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Published in final edited form as:

Title: Nitrosative stress and pharmacological modulation of heart failure.

Authors: Pacher P, Schulz R, Liaudet L, Szabó C

Journal: Trends in pharmacological sciences

Year: 2005 Jun

Volume: 26

Issue: 6

Pages: 302-10

DOI: 10.1016/j.tips.2005.04.003

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Published in final edited form as:

Trends Pharmacol Sci. 2005 June ; 26(6): 302–310.

Nitrosative stress and pharmacological modulation of heart failure

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Abstract

Dysregulation of nitric oxide (NO) and increased oxidative and nitrosative stress are implicated in the pathogenesis of heart failure. Peroxynitrite is a reactive oxidant that is produced from the reaction of nitric oxide with superoxide anion and impairs cardiovascular function through multiple mechanisms, including activation of matrix metalloproteinases (MMPs) and nuclear enzyme poly (ADP-ribose) polymerase (PARP). Recent studies suggest that the neutralization of peroxynitrite or pharmacological inhibition of MMPs and PARP are promising new approaches in the experimental therapy of various forms of myocardial injury. In this article, the role of nitrosative stress and downstream mechanisms, including activation of MMPs and PARP, in various forms of heart failure are discussed and novel emerging therapeutic strategies offered by neutralization of peroxynitrite and inhibition of MMPs and PARP in these pathophysiological conditions are reviewed.

Role of increased oxidative stress in heart failure

Acute heart failure and chronic heart failure (CHF) are major causes of hospitalization, morbidity and mortality worldwide. Several different mechanisms can lead to cardiac pump failure (Figure 1). These mechanisms result in a mismatch between the load applied to the heart and the energy needed for contraction, which leads to reduced contractile efficiency (Figure 1).

The pathomechanism of heart failure is complex and involves the activation of numerous secondary pathways (e.g. pathways involving neurohormones, cytokines, oxidative stress and nitrosative stress), which leads to: (i) abnormalities in various signaling processes, cardiac receptors and Ca²⁺ homeostasis; (ii) contractile protein desensitization; and (iii) endothelial dysfunction. The resulting structural alterations can involve cardiac and vascular remodeling with hypertrophy, fibrosis, cardiac dilation and myocardial necrosis. The adverse remodeling and increased peripheral resistance further aggravate heart failure (Figure 1).

Experimental and clinical studies demonstrate increased production of reactive oxygen species (ROS) in the pathogenesis of acute heart failure and CHF. Plasma malondialdehyde-like activity (MDA), a marker of lipid peroxidation, is increased in patients with ischemic and non-ischemic dilated cardiomyopathy, and has an inverse relationship with ejection fraction (an

index of cardiac function) and exercise capacity. The pericardial concentration of 8-iso-prostaglandin F₂ (a marker for ROS production) in these patients also correlates closely with the degree of contractile dysfunction (reviewed in [1]).

The generation of ROS in the myocardium is triggered by ischemia and reperfusion and by exposure of the heart to inflammatory cytokines [e.g. tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6)]. Impaired antioxidant defense mechanisms (superoxide dismutase, catalase and glutathione peroxidase) or reduced concentrations of endogenous antioxidants (vitamin E, ascorbic acid and glutathione) can also increase ROS levels within the myocardium. Sources of ROS in failing myocardium include, among many others, xanthine and NAD(P)H oxidoreductases, the mitochondrial electron transport chain and activated neutrophils [1–4].

Role of increased nitrosative stress and dysregulation of nitric oxide synthase in heart failure

Cardiomyocytes, endocardial endothelium, coronary endothelium and cardiac nerves are all sources of nitric oxide (NO) produced by Ca²⁺-dependent NO synthase (NOS) enzymes. NO serves several important physiological roles in the regulation of cardiac function, including coronary vasodilatation, inhibition of platelet and neutrophil adhesion and activation, modulation of cardiac contractile function, and inhibition of cardiac oxygen consumption (reviewed in [4–6]).

NO is necessary for normal cardiac physiology and has a protective role in the ischemic heart. This protective role is mediated by several mechanisms, including the stimulation of soluble guanylyl cyclase, which leads to a decrease of the concentration of intracellular Ca²⁺, and the termination of chain-propagating lipid radical reactions caused by oxidative stress [7]. However, reactive nitrogen species can also exert cytotoxic effects. Many of the toxic actions of NO are not due directly to NO itself but are mediated by the highly reactive oxidant peroxynitrite, which is produced following interaction of NO with superoxide anions (reviewed in [8,9]) (Table 1).

NO is overproduced in the failing myocardium as a result of the increased expression and activity of the inducible isoform of NOS (iNOS) [9,10]. There is a correlation between myocardium-specific, chronic over-expression of iNOS and peroxynitrite generation and cardiac enlargement, conduction defects, sudden cardiac death and, less commonly, heart failure (HF) in mice [11]. Myocardial iNOS is induced in rats that have volume-overload HF, and increased iNOS activity in these rats leads to: (i) a loss of myocardial contractility; and (ii) β -adrenoceptor hyporesponsiveness [12]. NO derived from neuronal NOS is also present in the failing human heart [13]. It is likely that it is not the net amount of NO produced *per se* that determines cardiac cytotoxicity, but instead the type of reactive nitrogen species present (e.g. the toxic oxidant peroxynitrite produced following the interaction of NO and superoxide) [8,9,14]. The generation of peroxynitrite has been demonstrated in various forms of acute heart failure and CHF in both animals and humans (Table 2) [11,15–30].

CHF is also characterized by impaired endothelium-dependent vasodilatation, which might contribute to increased peripheral vascular resistance. The pathogenesis of endothelial dysfunction associated with CHF involves enhanced oxidative stress from various local sources. A potential additional source of ROS is iNOS, which, paradoxically, can produce superoxide instead of NO if L-arginine (from which NO is normally produced) is depleted or there is a lack of the important cofactor tetrahydrobiopterin (BH₄). The importance of this mechanism has been demonstrated in cultured cells, and in vascular tissues from CHF animals or humans [31,32], but has not yet been studied in hearts from CHF animals or patients.

Mechanisms of peroxynitrite-induced cardiac and vascular dysfunction

There are multiple possible downstream targets of peroxynitrite (Table 1, Figure 2) (reviewed in [8,9,14]). For example, peroxynitrite-induced tyrosine nitration can lead to dysfunctional nitrated proteins whereas peroxynitrite-induced oxidation of crucial sulfhydryl groups can inhibit important enzymes in the mitochondrial respiratory chain. Indeed, peroxynitrite-mediated nitration of myofibrillar creatine kinase impairs myocardial contractility [10]. Furthermore, peroxynitrite-modified cellular proteins are subject to accelerated degradation via the proteasome and peroxynitrite inhibits a variety of ion pumps, including Ca^{2+} pumps, Ca^{2+} -activated K^+ channels and Na^+-K^+ -ATPase [8,9].

Peroxyntirite also potently oxidizes various biomolecules including BH_4 , a crucial cofactor of NOS, to quinonoid 5,6-dihydrobiopterin. A large proportion of the quinonoid isomer readily loses its side-chain to form 7,8-dihydropterin, which does not serve as a cofactor for NOS. Low cellular levels of BH_4 can promote a cycle of its own destruction mediated by NOS-dependent formation of peroxynitrite. This mechanism might contribute to vascular endothelial dysfunction. The generation of peroxynitrite also decreases the availability of NO for G-protein stimulation and vasodilatation, thus further contributing to endothelial dysfunction. In addition, peroxynitrite can inhibit superoxide dismutase, glutaredoxin and other antioxidant molecules and systems, which leads to positive feedback cycles of intracellular oxidant generation and oxidative injury [8,9]. As overviewed in subsequent sections, peroxynitrite can also activate matrix metalloproteinases (MMPs) [33,34] and the nuclear enzyme poly(ADP-ribose) polymerase (PARP) [8,35] in HF. Peroxynitrite also triggers the expression of P-selectin, intercellular adhesion molecule 1 (ICAM-1) and Mac-1 (CD11b/CD18), and mediates the cytokine-induced expression of IL-8 in human leukocytes via enhancement of nuclear factor κB (NF- κB) activation [8], thereby promoting pro-inflammatory responses.

Role of peroxynitrite-induced activation of matrix metalloproteinases

MMPs are a family of proteolytic enzymes that are best known for their ability to degrade and remodel the extracellular matrix. MMPs are now also recognized to have a variety of novel actions as proteases on substrates other than extracellular matrix proteins. They have important roles both under physiological (e.g. angiogenesis and remodeling of the endometrium) and pathophysiological (e.g. neutrophil infiltration, cancer cell invasion and connective tissue remodeling) conditions. In the heart, collagens are the major extracellular matrix proteins that surround and interconnect myocytes and muscular fibers to provide mechanical support. Loss of collagen leads to myocyte slippage, ventricular dilation, and progressive contractile dysfunction. Importantly, modulation of the balance between the synthesis and degradation of matrix proteins is implicated in the process of ventricular remodeling during CHF (reviewed in [34,36]). The type of MMP involved is important. For example, changes in gelatinases (MMP-2 and MMP-9) are not directly involved in fibrillar collagen breakdown or the cardiac remodeling produced by catabolism of type I and III collagens, whereas MMP-1 (interstitial collagenase), MMP-8 (neutrophil collagenase), MMP-13 (collagenase type 3) and MMP-14 [MT1 (membrane type 1)-MMP] are the only MMPs that cleave triple helical collagen [37].

MMPs are thought to be secreted in an inactive form from cells and activated at the cell membrane via a proteolytic mechanism. However, this paradigm of only extracellular activation and action of MMPs, particularly that of MMP-2, has been challenged by recent evidence.

Increased oxidative and nitrosative stress have been proposed to have an important role in the activation of MMPs both *in vitro* and *in vivo* (reviewed in [34,36,37]). Oxidative stress causes tissue injury through activation of pro-MMPs, which is triggered by the interaction of peroxynitrite with cytosolic glutathione and subsequent S-glutathiolation of a crucial cysteine

residue in the autoinhibitory domain of the pro-MMP, resulting in a catalytically active pro-MMP [33]. MMP-2 is activated and released from the reperfused heart following ischemia, and inhibition of MMP-2 activity functionally protects the heart from ischemia–reperfusion injury [38]. Infusion of peroxynitrite into isolated rat hearts results in MMP-2 activation, which precedes the onset of mechanical dysfunction, an effect that is abolished by MMP inhibitors [39]. Upon ischemia–reperfusion injury MMP-2 was found to be localized to the sarcomeric thin myofilaments and was responsible for the proteolytic cleavage of troponin I [40], which contributes to the early reduction of myocardial contractility in acute myocardial reperfusion injury.

MMP-2 activity is increased in the myocardium of spontaneously hypertensive rats with CHF and correlates with ventricular dilation and age-dependent dysfunction. Inhibition of MMP-2 activity ameliorates these changes [41]. Targeted deletion of MMP-9 also attenuates left ventricular remodeling after experimental myocardial infarction in mice [42]. Additional evidence for the importance of MMP-2 and -9 in the heart is provided by the demonstration that, in a porcine model, MMP inhibitors ameliorate ventricular dysfunction induced by rapid ventricular pacing [43]. Peroxynitrite-dependent early activation of MMP-2 also occurs in rat hearts exposed to pro-inflammatory cytokines [26] and in a doxorubicin-induced murine heart failure model [15,16]. Contractile failure of the heart was reduced either by MMPs inhibitors [26] or by a peroxynitrite decomposition catalyst [15,16].

MMP-2 and MMP-9 have pivotal roles in a variety of human cardiovascular disorders. The activities of both these MMPs are increased in the ventricles of patients suffering from dilated cardiomyopathy [44] and in atrial biopsies of patients undergoing cardiopulmonary bypass [45]. The concentration of plasma MMP-9 might predict adverse cardiovascular events [46]. Furthermore, a particular polymorphism of MMP-9 (R279Q) is highly associated with the occurrence of stable angina in humans [46].

Role of poly(ADP-ribose) polymerase (PARP) activation

Poly(ADP-ribose) polymerase 1 (PARP-1) is the major isoform of a family of nuclear enzymes with multiple regulatory functions. PARP cleaves NAD⁺ to nicotinamide and ADP-ribose to form long branches of ADP-ribose polymers on nuclear target proteins. PARP activation exerts its pathophysiological effects via two principal mechanisms (reviewed in [35]). First, when PARP is activated by single-strand breaks in DNA, it catalyzes the cleavage of NAD⁺ into nicotinamide. As a result, in oxidatively or nitrosatively stressed cells NAD becomes depleted, impairing glycolysis, Krebs cycle and mitochondrial electron transport, eventually resulting in ATP depletion. Second, PARP-1 regulates the expression of various proteins, including iNOS, ICAM-1, and various cytokines and chemokines, at the transcriptional level. Pharmacological inhibition or genetic deletion of PARP attenuates the production of a variety of inflammatory mediators *in vivo* [35]. The relative contribution of the two mechanisms to acute heart failure and CHF has not yet been clarified completely. In acute hypoxic-reoxygenated hearts, there is a massive drop in NAD and ATP levels, compared with in control hearts, and PARP inhibitors accelerate the recovery of these energetic pools during reperfusion [47]. The effect of PARP inhibitors on myocardial energetics in CHF has not yet been studied. The triggers of PARP activation, in general, are DNA strand breakage and increased intracellular concentrations of Ca²⁺ [35]; both of these triggers have been demonstrated in acute heart failure and CHF [1, 48,49].

Pharmacological inhibition or genetic deletion of PARP markedly improves the outcome of myocardial ischemia–reperfusion injury [35,48] and various forms of cardiomyopathy and CHF (Table 3) [15,21,50–68]. In diabetic cardiomyopathy models the depression of myocardial contractile function was associated with a significant increase in poly(ADP-ribosyl)ation in

cardiac myocytes [54]. In rat models of CHF induced by chronic coronary artery ligation and advanced aging, increased nitrosative stress and poly(ADP-ribose)ation were found in both myocytes and endothelial cells. Pharmacological inhibition of PARP attenuated the myocardial hypertrophy and improved endothelial function in these rat models [21,62]. Using a dual approach of PARP-1 suppression by genetic deletion or pharmacological inhibition, the role of oxidative and nitrosative stress-induced PARP activation in the development of cardiac dysfunction induced by doxorubicin was also demonstrated [15,52,69]. Most recently, two studies demonstrated increased poly(ADP-ribose)ation and overexpression of PARP-1 in the myocardium of mice with aortic banding-induced CHF [49,53]. In these studies inhibition or genetic inactivation of PARP protected against a hypertrophy response and heart failure induced by aortic banding. There is also evidence for overexpression of PARP-1 in biopsies from human subjects with CHF [49]. Pharmacological inhibition of PARP or genetic PARP-1 deficiency also prevents the mitochondrial-to-nuclear translocation of the cell death factor apoptosis-inducing factor (AIF) in a banding-induced murine heart failure model [53].

Angiotensin II might also be important in PARP activation and the pathogenesis of heart failure. Angiotensin II is a known factor in the pathogenesis of CHF, and can induce endothelial peroxynitrite formation [70] and PARP activation [58]. Angiotensin II triggers the activation of PARP in cultured endothelial cells *in vitro* and chronic infusion of sub-pressor doses of angiotensin II triggers endothelial dysfunction *in vivo*, which can be prevented or reversed by PARP inhibition [58]. A recent study also implicates PARP in regulating the expression of endothelin receptors [71], and the dysregulation of endothelin signaling is known to be important in CHF.

Although the exact molecular triggers of PARP activation in CHF are unknown, we propose that following initial damage (e.g. myocardial infarction) the compensatory increased sympathetic activity, activated renin-angiotensin system and increased workload of the non-infarcted regions of the myocardium leads to myocardial hypertrophy, myocardial ischemia or functional hypoxia, increased oxidative and nitrosative stress and PARP activation, which in turn results in alterations in cellular energetics and the activation of pro-inflammatory pathways (Figures 1 and 2).

Possibilities for future pharmacological interventions for the experimental therapy of heart failure

Although there is strong experimental evidence for the role of ROS and reactive nitrogen species in the development of the structural and functional changes of the failing myocardium, the results of clinical trials with antioxidants such as vitamin C, vitamin E and coenzyme Q10 are equivocal [1]. The reason for these findings might be related to the type of antioxidant used (relatively low reaction rate with the reactive species or non-catalytic antioxidant) and the dose of the antioxidant used (less than the doses used in preclinical animal studies).

Approaches that use more-effective antioxidants (catalytic antioxidants with high reaction rates) or that specifically target myocardial oxidant-producing enzymes or specific downstream targets of oxidative or nitrosative stress might be more fruitful approaches in the future. For example, xanthine oxidase, which is an important source of superoxide in the failing heart, can be inhibited by pharmacological agents (e.g. allopurinol and oxypurinol). Both preclinical and clinical studies demonstrate that this approach improves cardiac efficiency and might become a future therapeutic modality. Furthermore, the data obtained so far in short-term clinical studies with allopurinol in CHF patients are encouraging (reviewed in [3]). In theory, mitochondrial uncoupling proteins might reduce superoxide formation in mitochondria in the heart [72], but such an approach might induce several unwanted effects. The pathogenetic role of NADPH oxidase in CHF might be exploited with apocynin (a weak nonselective NADPH

oxidase inhibitor) [73] or with yet to be identified more-potent or more-selective NADPH oxidase inhibitors.

Peroxynitrite decomposition catalysts appear to improve cardiac function and overall outcome in animal models of CHF. For example, the metalloporphyrin peroxynitrite decomposition catalyst FP15 reduced myocardial necrosis in rat [15] and pig [68] models of acute myocardial infarction, and improved mechanical dysfunction in rat hearts exposed to pro-inflammatory cytokines [74]. Furthermore, catalytic decomposition of peroxynitrite significantly improved cardiac function in models of diabetic and doxorubicin-induced cardiomyopathy [15,55,75].

Both ROS and reactive nitrogen species trigger downstream cellular suicide pathways. As discussed earlier, MMP activation and PARP activation might be two examples of such pathways, and in both cases there are significant preclinical data demonstrating their efficacy in HF models (see earlier and Table 3). With respect to MMP inhibitors, there are several compounds (including the tetracycline-class antibiotics, which possess the ability to inhibit MMP activity independently of their antibacterial action [38]) that might be used clinically and might be suitable for controlled clinical investigations. In fact, recent preclinical studies demonstrate the cardioprotective effects of doxycycline in myocardial infarction [76,77], and in a small clinical trial a sub-antimicrobial dose of doxycycline (the most potent tetracycline inhibitor of MMP) attenuated the levels of several inflammatory markers in post-myocardial infarction patients [78]. In addition, potent pharmacological PARP inhibitors have recently undergone clinical investigations for cardioprotective indications [79].

It must be kept in mind that, at present, CHF patients receive a variety of therapeutic agents, some of which have known antioxidant effects (e.g. the β -adrenoceptor antagonist carvedilol). In the case of diabetic heart failure, it must also be kept in mind that acute (in addition to chronic) hyperglycemia are strong triggers of ROS and reactive nitrogen species formation in the cardiovascular system [75,80]. Therefore, tight control of hyperglycemia in diabetics might be one of the best ways to prevent the production of reactive species and the initiation of downstream pathways of myocardial injury. Whether conventional interventions (e.g. antioxidant therapies, statins, β -adrenoceptor antagonists, angiotensin-converting enzyme inhibitors, insulin or oral anti-diabetic agents) are able to suppress the activation of PARP or MMPs during CHF remains to be investigated. Overall, we expect that recent advances in our understanding of the role of reactive species in the pathogenesis of heart failure will, in the not-so-distant future, yield novel therapeutic approaches (monotherapy or combination approaches) that will be exploited for the benefit of patients with heart failure.

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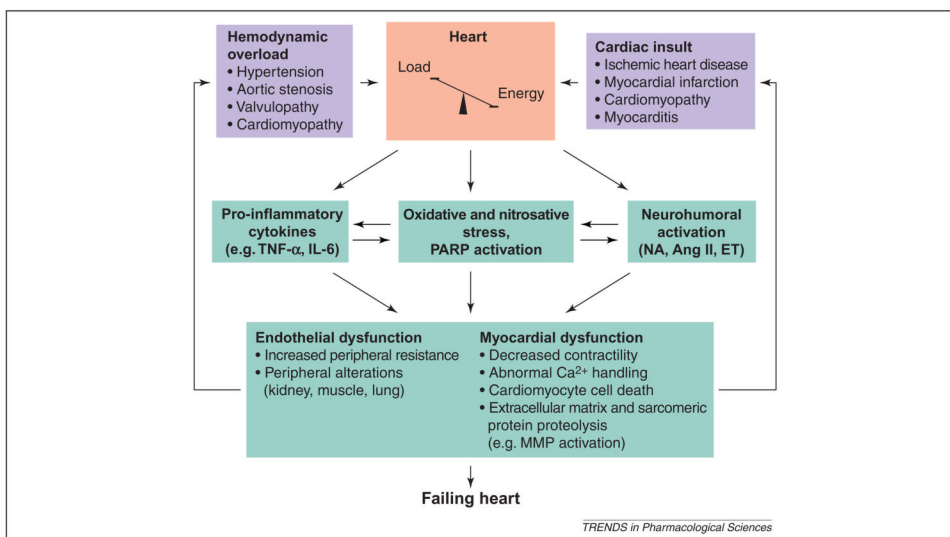


Figure 1.

Progression of heart failure and the role of oxidative and nitrosative stress. The mechanisms that lead to heart failure are of multiple origins and include acute and chronic ischemic heart disease, cardiomyopathies, myocarditis and pressure overload. These diseases result in a mismatch between the load applied to the heart and the energy needed for contraction, leading to mechanoenergetic uncoupling. Following initial insult, secondary mediators such as angiotensin II (Ang II), noradrenaline (NA), endothelin (ET) and pro-inflammatory cytokines [e.g. tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6)], in concert with oxidative and nitrosative stress, act directly on the myocardium or indirectly via changes in hemodynamic loading conditions to cause endothelial and myocardial dysfunction, cardiac and vascular remodeling with hypertrophy, fibrosis, cardiac dilation and myocardial necrosis, leading eventually to heart failure. The adverse remodeling and increased peripheral resistance further aggravate heart failure. Abbreviations: MMP, matrix metalloproteinase; PARP, poly(ADP-ribose) polymerase.

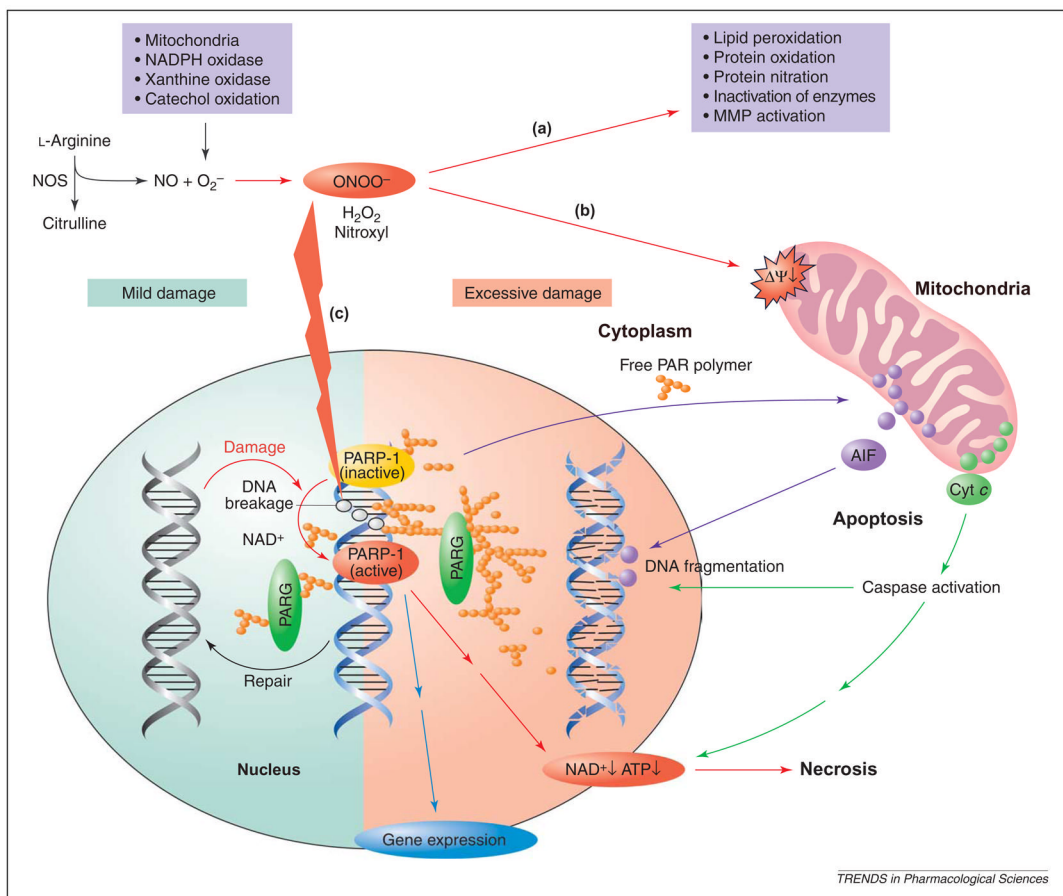


Figure 2.

Proposed role of the oxidative and nitrosative stress–poly(ADP-ribose) polymerase 1 (PARP-1) pathway in oxidant-induced cellular dysfunction and necrosis. Nitric oxide (NO) and superoxide (O_2^-) (derived from NADPH oxidase, mitochondria, xanthine oxidase and catechol oxidation) react to form peroxynitrite ($ONOO^-$), which induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and activation of matrix metalloproteinases (MMPs) among others (a) (Table 1). Peroxynitrite also acts on mitochondria [decreasing the membrane potential (Ψ)], triggering the release of pro-apoptotic factors such as cytochrome *c* (Cyt *c*) and apoptosis-inducing factor (AIF) (b). These factors mediate caspase-dependent and caspase-independent apoptotic death pathways, respectively. Moreover, peroxynitrite, in concert with other oxidants [e.g. hydrogen peroxide (H_2O_2)], causes strand breaks in DNA, activating PARP-1 (c). Mild damage to DNA activates the DNA repair machinery. By contrast, once excessive oxidative and nitrosative stress-induced DNA damage occurs, as in various forms of myocardial reperfusion injury and heart failure, overactivated PARP-1 initiates an energy-consuming cycle by transferring ADP-ribose units (small orange spheres) from NAD^+ to nuclear proteins, resulting in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, and eventually leading to cellular dysfunction and death. Poly (ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose. PARP-1 also regulates the expression of a variety of inflammatory mediators, which might facilitate the progression of heart failure.

Table 1Cytotoxic processes initiated by peroxynitrite *in vitro*

Action	Mechanism(s)
Lipid peroxidation	Peroxidation
Inhibition and depletion of antioxidant enzymes	Oxidation and nitration of glutathione and cysteine (required by antioxidant enzymes)
Activation of matrix metalloproteinases (MMPs)	S-Glutathiolation of pro-matrix metalloproteinases
Disturbances of signal transduction pathways	Oxidation and nitration of various proteins
Mitochondrial dysfunction	Inhibition of, for example, cytochromes and NADH-CoQ1 reductase (complex I)
Inhibition of cytosolic enzymes and membrane pumps	Oxidation and nitration of various proteins
Ca ²⁺ dysregulation	Inhibition of ion pumps (e.g. Ca ²⁺ pumps, Ca ²⁺ -activated K ⁺ channels and Na ⁺ -K ⁺ -ATPase, leading to dysfunctional cell energetics
Contractile protein dysfunction	Oxidation and nitration of contractile proteins
Upregulation of adhesion receptors	Activation of NF-κB
DNA injury and PARP activation	Oxidation and nitration of various proteins
Inhibition of NAD-dependent enzymes	NAD oxidation, and NAD depletion via PARP
Apoptosis	Mitochondrial injury, DNA injury, caspase activation, signal transduction disturbances and Ca ²⁺ dysregulation
Cell necrosis	Mitochondrial injury, energetic collapse, oxidation, nitration, antioxidant depletion and Ca ²⁺ dysregulation

Table 2Evidence for nitrosative stress and peroxynitrite in various forms of cardiac injury^a

Experimental model	Disease or trigger	Main findings	Refs
Mouse	Chronic myocardial ischemia	Increased myocardial iNOS expression, plasma nitrate and nitrite concentrations, and myocardial and plasma nitrotyrosine levels in wild-type compared with iNOS ^{-/-} mice; improved left ventricular function in iNOS ^{-/-} mice compared with wild-type mice	^b
Mouse	Cardiac-specific iNOS overexpression	Increased peroxynitrite production, inflammation, cardiac fibrosis, hypertrophy, dilatation and sudden cardiac death compared with wild-type mice	[11]
Mouse	Doxorubicin	Increased myocardial iNOS expression, nitrotyrosine formation and matrix metalloproteinase activation, which correlates with cardiac dysfunction	[15,16] ^b
Mouse	LPBM5 retrovirus (murine AIDS model)	Progressive cardiac dysfunction correlated with increased myocardial inflammation and protein nitration	[17]
Mouse, rat heart	High glucose, STZ-induced diabetes	Increased levels of apoptosis, hydrogen peroxide, superoxide, angiotensin II, NO and nitrotyrosine formation and increased iNOS expression in myocytes	[18] ^b
Rat cardiac myofibrils, trabeculae	Peroxynitrite	Concentration-dependent nitration of myofibrillar creatine kinase; decreased activity of creatine kinase and impaired contractile force generation	[19,20] ^b
Rat	Chronic myocardial ischemia	Increased myocardial nitrotyrosine formation and decreased myofibrillar creatine kinase activity	[21] ^b
Rat heart	Global ischemia– reperfusion	Cardiac dysfunction; decreased efficiency of oxygen utilization and ATP and membrane-bound creatine kinase activity; increased iNOS expression; increased formation of superoxide and myocardial peroxynitrite; NOS inhibition, superoxide dismutase and urate (a peroxynitrite scavenger) greatly enhanced the recovery of contractile function in post-ischemic hearts	[22] ^b
Rat heart	Endotoxin	Enhanced generation of NO, superoxide and peroxynitrite in dysfunctional hearts from endotoxemic rats	[23,24]
Rat, mouse	Porcine cardiac myosin	Autoimmune myocarditis characterized by myocardial destruction, inflammation and increased iNOS expression and nitrotyrosine formation, which is decreased by the iNOS inhibitor aminoguanidine	^b
Rat heart, rat cardiac myocytes	Mixture of pro- inflammatory cytokines	Increased production of superoxide and NO, increased peroxynitrite formation and increased activation of matrix metalloproteinase 2 (MMP-2); contractile dysfunction, which is prevented by superoxide and peroxynitrite scavengers, an MMP-2 antibody or MMP inhibitors	[25,26] ^b
Dog	Pacing, intracoronary inflammatory cytokine	Cardiac dysfunction, nitrotyrosine formation and cell death in myocytes, which is prevented by the iNOS inhibitor aminoguanidine or a superoxide scavenger	^b
Dog, pig	Regional ischemia– reperfusion	Increased nitrotyrosine formation and cardiac dysfunction	[27] ^b
Human	Coronary artery disease, atrial fibrillation, cardiopulmonary bypass surgery	Increased NOS activity and nitrotyrosine formation in myocytes of patients	[28–30] ^b
Human	Type 2 diabetes mellitus, hypertension	Increased apoptosis, necrosis and formation of angiotensin II and nitrotyrosine in myocytes	^b
Human	HIV	Increased myocardial nitrotyrosine formation in patients with HIV-induced dilated cardiomyopathy	[17]

^a Abbreviations: iNOS, inducible nitric oxide synthase; NO, nitric oxide; STZ, streptozotocin.

^b Studies published before 2002 are not included in the reference list, but are reviewed in [9,14].

Table 3Protection against cardiac dysfunction and injury by scavenging of peroxynitrite or by inhibition of PARP^a

Experimental model	Trigger of injury	Mode of peroxynitrite neutralization or PARP inhibition ^b	Effects of peroxynitrite neutralization or PARP inhibition	Refs
Mouse, mouse heart	Global or regional ischemia–reperfusion	PARP ^{-/-} phenotype	Improved left ventricular function; reduced myocardial damage and apoptosis; reduced neutrophil infiltration; reduced circulating TNF- α , IL-10, nitrate, ICAM-1 and P-selectin expression, NAD ⁺ consumption, NF- κ B activation and phosphorylative activity of JNK and AP-1 activation; increased HSF-1 and HSP70 activation	[50, 51] ^c
Mouse	Doxorubicin	PARP ^{-/-} phenotype, PJ34, FP15	Improved contractile function; reduced myocyte death; decreased mortality	[15, 52]
Mouse	Aortic banding	PARP ^{-/-} phenotype, INO1001	Decreased hypertrophy and improved contractile function; improved survival	[49, 53]
Mouse, rat	NOD diabetes, STZ diabetes, regional ischemia–reperfusion	PJ34, FP15, INO1001	Improved cardiac and vascular function; reduced infarct size and mitochondrial-to-nuclear translocation of the cell death effector apoptosis-inducing factor (AIF) in myocardial infarction	[54–56]
Mouse, rat	Endotoxin	PARP ^{-/-} phenotype, PJ34	Improved contractile function and decreased mortality	[57]
Mouse, rat	Angiotensin II infusion	PJ34, INO1001	Improved endothelial function	[58]
Rat cardiomyoblasts, rat and human cardiomyocytes	Hydrogen peroxide, peroxynitrite, hypoxia reoxygenation	3-AB, nicotinamide, 5-AIQ	Reduced cell death; improved mitochondrial respiration and cellular ATP levels	^c
Rat heart	Mixture of pro-inflammatory cytokines	Tiron, FeTPPS	Improved cardiac function in cytokine-induced myocardial contractile failure	^c
Rat, rat and rabbit heart	Regional and global ischemia–reperfusion	FP15, 3-AB, nicotinamide, 5-AIQ, BGP15, GPI6150	Reduction of infarct size and left ventricular dysfunction; preservation of myocardial ATP stores	[15, 22] ^c
Rat	Heart transplantation	3-AB, PJ34, 5-AIQ	Decreased myocardial damage; reduced oxidative stress; improved cellular NAD ⁺ and ATP levels; improved contractile and endothelial function; reduced ICAM-1 and P-selectin expression	[59–61]
Rat	Chronic myocardial ischemia and advanced aging	PJ34, INO1001	Improved left ventricular and vascular function; reduced hypertrophy	[21, 62]
Rat	Endotoxin	iNOS inhibitor mercaptoethylguanidine and peroxynitrite decomposition catalyst FeTPPS	Improved contractile function and decreased myocardial peroxynitrite accumulation; decreased degradation of the NF- κ B inhibitory protein I κ B; decreased levels of plasma TNF- α ; decreased vascular endothelial cell–leukocyte activation	[63]
Rat heart	High glucose	M40403	Decreased QT interval prolongation; decreased coronary perfusion pressure and lipid peroxidation; decreased nitrotyrosine formation and PARP activation	[64]
Dog	Cardiopulmonary bypass; crystalloid cardioplegia and extra-corporal circulation	PJ34, INO1001	Better recovery of left and right ventricular systolic function; increased coronary blood flow; improved vascular and pulmonary function	[65–67]
Pig	Regional ischemia–reperfusion	FP15, 3-AB, PJ34	Reduced infarct size; improved left ventricular function	[68] ^c

^a Abbreviations: AP-1, activator protein 1; HSF-1, heat shock transcription factor 1; HSP70, heat shock protein 70; ICAM-1, intercellular adhesion molecule 1; IL-10, interleukin 10; iNOS, inducible nitric oxide synthase; JNK, Jun N-terminal kinase; NF- κ B, nuclear factor κ B; NOD, non-obese diabetic; PARP, poly(ADP-ribose) polymerase; STZ, streptozotocin; TNF- α , tumor necrosis factor α .

^bThe following drugs were used in these studies: (i) early PARP inhibitors of low potency [3-aminobenzamide (3-AB) and nicotinamide]; (ii) a potent new generation of selective PARP inhibitors {e.g. GPI6150 (1,11*b*-dihydro-[2H]benzopyrano[4,3,2-*de*]isoquinolin-3-one), BGP15 (O-(3-piperidino-2-hydroxy-1-propyl)nicotinic amidoxime), 5-AIQ (5-aminoisoquinolinone), PJ34 (N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide HCl) and INO1001 (isoindolinone-based PARP inhibitor)}; (iii) antioxidants (tiron); (iv) peroxynitrite decomposition catalysts {FeTPPS [5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron (III)], FP15 [FeCl tetrakis-2-(triethylene glycol monomethyl ether) pyridyl porphyrin]}; and (v) the superoxide dismutase (SOD) mimetic M40403 [a manganese (II) complex with a bis(cyclohexylpyridine-substituted) macrocyclic ligand].

^cStudies published before 2002 are not included in the reference list, but are reviewed in [35,48].