density lipoprotein; MPO, myeloperoxidase; SMCs, smooth muscle cells. Color images are available online.

uncoupling of the mitochondrial electron transport chain, further ATP depletion in the damaged myocardium, cell swelling, and cardiomyocyte necrosis (Fig. 2) (50, 114).

Many modes of cell death are observed after AMI, including apoptosis (*e.g.*, *via* intrinsic or extrinsic pathways), necrosis (*e.g.*, *via* opening of the mitochondrial permeability transition pore; MPTP), and the more recently identified necroptosis. Necroptosis is a form of programmed necrosis

that is elevated in failing human and rat hearts (94, 178) and can be induced by tumor necrosis factor (TNF) binding to the TNF Receptor 1 with a sequential activation of multiple intermediate mediators culminating in the activation of receptor-interacting protein 1 (RIP1), which activates receptor-interacting protein 3 (RIP3), which, in turn, activates mixed lineage kinase domain-like (MLKL). Thus, the RIP1-RIP3-MLKL axis is involved in necroptosis induction

FIG. 2. Ischemia and reperfusion injury to cardiomyocytes. On ischemia, there is a shift in energy production from aerobic mitochondrial respiration to anaerobic glycolysis due to low oxygen levels. As such, lactic acid is produced and hydrogen ions (H^+) build up, which reduces intracellular pH. This activates the NHE that exchanges an H^+ for a sodium ion (Na^+). The subsequent build-up of Na^+ activates the plasma membrane Na^+/Ca^{2+} exchanger (NCX), which exchanges 3 Na^+ for one calcium ion (Ca^{2+}) resulting in a build-up of Ca^{2+} ions within cells, which stimulates myocyte swelling, contractile dysfunction, and cardiomyocyte necrosis. On reperfusion, excessive H^+ is exchanged extracellularly via the NHE transporter, resulting in the intracellular accumulation of Na^+ . The Na^+ is subsequently exchanged for Ca^{2+} , which results in intracellular calcium overload and the elevated production of ROS to cause MPTP opening, mitochondrial depolarization, ATP depletion, and myocyte necrosis. Ca^{2+} overload also increases mitochondrial permeability, facilitating the release of cytochrome *c* and stimulation of apoptosis. Reperfusion also activates inflammasome signaling and mitochondrial electron transport chain disruption, resulting in leakage of electrons that react with molecular oxygen to produce $O_2^{\bullet-}$. Infiltrating neutrophils and damaged mitochondria produce excessive amounts of ROS, which stimulate the upregulation of redox-sensitive signaling pathways such as the transcription factor NF- κ B that induces the activation of inflammatory genes such as TNF, IL-1 β , and IL-6. ROS also mediate cellular damage to biomolecules that is discussed further in later sections. Finally, excessive infiltration of neutrophils and immune cells can contribute to microvascular blockage of coronary arteries. MPTP, mitochondrial permeability transition pore; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NHE, Na^+/H^+ exchanger; $O_2^{\bullet-}$, superoxide radical anion; ROS, reactive oxygen species; TNF, tumor necrosis factor. Color images are available online.

(219). It has been recently shown that RIP3 acts *via* Ca^{2+} -calmodulin-dependent protein kinase (CaMKII), to activate MPTP opening and subsequent cell death (217). A key study reported that inhibition of RIP3 or CaMKII ameliorates ischemia reperfusion (I/R) injury in a mouse model of experimental AMI (217). Also, the administration of the micro-RNA, miR-105, a dual-inhibitor of RIP3-induced necroptosis and BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3)-induced apoptosis, decreased the levels of both apoptosis and necroptosis and reduced infarct size in a rat model of experimental AMI and reperfusion (165). On the other hand, TNF receptor-associated factor 2 (Traf2) has been recently shown to possess protective mechanisms in the heart by negatively modulating necroptosis through the regulation of the RIP1-RIP3-MLKL pathway (59). Knockout of Traf2 in mice resulted in adverse cardiac remodeling and heart failure, suggesting to be due to a feedforward mechanism that increases the levels of TNF to promote cell death in the absence of Traf2. However, deletion of the TNF receptor 1 largely but not fully rescued the development of adverse cardiac remodeling signaling that although TNF plays a role in adverse cardiac remodeling post-AMI, other TNF receptor 1 independent pathways might also be involved in this pathological pathway (59).

Another recently identified form of cell death is ferroptosis, which involves the uptake of iron into the cell and into the mitochondria prompting the excessive production of ROS (113) that mediates damage to the myocardium as discussed in this review. Notably, these ROS oxidize membrane lipids, resulting in the formation of lipid peroxides, a feature of ferroptosis (113). Thus, higher levels of cardiac non-heme iron were detected in mice subjected to experimental AMI with reperfusion. Blocking ferroptosis in these animals by using a pharmacologic inhibitor or an iron chelator decreased infarct size, levels of serum cardiac enzymes (lactate dehydrogenase, aspartate transaminase, and creatine kinase-MB isoenzyme), cardiac remodeling, and the extent of fibrosis (42). Hence, many cellular targets involved in initiating cell death pathways are potential therapeutic targets for the reduction of cardiomyocyte cell death post-AMI.

The timely restoration of coronary artery blood flow after AMI halts progressive cardiomyocyte death from ischemia and potentially rescues 30%–50% of cardiac tissue in the area at risk surrounding the infarct (69). However, reperfusion *per se* imposes the risk of further myocardial injury *via* the interlinked processes of inflammation and oxidative stress that in the long term can lead to chronic heart failure (121). Thus, acute reperfusion reintroduces oxygen, inflammatory mediators, and various ions (*e.g.*, Na^+ and Ca^{2+}) that instigate a second wave of damaging oxidant formation; alters metabolic function(s); and/or activates immune responses that further damage cardiomyocytes (147). Accumulating ROS are involved in direct oxidative tissue damage and the redox activation of cell signaling pathways, including the key redox-sensitive transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which upregulates several key inflammatory genes such as TNF, IL-1 β , and IL-6 (122, 135). Augmented innate immune responses increase the local recruitment of activated leukocytes that can promote coronary microvascular blockage and impair NO bioactivity, resulting in endothelial dysfunction and heightened vascular inflammation (Fig. 2) (184).

Functional mitochondria are essential for cardiomyocyte viability, producing >90% of the ATP required for cardiac output (58). However, both ischemia and reperfusion induce mitochondrial damage and dysfunction that impact cardiomyocyte function and viability (88). For example, Ca^{2+} overload causes MPTP opening, leading to mitochondrial depolarization, ATP depletion, and myocyte necrosis (114). Also, excess Ca^{2+} and unregulated mitochondrial depolarization increase mitochondrial permeability, facilitating the release of cytochrome *c* and other cell death factors, thereby triggering myocyte apoptosis (162). Myocyte apoptosis can activate inflammasome signaling and proinflammatory cytokine production, for example, IL-1 β (164). Another consequence of ischemia-induced mitochondrial dysfunction is electron transport chain disruption, which leads to a decrease in oxidative phosphorylation and an increased leakage of electrons that react with molecular O_2 to form the superoxide radical anion ($\text{O}_2^{\bullet-}$) (Fig. 2). This increase in mitochondrial ROS can induce cellular oxidative and/or mitochondrial DNA (mtDNA) damage (166). Reperfusion after ischemia triggers a secondary phase of ROS production (88), which further limits ATP production and augments cellular ROS generation, events that, together with myocyte mitochondrial Ca^{2+} overload (114), provoke post-AMI cardiac dysfunction and eventual progression to heart failure (17).

Progression to Cardiac Dysfunction and Heart Failure After AMI

Although reperfusion of the ischemic myocardium is critical for patient survival post-ACS (193), AMI remains a leading cause of left ventricle (LV) dysfunction and cardiac-related death, with ~25% of survivors subsequently progressing to chronic or congestive heart failure with 12 months after suffering an AMI (196). This secondary phase of pathology is characterized by cellular responses to the initial myocardial ischemia and, in particular, to the extent of cardiomyocyte necrosis within the infarct (47, 67). Adaptive ventricular remodeling after AMI involves the reorganization of supporting structures, myocyte hypertrophy, and collagen deposition (leading to scar formation). This latter feature is primarily the result of resident cardiac fibroblast activation, which responds to inflammatory stimuli by differentiating into a myofibroblast phenotype, proliferating, and subsequently causing maladaptive extracellular matrix (ECM) turnover; these adaptive processes lead to adverse matrix remodeling (111) and infarct expansion that is conducive to heart failure (191, 204).

Role of Innate Immune Cells After AMI and Reperfusion

The innate immune response after ischemic myocardial injury is aimed at facilitating beneficial tissue remodeling and repair of the damaged cardiac muscle (48). Neutrophils are the predominant early responder cells recruited into the myocardial infarct zone during the first 24–48 h post-AMI and reperfusion (Fig. 3). Neutrophils aggregate around the infarcted tissue in the area at risk, yielding a definitive hyperemic border, where these leukocytes play a role in phagocytosing necrotic cells, which can further augment the inflammatory response (209). Notably, neutrophils contain potent ROS-generating and proteolytic enzyme systems

FIG. 3. Timeline of innate immune cell infiltration into the myocardium post-AMI and reperfusion. Circulating N1 neutrophils are the first immune responder cells that infiltrate the myocardium on postinfarction of the affected myocardium. This cell type is activated by the release of DAMPs from the damaged myocardium that bind to TLR4 and activate N1 neutrophils. Recruited N1 neutrophils are mobilized to the infarct region within 24 h and may last up to 5 days; however, their numbers decrease over days 2–5. Infiltrating neutrophils release various mediators such as proinflammatory cytokines (TNF and IL-1 β), MPO, and MMP to remove necrotic tissue and ECM debris. Ly6C^{hi} monocytes infiltrate at day 1 postinfarction and remain until day 3. This monocyte population is characterized by high expression levels of the chemokine receptor CCR2 and are stimulated to differentiate into proinflammatory macrophages that release high levels of inflammatory cytokines (*e.g.*, TNF and IL-6), phagocytose and remove necrotic cells. From day 5 post-AMI, the monocyte/macrophage phenotype changes and Ly6C^{lo} monocytes characterized by high expression of the chemokine receptor CXCR1 prevail. Ly6C^{lo} monocytes differentiate into reparative macrophages, which secrete anti-inflammatory IL-10 cytokines (to resolve the inflammatory response) and proangiogenic mediators (such as the VEGF) to increase vascular supply and aid activated myofibroblasts in depositing the collagen scar that replaces the necrotic myocytes. Around day 7, N2 neutrophils that possess anti-inflammatory activity begin to increase in numbers to assist with the resolution of the infarct wound. AMI, acute myocardial infarction; DAMP, damage-associated molecular pattern; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor. Color images are available online.

involving the coordinated activation of NADPH oxidase 2 (NOX2) and myeloperoxidase (MPO), as well as the release of MMPs and other proteolytic enzymes that initiate tissue remodeling and repair (116). In addition, the presence of cytokines (*e.g.*, TNF, IL-1, IL-6) (132) and alarmin binding to neutrophil receptors activates signaling pathways involving mitogen activated protein kinases (MAPK) and NF- κ B, further enhancing the local production of inflammatory cytokines (*e.g.*, TNF, IL-1; IL-8) that recruit circulating Ly6C^{hi} monocytes into the injured site from days 1 to 3 postinjury (118, 209) (Fig. 3). The population of infiltrating Ly6C^{hi} monocytes then differentiate into macrophages of the M1 phenotype (between 3 and 5 days post-AMI) that secrete proteolytic enzymes and inflammatory cytokines such as IL-6 and IL-1 β in an attempt to remove dying neutrophils and necrotic debris (118, 209).

Changes in monocyte and macrophage phenotype under the influence of neutrophils occur after the 5th day from AMI onset and help in the transition from the innate immune response to the repair phase of the acute response (47, 209).

Thus, Ly6C^{lo} monocytes, characterized by high expression levels of the chemokine receptor CX3CR1, predominate from day 5 post-AMI and differentiate into macrophages of the reparative M2 phenotype, which are an important source of anti-inflammatory mediators such as IL-10 and transforming growth factor beta (TGF- β) (209) (Fig. 3). These reparative macrophages further modulate ECM deposition and metabolism *via* regulating the expression of matrix-degrading MMPs and tissue inhibitors of MMPs (TIMPs) while stimulating the parallel activation, proliferation, and differentiation of resident fibroblasts into myofibroblasts. Recent studies have reported that similar to monocytes, different neutrophil populations are mobilized into the infarcted myocardium. Myocardial tissue injury stimulates the release of endogenous agents known as damage-associated molecular patterns (DAMPs), which activate TLR4 expressed on neutrophils and which promote the mobilization of inflammatory N1 neutrophils and the release of inflammatory cytokines (TNF and IL-1 β) and matrix degrading enzymes such as MMPs that contribute to thinning of the LV wall. This

front-line population of neutrophils can persist in the infarcted region over the first 5 days after injury, although their levels decrease over days 1–5 (Fig. 3). Another population of neutrophils that elicit anti-inflammatory characteristics is reported to increase around day 7 (101) (Fig. 3). As such, these changes in neutrophil population phenotype from inflammatory to anti-inflammatory parallel the changes observed in the monocyte phenotype post-AMI.

Fibroblasts make up ~27% of all cells in the heart, with this number greatly increasing during injury (15). They also release angiogenic mediators such as vascular endothelial growth factor (VEGF), which initiates angiogenesis (118). These tissue repair processes are important in promoting scar formation and preventing cardiac rupture, adverse LV remodeling (208), and progression to heart failure (204). Further, TGF- β stimulates myofibroblasts to deposit collagen type I/III to yield scar formation [reviewed in Czubryt (30) and Van Amerongen *et al.* (192)], which acts to replace dying cardiomyocytes within the infarct zone with connective tissue that leaves a firm but thinned, noncontractile scar (118).

Studies with animal models have highlighted the importance of Ly6C^{lo} monocytes in the resolution of inflammation and ventricular remodeling; for example, atherosclerosis-prone ApoE^{-/-} mice characterized by a Ly6C^{hi} monocytosis displayed elevated inflammation and deteriorated ventricular function 5 days post-AMI (134). Therefore, a balance is required between inflammatory Ly6C^{hi} and reparative Ly6C^{lo} monocytes to control the resolution of inflammation, myocardial healing, and repair (52). Neutrophils influence the recruitment and polarization of monocyte/macrophage populations post-AMI, and therefore, they can be involved in governing tissue repair and fibrosis. For example, an aberrant wound-healing response in neutrophil-depleted mice was characterized by a reduction in the removal of dying cardiomyocytes [a critical precursor for wound healing and collagen deposition (197)], excessive fibrosis, and ventricular dysfunction post-AMI, which were linked to a decreased recruitment of inflammatory M1 macrophages and reparative M2c macrophages and an increase in classic anti-inflammatory M2 macrophages (70).

These data suggest that neutrophils play dual roles, both positive and detrimental, in the post-AMI innate immune response involved in infarct wound resolution. This complexity of action indicates that neutrophils are a functionally and phenotypically heterogeneous population of innate immune cells that depending on the local environment are capable of exhibiting a longer lifespan, which permits interaction with multiple immune cell types to shape innate and adaptive immune responses (167). Therefore, rather than broadly targeting neutrophils *per se* to limit post-AMI tissue damage, it may be more important to selectively target tissue-damaging neutrophil proteins, for example, the homodimeric and oxidative heme protein MPO.

Given the limited regenerative capacity of cardiomyocytes, fine-tuning of the reparative innate immune response by heterogeneous populations of neutrophils, dendritic cells, monocytes, and macrophages is vital for the successful restoration or preservation of adequate cardiac function (168). Endogenous anti-inflammatory signals and processes that suppress an excessive inflammatory response are important for transitioning away from further tissue injury both within and surrounding the infarct zone (47). A prolonged

proinflammatory phase and/or limited reparative anti-inflammatory phase can exacerbate reperfusion injury and contribute to adverse tissue remodeling post-AMI, thereby increasing the risk of subsequent heart failure (7).

Inflammation Mediates Reperfusion Injury Post-AMI

A multitude of studies have shown that the degree of inflammation, both locally within the injured myocardium and systemically, is upregulated after AMI, with the latter response in some cohorts being predictive of adverse clinical outcomes, including a recurrent heart attack, heart failure, and death (10, 191). Various proinflammatory chemokines and cytokines are elevated in heart failure patients, suggesting that endogenous cytokines exert deleterious effects on the heart and peripheral circulation (105). Accordingly, preclinical studies support the fact that antagonism of innate immune receptors, signaling pathways, or proinflammatory cytokines attenuates adverse cardiac alterations post-AMI (52, 105). For example, IL-6 levels are elevated in heart failure patients and inversely correlate with LV function (140), whereas an IL-6 receptor antagonist alleviates inflammatory damage in patients with non-ST-elevation myocardial infarction (NSTEMI) (82).

Excessive or dysregulated reperfusion-induced inflammation promotes aberrant ECM degradation and deposition to yield excessive tissue fibrosis. Thus, increased levels of active MMPs, coupled with a downregulation of TIMP levels, are found in failing human hearts, which facilitate dysfunctional ECM remodeling (91, 190). Although coordinated ECM turnover is necessary for the removal of necrotic debris, an augmented or prolonged inflammatory response leads to excessive ECM degradation by MMPs that elevates fibrotic scar formation (36) that is associated with elevated immune cell infiltration in heart failure patients (204). Preclinical studies show that antifibrotic therapy affords a decrease in total and nonscar fibrosis, which improves LV function post-AMI (124).

ROS and Mitochondrial Dysfunction Contribute to Inflammation in Reperfusion Injury

The coordinated production of low levels of ROS, in particular hydrogen peroxide (H₂O₂), can act as endogenous redox cell signaling molecules that regulate immune and inflammatory responses (*e.g.*, via activation of redox-sensitive transcription factors such as NF- κ B and nuclear factor erythroid-2 related factor 2 [Nrf2]) (184). However, excessive or aberrant ROS production can promote dysfunctional signaling responses and directly mediate oxidative damage to proteins, lipids, and nucleic acids in inflammatory tissues, thereby adversely affecting cell function and stimulating the further production of oxidants that can result in positive feedback to augment inflammation (114).

A key factor orchestrating the postreperfusion inflammatory response is the elevated production of ROS and associated redox signaling. In the infarcted myocardium, a principal source of ROS is the oxidative burst of infiltrating activated leukocytes (neutrophils and monocytes) that involves the concerted activation of NOX2 and MPO (47). In addition to the phagocytic oxidative burst, local parenchymal cells also represent a significant source of ROS in response to I/R injury. For example, activated cardiomyocytes,

myofibroblasts, or endothelial cells can produce ROS *via* various endogenous sources, including activation of several NOX isoforms including NOX 1, 2, 4, and 5, electron leakage from the mitochondrial respiratory chain, uncoupled NOS, or xanthine oxidase (57, 184).

Accumulation of ROS and resultant oxidative stress are implicated as drivers of chronic diseases characterized by a prolonged dysregulated inflammatory response, including atherosclerosis (136, 175), and they are associated with the severity of reperfusion injury post-AMI and progression to heart failure (103, 189, 201). For example, 8-iso-prostaglandin F_{2α}, a commonly measured marker of *in vivo* oxidative stress, is elevated in patients with severe heart failure (103). Further, the tissue-specific overexpression of superoxide dismutase (SOD) in cardiac myocytes protects against myocardial reperfusion injury and results in a decrease in infarct size through a mechanism involving enhanced NO bioavailability (128). These studies support attempts at bolstering the myocardium's antioxidant capacity to provide cardioprotection in AMI.

Studies with mice deficient in p47^{phox} (an essential subunit for NOX enzymes) displayed decreased fibrosis, apoptosis, and ventricular dilation in response to MI, identifying a pathogenic role for NOX-derived ROS post-AMI (40). Interestingly, highlighting their complex role in AMI, other studies report that NOX-derived ROS can also signal for protective adaptive responses to I/R injury, involving the activation of the HIF1 α and Nrf2 transcription factor pathways (107) and signaling for the polarization of macrophages into a reparative M2 phenotype (117). The studies just cited indicate that the role of NOX-derived ROS in AMI is complex and that targeted, temporal NOX inhibition rather than global, persistent NOX inhibition is a more viable therapeutic value.

Unregulated ROS production during I/R injury can act to promote cardiac dysfunction in several ways. For example, ROS can selectively oxidize amino acid residues or active-site metal centers to target proteins that modulate cardiomyocyte function such as myoglobin (201) and electrical conductance such as the sarco-endoplasmic reticulum Ca²⁺ ATPase that are essential for the maintenance of intracellular Ca²⁺-homeostasis (28). Interestingly, hypochlorous acid (HOCl), an oxidant produced by the neutrophilic enzyme MPO on reaction with Cl⁻ ions and H₂O₂, affects the peroxidase activity of myoglobin but does not impact its catalase-like activity (3). Dysregulated ROS production can also promote cardiomyocyte death *via* activating proapoptotic signaling kinases such as the MAPK p38 and jun N-terminal kinase (202, 211), inducing mitochondrial dysfunction involving the opening of the MPTP and/or through oxidatively damaging DNA that can activate poly(ADP-ribose) polymerase-1, which is intricately involved in cell death (189).

In addition to the NOX enzymes, mitochondria also yield ROS in response to I/R injury post-AMI. During ischemia, succinate from the citric acid cycle accumulates and is oxidized by succinate dehydrogenase on reperfusion, producing mitochondrial ROS by reverse electron transport at complex I (26). The ketoglutarate complex, an enzyme in the citric acid cycle, is inactivated in reperfusion, resulting in a decline in NADH-linked respiration (158). However, it is not studied widely in the field of myocardial I/R injury, with some studies

reporting that although I/R injury involves mitochondrial damage, the damage is primarily attributed to changes in the mitochondrial electron transport chain and not the citric acid cycle enzymes (68). However, other studies have reported that progressive changes to the citric acid cycle can occur in the longer term after MI, as a maladaptive response to MI-induced metabolic alterations. These changes are proportional to the degree of impairment in the myocardium as a result of MI (39). Nonetheless, it is not a focus of current preclinical or clinical trials in AMI research.

Moreover, it is increasingly recognized that ROS produced by different NOX isoforms and mitochondria cross-talk to augment overall cellular production of ROS (37). A paradigm linking oxidative stress, mitochondrial dysfunction, and chronic inflammation is supported by recent studies reporting that mitochondrial-derived ROS stimulate inflammation by triggering proinflammatory redox-sensitive signaling events such as NF- κ B activation or directly activating the NLRP3 inflammasome (164, 222). This is relevant to resident cardiac fibroblast cells; early inflammasome activation in cardiac fibroblasts has been shown to contribute to early I/R injury, and animal knockout studies targeting various components of the inflammasome system alleviated injury (77, 159). NLRP3 inflammasome activation has also been associated with atrial fibrillation (AF) in human patients (210), and NLRP3 inhibitors have alleviated AF and I/R injury in *in vivo* and *ex vivo* mouse models of experimental acute myocardial infarction and reperfusion (AMI/R) and AF (120, 210).

Mitochondrial dysfunction is a key event in AMI and resulting complications such as ventricular dysfunction, increased ROS production, and loss of ATP are features of failing hearts (19) (Fig. 2). On reperfusion, increased mitochondrial levels of ROS and Ca²⁺, together with the associated changes to pH, stimulate the opening of the MPTP channel (162), resulting in membrane swelling, subsequent mitochondrial release of cytochrome c into the cytoplasm, and eventual cell death (60). Blockage of the mitochondrial electron transport chain with reversible inhibitors, such as amobarbital, during ischemia reduces reperfusion-induced Ca²⁺-overload and ROS generation, thereby protecting against cardiomyocyte injury (24, 180). Further, damaged mitochondria and necrotic cells release DAMPs, such as DNA and ATP (215, 218), that stimulate sterile immune responses, the inflammasome, and the time- and stage-dependent activation of neutrophils and their polarization into inflammatory N1 neutrophils (7, 101, 139) (Fig. 3). Preclinical animal studies have linked mitochondrial DAMP production with cardiac inflammation and heart failure (119).

Therapies Targeting Inflammation and ROS in Reperfusion Injury Post-AMI

Current interventions for AMI focus on timely restoration of coronary flow *via* percutaneous coronary intervention (PCI). However, PCI stimulates reperfusion-tissue injury in the local coronary artery wall and adjacent cardiac tissue, which is associated with poor clinical outcomes (142). This supports the need for adjunct therapies that target the local inflammatory and oxidative response to reduce the accompanying reperfusion-stimulated injury subsequent to PCI.

Current data show that limiting the degree of inflammation, ROS production and mitochondrial dysfunction can

ameliorate I/R injury and preserve cardiac function in both the early and late phases post-AMI (55, 104, 160). However, adjunct therapies that have proven beneficial in preclinical models of reperfusion injury have not yielded translational benefits in the clinic, especially in terms of reducing infarct size. Of particular note is the relative failure of translating positive phase I/II trial outcomes for interventions, such as cyclosporine A (CsA) and the MPTP inhibitor TRO40303, into long-term clinical benefit in large, multicenter trials (63, 65). Targeting inflammation or ROS-mediated oxidative damage through the use of broad-spectrum antioxidant therapies, such as vitamins E and C, has also proven largely unsuccessful in improving cardiovascular endpoints in large and long-term follow-up human trials in nondiabetic CAD patients (75, 163). Reasons for these disappointing clinical outcomes include the underlying incongruity between animal disease models and human pathology, reflecting differences in the timing of drug delivery, that is, prophylaxis delivery in animal models *versus* treatment of established disease in humans.

The poor outcomes in large cohort trials to date may arise from inadequate pharmacological targeting of the most relevant sources and specific types of ROS that induce cardiovascular dysfunction. Thus, although mitochondrial aberrations and activation of different NOX isoforms are pivotal in driving myocardial ROS production/inflammation post-I/R, nutritional and therapeutic antioxidants may not be accessible to the relevant intracellular sites or organelles to effectively target the locally produced ROS. In support of this, mitochondrial-targeted antioxidants, such as mitochondrial-targeted forms of coenzyme Q (MitoQ) or the nitroxide tempol (Mito-TEMPO) (2, 100), have been shown to provide cardioprotection (32) through limiting oxidative damage and inflammation, as well as maintaining mitochondrial function in animal models, although their efficacy remains to be verified in humans.

Studies have also characterized the changes to mitochondrial function/structure during reperfusion injury (65). For example, CsA, a natural product and immunosuppressant, binds to cyclophilin D in the MPTP, inhibiting its opening (157). Preclinical studies show improved ventricular functioning with CsA administration after myocardial I/R (212). However, these promising preclinical findings have to date not been translated into the clinical setting. Thus, the recent double-blind randomized CIRCUS trial reported that patients presenting with ST-elevated myocardial infarction (STEMI) who received intravenous CsA before reperfusion (PCI) therapy showed no alteration in circulating creatine kinase levels (a marker of cardiomyocyte death), LV ejection fraction, end diastolic volume, or heart failure incidence (29), an outcome validated by the subsequent clinical trial (132).

Another molecular target for ameliorating reperfusion injury post-MI that has received recent interest is the inflammasome that is activated by DAMPs released on myocardial damage (164). For example, an NLRP3 inflammasome inhibitor decreased infarct size in a mouse model of postischemic injury (106, 187). Studies have also investigated targeting IL-1, the major product of the activated inflammasome. For example, the VCU-ART2 trial assessed the potential of an IL-1 receptor antagonist, Anakinra, to alleviate inflammation and adverse events after AMI. Although Anakinra failed to decrease infarct size, the drug

improved LV end-volume indices together with decreasing inflammation and rates of heart failure 48 months post-AMI (1). Nonetheless, this study employed a small cohort ($n=10$) and, as such, the results may be difficult to interpret and extrapolate to the broader community, with a phase II trial currently underway (194). The recent CANTOS study utilized another agent (Canakinumab) to neutralize the pro-inflammatory IL-1 in a long-term, large patient cohort with previous MI. The trial authors reported positive reductions in the levels of the inflammatory marker C-reactive protein (CRP) and the incidence of cardiovascular events and death, although the trial was primarily designed to test outcomes of immunomodulation on atherosclerosis and did not explore the direct impact on cardiac function and health outcomes (149).

Modulation of proinflammatory signaling pathways also showed promise in preclinical trials. For example, a p38 MAP kinase inhibitor decreased infarct size in an adult rat model of ischemia, although cardioprotection was only observed when the drug was administered before reperfusion, thereby questioning the drugs' clinical utility (177). The SOLSTICE phase II trial reported that another p38 MAPK inhibitor, Losmapimod, attenuated inflammation markers and improved LV function after PCI in NSTEMI patients (123). However, these cardioprotective outcomes were not observed in the larger and more recently conducted phase III randomized LATITUDE-TIMI trial that evaluated Losmapimod in AMI before reperfusion therapy, whereby the drug did not reduce the risk of major ischemic cardiovascular events despite having a positive impact on reducing circulating levels of inflammatory (hsCRP) and heart failure (NT-proBNP) biomarkers (129).

Beta-blockers have been successful in early clinical trials (56, 131). The METOCARD-CNIC randomized clinical trial reported a significant decrease in infarct size and a significant increase in LV ejection fraction in patients who received an oral or intravenous dose of metoprolol before reperfusion (74). However, decreased infarct size was only significant in patients with no or incomplete perfusion beyond the site of occlusion and infarct size was not improved significantly in patients with partially or completely restored blood flow in the distal regions beyond blood flow occlusion (74). Conversely, the EARLY-BAMI trial reported no significant change in infarct size in patients who received intravenous metoprolol before PCI (150), although differences in drug dosage and timing between the two studies may explain these apparent discrepancies (69). The mechanism underlying β 1-adrenergic-receptor cardioprotection in reperfusion remains unclear, as the majority of these studies were performed before PCI was implemented as a routine clinical treatment for AMI. However, adrenergic signaling is linked to inflammation, in particular the activation of the neutrophil oxidative burst, adhesion to endothelium, and tissue migration (161). Interestingly, metoprolol's ability to reduce the infarct size in a pig model of AMI was reported to be independent of the drug's effect on heart rate and to instead correlate with a reduction in neutrophil infiltration (measured as MPO activity) and apoptosis in the postischemic myocardium (73). More recently, metoprolol has been demonstrated to reduce infarct size by altering neutrophil activity and neutrophil-platelet interactions in AMI patients (51). Hence, targeting neutrophil function and ROS production is a promising

strategy in limiting the degree of chronic inflammation and reperfusion injury occurring after AMI. The next section discusses the role of the abundant neutrophil-derived oxidative enzyme MPO in promoting cardiac dysfunction post-MI and the extent to which it represents a useful therapeutic target.

Myeloperoxidase

Neutrophils are the first immune responders to tissue injury and infection. Neutrophil activation stimulates the release of MPO from its azurophilic storage granules into the phagosome or extracellular environment where MPO employs its co-substrate H_2O_2 (from the plasma membrane NOX2 enzyme) to catalyze the halogenation and peroxidase redox cycles (81, 206). On reaction with H_2O_2 , the heme-iron undergoes a 2-electron oxidation to yield MPO-compound I, a ferryl-oxo ($Fe^{IV}=O$) species, and a heme porphyrin free radical (Fig. 4, Reaction A). MPO-Compound I catalyzes the two-electron halide oxidation to yield their respective hypohalous acids (predominantly HOCl) (Fig. 4, Reaction B).

FIG. 4. MPO enzymatic cycle. The heme-iron active site in native MPO exists in the ferric (Fe^{III}) form. Reaction with the co-substrate H_2O_2 generates MPO compound I, which consists of a ferrous heme ($O=Fe^{VI}$) and highly reactive porphyrin radical ($por^{+•}$) (Reaction A). MPO compound I catalyzes the two-electron oxidation of halide anions into their respective hypohalous acids (Reaction B). The most commonly formed hypohalous acid is HOCl. In this reaction cycle termed the halogenation cycle, MPO compound I is reduced back to its native form, thereby recycling the enzyme. MPO also exhibits a peroxidase cycle referring to two successive one-electron oxidation reactions of small-molecule substrates mediated by MPO compound I and MPO compound II, which refers to the ferryl-oxo heme MPO species ($O=Fe^{IV}$) (Reactions C and D). The peroxidase cycle is accompanied by the generation of reactive small-molecule radicals and culminates in the formation of native ferric MPO. Finally, the ferric iron in native MPO can be reduced by $O_2^{•-}$ generating the ferrous ($O_2=Fe^{II}$)-heme MPO compound III (Reaction E), which is reduced by $O_2^{•-}$ into ferric MPO generating oxygen and H_2O_2 (Reaction F). Compound III is also reduced by serotonin (HT) or another biological reductant to yield ferric MPO to complete the catalytic cycle, while generating serotonin radicals ($HT^•$ /oxidized reductant) (Reaction G). H_2O_2 , hydrogen peroxide. Color images are available online.

The potent oxidant HOCl reacts with a large array of biomolecules, including protein amino acids and amines, heme proteins, thiols, iron-sulfur complexes, nucleic acids, and lipids (34). Due to its pKa of 7.53 (6), HOCl is primarily protonated and membrane permeable under the postischemic acidic conditions in the myocardium. HOCl chlorinates biomolecules forming chloroamines, chlorinated lipids, or chlorinated amino acid residues including tyrosine to form 3-chlorotyrosine (3-Cl-Tyr), which is commonly assayed by using quantitative mass spectrometry and employed as a specific *in vivo* biomarker for MPO-derived production of HOCl (34).

The porphyrin radical of MPO-compound I can also mediate the one-electron oxidation of various low-molecular-weight substrates (*e.g.*, NO, nitrite, tyrosine, urate, ascorbate) (Fig. 4, Reaction C) to form compound II (referring to the remaining ferryl-oxo species) and the one-electron oxidation product of the substrate. Compound II can mediate a second one-electron oxidation reaction to cycle the enzyme back to its native state (Fig. 4, Reaction D). A notable biological reductant for MPO-compound II is $O_2^{•-}$ that yields ferric MPO, which is then able re-enter halogenation/peroxidation cycles. Also, $O_2^{•-}$ can react with ferric-MPO to form MPO-compound III (formerly a ferrous iron-oxygen [$Fe^{II}-O_2$] complex) (Fig. 4, Reaction E). In addition to the complexity of MPO's catalytic cycle, $O_2^{•-}$ can also react with MPO-compound III to re-form native ferric-MPO, while generating oxygen and H_2O_2 (78) (Fig. 4, Reaction F). Compound III is also a substrate for serotonin (HT), which also reduces it back to ferric MPO (207) (Fig. 4, Reaction G).

With respect to the physiologically relevant peroxidase substrates, nitrite is of considerable significance. On reaction with MPO compound I or II, nitrite is converted into the highly reactive nitrogen dioxide radical ($•NO_2$), which is capable of initiating lipid peroxidation (216) and reacting with tyrosines to form 3-nitrotyrosine; a biomarker of oxidative tissue damage in disease (170).

Neutrophils and MPO in Cardiac Dysfunction Post-AMI

As indicated earlier, neutrophils are recruited early into the infarcted myocardium post-AMI (209), where they respond to DAMPs derived from damaged mitochondria and dying cardiomyocytes and release mediators that augment inflammation to resolve the ongoing tissue injury (139). Activated neutrophils also secrete an array of proteolytic and oxidative substances, including proteinases and MPO, with the potential to further promote myocardium injury, DAMP production and establish a chronic, low-grade inflammation (61). Hence, there is a potential for a pathogenic role of neutrophils in CAD and cardiac inflammation (127). Consistent with this, neutrophil recruitment into the infarct zone and surrounding areas is a prominent feature of ischemic cardiac injury (209) and neutrophils are causally linked to the microvascular dysfunction occurring during reperfusion injury and the microvascular disturbances associated with the no-reflow phenomenon, defined as the incomplete reperfusion of blood to areas of the myocardium (95). Also, poor prognosis of CAD patients is associated with elevated systemic neutrophil levels (8, 53), MPO (182) and neutrophilia correlates with the subsequent development of chronic heart failure post-AMI (86).

Neutrophil-derived MPO has received considerable attention as a mediator of cardiovascular disorders. MPO is mechanistically linked to several key processes in the pathogenesis of CAD, including atherosclerosis, myocardial injury, endothelial and microvascular dysfunction, and plaque formation and vulnerability (33, 125). Clinical studies have shown that elevated circulating extracellular MPO is a valuable prognostic index for adverse events after AMI (79, 115), chest pain (18), and chronic heart failure (181). Notably, leukocyte MPO content significantly decreases on traversing the coronary circulation in ACS patients with unstable angina, consistent with intraluminal leukocyte degranulation and extracellular MPO release (20). Also, MPO levels in AMI patients increase acutely and remain elevated 4 h after AMI onset in patients, thereby supporting the acute activation of neutrophils in response to ischemia and before myocardial damage (54).

Importantly, MPO and biomarkers indicative of its oxidants (e.g., 3-Cl-Tyr, 3-NO₂-Tyr, HOCl-oxidized and carbamylated proteins) are detected in diseased tissue, including atherosclerotic lesions of CAD patients and the atrium of AF patients (33, 66, 125, 155, 220). Further, infarcted myocardium exhibits increased levels of MPO-derived oxidation products, including chlorinated plasminogen activated inhibitor 1 (PAI-1) (9), amino acid-derived aldehydes (195), chlorinated lipids (185), and oxidized myoglobin (201).

Preclinical studies with MPO genetically modified mice indicate a role for leukocytes and MPO-generated oxidants in the pathophysiologic consequences of atherosclerosis (109), plaque rupture (143), and myocardial remodeling after ischemia (9, 195). With respect to the myocardial ischemia, MPO's pathogenic actions have been linked to the elevated infiltration of myocardial leukocytes, oxidative inactivation of PAI-1, and impaired LV function and potentially heart failure post-AMI (9) involving the MPO-dependent formation of cytotoxic aldehydes (195). Interestingly, the significant LV dysfunction induced by MPO-derived oxidants post-AMI is independent of changes in infarct size (195). Human AF, which is associated with ongoing inflammation and cardiac dysfunction occurring after an AMI, is also linked to increased levels of MPO in the plasma and cardiac tissue of AF patients (155). Moreover, in a mouse model of angiotensin II-induced atrial fibrosis and fibrillation, wild-type mice exhibited elevated MPO deposition, HOCl production, MMP activity, and atrial fibrosis/fibrillation, events abrogated in MPO gene-deficient mice (116, 155).

Mechanistically, MPO-derived oxidants react with bystander cells in the heart (including endothelial cells, resident cardiac fibroblasts, and cardiomyocytes) to potentiate cardiac dysfunction and promote endothelial/vasomotor dysfunction, adverse tissue remodeling, high-density lipoprotein (HDL) dysfunction, and cytotoxicity, all components underlying progression to heart failure (49, 145, 154, 203, 220).

A characteristic of CAD and ACS is the increased intravascular release of MPO by circulating leukocytes that has the potential to bind to and undergo transcytosis (13) into the vascular endothelium of local infarct-related coronary arteries (12) and cardiac tissue of atrial fibrosis patients (155). Within arteries, the transcytosed MPO accumulates in the sub-endothelium (12, 13), where it is positioned to catalyze local oxidative reactions that can promote endothelial dysfunction and impair vasomotor tone (145). For example,

various studies have shown that MPO negatively modulates vascular tone by binding to the endothelium and impairing vasorelaxation by decreasing NO bioavailability (41, 154). Mechanistically, endothelial-transcytosed MPO can impair endothelial-derived NO bioactivity by producing HOCl or *via* its NO oxidase activity that limits bioavailable NO within the vessel wall (184). With respect to HOCl, exposure of the endothelium to this oxidant promotes the uncoupling of electron flow through endothelial nitric oxide synthase (eNOS), which stimulates endothelial O₂^{•-} production that rapidly reacts with NO to impair its bioactivity (174). HOCl also limits NO production by oxidatively inactivating soluble guanylate cyclase that transmits/amplifies NO signaling in the vessel wall (25).

The NO oxidase function refers to the catalytic consumption of NO *via* the peroxidase cycle of MPO that can occur *via* direct NO oxidation by MPO compounds I and II or indirectly by the MPO-catalyzed one-electron oxidation of physiological substrates tyrosine, ascorbate, and urate that form NO-consuming substrate-derived radicals (41, 102, 110, 146). A role for the NO oxidase function as a cause of impaired NO bioactivity has been provided by experimental and clinical studies. For example, Eiserich *et al.* documented that endothelial-sequestered MPO in the presence of H₂O₂ catalytically consumed NO to compromise endothelial-dependent vasorelaxation of isolated arteries (41). Rudolph *et al.* reported that nicotine infusion into humans induced the intravascular release of MPO by circulating leukocytes and the acute impairment of endothelial-dependent vasomotor function (154). Notably, the nicotine-induced endothelial dysfunction was abrogated in MPO-deficient humans. The increased propensity for human AMI patients to consume NO has also been attributed to the NO oxidase activity of circulating MPO, which correlated with impaired endothelium-dependent microvascular dysfunction in patients with CAD (14).

Neutrophil-MPO-catalyzed oxidation reactions can also promote endothelial dysfunction *via* mechanisms independent of their effects on NO bioactivity. For example, HOCl derived from sub-endothelial MPO oxidatively modifies ECM proteins, such as fibronectin, which activates a novel mode of redox-sensitive "outside-in" signaling that activates focal adhesion and actomyosin signaling pathways to promote endothelial cell contractile responses and increased endothelial permeability (Fig. 1) (145). Also, exposure of endothelial cells to low, nontoxic concentrations of HOCl stimulates the expression of the potent thrombogenic tissue factor protein (176).

Chlorinated lipids result from the HOCl-mediated modification of plasmalogen phospholipids, which contain a vinyl ether bond and are present in endothelial cells and cardiomyocytes (171). Chlorination of the vinyl ether yields α -chloro fatty aldehydes such as 2-chlorohexadecanal (2-CIHDA) (4). Elevated levels of 2-CIHDA have been reported in the heart of rat models of experimental AMI and absent in infarcted hearts of neutropenic rats (185). These modified lipids can react with endothelial cells to promote endothelial cell dysfunction that contributes to atherogenesis by increasing (i) the expression of P-selectin to elevate endothelial-leukocyte interactions, (ii) the release of von Willebrand factor that can promote platelet adhesion, and (iii) angiopoietin-2 that increases endothelial permeability

(Fig. 1B) (62). Hence, chlorinated lipids present another potential mediator of post-AMI and I/R cellular injury and dysfunction that is linked to the activity of MPO.

In addition to HOCl, MPO can produce hypothiocyanous acid (HOSCN) that promotes endothelial inflammation and dysfunction by upregulating the NF- κ B-dependent expression of endothelial tissue factor or leukocyte adhesion molecules (198, 199), as well as causing mitochondrial dysfunction and release of proapoptotic factors, including cytochrome *c*, apoptosis-inducing factor, and endonuclease G (97).

Neutrophil-derived MPO is also linked with adverse tissue remodeling through the ability of HOCl to oxidize critical amino acid residues of proteins. For example, HOCl oxidizes cysteine thiol residues within the cysteine switch domain of MMPs, resulting in activation of the enzyme's proteolytic activity (49). Conversely, HOCl inhibits TIMPs by oxidizing the N-terminal cysteine, thereby impairing their inhibitory activity toward MMPs (203). Together, MPO-derived HOCl

results in the unregulated activation of MMPs (Fig. 5), thereby facilitating dysregulated ECM turnover and potentially contributing to excessive fibrosis and tissue remodeling within the inflamed heart. Consistent with this, an increased colocalization of atrial MPO and the HOCl-biomarker 3-Cl-Tyr in human AF patients is linked to aberrant tissue remodeling and fibrosis, as well as electrical dysfunction in the heart atrium (155).

Characteristically, CAD and ACS/AMI patients display increased conversion of HDL into a dysfunctional form that exhibits a loss or impairment of the cardioprotective actions of HDL concomitant with a gain of specific proinflammatory properties (151, 172). During cardiovascular disease, apolipoprotein A-1 (ApoA-1; the major apo-protein component of HDL) is a major binding partner and oxidation target of MPO and oxidation/nitration of specific amino acid residues in ApoA-1 is a significant cause of impaired cholesterol efflux, antioxidant and anti-inflammatory activities of HDL (16, 220). Therefore, the MPO-catalyzed oxidation of HDL

FIG. 5. Neutrophil-mediated damage to cardiomyocytes on reperfusion. On degranulation, neutrophils release the enzyme MPO, which converts H_2O_2 and halide anions into hypohalous acids, including HOCl. This potent oxidant modulates multiple cellular parameters, disrupts homeostatic functioning, and induces myocardial necrosis. Thus, HOCl inhibits the activity of phosphatases and activates cellular kinases, thereby disrupting the phosphoproteome and enhancing cytokine production by MAPK p38-mediated transcription and translation of inflammatory genes. HOCl further oxidizes the N-terminal cysteine in the active site of the TIMP, thereby inactivating them and indirectly increasing the activity of MMP. HOCl also directly activates MMPs by oxidizing the cysteine switch in the enzyme's active site to yield excessive ECM degradation and subsequent tissue fibrosis. Further, HOCl oxidizes the thiol groups in the major intracellular antioxidant GSH, thereby rendering the cell more susceptible to oxidative stress. HOCl oxidizes HDL, impairing its antioxidant and anti-inflammatory activities. HOCl also induces the opening of the MPTP, resulting in increased permeability, mitochondrial damage, and release of cytochrome *c* and multiple other apoptotic factors, which lead to myocyte apoptosis. Finally, HOCl induces endothelial dysfunction and decreases NO bioactivity, thereby impairing vasorelaxation. GSH, glutathione; HDL, high-density lipoprotein; MAPK, mitogen activated protein kinases; NO, nitric oxide; TIMP, tissue inhibitors of matrix metalloproteinase. Color images are available online.

and loss of HDL cardioprotective actions represents a further mechanism by which MPO may exacerbate local ongoing cardiovascular inflammation in ACS/AMI patients.

Potent oxidants generated by MPO can contribute further to tissue damage during AMI by oxidizing intracellular thiols and antioxidants and promoting redox cell signaling that alters the function/phenotype of cardiomyocytes and non-cardiomyocyte bystander cells such as endothelial cells and promote cytotoxicity (206). Thus, HOCl readily oxidizes reduced glutathione (GSH) (Fig. 5), yielding oxidation products including GSH disulfide (22) and GSH sulfonamide (64). Major intracellular targets for HOCl also include thiol-containing enzymes, including GAPDH, peroxiredoxins (97, 141, 173), and iron-sulfur and heme-containing enzymes (179), including those present in the mitochondria.

The ability of HOCl/HOSCN to alter the redox status of labile amino acid residues (*e.g.*, cysteines, tyrosines, methionines) and active-site metal centers also modulates the activities of signaling proteins, including phosphatases, protein kinases, and transcription factors (23, 112) (Fig. 5). Specifically, the modulation of the cellular phosphoproteome involves promoting phosphorylation by activating MAPK (*e.g.*, p38) and deactivating protein tyrosine phosphatases (PTPs); the modulated phosphoproteome then shifts to a hyperphosphorylated state that ultimately decreases cell viability (Fig. 5). For example, the exposure of cardiomyocytes to exogenous HOCl inhibits phosphatase activity concomitant with the activation of kinase activity and promotion of apoptosis in a co-culture model of cardiomyocytes and activated HOCl-producing neutrophils (23). In endothelial cells, exposure to HOCl induces MAP kinase signaling involving the activation of Erk1/2 and p38, but not JNK, with the activation of Erk1/2 being a protective signaling response to the elevated oxidative stress (112). Activation of the Nrf2-ARE axis and AP-1 transcriptional responses have also been

identified as protective intracellular signal transduction responses on exposure to MPO-derived oxidants (148).

The decrease in intracellular levels of low-molecular-weight and enzymatic thiol-based antioxidants together with the induction of aberrant redox signaling and impairment of fundamental metabolic processes (mitochondrial respiration) leads to unregulated cytotoxicity. Exposure of cardiomyocytes to HOCl and HOSCN increases cytosolic calcium, depletion of protein thiols and GSH, alters mitochondrial membrane potential, and increases necrosis (148). In endothelial cells, exposure to HOCl or HOSCN promotes oxidation of intracellular thiols and antioxidants and loss of cell viability (97, 176). Clinical data indicate that circulating MPO is strongly associated with a higher incidence of endothelial erosion in coronary atherosclerotic plaques, supporting that MPO induces loss of endothelial viability *in vivo* (43).

Pharmacological Inhibition of MPO

In light of MPO's pathogenic potential in various inflammatory disorders, there is currently significant interest in the discovery and development of new potent and selective MPO inhibitors. Two major classes of inhibitors have received most attention: irreversible or suicide inhibitors and reversible inhibitors.

Irreversible MPO inactivators (186) act by rapidly reacting with MPO compound I to form a drug-derived radical that subsequently forms a covalent drug-heme adduct with the MPO active site to irreversibly inactivate the enzyme. Overall, 2-thioxanthines are a potent and selective class of irreversible MPO inhibitors (186) (Fig. 6). This nontoxic class of pharmacologic inhibitors of MPO potently inhibited the chlorination (186) and NO oxidase (102) activities of purified MPO, mitigated the formation of HOCl by activated

FIG. 6. Chemical structure of MPO inhibitors. The chemical structures of 2-thioxanthines, PF-06282999, PF-1355, paracetamol (acetaminophen), TEMPO, and TEMPOL are shown. These chemical agents show different mechanisms of action toward MPO.

neutrophils in a mouse model of peritonitis (186), and provided protection against endothelial dysfunction (25) and atherosclerotic plaque instability in mice (143). Due to these promising outcomes, 2-thioxanthines have entered phase I clinical trials for COPD and multiple sclerosis and phase IIA trials in Parkinson's disease (31).

Other irreversible mechanism-based MPO inhibitors that have received recent interest are thiouracil derivatives, of which PF-06282999 and PF-1355 are lead candidates (156) (Fig. 6). Inhibition of MPO by PF-1355 is reported to provide protection in murine models of immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis (221). Recent work has reported that although administration of PF-06282999 to atherosclerosis-prone mice did not slow lesion formation, it did reduce the extent of lesion inflammation and necrotic core size, a finding indicative of enhanced plaque stability (153). Notably, oral PF-1355 treatment also reduced MPO-mediated tissue damage in a mouse MI model, reducing the inflammatory cell infiltrate and improving myocardial function as judged by improved LV ejection fraction and reduced end-diastolic volume (5). This study, together with studies using MPO gene-deficient mice (9, 116, 195), highlight that targeting MPO is a viable and promising therapeutic option for treating post-AMI cardiac dysfunction.

Reversible MPO inhibitors are enzyme substrates that are able to competitively react with MPO's active site heme to reversibly inhibit enzyme activity. These inhibitors can act in several ways: (i) by reversibly binding to ferric-MPO to occupy the enzyme's active site and effectively inhibit access of physiological substrates (*e.g.*, aromatic hydroxamates) (46), (ii) effectively competing with physiological substrates for reaction with MPO compounds I and II (*e.g.*, paracetamol also known as acetaminophen) (84) (Fig. 6), (iii) by competitively reacting with MPO compound I, but not MPO compound II, thereby promoting the build-up of compound II in the peroxidase cycle (*e.g.*, nitroxides) (144), and (iv) promoting the formation of MPO compound III (*e.g.*, isoniazid) (45).

Promising classes of reversible MPO inhibitors with therapeutic potential include aromatic hydroxamates and cyclic nitroxides. With respect to the aromatic hydroxamates, these agents selectively dock into the active-site pocket of native MPO to retard subsequent substrate binding and potentially inhibit the enzyme's halogenation and peroxidase cycles (46). This reversible mode of inhibition has a potential advantage over substrate-based irreversible inhibitors, as MPO's oxidative capacity is selectively blocked without permanently inactivating the enzyme. To date, aromatic hydroxamates have not been trialed in preclinical inflammatory models for their therapeutic potential (46).

Cyclic nitroxides are stable free radicals characterized by a backbone containing a five-membered pyrrolidine, pyrroline, or oxazolidine or a six-membered piperidine ring with a nitroxyl group (89) (Fig. 6). Several classes of nitroxides have received wide interest as a versatile group of therapeutic antioxidant drugs that are capable of reducing the extent of inflammation and reperfusion injury in various tissues (205) such as the kidney (214), brain (76), intestinal (183), and ovarian tissue (138). Notably, cyclic nitroxides are a class of therapeutic radical scavengers that also exhibit potent MPO inhibition *via* a reversible mechanism (144). These compounds are cell permeable, display relatively low toxicity,

with low-millimolar concentrations achievable *in vivo* with no apparent acute or long-term toxic effects (169). Accordingly, nitroxides are currently used clinically in the treatment of radiation-induced alopecia and macular degeneration (213). Nitroxides exhibit multiple antioxidant properties, including an ability to directly scavenge free radicals and act as SOD mimetics (89, 130) with catalytic self-regeneration.

Nitroxides have been identified as reversible MPO inhibitors that inhibit HOCl production by rapidly reacting with MPO compound I to form the corresponding nitroxide oxoammonium cation and MPO compound II, thereby diverting the enzyme from its halogenation cycle (144). As nitroxides are poor substrates for compound II, this redox intermediate of MPO accumulates and HOCl production is effectively limited with an IC_{50} of $\sim 1 \mu M$ reported for 4-amino-TEMPO (144). A potential drawback for nitroxides as MPO inhibitors is that physiological small-molecule substrates (*e.g.*, ascorbate, $O_2^{\bullet -}$) can reduce MPO compound II back to native MPO, which can limit the inhibitory capacity of competitive substrate MPO inhibitors such as nitroxides in complex biological systems (144).

Despite this, nitroxides effectively inhibit the production of HOCl by activated neutrophils that also produce elevated levels of $O_2^{\bullet -}$, with an IC_{50} of $\sim 6 \mu M$ reported for 4-amino-TEMPO (144). The ability of nitroxides to maintain their inhibitory capacity in the presence of $O_2^{\bullet -}$ likely reflects their actions as SOD mimetics. As such, the pleiotropic antioxidant actions of nitroxides that include radical scavenging, SOD mimetic activity, and reversible MPO inhibition mean that they have greater potential in alleviating oxidative tissue damage within complex inflammatory tissues compared with selective MPO inhibitors.

Of the nitroxides, 4-aminoTEMPO and 4-hydroxy-TEMPO (tempol) represent the most potent inhibitors of HOCl production by MPO (144). Another nitroxide, 4-methoxy TEMPO, attenuated inflammatory damage in a cardiomyocyte cell line incubated with activated neutrophils (23). In this coculture system, exposure of cardiomyocytes to activated neutrophils as a cellular source of MPO-derived HOCl increased oxidative stress, redox cell signaling, and cardiomyocyte apoptosis. The addition of 4-methoxy TEMPO decreased the formation of 3-Cl-Tyr, indicating the successful inhibition of MPO catalytic activity, restored the balance of the intracellular phosphoproteome and protected cardiomyocytes against apoptosis (23). Nitroxides have also been reported to protect cardiomyocyte plasma membranes against rigidification *in vivo* in mice treated with the cardiotoxic anthracycline drugs aclarubicin and doxorubicin (83).

A limitation for use of nitroxides as pharmacological agents is the short half-life of the active nitroxide moiety that is reduced by biological reductants to the corresponding hydroxylamine, which may exhibit variable pharmacokinetics (85, 90). Therefore, current studies of nitroxides have focused on designing approaches to ensure the longevity of the drug within the desired tissue. For example, an injection of 4-amino TEMPO covalently bonded to thermally responsive hydrogels (that enhance the nitroxide *in vivo* lifetime) at different infarct zones after coronary ligation/reperfusion in a rat model of AMI reduced the levels of apoptotic cells in the infarct and border regions and decreased oxidative stress in the infarcted hearts, resulting in improved overall cardiac functioning to a greater extent compared with an injection of 4-amino TEMPO alone (223). Although

hydrogels mitigated oxidative heart damage, a number of limitations impact their use, including inability to enter cells and scavenge intracellular ROS. Further, studies are required to assess whether the introduction of hydrogels disturbs cardiac electrical conductance or activates immune cascades in response to foreign material (223). Another approach to enhance the half-life of tempol *in vivo* is its administration in conjunction with polynitrated albumin, which ensures the recycling of the hydroxylamine, that is, conversion of the biodegraded form of tempol back into the active tempol form. This approach enhanced tempol's half-life to 30 min in an *in vivo* rat model of I/R injury and subsequently decreased infarct size post-AMI/R, whereas the administration of tempol alone failed to replicate this protective effect (90).

Together, what has been cited earlier identifies nitroxides as a promising drug class to target oxidative tissue damage mediated by activated neutrophils post-AMI. Further studies are required to determine the efficacy by which novel, tissue-targeted nitroxides protect the heart post-AMI and to determine the extent to which this cardioprotective activity can be attributed to targeting MPO *versus* other documented antioxidant activities.

Concluding Remarks

After AMI, neutrophils, the first immune cell infiltrators into the damaged myocardium, degranulate and release MPO. MPO catalyzes the production of various oxidants, including HOCl, which are capable of inducing oxidative stress, mitochondrial dysfunction, and decreased cardiomyocyte viability and activating MMPs, leading to increased ECM turnover, fibrosis, and infarct expansion. Neutrophils and MPO levels are associated with a worse prognosis in patients post-AMI, and studies with MPO gene-deficient mice and irreversible MPO inhibitors establish a pathogenic role for the enzyme during AMI. As such, targeting neutrophil-derived MPO by pharmacological inhibitors may be a promising therapeutic strategy to limit tissue damage post-AMI (particularly in the myocardial area at risk) and subsequently diminish heart failure rates in humans. For example, nitroxides show significant potential in combating I/R tissue injury as they are capable of simultaneous ROS scavenging, anti-inflammatory actions, and MPO inhibition and, therefore, represent a promising class of versatile redox-active drugs in treating post-AMI injury and preventing heart failure. Further studies are warranted to determine the efficacy by which novel MPO-targeted inhibitors such as cyclic nitroxides with improved cardiac tissue half-life can ameliorate post-AMI tissue injury and the therapeutic window for the administration of this drug class as an adjunct therapy to PCI.

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References

- Abbate A, Van Tassel BW, Biondi-Zoccai G, Kontos MC, Grizzard JD, Spillman DW, Oddi C, Roberts CS, Melchior RD, Mueller GH, Abouzaki NA, Rengel LR, Varma A, Gambill ML, Falcao RA, Voelkel NF, Dinarello CA, and Vetrovec GW. Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2) (VCU-ART2) pilot study]. *Am J Cardiol* 111: 1394–1400, 2013.
- Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP, and Sammut IA. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. *FASEB J* 19: 1088–1095, 2005.
- Ahmad G, Chami B, El Kazzi M, Wang X, Moreira MTS, Hamilton N, Maw AM, Hambly TW, and Witting PK. Catalase-like antioxidant activity is unaltered in hypochlorous acid oxidized horse heart myoglobin. *Antioxidants (Basel)* 8: e414, 2019.
- Albert CJ, Crowley JR, Hsu FF, Thukkani AK, and Ford DA. Reactive chlorinating species produced by myeloperoxidase target the vinyl ether bond of plasmalogens: identification of 2-chlorohexadecanal. *J Biol Chem* 276: 23733–23741, 2001.
- Ali M, Pulli B, Courties G, Tricot B, Sebas M, Iwamoto Y, Hilgendorf I, Schob S, Dong A, Zheng W, Skoura A, Kalgukar A, Cortes C, Ruggeri R, Swirski FK, Nahrendorf M, Buckbinder L, and Chen JW. Myeloperoxidase inhibition improves ventricular function and remodeling after experimental myocardial infarction. *JACC Basic Transl Sci* 1: 633–643, 2016.
- Alrabadi N, Chami B, Kim HB, Maw AM, Dennis JM, and Witting PK. Hypochlorous acid generated in the heart following acute ischaemic injury promotes myocardial damage: a new target for therapeutic development. *Trends Cell Mol Biol* 9: 1–17, 2014.
- Anders HJ and Schaefer L. Beyond tissue injury-damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis. *J Am Soc Nephrol* 25: 1387–1400, 2014.
- Arruda-Olson AM, Reeder GS, Bell MR, Weston SA, and Roger VL. Neutrophilia predicts death and heart failure after myocardial infarction: a community-based study. *Circ Cardiovasc Qual Outcomes* 2: 656–662, 2009.
- Askari AT, Brennan ML, Zhou X, Drinko J, Morehead A, Thomas JD, Topol EJ, Hazen SL, and Penn MS. Myeloperoxidase and plasminogen activator inhibitor 1 play a central role in ventricular remodeling after myocardial infarction. *J Exp Med* 197: 615–624, 2003.
- Azab B, Zaher M, Weiserbs KF, Torbey E, Lacossiere K, Gaddam S, Gobunsuy R, Jadonath S, Baldari D, McCord D, and Lafferty J. Usefulness of neutrophil to lymphocyte ratio in predicting short- and long-term mortality after non-ST-elevation myocardial infarction. *Am J Cardiol* 106: 470–476, 2010.
- Azam MA, Wagg CS, Masse S, Farid T, Lai PF, Kusha M, Asta J, Jaimes R, 3rd, Kuzmiak-Glancy S, Kay MW, Lopaschuk GD, and Nanthakumar K. Feeding the fibrillating heart: dichloroacetate improves cardiac contractile dysfunction following VF. *Am J Physiol Heart Circ Physiol* 309: H1543–H1553, 2015.
- Baldus S, Eiserich JP, Brennan ML, Jackson RM, Alexander CB, and Freeman BA. Spatial mapping of pulmonary and vascular nitrotyrosine reveals the pivotal role of myeloperoxidase as a catalyst for tyrosine nitration in inflammatory diseases. *Free Radic Biol Med* 33: 1010, 2002.

13. Baldus S, Eiserich JP, Mani A, Castro L, Figueroa M, Chumley P, Ma W, Tousson A, White CR, Bullard DC, Brennan ML, Lusis AJ, Moore KP, and Freeman BA. Endothelial transcytosis of myeloperoxidase confers specificity to vascular ECM proteins as targets of tyrosine nitration. *J Clin Invest* 108: 1759–1770, 2001.
14. Baldus S, Heitzer T, Eiserich JP, Lau D, Mollnau H, Ortak M, Petri S, Goldmann B, Duchstein HJ, Berger J, Helmchen U, Freeman BA, Meinertz T, and Munzel T. Myeloperoxidase enhances nitric oxide catabolism during myocardial ischemia and reperfusion. *Free Radic Biol Med* 37: 902–911, 2004.
15. Banerjee I, Fuseler JW, Price RL, Borg TK, and Baudino TA. Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse. *Am J Physiol Heart Circ Physiol* 293: H1883–H1891, 2007.
16. Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO, Singh P, Anantharamaiah GM, Chait A, Brunzell J, Geary RL, Oram JF, and Heinecke JW. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc Natl Acad Sci U S A* 101: 13032–13037, 2004.
17. Bertero E and Maack C. Calcium signaling and reactive oxygen species in mitochondria. *Circ Res* 122: 1460–1478, 2018.
18. Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, and Hazen SL. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med* 349: 1595–1604, 2003.
19. Brown DA, Perry JB, Allen ME, Sabbah HN, Stauffer BL, Shaikh SR, Cleland JG, Colucci WS, Butler J, Voors AA, Anker SD, Pitt B, Pieske B, Filippatos G, Greene SJ, and Gheorghiade M. Expert consensus document: mitochondrial function as a therapeutic target in heart failure. *Nat Rev Cardiol* 14: 238–250, 2017.
20. Buffon A, Biasucci LM, Liuzzo G, D'Onofrio G, Crea F, and Maseri A. Widespread coronary inflammation in unstable angina. *N Engl J Med* 347: 5–12, 2002.
21. Burke AP and Virmani R. Pathophysiology of acute myocardial infarction. *Med Clin North Am* 91: 553–572; ix, 2007.
22. Carr AC and Winterbourn CC. Oxidation of neutrophil glutathione and protein thiols by myeloperoxidase-derived hypochlorous acid. *Biochem J* 327 (Pt 1): 275–281, 1997.
23. Chami B, Jeong G, Varda A, Maw AM, Kim HB, Fong GM, Simone M, Rayner BS, Wang XS, Dennis JM, and Witting PK. The nitroxide 4-methoxy TEMPO inhibits neutrophil-stimulated kinase activation in H9c2 cardiomyocytes. *Arch Biochem Biophys* 629: 19–35, 2017.
24. Chen Q, Moghaddas S, Hoppel CL, and Lesnefsky EJ. Reversible blockade of electron transport during ischemia protects mitochondria and decreases myocardial injury following reperfusion. *J Pharmacol Exp Ther* 319: 1405–1412, 2006.
25. Cheng D, Talib J, Stanley CP, Rashid I, Michaelsson E, Lindstedt EL, Croft KD, Kettle AJ, Maghazal GJ, and Stocker R. Inhibition of MPO (myeloperoxidase) attenuates endothelial dysfunction in mouse models of vascular inflammation and atherosclerosis. *Arterioscler Thromb Vasc Biol* 39: 1448–1457, 2019.
26. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord ENJ, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa ASH, Brookes PS, Davidson SM, Duchon MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, and Murphy MP. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* 515: 431–435, 2014.
27. Clanachan AS. Contribution of protons to post-ischemic Na(+) and Ca(2+) overload and left ventricular mechanical dysfunction. *J Cardiovasc Electrophysiol* 17(Suppl 1): S141–S48, 2006.
28. Cook NL, Viola HM, Sharov VS, Hool LC, Schoneich C, and Davies MJ. Myeloperoxidase-derived oxidants inhibit sarco/endoplasmic reticulum Ca²⁺-ATPase activity and perturb Ca²⁺ homeostasis in human coronary artery endothelial cells. *Free Radic Biol Med* 52: 951–961, 2012.
29. Cung TT, Morel O, Cayla G, Rioufol G, Garcia-Dorado D, Angoulvant D, Bonnefoy-Cudraz E, Guerin P, Elbaz M, Delarche N, Coste P, Vanzetto G, Metge M, Aupetit JF, Jouve B, Motreff P, Tron C, Labeque JN, Steg PG, Cottin Y, Range G, Clerc J, Claeys MJ, Coussement P, Prunier F, Moulin F, Roth O, Belle L, Dubois P, Barragan P, Gilard M, Piot C, Colin P, De Poli F, Morice MC, Ider O, Dubois-Rande JL, Untersee T, Le Breton H, Beard T, Blanchard D, Grolhier G, Malquarti V, Staat P, Sudre A, Elmer E, Hansson MJ, Bergerot C, Boussaha I, Jossan C, Derumeaux G, Mewton N, and Ovize M. Cyclosporine before PCI in patients with acute myocardial infarction. *N Engl J Med* 373: 1021–1031, 2015.
30. Czubyryt MP. Common threads in cardiac fibrosis, infarct scar formation, and wound healing. *Fibrogenesis Tissue Repair* 5: 19, 2012.
31. Dao VT, Casas AI, Maghazal GJ, Seredenina T, Kaludercic N, Robledinos-Anton N, Di Lisa F, Stocker R, Ghezzi P, Jaquet V, Cuadrado A, and Schmidt HH. Pharmacology and clinical drug candidates in redox medicine. *Antioxid Redox Signal* 23: 1113–1129, 2015.
32. Dare AJ, Logan A, Prime TA, Rogatti S, Goddard M, Bolton EM, Bradley JA, Pettigrew GJ, Murphy MP, and Saeb-Parsy K. The mitochondria-targeted anti-oxidant MitoQ decreases ischemia-reperfusion injury in a murine syngeneic heart transplant model. *J Heart Lung Transplant* 34: 1471–1480, 2015.
33. Daugherty A, Dunn JL, Rateri DL, and Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 94: 437–444, 1994.
34. Davies MJ. Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention. *J Clin Biochem Nutr* 48: 8–19, 2011.
35. De Tombe PP. Altered contractile function in heart failure. *Cardiovasc Res* 37: 367–380, 1998.
36. Deleon-Pennell KY, Altara R, Yabluchanskiy A, Modesti A, and Lindsey ML. The circular relationship between matrix metalloproteinase-9 and inflammation following myocardial infarction. *IUBMB Life* 67: 611–618, 2015.
37. Dikalov S. Cross talk between mitochondria and NADPH oxidases. *Free Radic Biol Med* 51: 1289–1301, 2011.
38. Dirksen MT, Laarman GJ, Simoons ML, and Duncker DJ. Reperfusion injury in humans: a review of clinical trials on reperfusion injury inhibitory strategies. *Cardiovasc Res* 74: 343–355, 2007.

39. Dodd MS, Atherton HJ, Carr CA, Stuckey DJ, West JA, Griffin JL, Radda GK, Clarke K, Heather LC, and Tyler DJ. Impaired in vivo mitochondrial Krebs cycle activity after myocardial infarction assessed using hyperpolarized magnetic resonance spectroscopy. *Circ Cardiovasc Imaging* 7: 895–904, 2014.
40. Doerries C, Grote K, Hilfiker-Kleiner D, Luchtefeld M, Schaefer A, Holland SM, Sorrentino S, Manes C, Schieffer B, Drexler H, and Landmesser U. Critical role of the NAD(P)H oxidase subunit p47phox for left ventricular remodeling/dysfunction and survival after myocardial infarction. *Circ Res* 100: 894–903, 2007.
41. Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, and Freeman BA. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 296: 2391–2394, 2002.
42. Fang X, Wang H, Han D, Xie E, Yang X, Wei J, Gu S, Gao F, Zhu N, Yin X, Cheng Q, Zhang P, Dai W, Chen J, Yang F, Yang HT, Linkermann A, Gu W, Min J, and Wang F. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci U S A* 116: 2672–2680, 2019.
43. Ferrante G, Nakano M, Prati F, Niccoli G, Mallus MT, Ramazzotti V, Montone RA, Kolodgie FD, Virmani R, and Crea F. High levels of systemic myeloperoxidase are associated with coronary plaque erosion in patients with acute coronary syndromes: a clinicopathological study. *Circulation* 122: 2505–2513, 2010.
44. Fillmore N, Levasseur JL, Fukushima A, Wagg CS, Wang W, Dyck JRB, and Lopaschuk GD. Uncoupling of glycolysis from glucose oxidation accompanies the development of heart failure with preserved ejection fraction. *Mol Med* 24: 3, 2018.
45. Forbes LV, Furtmuller PG, Khalilova I, Turner R, Obinger C, and Kettle AJ. Isoniazid as a substrate and inhibitor of myeloperoxidase: identification of amine adducts and the influence of superoxide dismutase on their formation. *Biochem Pharmacol* 84: 949–960, 2012.
46. Forbes LV, Sjogren T, Auchere F, Jenkins DW, Thong B, Laughton D, Hemsley P, Pairaudeau G, Turner R, Eriksson H, Unitt JF, and Kettle AJ. Potent reversible inhibition of myeloperoxidase by aromatic hydroxamates. *J Biol Chem* 288: 36636–36647, 2013.
47. Frangogiannis NG. Cell biological mechanisms in regulation of the post-infarction inflammatory response. *Curr Opin Physiol* 1: 7–13, 2018.
48. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodeling. *Nat Rev Cardiol* 11: 255–265, 2014.
49. Fu X, Kassim SY, Parks WC, and Heinecke JW. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. *J Biol Chem* 276: 41279–41287, 2001.
50. Garcia-Dorado D, Ruiz-Meana M, Inerte J, Rodriguez-Sinovas A, and Piper HM. Calcium-mediated cell death during myocardial reperfusion. *Cardiovasc Res* 94: 168–180, 2012.
51. Garcia-Prieto J, Villena-Gutierrez R, Gomez M, Bernardo E, Pun-Garcia A, Garcia-Lunar I, Crainiciuc G, Fernandez-Jimenez R, Sreeramkumar V, Bourio-Martinez R, Garcia-Ruiz JM, Del Valle AS, Sanz-Rosa D, Pizarro G, Fernandez-Ortiz A, Hidalgo A, Fuster V, and Ibanez B. Neutrophil stunning by metoprolol reduces infarct size. *Nat Commun* 8: 14780, 2017.
52. Getts DR, Terry RL, Getts MT, Deffrasnes C, Muller M, van Vreden C, Ashhurst TM, Chami B, McCarthy D, Wu H, Ma J, Martin A, Shae LD, Witting P, Kansas GS, Kuhn J, Hafezi W, Campbell IL, Reilly D, Say J, Brown L, White MY, Cordwell SJ, Chadban SJ, Thorp EB, Bao S, Miller SD, and King NJ. Therapeutic inflammatory monocyte modulation using immune-modifying micro-particles. *Sci Transl Med* 6: 219ra7, 2014.
53. Ghaffari S, Nadiri M, Pourafkari L, Sepehrvand N, Movasagpoor A, Rahmatvand N, Rezazadeh Saatloo M, Ahmadi M, and Nader ND. The predictive value of total neutrophil count and neutrophil/lymphocyte ratio in predicting in-hospital mortality and complications after STEMI. *J Cardiovasc Thorac Res* 6: 35–41, 2014.
54. Goldmann BU, Rudolph V, Rudolph TK, Holle AK, Hillebrandt M, Meinertz T, and Baldus S. Neutrophil activation precedes myocardial injury in patients with acute myocardial infarction. *Free Radic Biol Med* 47: 79–83, 2009.
55. Gonzalez-Montero J, Brito R, Gajardo AI, and Rodrigo R. Myocardial reperfusion injury and oxidative stress: therapeutic opportunities. *World J Cardiol* 10: 74–86, 2018.
56. Gottlieb SS, McCarter RJ, and Vogel RA. Effect of beta-blockade on mortality among high-risk and low-risk patients after myocardial infarction. *N Engl J Med* 339: 489–497, 1998.
57. Granger DN and Kvietys PR. Reperfusion injury and reactive oxygen species: the evolution of a concept. *Redox Biol* 6: 524–551, 2015.
58. Griffiths EJ and Rutter GA. Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. *Biochim Biophys Acta* 1787: 1324–1333, 2009.
59. Guo X, Yin H, Li L, Chen Y, Li J, Doan J, Steinmetz R, and Liu Q. Cardioprotective role of tumor necrosis factor receptor-associated factor 2 by suppressing apoptosis and necroptosis. *Circulation* 136: 729–742, 2017.
60. Halestrap AP, Clarke SJ, and Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. *Cardiovasc Res* 61: 372–385, 2004.
61. Hansen PR. Role of neutrophils in myocardial ischemia and reperfusion. *Circulation* 91: 1872–1885, 1995.
62. Hartman CL, Duerr MA, Albert CJ, Neumann WL, McHowat J, and Ford DA. 2-Chlorofatty acids induce Weibel-Palade body mobilization. *J Lipid Res* 59: 113–122, 2018.
63. Hartman MHT, Groot HE, Leach IM, Karper JC, and van der Harst P. Translational overview of cytokine inhibition in acute myocardial infarction and chronic heart failure. *Trends Cardiovasc Med* 28: 369–379, 2018.
64. Harwood DT, Kettle AJ, and Winterbourn CC. Production of glutathione sulfonamide and dehydroglutathione from GSH by myeloperoxidase-derived oxidants and detection using a novel LC-MS/MS method. *Biochem J* 399: 161–168, 2006.
65. Hausenloy DJ, Botker HE, Engstrom T, Erlinge D, Heusch G, Ibanez B, Kloner RA, Ovize M, Yellon DM, and Garcia-Dorado D. Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: trials and tribulations. *Eur Heart J* 38: 935–941, 2017.

66. Hazell LJ, Arnold L, Flowers D, Waeg G, Malle E, and Stocker R. Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *J Clin Invest* 97: 1535–1544, 1996.
67. Hearse DJ, Humphrey SM, and Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release. *J Mol Cell Cardiol* 5: 395–407, 1973.
68. Heather LC, Carr CA, Stuckey DJ, Pope S, Morten KJ, Carter EE, Edwards LM, and Clarke K. Critical role of complex III in the early metabolic changes following myocardial infarction. *Cardiovasc Res* 85: 127–136, 2010.
69. Heusch G and Gersh BJ. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. *Eur Heart J* 38: 774–784, 2017.
70. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, Weber C, Soehnlein O, and Steffens S. Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. *Eur Heart J* 38: 187–197, 2017.
71. Huang Y, Hickey RP, Yeh JL, Liu D, Dadak A, Young LH, Johnson RS, and Giordano FJ. Cardiac myocyte-specific HIF-1 α deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart. *FASEB J* 18: 1138–1140, 2004.
72. Huynh LT, Rankin JM, Tideman P, Brieger DB, Erickson M, Markwick AJ, Astley C, Kelaher DJ, and Chew DP. Reperfusion therapy in the acute management of ST-segment-elevation myocardial infarction in Australia: findings from the ACACIA registry. *Med J Aust* 193: 496–501, 2010.
73. Ibanez B, Cimmino G, Prat-Gonzalez S, Vilahur G, Hutter R, Garcia MJ, Fuster V, Sanz J, Badimon L, and Badimon JJ. The cardioprotection granted by metoprolol is restricted to its administration prior to coronary reperfusion. *Int J Cardiol* 147: 428–432, 2011.
74. Ibanez B, Macaya C, Sanchez-Brunete V, Pizarro G, Fernandez-Friera L, Mateos A, Fernandez-Ortiz A, Garcia-Ruiz JM, Garcia-Alvarez A, Iniguez A, Jimenez-Borreguero J, Lopez-Romero P, Fernandez-Jimenez R, Goicolea J, Ruiz-Mateos B, Bastante T, Arias M, Iglesias-Vazquez JA, Rodriguez MD, Escalera N, Acebal C, Cabrera JA, Valenciano J, Perez de Prado A, Fernandez-Campos MJ, Casado I, Garcia-Rubira JC, Garcia-Prieto J, Sanz-Rosa D, Cuellas C, Hernandez-Antolin R, Albarran A, Fernandez-Vazquez F, de la Torre-Hernandez JM, Pocock S, Sanz G, and Fuster V. Effect of early metoprolol on infarct size in ST-segment-elevation myocardial infarction patients undergoing primary percutaneous coronary intervention: the Effect of Metoprolol in Cardio-protection During an Acute Myocardial Infarction (METOCARD-CNIC) trial. *Circulation* 128: 1495–1503, 2013.
75. Jaxa-Chamiec T, Bednars B, Herbaczynska-Cedro K, Maciejewski P, and Ceremuzynski L; MIVIT Trial Group. Effects of vitamins C and E on the outcome after acute myocardial infarction in diabetics: a retrospective, hypothesis-generating analysis from the MIVIT study. *Cardiology* 112: 219–223, 2009.
76. Kato N, Yanaka K, Hyodo K, Homma K, Nagase S, and Nose T. Stable nitroxide Tempol ameliorates brain injury by inhibiting lipid peroxidation in a rat model of transient focal cerebral ischemia. *Brain Res* 979: 188–193, 2003.
77. Kawaguchi M, Takahashi M, Hata T, Kashima Y, Usui F, Morimoto H, Izawa A, Takahashi Y, Masumoto J, Koyama J, Hongo M, Noda T, Nakayama J, Sagara J, Taniguchi S, and Ikeda U. Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. *Circulation* 123: 594–604, 2011.
78. Kettle AJ, Anderson RF, Hampton MB, and Winterbourn CC. Reactions of superoxide with myeloperoxidase. *Biochemistry* 46: 4888–4897, 2007.
79. Khan SQ, Kelly D, Quinn P, Davies JE, and Ng LL. Myeloperoxidase aids prognostication together with N-terminal pro-B-type natriuretic peptide in high-risk patients with acute ST elevation myocardial infarction. *Heart* 93: 826–831, 2007.
80. Kihara Y, Sasayama S, Inoko M, and Morgan JP. Sodium/calcium exchange modulates intracellular calcium overload during posthypoxic reoxygenation in mammalian working myocardium. Evidence from aequorin-loaded ferret ventricular muscles. *J Clin Invest* 93: 1275–1284, 1994.
81. King CC, Jefferson MM, and Thomas EL. Secretion and inactivation of myeloperoxidase by isolated neutrophils. *J Leukoc Biol* 61: 293–302, 1997.
82. Kleaveland O, Kunszt G, Bratlie M, Ueland T, Broch K, Holte E, Michelsen AE, Bendz B, Amundsen BH, Espevik T, Aakhus S, Damas JK, Aukrust P, Wiseth R, and Gullestad L. Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial. *Eur Heart J* 37: 2406–2413, 2016.
83. Koceva-Chyla A, Sokal A, Kania K, Gwoździński K, and Jóźwiak Z. The nitroxides pirolin and pirolid protect the plasma membranes of rat cardiomyocytes against damage induced by anthracyclines. *Cell Mol Biol Lett* 8: 171–177, 2003.
84. Koelsch M, Mallak R, Graham GG, Kajer T, Milligan MK, Nguyen LQ, Newsham DW, Keh JS, Kettle AJ, Scott KF, Ziegler JB, Pattison DI, Fu S, Hawkins CL, Rees MD, and Davies MJ. Acetaminophen (paracetamol) inhibits myeloperoxidase-catalyzed oxidant production and biological damage at therapeutically achievable concentrations. *Biochem Pharmacol* 79: 1156–1164, 2010.
85. Kuppusamy P, Wang P, Zweier JL, Krishna MC, Mitchell JB, Ma L, Trimble CE, and Hsia CJ. Electron paramagnetic resonance imaging of rat heart with nitroxide and polynitroxyl-albumin. *Biochemistry* 35: 7051–7057, 1996.
86. Kyne L, Hausdorff JM, Knight E, Dukas L, Azhar G, and Wei JY. Neutrophilia and congestive heart failure after acute myocardial infarction. *Am Heart J* 139: 94–100, 2000.
87. Lee JW, Bae SH, Jeong JW, Kim SH, and Kim KW. Hypoxia-inducible factor (HIF-1) α : its protein stability and biological functions. *Exp Mol Med* 36: 1–12, 2004.
88. Lesnefsky EJ, Chen Q, Tandler B, and Hoppel CL. Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. *Annu Rev Pharmacol Toxicol* 57: 535–565, 2017.
89. Lewandowski M and Gwoździński K. Nitroxides as antioxidants and anticancer drugs. *Int J Mol Sci* 18: e2990, 2017.
90. Li H, Ma L, Hsia CJ, Zweier JL, and Kuppusamy P. Polynitroxyl-albumin (PNA) enhances myocardial

- infarction therapeutic effect of tempol in rat hearts subjected to regional ischemia-reperfusion. *Free Radic Biol Med* 32: 712–719, 2002.
91. Li YY, Feldman AM, Sun Y, and McTiernan CF. Differential expression of tissue inhibitors of metalloproteinases in the failing human heart. *Circulation* 98: 1728–1734, 1998.
92. Libby P, Lichtman AH, and Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity* 38: 1092–1104, 2013.
93. Libby P, Ridker PM, and Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature* 473: 317–325, 2011.
94. Lichy M, Szobi A, Hrdlicka J, Horvath C, Kormanova V, Rajtik T, Neckar J, Kolar F, and Adameova A. Different signalling in infarcted and non-infarcted areas of rat failing hearts: a role of necroptosis and inflammation. *J Cell Mol Med* 23: 6429–6441, 2019.
95. Litt MR, Jeremy RW, Weisman HF, Winkelstein JA, and Becker LC. Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Evidence for neutrophil-mediated reperfusion injury. *Circulation* 80: 1816–1827, 1989.
96. Liu H, Cala PM, and Anderson SE. Na/H exchange inhibition protects newborn heart from ischemia/reperfusion injury by limiting Na⁺-dependent Ca²⁺ overload. *J Cardiovasc Pharmacol* 55: 227–233, 2010.
97. Lloyd MM, Grima MA, Rayner BS, Hadfield KA, Davies MJ, and Hawkins CL. Comparative reactivity of the myeloperoxidase-derived oxidants hypochlorous acid and hypothiocyanous acid with human coronary artery endothelial cells. *Free Radic Biol Med* 65: 1352–1362, 2013.
98. Lopez-Armada MJ, Riveiro-Naveira RR, Vaamonde-Garcia C, and Valcarcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. *Mitochondrion* 13: 106–118, 2013.
99. Luo F, Liu X, Yan N, Li S, Cao G, Cheng Q, Xia Q, and Wang H. Hypoxia-inducible transcription factor-1 α promotes hypoxia-induced A549 apoptosis via a mechanism that involves the glycolysis pathway. *BMC Cancer* 6: 26, 2006.
100. Luo M, Guan X, Luczak ED, Lang D, Kutschke W, Gao Z, Yang J, Glynn P, Sossalla S, Swaminathan PD, Weiss RM, Yang B, Rokita AG, Maier LS, Efimov IR, Hund TJ, and Anderson ME. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. *J Clin Invest* 123: 1262–1274, 2013.
101. Ma Y, Yabluchanskiy A, Iyer RP, Cannon PL, Flynn ER, Jung M, Henry J, Cates CA, Deleon-Pennell KY, and Lindsey ML. Temporal neutrophil polarization following myocardial infarction. *Cardiovasc Res* 110: 51–61, 2016.
102. Maiocchi SL, Morris JC, Rees MD, and Thomas SR. Regulation of the nitric oxide oxidase activity of myeloperoxidase by pharmacological agents. *Biochem Pharmacol* 135: 90–115, 2017.
103. Mallat Z, Philip I, Lebreton M, Chatel D, Maclouf J, and Tedgui A. Elevated levels of 8-iso-prostaglandin F₂ α in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure. *Circulation* 97: 1536–1539, 1998.
104. Manechote C, Palee S, Chattipakorn SC, and Chattipakorn N. Roles of mitochondrial dynamics modulators in cardiac ischaemia/reperfusion injury. *J Cell Mol Med* 21: 2643–2653, 2017.
105. Mann DL. Innate immunity and the failing heart: the cytokine hypothesis revisited. *Circ Res* 116: 1254–1268, 2015.
106. Marchetti C, Chojnacki J, Toldo S, Mezzaroma E, Tranchida N, Rose SW, Federici M, Van Tassell BW, Zhang S, and Abbate A. A novel pharmacologic inhibitor of the NLRP3 inflammasome limits myocardial injury after ischemia-reperfusion in the mouse. *J Cardiovasc Pharmacol* 63: 316–322, 2014.
107. Matsushima S, Kuroda J, Ago T, Zhai P, Ikeda Y, Oka S, Fong GH, Tian R, and Sadoshima J. Broad suppression of NADPH oxidase activity exacerbates ischemia/reperfusion injury through inadvertent downregulation of hypoxia-inducible factor-1 α and upregulation of peroxisome proliferator-activated receptor- α . *Circ Res* 112: 1135–1149, 2013.
108. Maxwell PH, Dachs GU, Gleadow JM, Nicholls LG, Harris AL, Stratford IJ, Hankinson O, Pugh CW, and Ratcliffe PJ. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc Natl Acad Sci U S A* 94: 8104–8109, 1997.
109. McMillen TS, Heinecke JW, and LeBoeuf RC. Expression of human myeloperoxidase by macrophages promotes atherosclerosis in mice. *Circulation* 111: 2798–2804, 2005.
110. Meotti FC, Jameson GN, Turner R, Harwood DT, Stockwell S, Rees MD, Thomas SR, and Kettle AJ. Urate as a physiological substrate for myeloperoxidase: implications for hyperuricemia and inflammation. *J Biol Chem* 286: 12901–12911, 2011.
111. Mewhort HE, Lipon BD, Svystonyuk DA, Teng G, Guzzardi DG, Silva C, Yong VW, and Fedak PW. Monocytes increase human cardiac myofibroblast-mediated extracellular matrix remodeling through TGF- β 1. *Am J Physiol Heart Circ Physiol* 310: H716–H724, 2016.
112. Midwinter RG, Vissers MC, and Winterbourn CC. Hypochlorous acid stimulation of the mitogen-activated protein kinase pathway enhances cell survival. *Arch Biochem Biophys* 394: 13–20, 2001.
113. Mishra PK, Adameova A, Hill JA, Baines CP, Kang PM, Downey JM, Narula J, Takahashi M, Abbate A, Piristine HC, Kar S, Su S, Higa JK, Kawasaki NK, and Matsui T. Guidelines for evaluating myocardial cell death. *Am J Physiol Heart Circ Physiol* 317: H891–H922, 2019.
114. Mittal M, Siddiqui MR, Tran K, Reddy SP, and Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 20: 1126–1167, 2014.
115. Mocatta TJ, Pilbrow AP, Cameron VA, Senthilmohan R, Frampton CM, Richards AM, and Winterbourn CC. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J Am Coll Cardiol* 49: 1993–2000, 2007.
116. Mollenhauer M, Friedrichs K, Lange M, Gesenberg J, Remane L, Kerkenpass C, Krause J, Schneider J, Ravekes T, Maass M, Halbach M, Peinkofer G, Saric T, Mehrkens D, Adam M, Deuschl FG, Lau D, Geertz B, Manchanda K, Eschenhagen T, Kubala L, Rudolph TK, Wu Y, Tang WHW, Hazen SL, Baldus S, Klinke A, and Rudolph V. Myeloperoxidase mediates postischemic arrhythmogenic ventricular remodeling. *Circ Res* 121: 56–70, 2017.

117. Mongue-Din H, Patel AS, Looi YH, Grieve DJ, Anilkumar N, Sirker A, Dong X, Brewer AC, Zhang M, Smith A, and Shah AM. NADPH oxidase-4 driven cardiac macrophage polarization protects against myocardial infarction-induced remodeling. *JACC Basic Transl Sci* 2: 688–698, 2017.
118. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, and Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med* 204: 3037–3047, 2007.
119. Nakayama H and Otsu K. Mitochondrial DNA as an inflammatory mediator in cardiovascular diseases. *Biochem J* 475: 839–852, 2018.
120. Nazir S, Gadi I, Al-Dabet MM, Elwakiel A, Kohli S, Ghosh S, Manoharan J, Ranjan S, Bock F, Braun-Dullaeus RC, Esmen CT, Huber TB, Camerer E, Dockendorff C, Griffin JH, Isermann B, and Shahzad K. Cytoprotective activated protein C averts Nlrp3 inflammasome-induced ischemia-reperfusion injury via mTORC1 inhibition. *Blood* 130: 2664–2677, 2017.
121. Neuzil J, Rayner BS, Lowe HC, and Witting PK. Oxidative stress in myocardial ischaemia reperfusion injury: a renewed focus on a long-standing area of heart research. *Redox Rep* 10: 187–197, 2005.
122. Neuzil J, Witting PK, Kontush A, and Headrick JP. Role of vitamins E and C in nuclear factor- κ B and nitric oxide signalling. In: *Encyclopedia of Vitamin E*, edited by Preedy VR and Watson RR. London, United Kingdom: CABI, 2006, pp. 880–902.
123. Newby LK, Marber MS, Melloni C, Sarov-Blat L, Aberle LH, Aylward PE, Cai G, de Winter RJ, Hamm CW, Heitner JF, Kim R, Lerman A, Patel MR, Tanguay JF, Lepore JJ, Al-Khalidi HR, Sprecher DL, and Granger CB; SOLSTICE Investigators. Losmapimod, a novel p38 mitogen-activated protein kinase inhibitor, in non-ST-segment elevation myocardial infarction: a randomised phase 2 trial. *Lancet* 384: 1187–1195, 2014.
124. Nguyen DT, Ding C, Wilson E, Marcus GM, and Olgin JE. Pirfenidone mitigates left ventricular fibrosis and dysfunction after myocardial infarction and reduces arrhythmias. *Heart Rhythm* 7: 1438–1445, 2010.
125. Nicholls SJ and Hazen SL. Myeloperoxidase and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 25: 1102–1111, 2005.
126. Nossuli TO, Frangogiannis NG, Knuefermann P, Lakshminarayanan V, Dewald O, Evans AJ, Peschon J, Mann DL, Michael LH, and Entman ML. Brief murine myocardial I/R induces chemokines in a TNF- α -independent manner: role of oxygen radicals. *Am J Physiol Heart Circ Physiol* 281: H2549–H2558, 2001.
127. Nussbaum C, Klinke A, Adam M, Baldus S, and Sperandio M. Myeloperoxidase: a leukocyte-derived protagonist of inflammation and cardiovascular disease. *Antioxid Redox Signal* 18: 692–713, 2013.
128. Obal D, Dai S, Keith R, Dimova N, Kingery J, Zheng YT, Zweier J, Velayutham M, Prabhu SD, Li Q, Conklin D, Yang D, Bhatnagar A, Bolli R, and Rokosh G. Cardiomyocyte-restricted overexpression of extracellular superoxide dismutase increases nitric oxide bioavailability and reduces infarct size after ischemia/reperfusion. *Basic Res Cardiol* 107: 305, 2012.
129. O'Donoghue ML, Glaser R, Cavender MA, Aylward PE, Bonaca MP, Budaj A, Davies RY, Dellborg M, Fox KA, Gutierrez JA, Hamm C, Kiss RG, Kovar F, Kuder JF, Im KA, Lepore JJ, Lopez-Sendon JL, Ophuis TO, Parkhomenko A, Shannon JB, Spinar J, Tanguay JF, Ruda M, Steg PG, Theroux P, Wiviott SD, Laws I, Sabatine MS, and Morrow DA; LATITUDE-TIMI Investigators. Effect of losmapimod on cardiovascular outcomes in patients hospitalized with acute myocardial infarction: a randomized clinical trial. *JAMA* 315: 1591–1599, 2016.
130. Offer T, Mohsen M, and Samuni A. An SOD-mimicry mechanism underlies the role of nitroxides in protecting papain from oxidative inactivation. *Free Radic Biol Med* 25: 832–838, 1998.
131. Olsson G, Rehnqvist N, Sjogren A, Erhardt L, and Lundman T. Long-term treatment with metoprolol after myocardial infarction: effect on 3 year mortality and morbidity. *J Am Coll Cardiol* 5: 1428–1437, 1985.
132. Ottani F, Latini R, Staszewsky L, La Vecchia L, Locuratolo N, Sicuro M, Masson S, Barlera S, Milani V, Lombardi M, Costalunga A, Mollicelli N, Santarelli A, De Cesare N, Sganzerla P, Boi A, Maggioni AP, and Limbruno U; CYCLE Investigators. Cyclosporine A in reperfused myocardial infarction: the multicenter, controlled, open-label CYCLE trial. *J Am Coll Cardiol* 67: 365–374, 2016.
133. Pagidipati NJ and Gaziano TA. Estimating deaths from cardiovascular disease: a review of global methodologies of mortality measurement. *Circulation* 127: 749–756, 2013.
134. Panizzi P, Swirski FK, Figueiredo JL, Waterman P, Sosnovik DE, Aikawa E, Libby P, Pittet M, Weissleder R, and Nahrendorf M. Impaired infarct healing in atherosclerotic mice with Ly-6C(hi) monocytosis. *J Am Coll Cardiol* 55: 1629–1638, 2010.
135. Pantano C, Reynaert NL, van der Vliet A, and Janssen-Heininger YM. Redox-sensitive kinases of the nuclear factor- κ B signaling pathway. *Antioxid Redox Signal* 8: 1791–1806, 2006.
136. Panth N, Paudel KR, and Parajuli K. Reactive oxygen species: a key hallmark of cardiovascular disease. *Adv Med* 2016: 9152732, 2016.
137. Pike MM, Kitakaze M, and Marban E. ^{23}Na -NMR measurements of intracellular sodium in intact perfused ferret hearts during ischemia and reperfusion. *Am J Physiol* 259: H1767–H1773, 1990.
138. Pinar N, Soylu Karapinar O, Ozcan O, Atik Dogan E, and Bayraktar S. Protective effects of tempol in an experimental ovarian ischemia-reperfusion injury model in female Wistar albino rats. *Can J Physiol Pharmacol* 95: 861–865, 2017.
139. Prabhu SD and Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ Res* 119: 91–112, 2016.
140. Ptaszynska-Kopczynska K, Szpakowicz A, Marcinkiewicz-Siemion M, Lisowska A, Waszkiewicz E, Witkowski M, Jakim P, Galar B, Musial WJ, and Kaminski KA. Interleukin-6 signaling in patients with chronic heart failure treated with cardiac resynchronization therapy. *Arch Med Sci* 13: 1069–1077, 2017.
141. Pullar JM, Winterbourn CC, and Vissers MC. Loss of GSH and thiol enzymes in endothelial cells exposed to sublethal concentrations of hypochlorous acid. *Am J Physiol* 277: H1505–H1512, 1999.
142. Rajagopal V, Gurm HS, Bhatt DL, Lincoff AM, Tcheng JE, Kereiakes DJ, Kleiman NS, Jia G, and Topol EJ.

- Relation of an elevated white blood cell count after percutaneous coronary intervention to long-term mortality. *Am J Cardiol* 94: 190–192, 2004.
143. Rashid I, Maghzal GJ, Chen YC, Cheng D, Talib J, Newington D, Ren M, Vajandar SK, Searle A, Maluenda A, Lindstedt EL, Jabbour A, Kettle AJ, Bongers A, Power C, Michaelsson E, Peter K, and Stocker R. Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque. *Eur Heart J* 39: 3301–3310, 2018.
 144. Rees MD, Bottle SE, Fairfull-Smith KE, Malle E, Whitelock JM, and Davies MJ. Inhibition of myeloperoxidase-mediated hypochlorous acid production by nitroxides. *Biochem J* 421: 79–86, 2009.
 145. Rees MD, Dang L, Thai T, Owen DM, Malle E, and Thomas SR. Targeted subendothelial matrix oxidation by myeloperoxidase triggers myosin II-dependent de-adhesion and alters signaling in endothelial cells. *Free Radic Biol Med* 53: 2344–2356, 2012.
 146. Rees MD, Maiocchi SL, Kettle AJ, and Thomas SR. Mechanism and regulation of peroxidase-catalyzed nitric oxide consumption in physiological fluids: critical protective actions of ascorbate and thiocyanate. *Free Radic Biol Med* 72: 91–103, 2014.
 147. Reichelt ME, Shanu A, Willems L, Witting PK, Ellis NA, Blackburn MR, and Headrick JP. Endogenous adenosine selectively modulates oxidant stress via the A1 receptor in ischemic hearts. *Antioxid Redox Signal* 11: 2641–2650, 2009.
 148. Reyes L, Hawkins CL, and Rayner BS. Characterization of the cellular effects of myeloperoxidase-derived oxidants on H9c2 cardiac myoblasts. *Arch Biochem Biophys* 665: 132–142, 2019.
 149. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, and Glynn RJ; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 377: 1119–1131, 2017.
 150. Roolvink V, Ibanez B, Ottavanger JP, Pizarro G, van Royen N, Mateos A, Dambrink JE, Escalera N, Lipsic E, Albarran A, Fernandez-Ortiz A, Fernandez-Aviles F, Goicolea J, Botas J, Remkes W, Hernandez-Jaras V, Kedhi E, Zamorano JL, Navarro F, Alfonso F, Garcia-Lledo A, Alonso J, van Leeuwen M, Nijveldt R, Postma S, Kolkman E, Gosselink M, de Smet B, Rasoul S, Piek JJ, Fuster V, and van 't Hof AWJ; EARLY-BAMI Investigators. Early intravenous beta-blockers in patients with ST-segment elevation myocardial infarction before primary percutaneous coronary intervention. *J Am Coll Cardiol* 67: 2705–2715, 2016.
 151. Rosenson RS, Brewer HB, Jr., Ansell BJ, Barter P, Chapman MJ, Heinecke JW, Kontush A, Tall AR, and Webb NR. Dysfunctional HDL and atherosclerotic cardiovascular disease. *Nat Rev Cardiol* 13: 48–60, 2016.
 152. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 340: 115–126, 1999.
 153. Roth Flach RJ, Su C, Bollinger E, Cortes C, Robertson AW, Opsahl AC, Coskran TM, Maresca KP, Keliher EJ, Yates PD, Kim AM, Kalgutkar AS and Buckbinder L. Myeloperoxidase inhibition in mice alters atherosclerotic lesion composition. *PLoS One* 14: e0214150, 2019.
 154. Rudolph TK, Wipper S, Reiter B, Rudolph V, Coym A, Detter C, Lau D, Klinke A, Friedrichs K, Rau T, Pekarova M, Russ D, Knoll K, Kolk M, Schroeder B, Wegscheider K, Andresen H, Schwedhelm E, Boeger R, Ehmke H, and Baldus S. Myeloperoxidase deficiency preserves vasomotor function in humans. *Eur Heart J* 33: 1625–1634, 2012.
 155. Rudolph V, Andrie RP, Rudolph TK, Friedrichs K, Klinke A, Hirsch-Hoffmann B, Schwoerer AP, Lau D, Fu X, Klingel K, Sydow K, Didie M, Seniuk A, von Leitner EC, Szoecs K, Schrickel JW, Treede H, Wenzel U, Lewalter T, Nickenig G, Zimmermann WH, Meinertz T, Boger RH, Reichenspurner H, Freeman BA, Eschenhagen T, Ehmke H, Hazen SL, Willems S, and Baldus S. Myeloperoxidase acts as a profibrotic mediator of atrial fibrillation. *Nat Med* 16: 470–474, 2010.
 156. Ruggeri RB, Buckbinder L, Bagley SW, Carpino PA, Conn EL, Dowling MS, Fernando DP, Jiao W, Kung DW, Orr ST, Qi Y, Rocke BN, Smith A, Warmus JS, Zhang Y, Bowles D, Widlicka DW, Eng H, Ryder T, Sharma R, Wolford A, Okerberg C, Walters K, Maurer TS, Zhang Y, Bonin PD, Spath SN, Xing G, Hepworth D, Ahn K, and Kalgutkar AS. Discovery of 2-(6-(5-Chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)acet amide (PF-06282999): a highly selective mechanism-based myeloperoxidase inhibitor for the treatment of cardiovascular diseases. *J Med Chem* 58: 8513–8528, 2015.
 157. Ruiz-Meana M, Abellan A, Miro-Casas E, and Garcia-Dorado D. Opening of mitochondrial permeability transition pore induces hypercontracture in Ca²⁺ overloaded cardiac myocytes. *Basic Res Cardiol* 102: 542–552, 2007.
 158. Sadek HA, Humphries KM, Szweda PA and Szweda LI. Selective inactivation of redox-sensitive mitochondrial enzymes during cardiac reperfusion. *Arch Biochem Biophys* 406: 222–228, 2002.
 159. Sandanger O, Ranheim T, Vinge LE, Bliksoen M, Alfsnes K, Finsen AV, Dahl CP, Askevold ET, Florholmen G, Christensen G, Fitzgerald KA, Lien E, Valen G, Espevik T, Aukrust P, and Yndestad A. The NLRP3 inflammasome is up-regulated in cardiac fibroblasts and mediates myocardial ischaemia-reperfusion injury. *Cardiovasc Res* 99: 164–174, 2013.
 160. Sanganalmath SK, Stein AB, Guo Y, Tiwari S, Hunt G, Vincent RJ, Huang Y, Rezazadeh A, Ildstad ST, Dawn B, and Bolli R. The beneficial effects of postinfarct cytokine combination therapy are sustained during long-term follow-up. *J Mol Cell Cardiol* 47: 528–535, 2009.
 161. Scanzano A and Cosentino M. Adrenergic regulation of innate immunity: a review. *Front Pharmacol* 6: 171, 2015.
 162. Seidlmayer LK, Juettner VV, Kettlewell S, Pavlov EV, Blatter LA, and Dedkova EN. Distinct mPTP activation mechanisms in ischaemia-reperfusion: contributions of Ca²⁺, ROS, pH, and inorganic polyphosphate. *Cardiovasc Res* 106: 237–248, 2015.
 163. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, Bubes V, Manson JE, Glynn RJ, and Gaziano JM. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 300: 2123–2133, 2008.
 164. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, Ramanujan VK, Wolf AJ, Vergnes L, Ojcius DM, Rentsendorj A, Vargas M, Guerrero C, Wang Y,

- Fitzgerald KA, Underhill DM, Town T, and Arditi M. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36: 401–414, 2012.
165. Shin S, Choi JW, Moon H, Lee CY, Park JH, Lee J, Seo HH, Han G, Lim S, Lee S, Kim SW, and Hwang KC. Simultaneous suppression of multiple programmed cell death pathways by miRNA-105 in cardiac ischemic injury. *Mol Ther Nucleic Acids* 14: 438–449, 2019.
 166. Shokolenko I, Venediktova N, Bochkareva A, Wilson GL, and Alexeyev MF. Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Res* 37: 2539–2548, 2009.
 167. Silvestre-Roig C, Hidalgo A, and Soehnlein O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood* 127: 2173–2181, 2016.
 168. Smith JG. Molecular epidemiology of heart failure: translational challenges and opportunities. *JACC Basic Transl Sci* 2: 757–769, 2017.
 169. Soule BP, Hyodo F, Matsumoto K, Simone NL, Cook JA, Krishna MC, and Mitchell JB. The chemistry and biology of nitroxide compounds. *Free Radic Biol Med* 42: 1632–1650, 2007.
 170. Souza JM, Peluffo G, and Radi R. Protein tyrosine nitration—functional alteration or just a biomarker?. *Free Radic Biol Med* 45: 357–366, 2008.
 171. Spickett CM. Chlorinated lipids and fatty acids: an emerging role in pathology. *Pharmacol Ther* 115: 400–409, 2007.
 172. Sposito AC, de Lima-Junior JC, Moura FA, Barreto J, Bonilha I, Santana M, Virginio VW, Sun L, Carvalho LSF, Soares AAS, Nadruz W, Feinstein SB, Nofer JR, Zanotti I, Kontush A, and Remaley AT. Reciprocal multifaceted interaction between HDL (high-density lipoprotein) and myocardial infarction. *Arterioscler Thromb Vasc Biol* 39: 1550–1564, 2019.
 173. Stacey MM, Vissers MC, and Winterbourn CC. Oxidation of 2-cys peroxiredoxins in human endothelial cells by hydrogen peroxide, hypochlorous acid, and chloramines. *Antioxid Redox Signal* 17: 411–421, 2012.
 174. Stocker R, Huang A, Jeranian E, Hou JY, Wu TT, Thomas SR, and Keaney JF, Jr. Hypochlorous acid impairs endothelium-derived nitric oxide bioactivity through a superoxide-dependent mechanism. *Arterioscler Thromb Vasc Biol* 24: 2028–2033, 2004.
 175. Stocker R and Keaney JF, Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev* 84: 1381–1478, 2004.
 176. Sugiyama S, Kugiyama K, Aikawa M, Nakamura S, Ogawa H, and Libby P. Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression: involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. *Arterioscler Thromb Vasc Biol* 24: 1309–1314, 2004.
 177. Surinkaew S, Kumphune S, Chattipakorn S, and Chattipakorn N. Inhibition of p38 MAPK during ischemia, but not reperfusion, effectively attenuates fatal arrhythmia in ischemia/reperfusion heart. *J Cardiovasc Pharmacol* 61: 133–141, 2013.
 178. Szobi A, Goncalvesova E, Varga ZV, Leszek P, Kusmierczyk M, Hulman M, Kyselovic J, Ferdinandy P, and Adameova A. Analysis of necroptotic proteins in failing human hearts. *J Transl Med* 15: 86, 2017.
 179. Talib J, Cook N, Pattison D, and Davies M. Disruption of the iron-sulfur cluster of aconitase by myeloperoxidase-derived oxidants. *Free Radic Biol Med* 75(Suppl 1): S27–S28, 2014.
 180. Tanaka-Espósito C, Chen Q, and Lesnfsky EJ. Blockade of electron transport before ischemia protects mitochondria and decreases myocardial injury during reperfusion in aged rat hearts. *Transl Res* 160: 207–216, 2012.
 181. Tang WH, Brennan ML, Philip K, Tong W, Mann S, Van Lente F, and Hazen SL. Plasma myeloperoxidase levels in patients with chronic heart failure. *Am J Cardiol* 98: 796–799, 2006.
 182. Tang WH, Wu Y, Nicholls SJ, and Hazen SL. Plasma myeloperoxidase predicts incident cardiovascular risks in stable patients undergoing medical management for coronary artery disease. *Clin Chem* 57: 33–39, 2011.
 183. Teke Z, Kabay B, Ozden A, Yenisey C, Bir F, Demirkan NC, Bicakci T, and Erdem E. Effects of tempol, a membrane-permeable radical scavenger, on local and remote organ injuries caused by intestinal ischemia/reperfusion in rats. *J Surg Res* 149: 259–271, 2008.
 184. Thomas SR, Witting PK, and Drummond GR. Redox control of endothelial function and dysfunction: molecular mechanisms and therapeutic opportunities. *Antioxid Redox Signal* 10: 1713–1765, 2008.
 185. Thukkani AK, Martinson BD, Albert CJ, Vogler GA, and Ford DA. Neutrophil-mediated accumulation of 2-CIHDA during myocardial infarction: 2-CIHDA-mediated myocardial injury. *Am J Physiol Heart Circ Physiol* 288: H2955–H2964, 2005.
 186. Tiden AK, Sjogren T, Svensson M, Bernlind A, Senthilmohan R, Auchere F, Norman H, Markgren PO, Gustavsson S, Schmidt S, Lundquist S, Forbes LV, Magon NJ, Paton LN, Jameson GN, Eriksson H, and Kettle AJ. 2-Thioxanthines are mechanism-based inactivators of myeloperoxidase that block oxidative stress during inflammation. *J Biol Chem* 286: 37578–37589, 2011.
 187. Toldo S, Marchetti C, Mauro AG, Chojnacki J, Mezzaroma E, Carbone S, Zhang S, Van Tassell B, Salloum FN, and Abbate A. Inhibition of the NLRP3 inflammasome limits the inflammatory injury following myocardial ischemia-reperfusion in the mouse. *Int J Cardiol* 209: 215–220, 2016.
 188. Tsuji T, Ohga Y, Yoshikawa Y, Sakata S, Abe T, Tabayashi N, Kobayashi S, Kohzaki H, Yoshida KI, Suga H, Kitamura S, Taniguchi S, and Takaki M. Rat cardiac contractile dysfunction induced by Ca²⁺ overload: possible link to the proteolysis of alpha-fodrin. *Am J Physiol Heart Circ Physiol* 281: H1286–H1294, 2001.
 189. Tsutsui H, Kinugawa S, and Matsushima S. Oxidative stress and heart failure. *Am J Physiol Heart Circ Physiol* 301: H2181–H2190, 2011.
 190. Tyagi SC, Campbell SE, Reddy HK, Tjahja E, and Voelker DJ. Matrix metalloproteinase activity expression in infarcted, noninfarcted and dilated cardiomyopathic human hearts. *Mol Cell Biochem* 155: 13–21, 1996.
 191. Tziakas D, Chalikias G, Parissis JT, Hatzinikolaou H, Stakos D, Papadopoulou E, Kortsaris A, and Hatzeras D. Prolonged activation of tumor necrosis factor (TNF)-alpha and its soluble receptors in chronic heart failure patients both in the compensated and decompensated state. Interplay between their levels and metalloproteinase-3. *Eur Cytokine Netw* 15: 231–239, 2004.
 192. Van Amerongen MJ, Harmsen MC, van Rooijen N, Petersen AH, and van Luyn MJ. Macrophage depletion impairs wound healing and increases left ventricular

- remodeling after myocardial injury in mice. *Am J Pathol* 170: 818–829, 2007.
193. Van de Werf F. The history of coronary reperfusion. *Eur Heart J* 35: 2510–2515, 2014.
 194. Van Tassell BW, Lipinski MJ, Appleton D, Roberts CS, Kontos MC, Abouzaki N, Melchior R, Mueller G, Garnett J, Canada J, Carbone S, Buckley LF, Wohlford G, Kadariya D, Trankle CR, Oddi Erdle C, Sculthorpe R, Puckett L, DeWilde C, Shah K, Angiolillo DJ, Vetrovec G, Biondi-Zoccai G, Arena R, and Abbate A. Rationale and design of the Virginia Commonwealth University-Anakirna Remodeling Trial-3 (VCU-ART3): a randomized, placebo-controlled, double-blinded, multicenter study. *Clin Cardiol* 41: 1004–1008, 2018.
 195. Vasilyev N, Williams T, Brennan ML, Unzek S, Zhou X, Heinecke JW, Spitz DR, Topol EJ, Hazen SL, and Penn MS. Myeloperoxidase-generated oxidants modulate left ventricular remodeling but not infarct size after myocardial infarction. *Circulation* 112: 2812–2820, 2005.
 196. Velagaleti RS, Pencina MJ, Murabito JM, Wang TJ, Parikh NI, D'Agostino RB, Levy D, Kannel WB, and Vasan RS. Long-term trends in the incidence of heart failure after myocardial infarction. *Circulation* 118: 2057–2062, 2008.
 197. Wan E, Yeap XY, Dehn S, Terry R, Novak M, Zhang S, Iwata S, Han X, Homma S, Drosatos K, Lomasney J, Engman DM, Miller SD, Vaughan DE, Morrow JP, Kishore R, and Thorp EB. Enhanced efferocytosis of apoptotic cardiomyocytes through myeloid-epithelial-reproductive tyrosine kinase links acute inflammation resolution to cardiac repair after infarction. *Circ Res* 113: 1004–1012, 2013.
 198. Wang JG, Mahmud SA, Nguyen J, and Slungaard A. Thiocyanate-dependent induction of endothelial cell adhesion molecule expression by phagocyte peroxidases: a novel HOSCN-specific oxidant mechanism to amplify inflammation. *J Immunol* 177: 8714–8722, 2006.
 199. Wang JG, Mahmud SA, Thompson JA, Geng JG, Key NS, and Slungaard A. The principal eosinophil peroxidase product, HOSCN, is a uniquely potent phagocyte oxidant inducer of endothelial cell tissue factor activity: a potential mechanism for thrombosis in eosinophilic inflammatory states. *Blood* 107: 558–565, 2006.
 200. Wang W, Zhang L, Battiprolu PK, Fukushima A, Nguyen K, Milner K, Gupta A, Altamimi T, Byrne N, Mori J, Alrob OA, Wagg C, Fillmore N, Wang SH, Liu DM, Fu A, Lu JY, Chaves M, Motani A, Ussher JR, Reagan JD, Dyck JRB, and Lopaschuk GD. Malonyl CoA decarboxylase inhibition improves cardiac function post-myocardial infarction. *JACC Basic Transl Sci* 4: 385–400, 2019.
 201. Wang XS, Kim HB, Szuchman-Sapir A, McMahon A, Dennis JM, and Witting PK. Neutrophils recruited to the myocardium after acute experimental myocardial infarct generate hypochlorous acid that oxidizes cardiac myoglobin. *Arch Biochem Biophys* 612: 103–114, 2016.
 202. Wang Y, Huang S, Sah VP, Ross J, Jr., Brown JH, Han J, and Chien KR. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 273: 2161–2168, 1998.
 203. Wang Y, Rosen H, Madtes DK, Shao B, Martin TR, Heinecke JW, and Fu X. Myeloperoxidase inactivates TIMP-1 by oxidizing its N-terminal cysteine residue: an oxidative mechanism for regulating proteolysis during inflammation. *J Biol Chem* 282: 31826–31834, 2007.
 204. Westermann D, Lindner D, Kasner M, Zietsch C, Savvatis K, Escher F, von Schlippenbach J, Skurk C, Steendijk P, Riad A, Poller W, Schultheiss HP, and Tschope C. Cardiac inflammation contributes to changes in the extracellular matrix in patients with heart failure and normal ejection fraction. *Circ Heart Fail* 4: 44–52, 2011.
 205. Wilcox CS. Effects of tempol and redox-cycling nitroxides in models of oxidative stress. *Pharmacol Ther* 126: 119–145, 2010.
 206. Winterbourn CC, Kettle AJ, and Hampton MB. Reactive oxygen species and neutrophil function. *Annu Rev Biochem* 85: 765–792, 2016.
 207. Ximenes VF, Maghzal GJ, Turner R, Kato Y, Winterbourn CC, and Kettle AJ. Serotonin as a physiological substrate for myeloperoxidase and its superoxide-dependent oxidation to cytotoxic tryptamine-4,5-dione. *Biochem J* 425: 285–293, 2009.
 208. Yabluchanskiy A, Li Y, Chilton RJ, and Lindsey ML. Matrix metalloproteinases: drug targets for myocardial infarction. *Curr Drug Targets* 14: 276–286, 2013.
 209. Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, Endo J, Yamamoto T, Takeshima A, Shinmura K, Shen W, Fukuda K, and Sano M. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *J Mol Cell Cardiol* 62: 24–35, 2013.
 210. Yao C, Veleza T, Scott L, Jr., Cao S, Li L, Chen G, Jeyabal P, Pan X, Alsina KM, Abu-Taha ID, Ghezelbash S, Reynolds CL, Shen YH, LeMaire SA, Schmitz W, Muller FU, El-Armouche A, Tony Eissa N, Beeton C, Nattel S, Wehrens XHT, Dobrev D, and Li N. Enhanced cardiomyocyte NLRP3 inflammasome signaling promotes atrial fibrillation. *Circulation* 138: 2227–2242, 2018.
 211. Yin T, Sandhu G, Wolfgang CD, Burrier A, Webb RL, Rigel DF, Hai T, and Whelan J. Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. *J Biol Chem* 272: 19943–19950, 1997.
 212. Zalewski J, Claus P, Bogaert J, Driessche NV, Driesen RB, Galan DT, Sipido KR, Buszman P, Milewski K, and Van de Werf F. Cyclosporine A reduces microvascular obstruction and preserves left ventricular function deterioration following myocardial ischemia and reperfusion. *Basic Res Cardiol* 110: 18, 2015.
 213. Zarling JA, Brunt VE, Vallerga AK, Li W, Tao A, Zarling DA, and Minson CT. Nitroxide pharmaceutical development for age-related degeneration and disease. *Front Genet* 6: 325, 2015.
 214. Zhang G, Wang Q, Zhou Q, Wang R, Xu M, Wang H, Wang L, Wilcox CS, Liu R, and Lai EY. Protective effect of tempol on acute kidney injury through PI3K/Akt/Nrf2 signaling pathway. *Kidney Blood Press Res* 41: 129–138, 2016.
 215. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, and Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464: 104–107, 2010.
 216. Zhang R, Brennan ML, Shen Z, MacPherson JC, Schmitt D, Molenda CE, and Hazen SL. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. *J Biol Chem* 277: 46116–46122, 2002.
 217. Zhang T, Zhang Y, Cui M, Jin L, Wang Y, Lv F, Liu Y, Zheng W, Shang H, Zhang J, Zhang M, Wu H, Guo J,

- Zhang X, Hu X, Cao CM, and Xiao RP. CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. *Nat Med* 22: 175–182, 2016.
218. Zhang W, Lavine KJ, Epelman S, Evans SA, Weinheimer CJ, Barger PM, and Mann DL. Necrotic myocardial cells release damage-associated molecular patterns that provoke fibroblast activation in vitro and trigger myocardial inflammation and fibrosis in vivo. *J Am Heart Assoc* 4: e001993, 2015.
219. Zhao J, Jitkaew S, Cai Z, Choksi S, Li Q, Luo J, and Liu ZG. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc Natl Acad Sci U S A* 109: 5322–5327, 2012.
220. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, Schmitt D, Fu X, Thomson L, Fox PL, Ischiropoulos H, Smith JD, Kinter M, and Hazen SL. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 114: 529–541, 2004.
221. Zheng W, Warner R, Ruggeri R, Su C, Cortes C, Skoura A, Ward J, Ahn K, Kalgutkar A, Sun D, Maurer TS, Bonin PD, Okerberg C, Bobrowski W, Kawabe T, Zhang Y, Coskran T, Bell S, Kapoor B, Johnson K, and Buckbinder L. PF-1355, a mechanism-based myeloperoxidase inhibitor, prevents immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis. *J Pharmacol Exp Ther* 353: 288–298, 2015.
222. Zhou R, Yazdi AS, Menu P, and Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469: 221–225, 2011.
223. Zhu Y, Matsumura Y, Velayutham M, Foley LM, Hitchens TK, and Wagner WR. Reactive oxygen species scavenging with a biodegradable, thermally responsive hydrogel compatible with soft tissue injection. *Biomaterials* 177: 98–112, 2018.

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Abbreviations Used

2-CIHDA = 2-chlorohexadecanal
 3-Cl-Tyr = 3-chlorotyrosine
 ACS = acute coronary syndrome
 AF = atrial fibrillation
 AMI = acute myocardial infarction
 AMI/R = acute myocardial infarction and reperfusion
 ApoA-1 = apolipoprotein A-1
 CAD = coronary artery disease
 CaMKII = Ca²⁺-calmodulin-dependent protein kinase
 CsA = cyclosporine A
 DAMP = damage-associated molecular patterns
 ECM = extracellular matrix
 GSH = glutathione
 H₂O₂ = hydrogen peroxide
 HDL = high-density lipoprotein
 HIF = hypoxia inducible factor
 HOCl = hypochlorous acid
 HOSCN = hypothiocyanous acid
 I/R = ischemia reperfusion
 LV = left ventricle
 MAPK = mitogen activated protein kinases
 MI = myocardial infarction
 MLKL = mixed lineage kinase domain-like
 MMP = matrix metalloproteinase
 MPO = myeloperoxidase
 MPTP = mitochondrial permeability transition pore
 NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
 NO = nitric oxide
 NOX2 = NADPH oxidase 2
 Nrf2 = nuclear factor erythroid-2 related factor 2
 NSTEMI = non-ST-elevation myocardial infarction
 O₂^{•-} = superoxide radical anion
 PAI-1 = plasminogen activated inhibitor 1
 PCI = percutaneous coronary intervention
 RIP1 = receptor-interacting protein 1
 RIP3 = receptor-interacting protein 3
 ROS = reactive oxygen species
 SOD = superoxide dismutase
 TGF-β = transforming growth factor beta
 TIMP = tissue inhibitors of MMP
 TNF = tumor necrosis factor
 Traf2 = TNF receptor-associated factor 2