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EDITORIAL COMMENT

Targeting Mitochondrial Oxidative Stress in Heart Failure

Throttling the Afterburner*

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Oxidative stress contributes to various cardiovascular diseases and aging and was causally linked to the progression of heart failure (1,2). Among the most relevant sources for reactive oxygen species (ROS) are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and mitochondria (1,3–7). The ROS impair excitation-contraction coupling, cause arrhythmias, and contribute to cardiac remodelling by inducing cardiac hypertrophy, apoptosis, necrosis, and fibrosis (1,8,9). However, antioxidative interventions (e.g., vitamin E) in patients with cardiovascular diseases have yielded disappointing results so far (10).

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In this issue of the *Journal*, Dai et al. (11) report that specifically targeting mitochondrial ROS production with the synthetic Szeto-Schiller peptide SS-31 prevents cardiac hypertrophy, fibrosis, and left ventricular diastolic dysfunction in a model of angiotensin II (and hypertension) induced cardiomyopathy. SS-31 is a dimethyltyrosine-containing peptide that scavenges various ROS (i.e., hydrogen peroxide [H_2O_2], superoxide [$\cdot O_2^-$], and hydroxyl radicals) (12). Due to its positive charge, it accumulates >1,000-fold in (negatively charged) mitochondria (12). After initial in vitro studies, in which the antioxidative capacity of SS-31 was protected from ROS-induced mitochondrial dysfunction and apoptosis, SS-31 was used in several in vivo models of diseases associated with mitochondrial oxidative stress, such as neurodegenerative and metabolic diseases, renal dysfunction, and myocardial ischemia/reperfusion injury (12,13).

Two aspects about this elegant study are particularly remarkable and deserve further discussion. First, cardiac hypertrophy and dysfunction were induced by infusion of angiotensin II, a canonical activator of NADPH oxidase, and yet, cardiac remodeling was prevented by inhibiting mitochondrial ROS production, suggesting that mitochondria might "amplify" NADPH oxidase-derived ROS production. Second, nontargeted ROS scavenging with N-acetyl cysteine (NAC) was less effective than mitochondria-targeted scavenging with SS-31, implying that primarily the mitochondrial component of cellular ROS production induces maladaptive cardiac remodeling.

How can these results be explained? In mitochondria, the Krebs cycle generates NADH, which delivers electrons to the electron transport chain (ETC) (Fig. 1), inducing translocation of protons across the inner mitochondrial membrane. This establishes a membrane potential ($\Delta\Psi_m$) that fuels the F_1F_0-ATPase to generate adenosine triphosphate (ATP). At the ETC, electrons can leak to oxygen to produce $\cdot O_2^-$, which is dismutated to H_2O_2 by manganese-dependent superoxide dismutase (Mn-SOD). H_2O_2, in turn, is detoxified by enzymes that require NADPH, and NADPH is regenerated by enzymes that derive their substrates from the Krebs cycle (Fig. 1). Accordingly, an equilibrium exists between NADH/NAD^+ and NADPH/$NADP^+$ (14).

Two principal scenarios can be envisioned that favor increased mitochondrial ROS-formation: 1) increased formation of $\cdot O_2^-$ at the ETC; and 2) decreased elimination of $\cdot O_2^-$ or H_2O_2 in the mitochondrial matrix. In heart failure, the first scenario occurs when modifications of ETC complexes (disturbed stochiometry, posttranslational modifications, and the like) hamper electron flux along the ETC, prematurely "deviating" electrons from reduced ETC complexes to oxygen to provoke excess $\cdot O_2^-$ formation (2). In fact, Ide et al. (5) reported a functional block at complex I in mitochondria of failing dog hearts, giving rise to increased $\cdot O_2^-$ formation. Considering the second scenario, activity of Mn-SOD is reduced in heart failure, and although glutathione peroxidase activity was unchanged in homogenized samples of human failing hearts (15), its activity in intact cells and mitochondria is dynamically governed by the availability of NADPH and glutathione (3,14). Because NADPH regeneration depends on Krebs cycle products (Fig. 1) and rate-limiting Krebs cycle enzymes are activated by calcium (Ca^{2+}), mitochondrial Ca^{2+} uptake dynamically controls the redox state of NAD(P)H in working cardiac myocytes and thus H_2O_2 formation (3). Because in failing cardiac myocytes, mitochondrial Ca^{2+} uptake is hampered, NAD(P)H is more oxidized and H_2O_2 formation is increased (3,16).

Activation of ion channels in the inner mitochondrial membrane—such as: 1) the permeability transition pore (PTP); 2) the inner membrane anion channel (IMAC);

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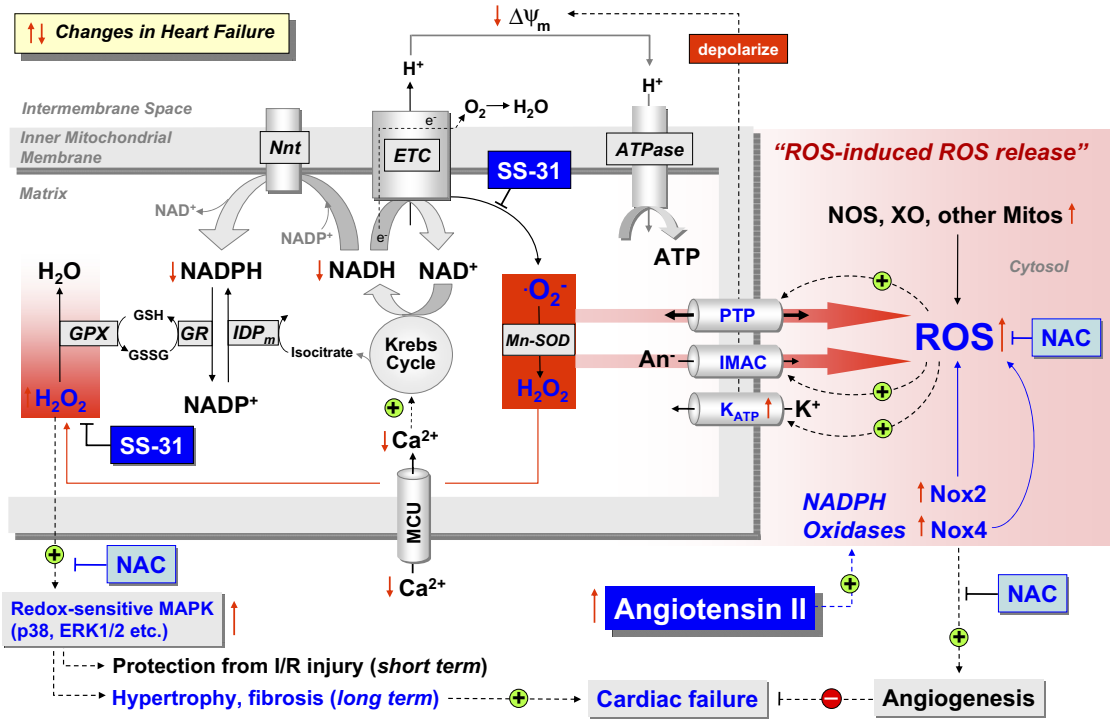

Figure 1 **SS-31 Specifically Targets Mitochondria as "Afterburners" of Cellular ROS Production**

Cytosolic reactive oxygen species (ROS) from various sources can activate (+) the permeability transition pore (PTP), the inner membrane anion channel (IMAC), and the K_{ATP}-channel in the inner mitochondrial membrane. Although the PTP is a large pore that is permeable for ions (along their respective electrochemical gradients) and larger molecules, the IMAC is permeable for anions (An^-) that are released from negatively polarized mitochondria to the cytosol. Thus, both channels can release anionic $\cdot O_2^-$ in a process termed "ROS-induced ROS release." Activation of PTP, IMAC, and/or K_{ATP} dissipates mitochondrial membrane potential ($\Delta\Psi_m$), necessitating increased electron (e^-) flux along the electron transport chain (ETC) to maintain $\Delta\Psi_m$ and adenosine triphosphate (ATP) production. NADPH is required to detoxify hydrogen peroxide (H_2O_2) via glutathione reductase (GR) and peroxidase (GPX). NADH is in equilibrium with NADPH, and thus oxidation of NADH (by $\Delta\Psi_m$ dissipation) principally favors H_2O_2 formation. Mitochondrial ROS activate mitogen-activated protein (MAP) kinases, such as p38 or ERK1/2, which on the one hand protect from ischemia/reperfusion (I/R) but on the other hand induce hypertrophy and fibrosis in the long run. In the present study, angiotensin II increased mitochondrial ROS formation via ROS-induced ROS release, which induced maladaptive cardiac remodelling. Targeted inhibition of mitochondrial ROS by SS-31 but not nontargeted N-acetyl cysteine (NAC) prevented remodelling. Changes in heart failure are indexed with **red arrows (↑ ↓)**. Novel aspects of the present study are highlighted in **blue**. GSH/GSSG = reduced/oxidized glutathione; IDP_m = mitochondrial $NADP^+$-dependent isocitrate dehydrogenase; MCU = mitochondrial Ca^{2+} uniporter; Mn-SOD = Mn^{2+}-dependent superoxide dismutase; Nnt = nicotinamide nucleotide transhydrogenase; NOS = uncoupled nitric oxide synthase; Nox = NADPH oxidase; XO = xanthine oxidase.

or 3) the mitochondrial ATP-dependent K^+-channel (mitoK_{ATP})—depolarizes $\Delta\Psi_m$, necessitating increased electron flux along the ETC (at the cost of NADH) to maintain $\Delta\Psi_m$ and ATP production (Fig. 1). Thus, opening of these channels could potentially increase ROS formation due to oxidation of NAD(P)H. Interestingly, these 3 channels have in common that they can be activated by ROS themselves. Furthermore, anionic $\cdot O_2^-$ can be released from mitochondria via the PTP or the IMAC but not via mitoK_{ATP} (Fig. 1). Accordingly, the PTP and IMAC play central roles in a phenomenon coined "ROS-induced ROS release," in which ROS trigger oscillations of $\Delta\Psi_m$ that can induce reperfusion arrhythmias and, when sustained, lead to cell death (9,17,18). By contrast, activation of mitoK_{ATP} during ischemic preconditioning transiently increases mitochondrial ROS formation that protects from ischemia/reperfusion-induced damage by activating redox-sensitive signaling pathways that converge on preventing PTP activation (19,20).

In this context, Kimura et al. (21) recently proposed that angiotensin II, by stimulating NADPH oxidase-derived $\cdot O_2^-$ production, could activate mitoK_{ATP} to trigger mitochondrial ROS production that provided protection against ischemia/reperfusion injury in the short term. Here, Dai et al. (11) demonstrate that sustained angiotensin II-induced ROS production triggers maladaptive remodelling that induces diastolic dysfunction. Because blockade of either the PTP or IMAC reduced angiotensin II-induced ROS production in isolated cardiac myocytes, the authors concluded that "ROS-induced ROS release" from mitochondria was the mechanism by which NADPH oxidase-derived ROS production is amplified by mitochondria. In this context, it is somewhat surprising that activation (rather than inhibition) of mitoK_{ATP} reduced ROS production. This is in

contrast to previous results (21) and might require further investigation. Notwithstanding, in human failing myocardium, NADPH oxidase activity is increased (6) and mitoK$_{ATP}$ are endogenously activated (8), which would support the concept of ROS-induced activation of mitoK$_{ATP}$. Although this endogenous activation protected from acute ROS-induced contractile dysfunction (8) (a scenario that occurs during ischemia/reperfusion and induces "myocardial stunning"), energetic mismatch and mitochondrial oxidative stress might be adverse long-term consequences of mitoK$_{ATP}$-induced dissipation of $\Delta\Psi_m$ that could trigger maladaptive remodelling, as suggested by the present study.

The second important aspect of the present study is that targeted mitochondrial ROS scavenging with SS-31 or mitochondria-targeted catalase reduced remodelling, whereas nontargeted (ubiquitous) ROS scavenging with NAC had no effect. This is in agreement with the lack of benefit provided by nontargeted vitamin E treatment in the clinical arena (10) and supports the general concept of compartmentalized signaling, which is increasingly recognized for many second messengers, such as Ca^{2+} or cyclic nucleotides, and might apply to NADPH oxidase-related ROS signaling as well. This concept implies that close vicinity of signaling molecules provides local control over second messengers that might not necessarily reflect changes in their global intracellular concentrations. In this regard, nontargeted ROS scavenging with NAC might be less efficient to scavenge ROS in such microdomains, whereas SS-31—which accumulates manifold at the site of ROS formation in mitochondria (at the inner mitochondrial membrane [12])—might be more efficient.

Along similar lines, 2 isoforms of NADPH oxidase exist in the heart, Nox2 and Nox4. Although Nox2 is located in the cell membrane, Nox4 is localized in perinuclear endoplasmic reticulum and/or mitochondria (4,7). Interestingly, although Nox2 mediates maladaptive cardiac remodelling in response to angiotensin II (22), Nox4 upregulation in heart failure might play a rather protective role by enhancing angiogenesis (Fig. 1) (4), although conflicting data exist (7). In the present study, Nox4 was upregulated in response to angiotensin II infusion, in agreement with other cardiac stresses in previous studies (4,7). If Nox4-related ROS production indeed protects from the development of heart failure (4), this might be another potential explanation why nontargeted ROS scavenging with NAC was less effective than mitochondria-targeted scavenging with SS-31, because NAC might scavenge both maladaptive (Nox2- and mitochondria-related) and protective (Nox4-derived) ROS. Clearly, these issues require further investigation, particularly concerning the cellular localization of Nox4 signaling and its role for cardiac remodeling (4,7).

In the study by Dai et al. (11), chronic angiotensin II treatment induced diastolic dysfunction with preserved systolic function. Clinically, morbidity and mortality is similarly adverse for patients with heart failure with either preserved (HFpEF) or reduced ejection fraction (HFrEF), and yet no effective treatment for HFpEF has been identified (23). Thus, there is an urgent need for novel therapeutic options especially for patients with HFpEF. Although the pathophysiological issues discussed in the preceding text might apply to both systolic and diastolic heart failure, the current data suggest that targeting mitochondrial ROS might be a particularly interesting option in patients with HFpEF.

Taken together, specific targeting of cellular microdomains of ROS signaling might be a promising therapeutic approach in heart failure. Future research should be directed at further testing the therapeutic efficiency of SS-31 in larger animal studies to translate the promising results from rodents to heart failure in humans.

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