Role of Oxidative Stress in Cardiac Hypertrophy and Remodeling

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Cardiac adaptation in response to intrinsic or external stress involves a complex process of chamber remodeling and myocyte molecular modifications. A fundamental response to increased biomechanical stress is cardiomyocyte and chamber hypertrophy. Although this may provide initial salutary compensation to the stress, sustained hypertrophic stimulation becomes maladaptive, worsening morbidity and mortality risks because of congestive heart failure and sudden death.1 Growing evidence highlights oxidative and nitrosative stresses as important mechanisms for this maladaptation.2–9

Oxidative stress occurs when excess reactive oxygen species (ROS) are generated that cannot be adequately countered by intrinsic antioxidant systems. Superoxide anion (O$_2^-$) can further combine with NO, forming reactive compounds such as peroxynitrite, generating nitroso-redox imbalance.4 ROS generation is a normal component of oxidative phosphorylation and plays a role in normal redox control of physiological signaling pathways.5,8,9 However, excessive ROS generation triggers cell dysfunction, lipid peroxidation, and DNA mutagenesis and can lead to irreversible cell damage or death.5,8,9 In this review, we discuss recent experimental evidence for the role of oxidant stress on cardiac remodeling, focusing on pressure-overload–induced hypertrophy and dilation.

ROS, Antioxidant Enzymes, and Nitroso-Redox Balance

ROS include free radicals such as superoxide (O$_2^-$) and hydroxyl radical and compounds such as hydrogen peroxide (H$_2$O$_2$) that can be converted to radicals, and they participate in both normal and pathologic biochemical reactions.9 O$_2^-$ is formed intracellularly (Figure 1) by activation of nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase or xanthine oxidase ( XO), uncoupling of NO synthase (NOS), and electron transport and “leakage” during oxidative phosphorylation in the mitochondria.5,8,9 H$_2$O$_2$ can generate the highly reactive hydroxyl radical via Fenton chemistry under pathological conditions.9

Cells also have intrinsic antioxidant systems that counter ROS accumulation. These include enzymes such as catalase, glutathione peroxidases, and superoxide dismutase, and nonenzymatic antioxidants, such as vitamins E, C, beta carotene, ubiquinone, lipotic acid, and urate.9,10 Superoxide dismutase converts O$_2^-$ to H$_2$O$_2$, which is further converted by catalase and glutathione peroxidase to water. The thioredoxin system, including thioredoxin, thioredoxin reductase, and NADPH, forms an additional integrated antioxidant defense system, which operates as a powerful protein–disulfide oxidoreductase.10–12

NO is another important reactive molecule controlling cardiovascular homeostasis. NO stimulates the synthesis of intracellular cGMP by activating soluble guanylyl cyclase, and cGMP and its target kinase cGK-1 (protein kinase G 1), in turn, modulate myocyte function, growth, and remodeling.13 NO also interacts with proteins via S-nitrosylation at specific cysteine residues to alter their function.14,15 S-nitrosylation is facilitated by O$_2^-$ at physiological levels; however, this process is inhibited at high levels of O$_2^-$9. As noted, increased O$_2^-$ interacts with NO to form peroxynitrite, a reactive species that is capable of triggering an array of cytotoxic processes, including lipid peroxidation, protein oxidation, and nitration (altering excitation–contraction coupling),16 and activation of matrix metalloproteinases (MMPs) contributing to chamber remodeling (reviewed in References17–18). NO can act as an antioxidant, inhibiting activation of XO19,20 and NADPH oxidase21,22 and maintaining normal O$_2^-$/NO homeostasis. Thus, increasing peroxynitrite levels means that normal NO bioavailability and physiology would be compromised.

Mechanisms for ROS Stimulation of Cardiac Hypertrophy/Remodeling

Figure 2 summarizes the mechanisms by which ROS stimulate myocardial growth, matrix remodeling, and cellular dysfunction. ROS activate a broad variety of hypertrophy signaling kinases and transcription factors.23 In rat neonatal cardiomyocytes, H$_2$O$_2$ stimulates the tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinases (extracellular response kinase and extracellular signal–regulated kinase), and Jun-nuclear kinase.24–26 Phosphoinositol 3-kinase also plays an important role in G protein–coupled hypertrophic stimulation by angiostatin II and a-adrenergic stimulation,29–33 the latter involving oxidative modulation of Ras thiol.s34 ROS also stimulate...
cellular apoptosis signaling kinase-1, a redox-sensitive kinase upstream of Jun-nuclear kinase and p38. Apoptosis signaling kinase-1 overexpression activates nuclear factor κB to stimulate hypertrophy, whereas genetic silencing of apoptosis signaling kinase-1 inhibits hypertrophy induced by angiotensin II, norepinephrine, and endothelin 1.35

ROS also have potent effects on the extracellular matrix, stimulating cardiac fibroblast proliferation36 and activating MMPs,37–39 effects central to fibrosis and matrix remodeling. MMPs are generally secreted in an inactive form and are activated posttranslationally by ROS from targeted interactions with critical cysteines in the propeptide autoinhibitory domain.40 ROS also stimulate transcription factors nuclear factor κB, Ets, and activator protein-1 to stimulate MMP expression.38

Cardiomyocyte apoptosis is another important contributor to hypertrophic remodeling and cell dysfunction.41 For example, mice lacking apoptosis signaling kinase-1 display both reduced ventricular remodeling in response to pressure load or after myocardial infarction (MI) and less cellular apoptosis.42 Apoptosis is inhibited in cells at low levels of ROS stimulation, whereas the opposite occurs at higher levels.28 Mechanisms include DNA and mitochondrial damage and activation of proapoptotic signaling kinases.

Lastly, ROS directly influence contractile function by modifying proteins central to excitation–contraction coupling (reviewed in Reference 43). This includes modification of critical thiol groups (–SH) groups on the ryanodine receptor to enhance its open probability,44 suppression of L-type calcium channel current,45 and oxidative and nitrosative interaction with the sarcoplasmic reticular Ca\(^{2+}\) ATPase to inhibit Ca\(^{2+}\) uptake.46,47

**NOS3 Uncoupling: A Pathophysiologic ROS Generator**

NOS3, or endothelial NOS, has not been traditionally considered a major oxidase, yet recent evidence suggests this function in cardiovascular pathologic remodeling (Figure 3).48–51 NOS3 normally generates NO to stimulate cGMP and cGK-1, which blunt cellular cardiac hypertrophy and fibrosis via transcriptional regulation, phosphorylation, and suppression of targeted signaling, such as from G\(_\text{q}\) stimulation.52–58 In mice exposed to sustained pressure overload, chronic inhibition of cGMP hydrolysis by phosphodiesterase 5A induced protein kinase G activation and attenuated chamber and myocyte hypertrophy and fibrosis coupled to inhibition of multiple hypertrophic cascades.59 ROS impede this regulation by reacting with NO to form peroxynitrite, stimulating nitrosative stress and reducing NO bioactivity, limiting soluble guanylate cyclase activity and expression.60 In this setting, NOS3 can generate O\(_2^-\) instead of NO.61

Under normal conditions, NOS3 consumes NADPH and generates NO and L-citrulline from L-arginine and O\(_2\). In this process, electrons are passed from a reductase domain to the heme-containing oxygenase domain (catalytic core). The cofactor tetrahydrobiopterin (BH4) is essential for donating an electron and proton to versatile intermediates in this
reaction cycle. Calmodulin controls the shuffling of the electrons, and a zinc-thiolate complex, as well as BH4, is required for NOS dimer formation and stability of the oxidase domain.\textsuperscript{62,63} NOS functions normally as a homodimer, and BH4 is required to maintain its “coupled” state and, thus, to synthesize NO.

When exposed to oxidative or nitrosative stress or when deprived of BH4 or l-arginine, NOS3 becomes structurally unstable. On protein gels, it appears more as a monomer, and the NOS inhibitor, N\textsuperscript{\textcircled{O}}-nitro-l-arginine methyl ester, suggests that ROS were being generated by NOS itself. Similarly, animals genetically lacking NOS3 exposed to pressure overload developed more modest and compensated concentric hypertrophy, with little cavity dilation, less interstitial fibrosis, and far less oxidative stress.

A major factor that may mediate NOS3 uncoupling in pressure-overloaded hearts is a decline in BH4 levels. This is supported by both direct BH4 measurements and findings that BH4 supplementation offsets the hypertrophic/dilative phenotype.\textsuperscript{49} Hearts with increased ROS because of uncoupled NOS3 have increased MMP activation, which, in turn, degraded extracellular matrix, facilitating left ventricular dilatation\textsuperscript{49,71,72} and worsening cardiac function.

Given that NOS3 is expressed in vascular endothelium and myocytes, with the latter representing <20 of total myocardial NOS3, it is unclear which cell type contributes most to ROS generated by NOS3 uncoupling. Furthermore, the exact mechanisms leading to NOS3 uncoupling and a reduction in BH4 levels remain unknown. One possibility reported by Landmesser et al\textsuperscript{51} is that initial oxidant stress (O\textsuperscript{\textcircled{2}}-) from NADPH oxidase enhances BH4 oxidation resulting in NOS3 uncoupling. In their study, oral supplementation with BH4 or genetic depletion of NADPH oxidase prevented uncoupling. As NADPH-dependent ROS generation increases in pressure-overload hypertrophy,\textsuperscript{73} a similar scenario may apply. The interaction between NOS3 uncoupling and BH4 is somewhat circular; NOS3 can become an O\textsuperscript{2-} generator without BH4 depletion,\textsuperscript{48} and the consequent ROS can, in turn, oxidize BH4 to worsen the process. Evidence of the latter was shown by Bendall et al,\textsuperscript{74} who generated stoichiometric discordance between NOS3 protein and BH4 levels by comparing endothelial-targeted overexpression of GTP cyclohydrolase 1 (GTPCH-1) rate-limiting BH4 synthetic enzyme), NOS3, and their combination. Imbalance between BH4 and NOS3 resulted in NOS3 uncoupling. The relative role of these mechanisms may depend on the nature and/or stage of the pathology. For example, NOS3 expression increases in cardiomyopathic hamsters\textsuperscript{75} and decreases in ischemic cardiomyopathy.\textsuperscript{76} BH4 depletion can occur by reduced synthesis, particularly related to changes in GTPCH-1, or by the salvage pathway that uses sepiapterin as an intermediate.\textsuperscript{77} Neopterin, a byproduct of BH4 synthesis by GTPCH-1, declines with pressure-load hypertrophy, suggesting that BH4 biosynthesis is diminished.\textsuperscript{49} Although studies on the regulation of GTPCH-1 and its role in the myocardium have yet to be reported, recent data showing that signal transducer and activator of transcription-3 activation in endothelial cells lowers GTPCH-1 expression\textsuperscript{78} suggest a potential mechanism, because signal transducer and activator of transcription-3 is potently activated by pressure overload in the heart.\textsuperscript{79}

**NADPH Oxidases**

NADPH oxidases (Figure 4A) are multimeric enzymes that consist of the membrane-bound flavocytochrome composing a catalytic Nox subunit and p22phox subunit, and 4 cytosolic regulatory subunits, p40phox, p47phox, p67phox, and the small GTP-binding protein Rac.\textsuperscript{80} Electron transfer occurs from NADPH to molecular oxygen at the catalytic site, resulting in the formation of superoxide. There are 5 Nox isoforms (Nox1–5), expressed in a tissue-specific manner. Among these, Nox2 (known previously as gp91phox) and Nox4 are the main isoforms expressed in the myocardium.\textsuperscript{5} NADPH oxidase activity increases from stimuli such as angiotensin
II, 81, 82 cyclic load, 83, 84 α-adrenergic agonists, 85 and tumor necrosis factor-α, 86 NADPH oxidase activity and subunit expression increase during the development of pressure-overload hypertrophy in guinea pigs 73 and human heart failure. 87, 88 ROS derived from NADPH oxidases can induce NOS3 uncoupling (as discussed above) and activate XO, 89 thus, the NADPH oxidases may serve as priming sources for amplification of ROS generation.

ROS generated by NADPH oxidase seem to play a key role to angiotensin II–induced cardiac hypertrophy/remodeling. Subpressor doses of angiotensin II induce cardiac hypertrophy that is blunted in hearts lacking Nox2. 90 In addition, Rac1 null mice have reduced NADPH oxidase activity, associated with lower myocardial oxidative stress, blunted hypertrophy, and less activation of apoptosis signaling kinase-1 and nuclear factor κB in response to angiotensin II infusion. 91 Nox2 null hearts also display less remodeling after MI. 92 However, mice lacking Nox2 develop similar hypertrophic responses to pressure overload as in controls but with less fibrosis and better cardiac contractility, and this was reversible by allopurinol. The same group reported that NOs1 null hearts had worse remodeling and cardiac function than wild-type after myocardial infarct; however, surprisingly, tissue ROS levels increased similarly in either genotype. 103 Importantly, they found different NO levels in heart tissues (increase in wild-type but not in NOs1 null hearts), suggesting that the imbalance between NOs1-mediated NO signaling and ROS, rather than the ROS level itself, was more important. Increased XO activity has been reported in late-stage pressure-overload–induced right ventricular hypertrophy. 106

Mitochondrial ROS
Excessive ROS derived from mitochondria are also likely contributors to cardiac failure (reviewed in Reference 107) and have also been reported in experimental models of MI and heart failure (eg, tachypacing–induced failure 108, 109). This has been attributed to electron leakage associated with reduced activity in electron transport chain complexes and can be a source for O2−, H2O2, and OH−. This oxidative stress plays an initial role in damaging mitochondrial DNA and organelle function and can result in membrane potential abnormalities, ROS leakage, and cellular damage. Overexpression of the mitochondrial antioxidant, peroxiredoxin-3, ameliorates mitochondrial DNA damage and inhibits adverse left ventricular remodeling after MI. 101 However, the specific role of ROS from mitochondria in pressure-overload–induced hypertrophy has not been reported to date and awaits further elucidation.

Antioxidant Systems and Pressure Overload
Several intrinsic antioxidants have been shown to ameliorate the evolution of heart failure or hypertrophy in experimental models. For example, chronic treatment with the nonspecific antioxidant vitamin E improved cardiac function and blunted superoxide generating xanthine oxidoreductase lie in physical proximity in the sarcoplasmic reticulum of cardiac myocytes. Deficiency of NOs1 increased xanthine oxidoreductase–mediated superoxide production, negatively regulating cardiac contractility, and this was reversible by allopurinol. The same group reported that NOs1 null hearts had worse remodeling and cardiac function than wild-type after myocardial infarct; however, surprisingly, tissue ROS levels increased similarly in either genotype. 103 Importantly, they found different NO levels in heart tissues (increase in wild-type but not in NOs1 null hearts), suggesting that the imbalance between NOs1-mediated NO signaling and ROS, rather than the ROS level itself, was more important. Increased XO activity has been reported in late-stage pressure-overload–induced right ventricular hypertrophy. 106

Xanthine Oxidase
Recent experimental data suggest that XO and XO-related oxidant stress also play a role in the pathogenesis of chronic heart failure (Figure 4B and 4C). Elevated XO expression and activity have been demonstrated in end-stage human heart failure 96 and in the canine rapid pacing-induced heart failure model. 97, 98 Chronic treatment with allopurinol significantly reduced adverse left ventricular remodeling 99 and modestly improved survival 100 in mice after MI or rats with dilated cardiomyopathy. 101 Allopurinol also prevented myofibrillar protein oxidation and preserved cardiac function in transgenic mice harboring truncated troponin I, a model of myocardial stunning. 102 Interestingly, XO-derived superoxide seems to interfere with NO regulation of myocardial energetics. 103 Khan et al 104 reported that neuronal NOS (NOS1 or nNOS) and the superoxide generating xanthine oxidoreductase lie in physical proximity in the sarcoplasmic reticulum of cardiac myocytes. Deficiency of NOS1 increased xanthine oxidoreductase–mediated superoxide production, negatively regulating cardiac contractility, and this was reversible by allopurinol. The same group reported that NOS1 null hearts had worse remodeling and cardiac function than wild-type after myocardial infarct; however, surprisingly, tissue ROS levels increased similarly in either genotype. 103 Importantly, they found different NO levels in heart tissues (increase in wild-type but not in NOS1 null hearts), suggesting that the imbalance between NOS1-mediated NO signaling and ROS, rather than the ROS level itself, was more important. Increased XO activity has been reported in late-stage pressure-overload–induced right ventricular hypertrophy. 106

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heart failure in a guinea pig pressure-overload model, although an antihypertrophic effect was not observed. 2N-mercaptopyruvyl glycine or N-acetyl cysteine blunted cardiac hypertrophy in mice with pressure overload. The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor simvastatin also can act as an antioxidant and has been shown to prevent in vitro cardiomyocyte and in vivo pressure-overload–induced hypertrophy linked to inhibition of Rac1 and, thus, reduction of NADPH oxidase activity.

Enzyme antioxidant pathways have been genetically manipulated to reveal a prominent role in hypertrophic remodeling. Thioredoxin is a ubiquitous thiol oxidoreductase composed of thioredoxin, thioredoxin reductase, and NADPH and limits oxidative stress by direct ROS scavenging and by interaction with other signaling kinases. Inhibition of endogenous thioredoxin-1 results in enhanced cardiac hypertrophy with increased myocardial oxidative stress to pressure overload, whereas overexpression of the protein reduces hypertrophy and oxidative stress. Interestingly, thioredoxin is upregulated by cGMP/protein kinase G in human neuroblastoma cells, protecting cells from oxidative stress–induced apoptosis. A similar mechanism might play a role in NO/cGMP/protein kinase G–mediated amelioration of cardiac hypertrophy/remodeling (Figure 3). Another intrinsic antioxidant enzyme, glutathione peroxidase, important for removing H₂O₂ and detoxifying lipid hydroperoxides, has also been overexpressed in mouse heart, and this ameliorated post-MI remodeling.

**Targeting Oxidative/Nitrosative Stress: A Clinical Strategy**

Although recent basic experimental studies strongly support a key role of oxidative/nitrosative stress in the pathophysiology of cardiac hypertrophic remodeling and dysfunction, clinical data testing these findings remain scant. Although small, often uncontrolled clinical studies have been supportive, larger prospective and randomized, controlled trials have failed to show clinical benefit from antioxidants such as vitamin C and vitamin E. However, these studies have not examined hypertrophic heart disease or heart failure, per se, and the bulk of the data, which are from cancer trials, may not predict cardiac disease responses. Recent clinical trials of allopurinol and oxypurinol to treat class II to III congestive heart failure found reduced plasma urate yet no impact on clinical outcome. However, angiotensin-converting enzyme inhibitors, the β-blocker carvedilol, and angiotensin receptor blockers can block NADPH oxidase, whereas statins inhibit Rac1 and, thus, NADPH oxidase, and this may contribute to part of their efficacy. More specific targeting of the source of oxidative stress, such as recoupling of NOS or enhancing intrinsic antioxidants, may ultimately provide more effective approaches to reversing cardiac remodeling.

BH4 and its precursor sepiapterin are well tolerated and are currently used to treat some forms of the genetic disease phenylketonuria in humans. The experimental data seem compelling, and it seems premature to abandon this tactic for clinical treatment. Further studies using more potent and better-targeted agents will hopefully establish their use in the future.


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