Oxidative Stress in Heart Failure: The Role of Mitochondria

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Abstract

Recent experimental and clinical studies have suggested that oxidative stress is enhanced in heart failure. The production of oxygen radicals is increased in the failing heart whereas antioxidant enzyme activities are preserved. Mitochondrial electron transport is an enzymatic source of oxygen radical generation and also a target against oxidant-induced damage. Chronic increases in oxygen radical production in the mitochondria can lead to a catastrophic cycle of mitochondrial DNA damage as well as functional decline, further radical generation, and cellular injury. These cellular events might play an important role in the development and progression of myocardial remodeling and failure.

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Key words: reactive oxygen species, free radicals, antioxidants, electron transport, extracellular matrix, apoptosis

Introduction

Congestive heart failure (HF) is an important cause of morbidity and mortality in patients with various heart diseases. Despite extensive studies, the fundamental mechanisms responsible for the development and progression of left ventricular (LV) failure have not yet been fully elucidated. Reactive oxygen species (ROS) such as superoxide anions (O$_2^-$) and hydroxyl radicals (-OH) cause the oxidation of membrane phospholipids, proteins, and DNAs (1) and have been implicated in a wide range of pathological conditions including ischemia-reperfusion injury, neurodegenerative diseases, and aging. Under physiological conditions, their toxic effects can be prevented by such scavenging enzymes as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase as well as by other non-enzymatic antioxidants. However, when the production of ROS becomes excessive, oxidative stress might have a harmful effect on the functional and structural integrity of biological tissue. ROS cause contractile failure and structural damage in the myocardium. The importance of oxidative stress is increasingly emerging with respect to LV dysfunction and HF progression.

Oxidant Stress in Failing Hearts

Recent experimental and clinical investigations have suggested the generation of ROS to increase in chronic HF (2-5). Lipid peroxides and 8-iso-prostaglandin F$_2$ alpha, which are the major biochemical consequences of ROS generation, have been shown to be elevated in plasma and pericardial fluid of patients with HF and also positively correlated to the severity of HF (2, 3). However, all of these findings have provided only indirect evidence of ROS generation in the failing hearts. It is difficult to quantify the amount of ROS in the intact biological system since they are unstable and rapidly react with unoxidized adjacent molecules and thus their half life is very short. The only method to directly quantify ROS in biological tissue is electron spin resonance (ESR) spectroscopy. Using ESR combined with the nitroxide radical, 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-N-oxyl (hydroxy-TEMPO), as a spin probe, we first provided a definitive and direct demonstration of enhanced generation of ROS in the failing myocardium (6). -O$_2^-$ is a primary radical that could lead to the formation of other ROS, such as H$_2$O$_2$ and -OH, in the failing myocardium. -OH could arise from electron exchange between -O$_2^-$ and H$_2$O$_2$ via the Harber-Weiss reaction. In addition, -OH is also generated by the reduction of H$_2$O$_2$ in the presence of endogenous iron by means of the Fenton reaction. The generation of -OH implies a pathophysiological significance of ROS in HF since -OH radicals are the predominant oxidant species causing cellular injury.

The decreased antioxidant capacity could further aggravate the ROS accumulation in HF. However, there is no significant difference in the total SOD and catalase content between control and HF (7). GSHPx activity is even higher in failing hearts. These results thus indicate that oxidative stress in HF is primarily due to the enhancement of prooxidant generation rather than to the decline in antioxidant defenses. Moreover, the generation of ROS is greater than the scavenging capacity of endogenous antioxidants within the failing myocardium.
Enzymatic Source of ROS

Possible cellular sources of ROS generation include cardiac myocytes, endothelial cells, and neutrophils within the heart. Within cardiac myocytes, ROS can be produced by several mechanisms including mitochondrial electron transport and xanthine dehydrogenase/xanthine oxidase (Table 1). Mitochondria produce ROS through one electron carriers in the respiratory chain. Under physiological conditions, small quantities of ROS are formed during mitochondrial respiration, which, however, can be detoxified by the endogenous scavenging mechanisms of myocytes.

\(-\text{O}_2^\cdot\) can be assessed by using ESR spectroscopy with 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap, a standard method to detect ROS in the biological tissue. The inhibition of electron transport at the sites of complex I and complex III in the normal submitochondrial particles results in a significant production of \(-\text{O}_2^\cdot\) (Fig. 1) (8). HF mitochondria produce more \(-\text{O}_2^\cdot\) than normal mitochondria in the presence of NADH, but not succinate, indicating that complex I is the predominant source of such \(-\text{O}_2^\cdot\) production. Furthermore, HF mitochondria are also found to be associated with a decrease in complex I activity. Therefore, mitochondria are the predominant source of ROS, indicating a similar pathophysiological link between mitochondrial dysfunction and oxidative stress in failing hearts (9) as has been reported in other disease conditions including aging and neurodegenerative diseases such as Parkinson’s disease.

Even though mitochondrial electron transport plays an important role in the ROS production in HF, other enzymatic sources of ROS generation within the heart including vascular endothelial cells (via xanthine oxidase and/or NADPH oxidase) and activated leukocytes (via NADPH oxidase) could also contribute to oxidative stress in HF. Bauersachs et al have demonstrated that vascular NAD(P)H oxidase is activated in HF (10). This enzyme system is the major source of ROS in both the endothelium and vascular smooth muscle. They were able to generate ROS in response to angiotensin II, which stimulates the expression of NAD(P)H oxidase. Plasma renin activity as well as tissue ACE activity is activated in HF. Therefore, an enhanced formation of angiotensin II in HF may lead to oxidative stress via this enzyme system.

ROS and Mitochondria

ROS can damage mitochondrial macromolecules either at or near the site of their formation. Therefore, in addition to the role of mitochondria as a source of ROS, the mitochondria themselves can be damaged by ROS.

Mitochondria contain closed circular, double-strand DNA of \(\sim 16.5\) kb. Both strands of the mitochondrial DNA (mtDNA) are transcribed. The mitochondrial genome encodes 13 polypep-

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Table 1. Enzymatic Source of Oxygen Free Radicals

<table>
<thead>
<tr>
<th>Source</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADPH oxidase</td>
<td>Plasma membrane</td>
</tr>
<tr>
<td>Electron transport system</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Cytosol</td>
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Figure 1. Components of the respiratory chain in mitochondria. FAD: flavin adenine nucleotide, FMN: flavin mononucleotide, Fe-S: iron-sulfur protein, Q: ubiquinone, Cyt: cytochrome. (Reproduced with permission from Ide T et al. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circ Res 85: 357–363, 1999.)
tides involved in oxidative phosphorylation, including 7 subunits (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6) of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I), 1 subunit (cytochrome b) of ubiquinol-cytochrome c oxidoreductase (complex III), 3 subunits (COI, COII, and COIII) of cytochrome-c oxidase (complex IV), and 2 subunits (ATPases 6 and 8) of complex V along with 22 tRNAs and 2 rRNA (12S and 16S) subunits. The polypeptides are translated by mitochondrial ribosomes and consist of components of the electron transport chain (Fig. 2).

The mtDNA could be a major target for ROS-mediated damage for several reasons. First, mitochondria do not have a complex chromatin organization consisting of histone proteins, which may serve as a protective barrier against ROS. Second, mtDNA has a limited repair activity against DNA damage. Third, a large part of \( \cdot \text{O}_2^- \) which is formed inside the mitochondria can not pass through the membranes and, hence, ROS damage may be contained largely within the mitochondria. In fact, mtDNA accumulates significantly higher levels of the DNA oxidation product, 8-hydroxydeoxyguanosine, than nuclear DNA (11). As opposed to nuclear-encoded genes, mitochondrial-encoded gene expression is largely regulated by the copy number of mtDNA (12). Therefore, mitochondrial injury is reflected by mtDNA damage as well as by a decline in the mitochondrial RNA (mtRNA) transcripts, protein synthesis, and mitochondrial function (13, 14). We have recently shown that the increased generation of ROS was associated with mitochondrial damage and a dysfunction in the failing hearts, which were characterized by an increased lipid peroxidation in the mitochondria, a decreased mtDNA copy number, a decrease in the number of mtRNA transcripts, and a reduced oxidative capacity due to low complex enzyme activities (15). Chronic increases in ROS production are associated with mitochondrial damage and dysfunction which thus can lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury (Fig. 3). MtDNA defects may thus play an important role in the development and progression of myocardial remodeling and failure.

A number of pathogenic mtDNA base substitution mutations, such as missense mutations and mtDNA rearrangement mutations (deletions and insertions), have been identified in patients with mitochondrial diseases (16). An accumulation of the deleted forms of mtDNA in the myocardium frequently results in either cardiac hypertrophy, conduction block, or HF (17). Furthermore, there is now a consensus view that mutations in mtDNA and abnormalities in mitochondrial function are associated with common forms of cardiac diseases such as ischemic heart disease (18) and dilated cardiomyopathy (19). In these conditions, however, the strict causal relationships between abnormalities in mtDNA and cardiac dysfunction have yet to be fully elucidated (20). Even though the mechanisms by which mtDNA damage arises in these conditions have not been clarified, ROS have been proposed to be the primary contributing factor. We have provided direct evidence that mtDNA defects occur not only in a limited small subset of mitochondrial diseases but also in a more common HF phenotype occurring after myocardial infarction. This is further supported by the studies on mice lacking MnSOD which show an accumulation of oxidative damage of mtDNAs and electron transport complexes (21) in association with the development of dilated cardiomyopathy (22).

**Oxidative Stress and Myocardial Damage**

ROS have direct effects on cellular structure and function and may be integral signaling molecules in myocardial remodeling and failure (Fig. 4). ROS result in a phenotype characterized by hypertrophy and apoptosis in isolated cardiac myocytes (23). ROS have also been shown to activate matrix metalloproteinase (MMP) in cardiac fibroblasts (24). Myocardial MMP activity is increased in the failing hearts. Further, an MMP inhibitor has been shown to limit early LV dilatation in a murine model of myocardial infarction (25). Because MMP can
Figure 3. Schematic representation of an intimate link between ROS, mtDNA damage, and respiratory chain dysfunction in the mitochondria. Mitochondrial ROS generation may lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury. (Reproduced with permission from Ide T et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts following myocardial infarction. Circ Res 88: 529–535, 2001.)

Figure 4. ROS-mediated structural changes in the myocardium that may lead to HF progression.

be activated by ROS, one proposed mechanism of LV remodeling is the activation of MMP secondary to increased ROS production. Sustained MMP activation might therefore influence the structural properties of the myocardium by providing an abnormal extracellular environment with which the myocytes interact. We have demonstrated that -OH scavenger, dimethylthiourea, inhibits the activation of MMP in association with the development of LV remodeling and failure (26). These data raise the interesting possibility that increased ROS after myocardi infarction can be a stimulus for myocardial MMP activation, which might play an important role in the development of HF.

Oxidative Stress and Skeletal Muscle Dysfunction

Oxidative stress could be the mechanistic basis for muscle fatigue and reduced exercise tolerance in HF patients (27). This notion is supported by a positive correlation between ROS and exercise intolerance in these patients (28). The production of ROS was increased in the skeletal muscle homogenates obtained from a murine model of HF and increased ROS were identified as -OH originating from ·O₂, which was associated with a concomitant increase in the oxidation of lipids (29). These results are consistent with the previous studies that the oxidative capacity is reduced and O₂ utilization is inadequate in skeletal muscle mitochondria from HF patients (30). Skeletal muscle mitochondria from HF are associated with a decrease in the oxidative activities (29). As has been shown in the failing hearts (8), the defects in electron transfer function may lead to the ROS production. ROS may play an important role in the muscle atrophy commonly seen in HF patients through the induction of apoptosis. In addition, ROS impair myoplasmic Ca²⁺ homeostasis and inhibit the oxidative energy production in the mitochondria, both of which may con-
Oxidative Stress and Heart Failure

tribute to the muscle contractile dysfunction. An attempt to attenuate oxidative stress would improve, to some extent, the exercise capacity of patients with HF.

Future Perspectives

What is stimulating oxidative stress in HF? Several possible factors might be involved as the stimuli for increased ROS in HF. The activation of neurohumoral factors commonly seen in HF, including catecholamines and cardiac sympathetic tone, renin-angiotensin system, cytokines, and nitric oxide, can all contribute to the generation of ROS. If mitochondria are the principle source of ROS in response to cytokines such as TNF-α and NO, such stimuli may directly modify mitochondrial electron transport function and lead to superoxide production. This would further imply that chronic remodeling stimuli alter the level of mitochondrial oxidative stress. In addition, angiotensin II and cytokines can stimulate activity and/or expression of NAD(P)H oxidase (31). Catecholamines can also contribute to the generation of ROS by the enhanced autooxidation. Further investigation on this issue is extremely important and may provide further insight into the pathophysiology of HF (9).

Recent studies from our laboratory and those from others have demonstrated that oxidative stress is involved in certain cardiovascular disease states, including atherosclerosis, hypertension, and now heart failure. ROS may contribute to the functional and structural changes that lead to disease progression. Therapeutic strategies to modulate this maladaptive response should become a target for future research.

Conclusions

Oxidative stress is enhanced in HF. The production of ROS is increased in the failing hearts whereas antioxidant enzyme activities are preserved normal. Mitochondrial electron transport is an enzymatic source of ROS generation and also a target against ROS-induced damage. Chronic increases in ROS production in the mitochondria can lead to a catastrophic cycle of mitochondrial functional defects, further ROS generation, and cellular injury. These cellular events might play an important role in the development and progression of myocardial remodeling and failure.

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References


