REVIEW

Translational medicine

Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications

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Systolic and diastolic myocardial dysfunction has been demonstrated to be associated with an activation of the circulating and local renin−angiotensin−aldosterone system (RAAS), and with a subsequent inappropriately increased production of reactive oxygen species (ROS). While, at low concentrations, ROS modulate important physiological functions through changes in cellular signalling and gene expression, overproduction of ROS may adversely alter cardiac mechanics, leading to further worsening of systolic and diastolic function. In addition, vascular endothelial dysfunction due to uncoupling of the nitric oxide synthase, activation of vascular and phagocytic membrane oxidases or mitochondrial oxidative stress may lead to increased vascular stiffness, further compromising cardiac performance in afterload-dependent hearts. In the present review, we address the potential role of ROS in the pathophysiology of myocardial and vascular dysfunction in heart failure (HF) and their therapeutic targeting. We discuss possible mechanisms underlying the failure of antioxidant vitamins in improving patients' prognosis, the impact of angiotensin-converting enzyme inhibitors or AT1 receptor blockers on oxidative stress, and the mechanism of the benefit of combination of hydralazine/isosorbide dinitrate. Further, we provide evidence supporting the existence of differences in the pathophysiology of HF with preserved vs. reduced ejection fraction and whether targeting mitochondrial ROS might be a particularly interesting therapeutic option for patients with preserved ejection fraction.

Introduction

Heart failure (HF) is the leading cause of morbidity and mortality in industrialized countries with direct and indirect costs in 2012 across the globe of $108 billion per annum,[1] making this condition among the most resource-intensive. Since the incidence of HF increases with age, demographic trends in the world indicate that HF hospitalizations are likely to increase worldwide.

Traditionally, HF has been viewed as a consequence of a reduced ejection fraction (i.e. HF with reduced ejection fraction; HF-rEF) resulting from myocardial infarction (MI), valve disease, arterial hypertension, myocarditis, or other structural or myocardial diseases. More recently, following an improved understanding of cardiac dynamics, it has become clear that ∼50% of patients present with symptoms of HF that are due to diastolic dysfunction despite

preserved systolic function (HF with preserved ejection fraction; HF-pEF). Although the prognosis of HF-pEF is apparently less severe than HF-rEF,[2] the preserved ejection fraction variety of HF is associated with significant mortality and morbidity early after the first diagnosis (*Figure 1*).

The classic clinical syndrome of HF is characterized by fluid retention leading to pulmonary congestion and peripheral oedema (backward failure) and by low cardiac output (forward failure), that combined may cause a severe limitation of exercise capacity. The major pathophysiological cause for HF-rEF is coronary artery disease and myocardial infarction (MI). Following MI, the non-infarcted areas of the heart adapt to the increased wall stress, a process known as cardiac remodelling which involves changes in the structure and function of cardiac myocytes as well as the extracellular matrix in the non-infarcted myocardium. The main mechanisms

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Figure 2 Superoxide ($O_2^{·-}$) is produced from molecular oxygen (O_2) and may react with nitric oxide ($^·NO$) to form peroxynitrite ($ONOO^-$), thereby reducing the bioavailability of nitric oxide. Alternatively, superoxide may be dismutated by superoxide dismutases (SODs) to form hydrogen peroxide (H_2O_2), which is either degraded to water by catalases (CAT) and glutathione peroxidases (GPx), or reacts with free ferrous iron (Fe^{2+}), generated by superoxide from ferric iron (Fe^{3+}), to form hydroxyl radical ($^·OH$) in the Fenton reaction, one of the most reactive species in biological systems. The entire reaction cycle is called the Haber−Weiss reaction or cycle.
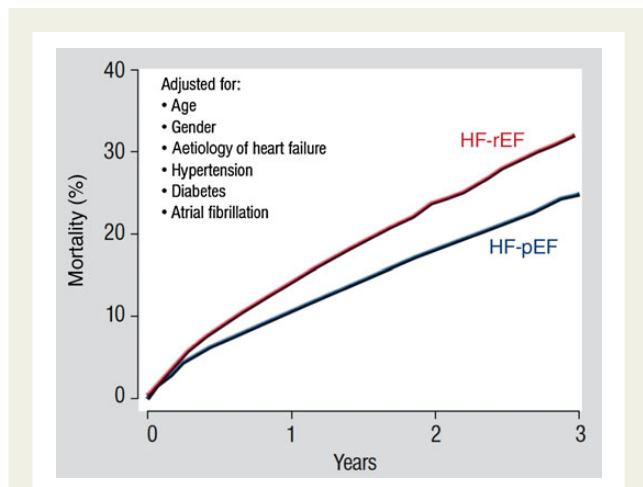
involved in these adaptive changes involve the activation of the sympathetic nervous system and the renin−angiotensin−aldosterone system (RAAS), which both drive maladaptive ventricular remodelling that produce the HF-rEF phenotype of the failing myocardium.

Notably, activation of the sympathetic nervous system and the RAAS both are associated with increased production of reactive oxygen species (ROS) in cardiovascular tissues. Increased ROS production, in turn, can result in excess oxidative stress, manifest as protein oxidation, lipid peroxidation, and DNA damage, that may contribute to cellular dysfunction. Indeed, a growing body of evidence supports the concept that myocardial systolic and diastolic dysfunction, as well as vascular/endothelial dysfunction, may at least, in part, be a consequence of this increased oxidative stress.

With the present review we wish to introduce the reader first to the complex free radical chemistry, discuss the enzymatic ROS sources that are active in the presence of systolic and diastolic HF and vascular dysfunction, their pathophysiological consequences with respect to the contraction and relaxation of the myocardium and to vascular stiffness and their therapeutic targeting. We will also discuss whether increased oxidative stress in HF-pEF has a different pathophysiology compared with HF-rEF and whether targeting mitochondrial ROS might be a particularly interesting option for patients with HF-pEF.

Free radical chemistry

Reactive oxygen species are oxygen-based chemical species that are able to react rapidly with molecules such as cellular lipids, proteins, and nucleic acids. They comprise a group of highly reactive molecules including the free radicals superoxide ($O_2^{·-}$), hydroxyl radical ($^·OH$), but also non-radical species such as hydrogen peroxide (H_2O_2) and hypochlorite (OCl^-). Superoxide is readily produced by reaction of excess electrons with molecular oxygen, and thus can be considered as a 'primary' ROS that with additional acquisition
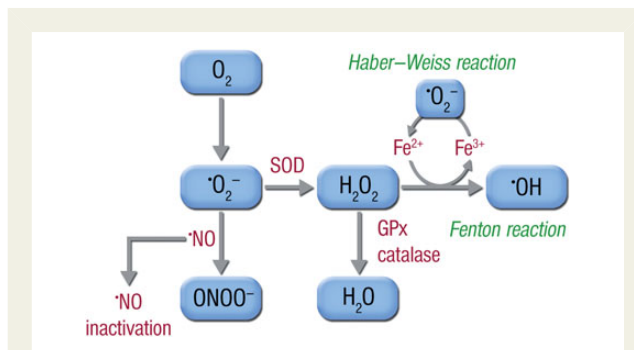
of electrons (reduction), gives rise to the formation of other ROS such as H_2O_2 and $^·OH$.

One means whereby superoxide can facilitate subsequent ROS generation is by reacting with Fe^{3+} to generate Fe^{2+}. This reduced form of iron can then react with H_2O_2 in the so-called Fenton reaction to produce $^·OH$ (*Figure 2*). The combined reactions of $O_2^{·-}$ and H_2O_2 to generate $^·OH$ in the presence of iron is known as the Haber−Weiss reaction (see *Figure 2*). Superoxide can also produce other ROS via its interaction with nitric oxide ($^·NO$). When the two species are formed simultaneously, these two radicals will react with each other at a diffusion-limited rate to form peroxynitrite ($ONOO^-$), a highly reactive intermediate that has important consequences for vascular tone and, as a consequence, ventricular afterload. For example, peroxynitrite produces tyrosine nitration of the prostacyclin synthase that leads to an inhibition of the vasodilator PGI_2.[3] Similarly, $ONOO^-$ can react with and inhibit the activity of the $^·NO$ target enzyme, soluble guanylyl cyclase.[4] Finally, peroxynitrite oxidizes tetrahydrobiopterin, an important cofactor for the endothelial nitric oxide synthase (eNOS), thereby impairing endothelial $^·NO$ production and even facilitating ROS production from eNOS, a phenomenon known as eNOS uncoupling.[5] Collectively, these consequences of ROS production can increase vascular tone resulting in reduced cardiac output and increased myocardial oxygen demand—deleterious effects that can exacerbate the clinical syndrome of HF.

Multiple antioxidant defence systems are in place to limit the damage of inappropriate ROS production. These include both non-enzymatic and enzymatic systems. The former consist mainly of antioxidants such as glutathione, vitamins C and E, bilirubin, uric acid, and beta carotene. The enzymatic systems include superoxide dismutase (SOD), catalase, peroxiredoxin (Prx), glutathione peroxidase (GPx), and thioredoxin (Trx). These antioxidant defence systems can become overwhelmed under certain circumstances and disease

states characterized by persistently high levels of ROS. Indeed, excessive ROS formation can deplete the low-molecular-weight antioxidants and peroxynitrite, in particular, is known to inactivate important antioxidant enzymes such as the mitochondrial MnSOD (by nitration and dityrosine formation)[6,7] and thiol-based antioxidant enzymes (by sulfoxidation). In fact, one can find increased 3-nitrotyrosine formation, a marker of excessive peroxynitrite generation *in vivo*, in the setting of inflammatory,[8] cardiovascular,[9] and neurodegenerative diseases.[10]

In general, there is a delicate balance between ROS formation and the inactivation of ROS via the antioxidant systems. Several ROS, particularly H_2O_2, are known to serve as local signalling molecules,[11,12] thus completely quenching ROS could have important adverse consequences via interruption of normal signalling cascades. It is thought that ROS are best suited for compartmentalized signalling and antioxidant defence systems may prevent their 'leaking' to unwanted areas resulting in cellular damage.[13] When a certain threshold of ROS formation and impaired ROS degradation is reached, redox signalling may be overcome by oxidative stress and the cell can accumulate oxidatively modified structures (e.g. proteins, DNA, lipids)[14] leading to disease states.

Enzymatic reactive oxygen species sources in the myocardium and the vasculature

A growing body of evidence suggests that in the setting of HF, ROS production within the myocardium and the vasculature is substantially increased.[15–19] This has been shown for several animal models of HF but also for patients with systolic and diastolic dysfunction and clinical signs of congestive HF. Various sources of ROS, all interacting with each other, were identified in HF (*Figure 3*).
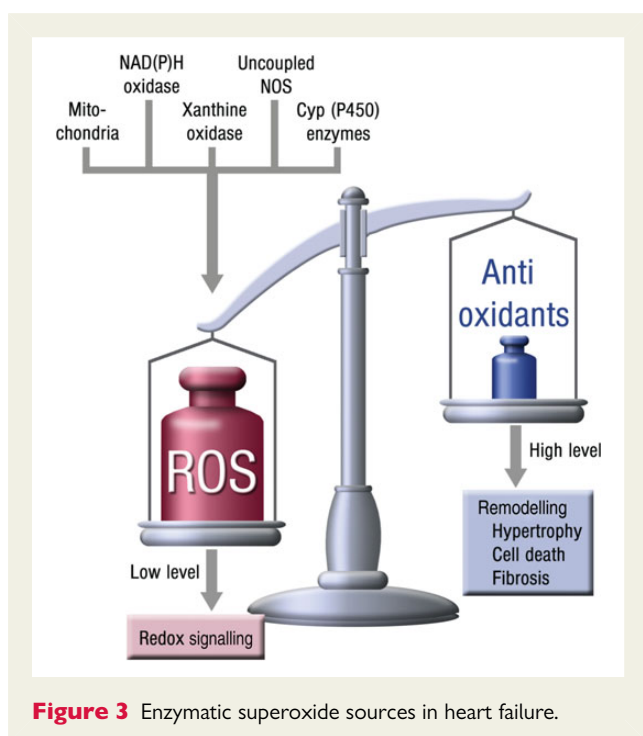

Figure 3 Enzymatic superoxide sources in heart failure.

Mitochondria

In mitochondria, the Krebs cycle generates NADH and $FADH_2$ which donate electrons to the electron transport chain (ETC) (*Figure 4*). Through sequential redox reactions along the respiratory chain, a proton gradient is formed, which drives ATP production.[20]

In the heart, the ATP pool of a cardiac myocyte is consumed in less than a minute, and in situations of exertion, cardiac output (and thus, ATP consumption) can be increased several fold.[21,22] Thus, matching ATP supply to demand is critical for normal cardiac myocyte function. One mechanism for ATP supply in the setting of increased demand (e.g. β-adrenergic stimulation) is an accelerated regeneration of oxidized NAD^+ to reduced NADH by increasing the amplitude and frequency of cytosolic Ca^{2+} transients and mitochondrial uptake of Ca^{2+},[23] which in turn activates key dehydrogenases of the Krebs cycle. Thus, typical variations in ATP demand and supply involve considerable modulation of oxidative phosphorylation and mitochondrial activity.

Depending on the respiratory state of the cell, between 0.2 and 2% of electrons can escape the respiratory chain to interact with oxygen thereby non-enzymatically generating $O_2^{\cdot-}$ during oxidative phosphorylation. In mitochondria, much of this superoxide is dismutated to H_2O_2 by Mn-SOD[24] and the resulting hydrogen peroxide eliminated by glutathione peroxidase and peroxiredoxin. Notably, these antioxidant processes also require reduced NADPH.[25,26] In summary, mitochondrial Ca^{2+} uptake is not only important to match ATP supply to demand (via NADH),[27] but also to regenerate the antioxidant capacity in the mitochondrial matrix (via NADPH).[28]

Unlike normal cardiac function, studies in HF identified increased $O_2^{\cdot-}$ generation from complex I of the respiratory chain, which consequently lead to excessive formation of H_2O_2 and $^{\cdot}OH$[29,30] as well as consumption of NAD(P)H. Further work suggested that excess ROS could lead to a feedforward cycle. In this scenario, ROS produced damage to mitochondrial DNA that lead to either downregulation (and/or mismatched stoichiometry) of respiratory complexes that exhibited reduced respiratory coupling and enhanced electron leakage from the respiratory chain to elevate ROS production.[31]

Besides these changes in ROS *production*, an increase in mitochondrial ROS *emission* might result from the pathological remodelling of cytosolic and mitochondrial ion handling that are hallmarks of HF.[25] During a cardiac action potential, Ca^{2+} enters myocytes triggering further release of Ca^{2+} from the sarcoplasmic reticulum (SR) and allowing contraction of the myofilaments. In HF, the dysfunction of the SR Ca^{2+} ATPase (SERCA2a) and an increased leak of Ca^{2+} from the SR via ryanodine receptors lead to a slowed kinetics of Ca^{2+} decay in the cytoplasm of myocytes and, therefore, to an impaired relaxation of the cardiac muscle during diastole. To compensate for the slowed uptake of Ca^{2+} into the SR, the expression and activity of the sarcolemmal $Na^+–Ca^{2+}$ exchanger are up-regulated, resulting however in an increased cytosolic Na^+ concentration.[32–36]

These changes in ionic homeostasis affect excitation−contraction coupling and have adverse effects on mitochondrial Ca^{2+} handling. Further, they affect mitochondrial energetics and ROS production through a reduced mitochondrial Ca^{2+} uptake and accelerated
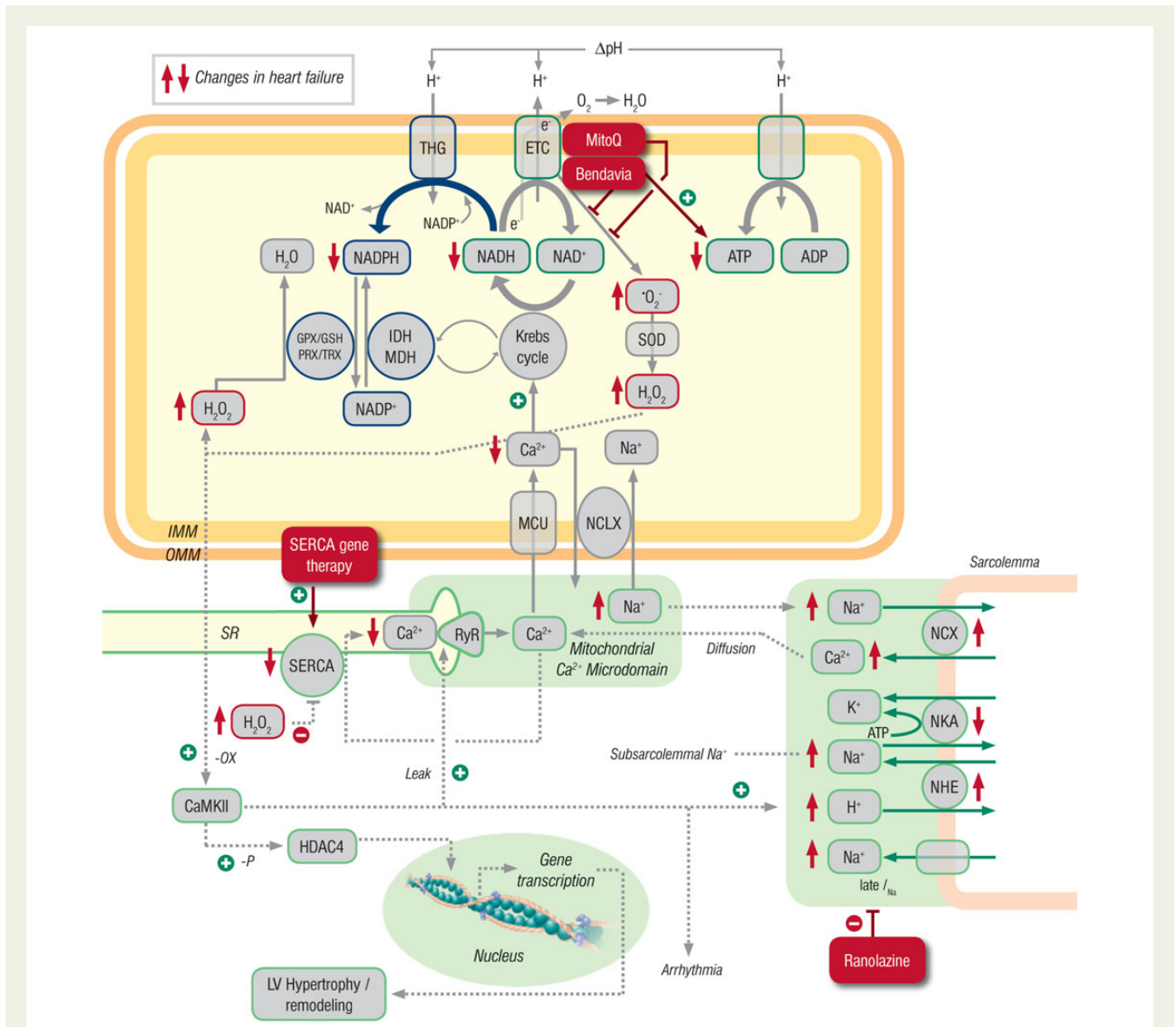
Figure 4 Interplay between excitation−contraction (EC) coupling, mitochondrial redox state, and oxidative stress in normal and failing hearts. The Krebs cycle produces NADH, which donates electrons (e^-) to the electron transport chain (ETC) to promote proton (H^+) translocation across the inner mitochondrial membrane (IMM). The proton gradient (ΔpH) is harnessed by the F_1F_o ATP synthase to generate ATP. Superoxide anion radicals ($^{\cdot}O_2^-$) are produced at the ETC and dismutated to hydrogen peroxide (H_2O_2) by Mn^{2+}-dependent superoxide dismutase (SOD). Hydrogen peroxide is eliminated by enzymes requiring NADPH (i.e. glutathione peroxidase, GPX; and peroxiredoxin, PRX). Reduced form of NADP is regenerated by enzymes facilitating products of the Krebs cycle (i.e. isocitrate dehydrogenase, IDH; malic enzyme, MDH; and transhydrogenase, THG). Ca^{2+} is taken up into mitochondria via a Ca^{2+} uniporter (MCU) and stimulates key enzymes of the Krebs cycle. Ca^{2+} is exported from mitochondria via a Na^+/Ca^{2+} exchanger (NCLX). During EC coupling, Ca^{2+} is released from the SR via ryanodine receptors (RyR), and due to the close vicinity of the SR to mitochondria, a mitochondrial Ca^{2+} microdomain with very high Ca^{2+} concentrations facilitates Ca^{2+} uptake into mitochondria. Mitochondrial Ca^{2+} uptake during increased physiological workload (i.e. during β-adrenergic stimulation) regenerates NADH and NADPH to match ATP supply to demand (through NADH) and regenerate the antioxidative capacity (through NADPH). In heart failure, decreased SR Ca^{2+} release and increased cytosolic Na^+ (in part related to an increased late Na^+ current, late I_{Na}) impairs mitochondrial Ca^{2+} uptake, which oxidizes NADH and NADPH and thus, provokes energetic deficit and oxidative stress (all changes in heart failure are marked with red arrows). This oxidizes Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) which in turn phosphorylates RyRs, thereby increasing SR Ca^{2+} leak, and also increases late I_{Na}, setting in motion a positive feedback loop of impaired EC coupling with contractile dysfunction and arrhythmias, impaired mitochondrial energetics and oxidative stress. Furthermore, CaMKII phosphorylates histone deacetylase 4 (HDAC4), which promotes cardiac hypertrophy through activation of pro-hypertrophic genes in the nucleus. Potential points of intervention in patients with heart failure are late I_{Na} (by ranolazine), improving SR Ca^{2+} ATPase (SERCA) activity through gene therapy, or by ameliorating mitochondrial ROS emission by Bendavia (SS-31) or MitoQ. GSH, glutathione; TRX, thioredoxin; $\Delta\Psi_m$, mitochondrial membrane potential. NCX, Na^+/Ca^{2+} exchanger; NKA, Na^+/K^+ ATPase; NHE, Na^+/H^+ exchanger; OMM, outer mitochondrial membrane.

Ca^{2+} extrusion via the mitochondrial Na^+/Ca^{2+} exchanger.[23,28,37] The decreased Ca^{2+} concentration hampers Krebs cycle activation and accounts for oxidation of NADH and NADPH, which may contribute to both an energetic deficit and oxidative stress.[28,38] Showing the importance of the maladaptive up-regulation of the Na−Ca exchanger in the pathophysiology of HF, pharmacological inhibition of this enzyme has been shown to restore mitochondrial Ca^{2+} levels, reducing mitochondrial ROS emission and improving remodelling in an animal model of HF.[39] Taken together, the changes in cytoplasmic Ca^{2+} handling may result in perturbations of mitochondrial Ca^{2+} and Na^+, which in turn limit NADH and NADPH levels needed for antioxidant activity thereby producing a pro-oxidative shift in the redox state of the mitochondrial matrix and an increased mitochondrial ROS emission.[25]

Since several ion handling proteins are redox-regulated, oxidative stress can further impact excitation−contraction coupling either directly or through the activation of so-called stress kinases, which may contribute to contractile dysfunction and arrhythmias.[40] A key mediator in this respect is the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a central regulator of proteins involved in excitation−contraction coupling. The expression and activity of the CaMKII are up-regulated in HF and thought to account for many pathological changes in the failing heart.[40] CaMKII is activated by oxidation[41] resulting in an increase in late I_{Na} as well as an increase in the open probability of ryanodine receptors that can initiate a feedforward mechanism of decreased sarcoplasmic Ca^{2+} load and release, elevated $[Na^+]_i$, mitochondrial ROS emission, and CaMKII activation.[42] Similar changes may result from ROS-mediated oxidative activation or inhibition of membrane ion transporters.[40] Other than Ca^{2+} handling, ROS have been shown to have important implications for contractile myofilaments as their Ca^{2+} sensitivity and elasticity are subject to redox modifications by reactions with titin and other proteins.[43,44]

Besides affecting excitation−contraction coupling, ROS are also known to contribute to maladaptive structural remodelling of the left ventricle through activation of CaMKII, HDAC4 and various mitogen-activated protein kinase pathways.[41,45,46] Finally, ROS are critical activators of the mitochondrial permeability transition pore, inducing apoptosis and/or necrosis.[47] Murine models of HF suggest that apoptosis may have an important role in determining the transition to dilated cardiomyopathy.[48] Thus, considerable evidence indicates that HF-rEF involves disturbances of cytosolic and mitochondrial ion handling that may account for an increase in mitochondrial ROS levels that has important implications for excitation−contraction coupling, leading to further impairments in myocardial contractility, energetics, and oxidative stress.

Evidence for mitochondrial ROS formation in HF comes from experimental work in animals with pressure overload-induced left- and right-ventricular failure.[49,50] In fact, recent experimental studies implicate mitochondrial ROS production as a causal feature of pressure-overload and also angiotensin II-induced systolic or diastolic HF.[51−53] In these models, mitochondria-targeted antioxidants[14,19,54,55] have been shown to prevent HF and cardiomyocyte dysfunction. Collectively, these studies suggested that mitochondria appear to integrate and even amplify ROS signalling from other non-mitochondrial sources in the cell.[19] In the following paragraphs, we will focus on other ROS sources and the interplay between ROS sources in various cell types.

NADPH oxidase

This enzyme was initially described in inflammatory cells like neutrophils and macrophages (phagocytic NADPH oxidase) but is known to also exist as seven distinct isoforms that each have distinct tissue distributions. In the cardiovascular system, specific isoforms have been demonstrated in endothelial, smooth muscle, and adventitial cells (vascular NADPH oxidase)[56−59] as well as cardiac myocytes[60] and NADPH oxidase activity is increased in failing human hearts.[16,61] Thus, vascular and myocardial tissue contain isoforms of the NADPH oxidase, suggesting that they could be involved in the regulation of cardiac function.

Over the last decade, evidence indicates that specific isoforms of the NADPH oxidase family have important roles in both the physiology and pathophysiology of the heart. Myocardial cells exposed to physiological stretch activate the sarcolemmal and t-tubule-localized Nox2 NADPH isoform to produce ROS in a process that requires intact microtubules (known as X-ROS signalling). Localized ROS, in turn, sensitize nearby ryanodine receptors in the SR to produce increased Ca^{2+} entry in the cytoplasm.[62,63] This process is called mechano-chemotransduction and also appears to involve nitric oxide synthase as well as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII),[63] raising the possibility that peroxynitrite and methionine oxidation are also involved.[64]

There is also evidence that Nox2 could be a target of therapy in HF. In murine models of ischaemic HF, genetic deletion of the Nox2 accessory protein, p47phox, improved infarct area and survival,[65] whereas gene therapy with Nox2−siRNA in nanoparticles improved cardiac function.[66] Whether the vascular or the phagocytic NADPH oxidase located in inflammatory cells prevail in determining the favourable impact of Nox2 inhibition in ischaemia-induced HF remains unclear. In vascular models of oxidative stress, such as angiotensin II infusion,[67,68] however, depletion of inflammatory cells attenuates some features of oxidative stress such as endothelial dysfunction, vascular ROS formation, and also arterial hypertension.[68] Thus, it is tempting to speculate that inhibition of the phagocytic NADPH oxidase may also have ameliorative effects on HF, but this remains to be investigated.[18]

The Nox4 isoform of NADPH oxidase has also been described in the myocardium. In contrast to Nox2, Nox4 is an unusual enzyme isoform as it does not require any regulatory subunits and it constitutively produces H_2O_2 rather than superoxide. In the cell, it is located in the endoplasmic reticulum and perhaps, also in the mitochondria.[69−71] Stimuli that activate Nox4 include ischaemia, hypoxia, and adrenergic stimuli, all present and enhanced in the setting of HF. The implications of Nox4 for HF syndromes are not yet clear, but evidence exists for some beneficial as well as negative effects. For example, mice lacking cardiac Nox4 were shown to either exhibit reduced or aggravated maladaptive remodelling in pressure overload-induced HF[72] are sensitized to ischaemic myocardial injury,[73] perhaps through reductive stress and down-regulation of stress−response pathways such as HIF-1α.[73,74] Since Nox4 is also known to promote angiogenesis, its absence in the myocardium could lead to capillary rarefaction and sensitivity to ischaemic injury.[75]

Crosstalk between mitochondria and the NADPH oxidase

A crosstalk between mitochondria and NADPH oxidases via ROS signalling appears to be of utmost importance for the cardiovascular system.[76,77] The release of ROS from the mitochondria into the cytosol may trigger further ROS-induced ROS production mediated by the activation of diverse 'redox-switches' within the cell (*Figure 5*). For example, oxidation of thiol residues in xanthine dehydrogenase promote conversion to its ROS-generating oxidase form[78] and oxidative events promote the uncoupling of NO synthase via multiple mechanisms including redox-sensitive kinases, BH_4 depletion, and disruption of the enzyme zinc-sulfur complex (summarized in ref.[76]). These mechanisms may be initiated by the 'escape' of mtROS that may occur in a burst-like fashion as a consequence of increased membrane permeability (summarized in refs.[25,77]), under de-energized conditions in the failing heart.
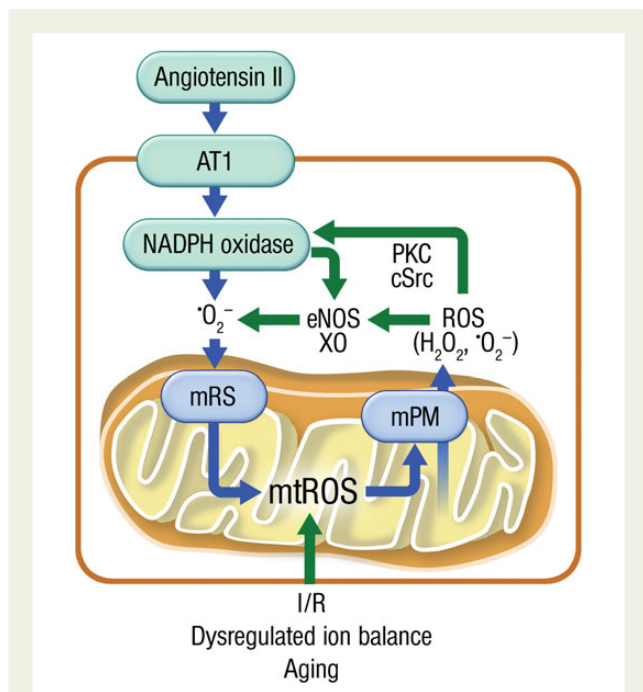

Figure 5 Crosstalk between different cardiovascular ROS sources. Upon agonist-driven activation of NADPH oxidase (e.g. via angiotensin-II-mediated AT1R activation), the enzyme produces superoxide and hydrogen peroxide leading to activation of mitochondrial ROS (mtROS) formation by mitochondrial redox switches (mRS). These mtROS can escape from mitochondria by increased mitochondrial permeability (mPM) involving several pores and channels. In the cytosol these mtROS can activate Nox2 (or Nox1) via redox-sensitive kinases such as protein kinase C (PKC) and tyrosine kinases (cSrc). Likewise Nox- and mitochondria-derived ROS can convert xanthine dehydrogenase to the xanthine oxidase (XO) or lead to eNOS uncoupling further contributing to this vicious circle. Alternatively, this crosstalk starts at the mitochondrial level by excessive mtROS formation during the aging process, a dysregulated ion balance (e.g. in the failing heart) or in response to ischaemia/reperfusion (I/R) damage.

Conversely, we also know that NADPH oxidase-derived ROS can augment ambient mitochondrial ROS levels via stimulation of ROS production (*Figure 5*) or simply by depletion of mitochondrial antioxidant systems (e.g. NADH/NADPH). Through this self-amplificating mechanism, focal cytosolic ROS production may be extended across the entire cellular mitochondrial network.[79–81] This mechanism may be particularly relevant in HF-pEF, since in a mouse model in which chronic angiotensin II infusion (a canonical Nox2 activator) induced diastolic (but not systolic) dysfunction and fibrosis, and this was completely ablated by mitochondrial over-expression of catalase or a mitochondria-targeted peptide (SS-31; see also below).[19,52]

Uncoupled endothelial nitric oxide synthase

Heart failure is associated with endothelial dysfunction,[82] an abnormality that can be corrected with oral or intra-arterial administration of vitamin C,[83] suggesting a role for oxidative stress in this phenomenon. Preclinical studies with experimental animal models of HF (both dilated cardiomyopathy and myocardial infarction) reveal both vascular and myocardial ROS production is up-regulated in these settings.[84,85] Further, inhibition of eNOS resulted in a decrease in ROS, demonstrating the existence of an uncoupled enzyme state where ˙NO production was replaced by superoxide production.[84] Similarly, several other enzymes downstream in the NO cascade (e.g. soluble guanylyl cyclase or cGMP-dependent kinase I) were also inhibited. Thus, ˙NO bioactivity was impaired and appeared related to activation of the RAAS, as inhibition with either captopril or the AT1 receptor blocker irbesartan improved the phenotype.[85] Collectively, these findings emphasize the role of the activated RAAS in the vascular abnormalities encountered in the setting of HF.

Xanthine oxidase and reactive oxygen species production in heart failure

Recent studies have demonstrated that serum uric acid levels >7 mg/dL are an important prognostic marker for all-cause mortality in HF.[86] In this context, serum uric acid levels are known to rise with increased purine catabolism resulting from tissue hypoxia, apoptosis, and/or enhanced or up-regulated xanthine oxidoreductase activity (reviewed in ref.[87]), raising the possibility that xanthine oxidase could contribute to the pathophysiology of HF. In this regard, increased xanthine oxidase activity and expression have been described in failing myocardium and inhibition of xanthine oxidase appeared to improve coronary and peripheral endothelial dysfunction, and myocardial contractility in HF patients.[88,89]

Despite these promising effects in smaller randomized trials, the results of a larger multicentre trial have been disappointing. Hare *et al.* randomized 405 class III or IV HF patients to oxypurinol or placebo and found no significant changes in clinical outcomes such as HF morbidity, mortality, and quality of life, with a non-significant trend in patients with serum uric acid levels >9.5 mg/dL.[90] Furthermore, in a very recent trial in patients with HF-rEF, allopurinol failed to improve clinical status, exercise capacity, quality of life, or left ventricular ejection fraction at 24 weeks.[91] Therefore, the most direct interpretation is that serum uric acid levels might not have a

causal role in HF but rather represent a marker of adverse prognosis indicative of oxidative damage within the myocardium and/or the vasculature.

Nitric oxide deficiency and heart failure with preserved ejection fraction

It is known for many years that ˙NO and NOS are having a role in cardiac relaxation. The endothelial and also the neuronal NOS are located in the heart and in particular the neuronal NOS has been demonstrated to be an important modulator of cardiac nitroso balance and function. E.g. it has been demonstrated that nNOS modulates cardiac relaxation via effects on phospholamban phosphorylation.[92] Interestingly, in the setting of arterial hypertension, Silberman et al.[93] demonstrated that decreased myocardial ˙NO levels lead to increased cytosolic calcium and diastolic dysfunction. These adverse effects were corrected by supplementation with BH_4, a NOS cofactor, but not NH_4 suggesting an uncoupling of NOS as the principle mechanism.[93]

While oxidative stress is common in patients with both HF-rEF and HF-pEF, different cellular and enzymatic sources of ROS production have been suggested. As recently outlined by Paulus and Tschöpe,[94] comorbidities induce a proinflammatory state in patients with HF-pEF (through elevated plasma levels of interleukin-6, soluble ST2, tumour necrosis factor-α, and pentraxin 3) that may induce ROS production in endothelial cells. Through paracrine mechanisms, this may then affect protein kinase G signalling in cardiac myocytes that induce sarcomere stiffness and pro-hypertrophic signalling. In contrast, in HF-rEF, most ROS production may occur in cardiac myocytes and induce maladaptive remodelling through the induction of programmed cell death, autophagy, and fibrosis. Such differential ROS signalling pathways may be one explanation why treatments that improved the outcome of patients with HF-rEF failed in patients with HF-pEF and should be subject to further research that enables us to identify better treatments of either syndrome.

Therapeutic strategies

The important contribution of ROS to the pathophysiology of HF may indicate that therapeutic strategies aimed at reducing oxidative stress within the myocardium and the vasculature may beneficially influence the course of the disease. In the next paragraphs we will discuss why so far simple antioxidant strategies, e.g. with vitamins failed to improve prognosis in patients with HF, discuss new approaches to reduce oxidative stress by the use of mitochondria targeting antioxidants and why ACE inhibitors or the combination therapy such as ISDN/hydralazine may be effective in restoring the impaired redox balance.

Antioxidants/vitamins

Despite a predominant role of ROS in the pathophysiology of HF, large clinical trials have failed to show any benefit of antioxidants such as vitamin E and vitamin C in any cardiovascular disease (reviewed in ref.[95]). Many explanations have been offered as to why antioxidants have proved ineffective in these studies, a prominent one being that agents such as vitamin E and vitamin C are not

targeted to sites of ROS generation that are most important in pathological conditions, such as mitochondria.

In addition, specific ROS often react much more quickly than can be quenched by antioxidants. For example, superoxide combines with ˙NO to form peroxynitrite in a diffusion-limited reaction ($k = 1.9 \times 10^{10}$ mol L^{-1} s^{-1}) that is almost $10\times$ faster than its degradation by SOD, suggesting that it is very difficult to prevent peroxynitrite formation. With regard to common antioxidants, superoxide and vitamin C react much slower than ˙NO, on the order of 10^5 mol L^{-1} s^{-1}, indicating that exceptionally high concentrations of this vitamin would be needed in specific tissue or even cell organelle to be effective. Since vitamin E is even less reactive ($\sim 10^3$ mol L^{-1} s^{-1}) the case is even stronger for its ineffectiveness. Further, the compartmentalization of ROS production makes non-targeted approaches much less effective. Thus, it is not likely that vitamins can scavenge superoxide in order to prevent its interaction with ˙NO and the subsequent formation of the highly reactive and toxic intermediate peroxynitrite, even when given in high concentrations.

Indeed, although vitamin E effectively scavenges lipid peroxyl radicals, it has been demonstrated to have a rather limited activity against other oxidants such as superoxide, peroxynitrite, and hypochlorous acid that have been implicated to play a major role in the pathophysiology of atherosclerosis.

There is also experimental evidence from animals and patients that suggests that lipid peroxidation occurs even in the presence of vitamin E and higher doses of the vitamin could be harmful (reviewed in ref.[95]). For example, further attempts to increase the effectiveness with higher doses of vitamin E resulted in worsening of atherosclerosis and vascular function,[96] which may be related to the formation of the pro-oxidative vitamin E radical[97] (Figure 6A and D). Enrichment of the vitamin E radical in the myocardium may exert negative inotropic effects, which may explain why long-term treatment with vitamin E does not prevent but rather induce HF and acute left heart decompensations as shown for the HOPE[98] and HOPE TOO trials[99] (Figure 6B and C).

Another important aspect why antioxidants may fail was presented by Ristow et al.[100] The authors demonstrated that antioxidant supplements such as vitamin C and E prevented the molecular regulators of insulin sensitivity and endogenous antioxidant defence induced by physical exercise,[100] and exercise was shown to improve symptoms and quality of life in patients with HF-rEF and also HF-pEF.[101–103]

Angiotensin-converting enzyme inhibitors or AT1-receptor blocker: are they the better 'antioxidants'?

Angiotensin-converting enzyme inhibitors inhibit kininase II, which leads to increased formation of bradykinin, which in turn enhances stimulation of the endothelial bradykinin receptor B2 prompting the release of ˙NO,[104] endothelium-derived hyperpolarizing factor and prostacyclin. These actions explain in part the combined vasodilator, antithrombotic, and anti-proliferative effects of ACE inhibitors. By inhibiting angiotensin II formation, they also beneficially influence inflammatory processes in the vessel wall,[105,106] prevent smooth muscle proliferation, and prevent the activation of the vascular
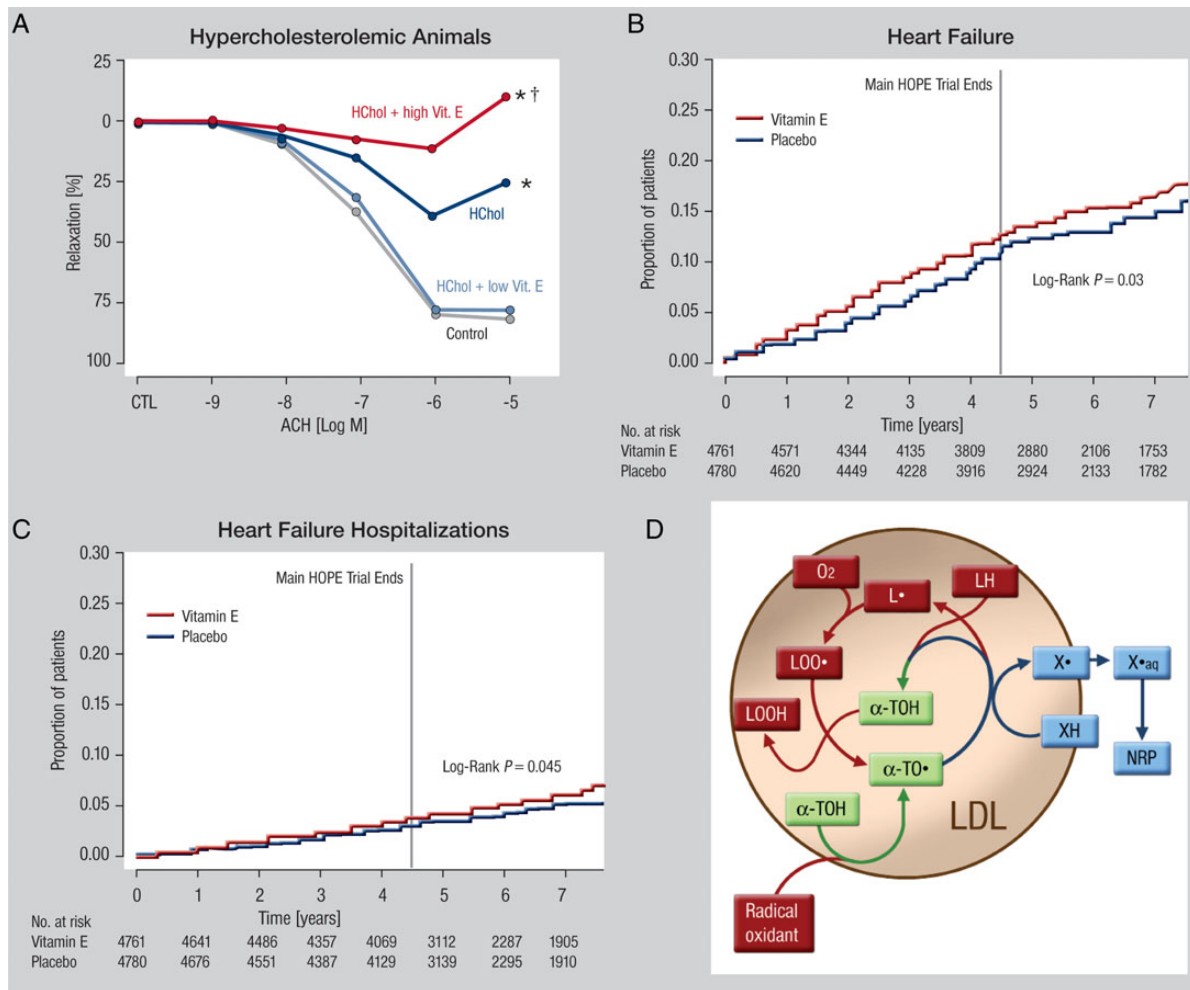
Figure 6 (*A*) Effects of Vitamin E high and low concentrations on endothelial function of cholesterol fed animals (with permission from ref.[93]). Copyright © 1994 American Society for Clinical Investigation. (*B*) Effects of Vitamin E treatment on the incidence of heart failure (with permission from ref.[113]). Copyright © 2005 American Medical Association. (*C*) Effects of Vitamin E treatment on left heart decompensations (with permission from ref.[113]). Copyright © 2005 American Medical Association. (*D*) Potential pro-oxidative effects of Vitamin E (with permission from ref.[94]). Copyright © 1999 Elsevier Science Ltd. All rights reserved.

and phagocytic NADPH oxidase, a dominant vascular source of superoxide.[84] By limiting the production of superoxide at its enzymatic source, many issues concerning the free radical scavenging efficacy of antioxidants (i.e. compartimentalization, pro-oxidant effects, etc.) are not a consideration.

Targeting mitochondrial reactive oxygen species

Recent studies indicate that targeted inhibition of ROS production in certain microdomains holds promise as a therapeutic strategy over the untargeted approach with classical antioxidants (*Figure 4*). In this regard, targeting mitochondria with their central role in cellular ROS formation has been a topic of great interest, particularly in cardiac myocytes. The Szeto-Schiller peptide (SS-31) is a tetrapeptide that accumulates several thousand-fold in mitochondria and

protects from apoptotic cell death.[107] While initial studies suggested that SS-31 was an antioxidant,[107] more recent studies revealed that it does not directly scavenge ROS, but rather prevents mitochondrial ROS formation via interaction with cardiolipin, an important lipid of the inner mitochondrial membrane critical for proper assembly of the respiratory complexes of the ETC.[108] Mitochondrial ROS production oxidizes cardiolipin and makes it dysfunctional, allowing dissociation of respiratory chain complexes which may give rise to further electron leakage promoting $O_2^{\cdot-}$ formation. SS-31 directly interacts with cardiolipin and prevents its oxidation, and this presumably interrupts a vicious cycle of cardiolipin oxidation, ROS production and deterioration of ATP production.[25,109]

The SS-31 peptide, but not the non-targeted *N*-acetyl cysteine, prevented the development of diastolic dysfunction in response to angiotensin II (a canonical activator of NADPH oxidase). These findings bolster the concept of NADPH oxidase/mitochondrial

crosstalk of ROS signalling.[51,52,76] and provide a rationale for microdomain-targeted therapies. Furthermore, SS-31 prevented HF development and maladaptive ventricular remodelling in a systolic HF model induced by pressure overload.[53] Also, in a large animal model of ischaemic cardiomyopathy, both short- and long-term treatment with SS-31 improved LV function, which was associated with improvement in mitochondrial respiration and ATP production. Under the name Bendavia, the drug is currently being tested clinically in a comprehensive programme involving patients with HF,[108] while a phase II study on patients with myocardial infarction showed safety and some promising signs towards improvement of cardiac and renal function (STEMI-EMBRACE trial, presented at ACC, Chicago, March 2015 and[110] for the study design).

Another class of compounds that appears attractive for targeting mitochondrial ROS are antioxidants linked to the lipophilic cation TPP$^+$[111] that accumulate in the mitochondrial matrix. Linkage of coenzyme Q, a respiratory chain component for ROS detoxification with TPP$^+$ (mitoQ), provided protection in various animal models of cardiovascular and neurodegenerative diseases.[25,111] For example, MitoQ improved cardiomyocyte function in a model of acute cardiac volume overload.[54] Clinically, MitoQ was safe in patients with neurodegenerative diseases but has not been tested in cardiovascular conditions as yet.[111] Interestingly, a recent clinical trial on 420 patients with HF with *un*conjugated coenzyme Q$_{10}$ showed a significant improvement of morbidity and mortality,[112] but its implications for future HF guidelines are unclear due to the rather small scale of the study.[113] The mitochondria-targeted antioxidant mitoTEMPO (as well as mitochondria-specific overexpression of human catalase) have been highly effective in preventing pressure-overload-induced HF in animals.[55] Considering the tight interaction between excitation−contraction coupling and mitochondrial energetics, attempts to improve cytosolic and/or mitochondrial ion handling may provide additional benefit by preventing mitochondrial ROS production. Two examples of this could be gene therapy to up-regulate SERCA (CUPID trial) or inhibiting late I_{Na} with ranolazine.[114] In this context, it is worth noting that both treatment with β-blockers and ACE inhibitors was associated with improvements of excitation−contraction coupling on the cellular level, pinpointing how these drugs may improve energetics and prevent oxidative stress as well (see preceding paragraph).

Cotherapy of hydralazine and nitrates improves the redox balance between nitric oxide and superoxide in patients with chronic heart failure

In patients with coronary artery disease (CAD), treatment with nitroglycerin (GTN) and isosorbide dinitrate (ISDN) cause rapid haemodynamic and anti-anginal tolerance,[115] an effect that is also seen in patients with chronic congestive HF where ISDN causes rapid tolerance development with respect to the left ventricular filling pressure.[116]

An interesting aspect is the interaction between the organic nitrate ISDN and the arteriolar dilator hydralazine in patients with severe HF. Several clinical studies have clearly established that the combination of ISDN with hydralazine is able to improve prognosis[117] compared with prazosin in the V-Heft-I and exercise capacity compared with enalapril in V-HeFT II trial.[118] Also, the African-American Heart Failure Trial (A-HeFT), a double-blind, randomized, trial demonstrated that the combination of ISDN and hydralazine markedly improved the composite endpoint of the trial, which included death from any cause, first hospitalization for HF and quality-of-life measures.[119] One potential mechanism for these observations is that this particular combination of ISDN and hydralazine is rather devoid of tolerance and its associated endothelial dysfunction. Indeed, more recent experimental studies demonstrated that hydralazine is a powerful inhibitor of GTN-induced formation of reactive oxygen species such as superoxide or peroxynitrite (ONOO$^-$) *in vitro* in the vasculature[120] and *in vivo*,[121] thus correcting the disrupted redox balance between superoxide and nitric oxide in the cardiovascular system of patients with chronic congestive HF.[122] Dulce *et al.*[123] demonstrated that by reducing sarcoplasmic Ca^{2+}-leak, hydralazine improves calcium cycling and contractility of the myocardium as well as restoring cardiac excitation−contraction coupling. Thus, these drugs in combination exert direct myocardial effects that provide a mechanistic basis for the favourable functional and structural responses in the treatment of congestive HF.

Phosphodiesterase 5 inhibition

According to a recent meta-analysis, the phosphodiesterase 5 (PDE5) inhibitor sildenafil improved haemodynamic parameters in HF-rEF patients when compared with placebo.[124] In contrast, administration of sildenafil for 24 weeks did not significantly improve exercise capacity or clinical status compared with placebo.[125]

Exercise training

Exercise training improves quality of life, symptoms, and possibly the outcome in patients with HF-pEF and HF-rEF. These benefits may be mediated by reducing central and systemic neuroendocrine activation, in particular the RAAS and sympathetic nervous systems. On a molecular level, exercise training may impact not only the heart directly, but also vessels, the kidney, skeletal muscles, and other organs, systemically interconnected through central sympatho-excitatory processes and mediated by reduced β-adrenergic- and angiotensin II-signalling that reduces oxidative stress and increases the bioavailability of NO from eNOS.[126]

Conclusions

While a large body of evidence supports an association between oxidative stress and vascular dysfunction, the relationship with myocardial contractile dysfunction has only been investigated recently. Reactive oxygen species are important mediators of physiological functions, but in higher concentrations they may modify cardiac metabolism and mechanics, triggering a feedforward mechanism that leads to further worsening of systolic and diastolic function.

Scavenging of ROS with antioxidants has however proved to have no effect in modifying patients' prognosis. While this evidence does not disprove the role of ROS in cardiovascular pathophysiology, it emphasizes the need of studies addressing ROS-dependent pathophysiology with highly specific interventions targeted at the cellular microcompartments in which these ROS are produced.

Funding

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