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Oxidative Stress in Heart Failure: What are we missing?

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Abstract

Over the past several decades investigations in humans and animal models of heart failure have provided substantial evidence that oxidative stress is increased in heart failure and contributes to disease progression. The requisite high metabolic activity of cardiac myocytes makes these cells active sources of reactive oxygen species. Work in cell and animal models clearly demonstrates that oxidative stress activates processes such as changes in gene expression and cell death that are now accepted components of myocardial remodeling and heart failure. Antioxidant appear to prevent progressive remodeling, and even improve cardiac function in animal models of heart failure. It is therefore disappointing that to date no antioxidant strategy has translated to therapeutic in the heart failure clinic. Possible explanations include inadequate appreciation of the critical disease-modifying sources of reactive oxygen species, the choice of the wrong antioxidant strategy, or incomplete understanding of individual variability in human antioxidant defenses. In this brief overview we will cover some of the evidence that has accumulated in this area of research, and discuss possible gaps to focus on in future investigations.

Keywords

Antioxidant enzymes; vitamin E; alpha-tocopherol; nitric oxide synthase; myocardial remodeling

Introduction

Over the past several decades investigations in humans and animal models of heart failure have provided substantial evidence that oxidative stress is increased in heart failure and contributes to disease progression. The high metabolic activity of the mitochondria-rich myocardium makes these findings seem intuitively obvious. Oxidative stress clearly activates processes in isolated heart cells such as changes in gene expression and cell death that are now accepted components of myocardial remodeling and heart failure. Moreover, many studies have been performed in animal models that demonstrate therapeutic effects of antioxidants on progression of heart failure. Yet we remain without any useful antioxidant strategy in the heart failure clinic. In this brief overview we will cover some of the evidence that has accumulated in this area of research, and discuss possible gaps to focus on in future investigations.

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Brief overview of reactive oxygen species, antioxidant systems, and oxidative stress

Reactive oxygen species (ROS) are byproducts of cellular metabolism, and oxidative stress occurs when ROS production overwhelms antioxidant defense systems. Cellular antioxidant systems balance the formation of ROS and protect cellular components from injury. Several distinct ROS have been identified that are the targets of specific enzymatic antioxidant enzymes, and a schematic outline is useful in organizing our thinking about ROS and oxidative stress in any context including heart failure. The one electron reduction of O_2 leads to formation of superoxide anion (O_2^-) an unstable free radical that reacts with itself and other oxygen containing species. Oxidative stress in human heart failure can be demonstrated by measuring formation of O_2^- or hydroxyl radical (OH) using electron paramagnetic resonance spectroscopy. In end-stage heart failure¹ and animals with experimental heart failure² an excess of ROS can be demonstrated using these methods.

Alternative methods to detect oxidative stress employ measures of biochemical derivatives of organic molecules that are products of reactions between cellular and extracellular constituents and ROS. Oxidative modifications of lipids, nucleic acids, and proteins ultimately lead to disruption of cellular functions. Clinical studies have demonstrated an increase in each of these in the setting of heart failure.³⁻⁷

Cellular antioxidant defenses include enzymes that catalyze their conversion ultimately to water. Superoxide dismutases (SOD) in eukaryotes each are organized around transition metals (MnSOD, CuZnSOD) that accept an electron from O_2^- and with H_2O create H_2O_2 . Glutathione peroxidase and catalase work in parallel with nonenzymatic antioxidants to convert H_2O_2 to H_2O .

The mitochondria are a potent source of O_2^- , and MnSOD (a.k.a. SOD2) and glutathione peroxidase (GPx) locate to the mitochondria to control ROS. Approximately 70% of the SOD activity in the heart, and 90% of that in the cardiac myocyte, is attributable to MnSOD.⁸ The remainder consists of cytosolic Cu/ZnSOD (a.k.a. SOD1), with less than 1% contributed by extracellular-SOD (also a CuZnSOD, a.k.a. SOD3).⁹ The importance of MnSOD in the regulation of oxidative stress in the myocardium, particularly during early post-natal development, is highlighted by the finding that MnSOD knockout mice develop normally *in utero*, but die soon after birth with dilated cardiomyopathy.¹⁰ In contrast, CuZnSOD or ECSOD knockout mice grow normally and have no overt myocardial phenotype.⁹

There are many 'ancillary' antioxidant enzymes that have been implicated in cardioprotection including glutathione reductase which maintains levels of reduced glutathione (GSH); glucose 6-phosphate dehydrogenase,¹¹ and other enzymes in the pentose phosphate required for reducing $NAD(P)^+$ to $NAD(P)H$; heme oxygenase I which breaks down the pro-oxidant heme, and forms the cytoprotectants carbon monoxide and bilirubin¹²; and haptoglobin, which binds free heme groups in the extracellular interstitium.¹³

Selenium is critical for the function of GPx,¹⁴ and Selenium deficiency is associated with increased oxidative stress and dilated cardiomyopathy, 'Keshan's disease'.¹⁵ Reduced levels of selenium have been observed in patients with heart failure, and is associated with disease severity.¹⁶ Glutathione is also critical for function of GPx, and acts as an important ROS scavenger. Other thiol antioxidant proteins that have been implicated in cardioprotection are metallothione,¹⁷ and thioredoxin.¹⁸

A cellular response to oxidative stress involves increased activity of antioxidant systems. There is increased mRNA expression of several antioxidant enzymes in the failing human heart.¹ Similarly, thioredoxin levels are increased in the setting of heart failure.^{19,20} Given the wealth of data demonstrating increased ROS generation in heart failure (e.g.¹) as well as increased oxidatively modified lipids (e.g.²¹), nucleic acids (e.g.²²), and proteins (e.g.⁷) in heart failure, it is clear that the increase in antioxidant expression is an inadequate response.

Effects of oxidative stress on myocardial structure and function - is it all bad?

There is substantial evidence that oxidative stress can activate many cellular responses that are characteristic of what occurs in heart failure, including cellular hypertrophy, changes in gene expression, and cell death,^{23,24} as well as alterations in the turnover and properties of the extracellular matrix.²⁵ Classic stimuli for ventricular remodeling including wall stress, inflammatory cytokines and neurohormones including catecholamines and angiotensin II all appear to induce cellular changes at least in part via oxidative or nitrosative stress.²⁶⁻³² The pathways for activation of cellular phenotypes of hypertrophy and apoptosis appear to involve one or more stress-responsive protein kinases,³³ many of which are activated by ROS.²⁴

Myocytes isolated from the failing heart show markedly abnormal intracellular Ca^{+2} transients along with alterations in the expression and/or activity of Ca^{+2} handling proteins,³⁴ and these changes may be due in part to oxidative stress. There are other effects of ROS on myocyte Ca^{+2} handling that can result in changes in contractile function. ROS alter Ca^{+2} transients and excitation-contraction coupling in isolated myocytes by increasing the activity of the $\text{Na}^{+}/\text{Ca}^{+2}$ exchanger, which in some situations may lead to Ca^{+2} overload.³⁵ A similar increase in $\text{Na}^{+}/\text{Ca}^{+2}$ exchanger activity can be seen in some models of heart failure. For example, late after MI in the rabbit heart there is increased $\text{Na}^{+}/\text{Ca}^{+2}$ exchanger activity together with a depressed force-frequency relationship.³⁶ Electrophysiological studies in isolated myocytes have also shown direct effects of reactive oxygen and nitrogen species on the voltage-dependent Ca^{2+} channel and the calcium release channel.^{37,38} These changes in Ca^{2+} handling may directly relate to the activation of kinase cascades and cell death pathways discussed above.³⁹ It remains to be seen whether these direct effects of ROS on Ca^{+2} handling proteins *in vitro* contribute to abnormal excitation-contraction coupling, changes in contractility, and/or progressive myocardial remodeling in the chronically failing heart.

These studies examining the isolated effects of ROS on signaling cascades highlight the increasing awareness that ROS are themselves signaling molecules that are an integral part of activating homeostatic pathways in all cells and tissues. While it is perhaps obvious that ROS-dependent progressive loss of myocytes is deleterious to the heart, the ROS dependent phenotype of sarcomere synthesis at some level may be compensatory. Similarly, ROS dependent changes in local Ca^{2+} handling may be important for regulation of local excitation-coupling as a component of normal myocyte physiology. If so, then completely suppressing ROS-dependent signaling in the heart may not be ideal.

Animals with heart failure are rescued by Vitamin E, why aren't humans?

The vitamin antioxidants α -tocopherol (vitamin E) and ascorbic acid (vitamin C) scavenge ROS and prevent free radical chain reactions, and have been studied in heart failure extensively. α -tocopherol levels change in heart failure indicative of oxidative stress,⁴⁰ and dietary supplements of α -tocopherol have a therapeutic effect in many animal models of heart failure.⁴¹⁻⁴³ Vitamin E supplementation clinical trials have been disappointing in heart

failure. Although short-term vitamin E supplementation reduces levels of oxidative stress biomarkers in subjects with heart failure,⁴⁴ there has been no discernable effect on heart failure symptoms or outcomes.⁴⁵ Long term vitamin E supplementation also produced no effects on primary prevention of cardiovascular events in humans,⁴⁶ and may in fact be associated with increased risk of heart failure exacerbation in large-scale clinical trials.^{46,47}

'Die-hard' advocates of vitamin E have offered reasonable explanations for this inability to translate the beneficial effects of vitamin E from animals to humans.⁴⁸ These include straightforward thoughts such as 1) a suboptimal dose and/or duration of vitamin E treatment was used, 2) studies did not include concurrent vitamin C, which has its own effects but also helps preserve vitamin E, 3) poor compliance with supplements, and lack of any monitoring of vitamin E levels in these trials. More intriguing explanations include the concept that supplementation with α -tocopherol might suppress the uptake of γ -tocopherol, the main form of vitamin E in the diet, and a more potent anti-oxidant.⁴⁹ Thus dietary supplementation via choice of foods may be more important in regulating oxidant stress than administration of vitamin supplements.

An additional explanation offered, that individual variability in levels of oxidant stress in human populations, and the inclusion of patients without biochemical evidence of increased oxidative stress dilutes the benefit, has some support from randomized clinical trials. Haptoglobin varies in its ability to prevent the pro-oxidant activity of free heme groups.¹³ This prompted a clinical trial that demonstrated that for individuals with the 2-2 haptoglobin haplotype vitamin E supplementation prevents the progression of heart disease in diabetics.⁵⁰ While the haptoglobin haplotype might help select a subset of people who will benefit from vitamin E, this does not explain why there were increased adverse outcomes in the GISSI and HOPE-TOO trials in the vitamin E treated groups. Furthermore, whether the haptoglobin haplotype determines vitamin E responses in heart failure remains to be examined.

Is there a better antioxidant than Vitamin C or E?

Mitochondria are a major source of ROS production, and this has prompted some investigators to develop strategies to develop antioxidants with enhanced mitochondrial uptake. Mitochondrially targeted vitamin C and E have been developed that use the unique features of mitochondrial biology to concentrate vitamin antioxidants, for example.⁵¹ To date these strategies have not been moved into preclinical and/or clinical studies. Increasing the availability of coenzyme Q, a critical mediator of mitochondrial electron transport, has also been proposed as a strategy to reduce the electron 'leakage' from the respiratory complexes to molecular O₂ to form O₂⁻.⁵² While randomized placebo controlled clinical trials show some benefit for symptoms of heart failure, there has been no clear effect on cardiac structure or function. Other antioxidants including probucol (e.g.⁵³), dimethylthiourea (DMTU),⁵⁴ and resveratrol⁵⁵ have been investigated with some success. In each case further work will be necessary to determine if any of these has a therapeutic effect in human heart failure.

Blocking formation of myocardial ROS: What is the right target?

An alternative to scavenging ROS is to block the source. Xanthine oxidoreductase has been proposed as a major source of ROS in human myocardium. Uric acid, the product of xanthine oxidoreductase, is increased in the failing human heart,⁵⁶ and is associated with a poor outcome.⁵⁷ These and other observations have led to the hypothesis that xanthine oxidase inhibition may improve heart failure.⁵⁸⁻⁶¹ In a long-term study of symptomatic systolic HF patients, however, there was very little effect of xanthine oxidase inhibition on heart failure endpoints except for modest improvement in heart failure symptoms in the

subgroup with elevated uric acid levels.⁶² Further prospective studies will be necessary to determine the value of xanthine oxidase inhibitors in the treatment of systolic heart failure.

The NAD(P)H oxidases are plasmalemmal enzymes that are part of the immune system where they generate toxic levels of ROS to destroy endocytosed microbes. In addition, these enzymes are expressed in other tissues including the cardiovascular system.⁶³ The activation of NAD(P)H oxidase occurs in response to many stimuli, and results in increased generation of O_2^- in the cytosol where it results in activation of many signaling pathways and biological responses.⁶⁴ In cardiac myocytes the NAD(P)H oxidases appear to regulate hypertrophic responses.³¹ Some aspects of cardiac remodeling appear to be dependent on the NAD(P)H oxidases, although there is little to support this in concept as of yet in clinical studies.⁶⁵

It would appear that mitochondria generation of ROS are an important source of myocardial ROS in the failing heart.⁶⁶ Ide et al. demonstrated increased mitochondrial formation of ROS in the animals with heart failure.⁶⁷ As discussed above, antioxidants modified to concentrate in the mitochondria may be worth further investigation.

Nitric oxide synthases as modulators of oxidative stress

Nitric oxide (NO) is synthesized in the conversion of L-arginine to L-citrulline by one of several nitric oxide synthases (NOS), and is a free radical with many biological functions including the modulation of tissue responses to oxidative stress. NOS3 is ubiquitously and constitutively expressed, and produces low levels of NO when activated. The activity of NOS3 is dynamically regulated by calcium and phosphorylation (for review see⁶⁸). In contrast, NOS2 is inducible (a.k.a. inducible NOS) is regulated by expression and is capable of producing high levels of cellular NO. Myocardial NOS2 expression is induced by exposure to stresses such as cytokines, hypoxia leads to a marked increase in the production of NO.⁶⁹ NOS2 expression is increased in the myocardium of patients with both idiopathic and ischemic dilated cardiomyopathies.⁷⁰

High levels of NO may lead to cytotoxicity through the generation of peroxynitrite ($ONOO^-$) through reaction with O_2^- .⁷¹ $ONOO^-$ can modify many cell constituents including proteins such as MnSOD, through irreversible modification of tyrosine to nitrotyrosine.⁷² This may explain why MnSOD expression is increased, but activity is decreased in the myocardium of end-stage heart failure patients.¹ The formation of $ONOO^-$ is favored when the levels of O_2^- and NO are high, and the level of SOD is low. Interestingly, NOS2 is also capable of catalyzing the formation of O_2^- ,⁷³ particularly when cells are depleted of the NOS substrate arginine. Thus, in addition to the indirect effect on $ONOO^-$ formation, NOS2 may also contribute directly to the formation of ROS (for review see⁷⁴).

Several lines of investigation support a role for NOS2 in progressive ventricular remodeling and failure. Chronic heart failure is a state of prolonged inflammation with elevated cytokines that are known to induce NOS2, and the cytotoxicity of cytokines in cardiac myocytes is mediated by NOS.^{27,75} Cytokine-induced myocyte apoptosis can be prevented by either NOS2 inhibition or an SOD-mimetic, implicating $ONOO^-$. This has been translated to in vivo models, where NOS2 knockout mice have reduced levels of myocyte apoptosis in association with improved cardiac function and survival late after myocardial infarction.⁷⁶ Similarly, the progressive myocardial damage in animal models of myocarditis is suppressed by aminoguanidine, an inhibitor of NOS2.⁷⁷

To date, NOS2 targeted therapies for myocarditis or progressive heart failure have not reached the level of clinical trial. Perhaps this is due to the concern that complete

suppression of NOS activity may have deleterious effects. For example, in mouse myocarditis model, there is an optimal level of NOS inhibition by L-NAME, above which there is decreased animal survival.⁷⁸ NO at low levels has many beneficial effects including reducing myocyte cell death.⁷⁹ This may occur via effects on specific enzymes in the programmed cell death pathway,⁸⁰ or reduced production of mitochondrial ROS production. Furthermore, mice lacking NOS3 have accelerated progressive ventricular remodeling late after MI,⁸¹ suggesting that NOS3-derived NO is beneficial for the failing heart. Perhaps development of a strategy to non-invasively detect the effects of NOS inhibition in the clinical setting allowing for selection of optimal NO levels, and/or development of specific and tolerable inhibitors of myocardial NOS2 will foster further work in this area.

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