EDITORIALS

Nitroso–Redox Balance in the Cardiovascular System

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In this issue of the *Journal*, Taylor and colleagues report the results of the African-American Heart Failure Trial (A-HeFT), a double-blind, randomized trial that evaluated the effect of the addition of isosorbide dinitrate and hydralazine to the best conventional therapy in patients with symptomatic congestive heart failure who identified themselves as black.[1] The results with the combination therapy were markedly positive, meeting the composite end point of the trial, which included death from any cause, a first hospitalization for heart failure, and quality-of-life measures. In addition, there was an impressive reduction in the rate of death from any cause of approximately 45 percent, leading to an early termination of the study.

This trial raises fascinating issues regarding heart-failure therapeutics and variations in drug susceptibility among populations. Attempting to understand these issues requires insight into the pharmacologic and biologic underpinnings of any drug combination that may enhance nitric oxide availability. This regimen of isosorbide dinitrate and hydralazine (which are available individually as inexpensive, generic formulations), initially selected for its vasodilatory properties in the Veterans Administration Heart Failure Trials (V-Heft) I and II,[2] turns out to be one of the most fortuitous combination therapies in cardiovascular medicine. Isosorbide dinitrate is an organic nitrate that stimulates nitric oxide signaling,[3] and hydralazine is a vasodilator and antioxidant that inhibits the enzymatic formation of reactive oxygen species such as superoxide (O_2^-) by NADH and NADPH oxidases.[4] The success of this therapy now brings to the forefront the need to understand the underlying biochemistry of nitric oxide and superoxide and their interaction, since these molecules are key determinants of cellular redox balance.

Nitric oxide and superoxide are physiologically produced, reactive molecules with unpaired electrons (i.e., free radicals). Nitric oxide is a ubiquitous signaling molecule that influences organ function by means of post-translational modification of effector molecules; this occurs most often at cysteine residues and is termed *S*-nitrosylation.[5] *S*-nitrosylation is a highly versatile signaling mechanism that affects hundreds of biologic events in a tightly regulated and reversible manner.[5] Superoxide plays a key role in this regulation by facilitating *S*-nitrosylation of proteins when it is present at physiologic levels but disrupting this signaling mechanism when it is present at elevated levels.[6] Specifically, superoxide reacts with the same cysteine thiol moieties on proteins that are regulated by *S*-nitrosylation, preventing this reaction from occurring[7] (Fig. 1). Excess superoxide also reacts directly with nitric oxide, disrupting its physiologic signaling and potentially leading to the production of other toxic and reactive molecules, notably peroxynitrite.[8] Thus, a central pathophysiological consequence of oxidative stress is the disruption of nitric oxide signaling, and it is therefore appropriate to think of cellular redox balance in terms of the nitroso–redox balance. From this perspective, the administration of a combination of a nitric oxide stimulator and an antioxidant turns out to be an excellent choice and one that addresses fundamental biochemical derangements in the failing cardiovascular system (Fig. 1).

S-nitrosylation regulates key processes in both the heart and the vascular tree, and thus, a nitroso–redox imbalance can affect both cardiac performance and vascular tone. In the heart, *S*-nitrosylation of ion channels maintains the calcium cycle within heart-muscle cells responsible for normal systolic and diastolic function.[9] In conductance vessels (those of medium-to-large size), nitric oxide

acts as the prototypical endothelium-derived relaxing factor by activating guanylyl cyclase to produce cyclic guanosine monophosphate. Finally, in the microcirculation, nitric oxide carried by *S*-nitrosohemoglobin regulates blood flow. It is important to note that nitric oxide is not produced by endothelial cells in the microcirculation but, rather, is carried there by hemoglobin itself.[10] In an elegant series of experiments carried out by Jonathan Stamler's group and recently confirmed by other laboratories,[11] red cells were shown to regulate the arteriolar microcirculation, coupling oxygen delivery with blood flow.[10]

A disruption in nitroso–redox signaling clearly has the potential to contribute to congestive heart failure. At an enzymatic level, oxidant-producing enzymes are up-regulated[12] and the level or spatial location of nitric oxide–producing enzymes — nitric oxide synthases — are altered within cells.[13] In addition, a deficiency of nitric oxide synthase may actually increase the activity of oxidases, since nitric oxide may be a physiologic down-regulator of superoxide production.[6] The levels of specific vascular NADPH oxidases are increased in the failing circulatory system, at least partly in response to increased levels of angiotensin II, suggesting a link between neurohormonal activation and a nitroso–redox imbalance.[14] In addition, the levels and activity of xanthine oxidase, an oxidase (for which NADH and oxygen are electron acceptors) produced in the liver, gut, and heart that circulates in the blood, are increased throughout the cardiovascular system, contributing to vasoconstriction and depressed cardiac function.[8,12,15]

With regard to the arteriolar vascular bed, the site that determines vascular resistance and therefore stress on the heart, Datta and colleagues recently demonstrated that the delivery of *S*-nitrosohemoglobin is impaired in the presence of both heart failure and one of the major risk factors for heart failure, diabetes.[11] Disruption of nitric oxide delivery to the microcirculation almost certainly contributes to the vasoconstriction and uncoupling of oxygen delivery in skeletal muscle that are characteristic of heart failure. Thus, although the sources of oxidative stress may differ and several different enzymatic and biochemical mechanisms can disrupt nitric oxide signaling, a central problem in the failing circulation appears to be a shift in the nitroso–redox balance away from physiologic *S*-nitrosylation to one of oxidative stress.

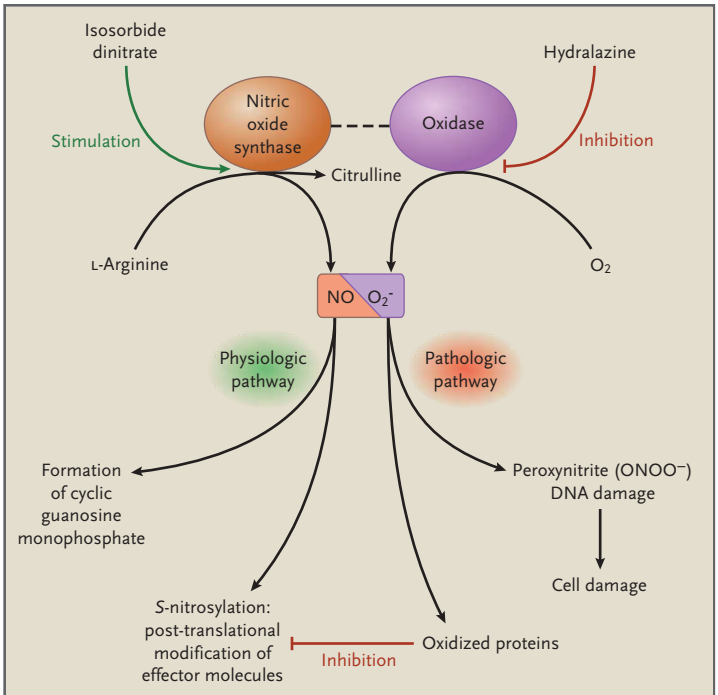

Figure 1. Consequences of Disrupting the Balance between Nitric Oxide and Superoxide Production in the Cardiovascular System of Patients with Congestive Heart Failure.

The interaction between nitric oxide (NO) and superoxide (O_2^-) production — the nitroso–redox balance — plays fundamental roles in cell and organ failure at key sites throughout the cardiovascular system. Physiologically, the level and location of nitric oxide and superoxide production are balanced within the cell, facilitating appropriate post-translational modification of effector proteins. In patients with heart failure, the production of superoxide is increased and the level or location of nitric oxide synthesis is disrupted, interrupting effector signaling and thus causing cellular dysfunction as a result of vasoconstriction of small and large blood vessels and cardiac contractility or, if prolonged, cell death or damage. Isosorbide dinitrate, a drug that stimulates the nitric oxide pathway, and hydralazine, an antioxidant that inhibits superoxide synthesis, may therefore restore the balance of nitric oxide and superoxide production, converting the pathologic pathways to physiologic pathways in both the heart and the blood vessels.

In addition to diminished nitric oxide production, alterations in the levels of nitric oxide synthases may also contribute directly to oxidative stress in patients with heart failure by producing superoxide when nitric oxide synthase cofactors are deficient. Finally, in some circumstances such as acute ischemia, sepsis, or heart failure, the production of nitric oxide synthase is abnormally elevated, usually as a result of the induction of a high-output isoform (inducible nitric oxide synthase), leading to an adverse situation of nitrosative stress.[9] Cellular damage in

this situation is often potentiated because the stimuli that lead to the formation of inducible nitric oxide synthase are also capable of up-regulating oxidases, and concomitant elevations in nitric oxide and superoxide lead to the formation of peroxynitrite.[8]

Insight into this pathophysiological signaling is highly relevant to therapeutics. Impairment in cardiac-energy use in patients with heart failure is reversible with the short-term use of xanthine oxidase inhibitors.[12] A prolonged nitroso–redox imbalance leads to the consequences more traditionally ascribed to oxidative stress — cell damage as a result of the oxidation of nucleic acids and proteins, cell loss owing to apoptosis, and phenotypic alteration as a result of the activation of abnormal gene programs (the fetal gene program and resultant cardiac hypertrophy are a prime example of this phenomenon).[8] The results of the A-HeFT strongly suggest that a regimen with the potential to restore the nitroso–redox balance probably also affects cardiac remodeling.

Viewed from the nitroso–redox perspective, it seems logical that the combination of isosorbide dinitrate and hydralazine should have potent cardiovascular effects in the failing circulation. Interestingly, although these are old drugs, the specific mechanism of action for these compounds has been identified only in the past decade. Munzel et al. demonstrated that hydralazine inhibits membrane-bound enzymatic sources of superoxide that use NADH and NADPH as electron acceptors[4]; the specific oxidase involved remains to be identified. Whether organic nitrates are themselves nitric oxide donors or require enzymatic biotransformation remains a matter of controversy. Recently, the metabolism of organic nitrates by mitochondrial aldehyde oxidase has been found to have a role in activating the vasodilatory nitric oxide pathway.[3] Ironically, it is this enzyme that not only induces nitric oxide bioactivity but also leads to nitrate tolerance, a circumstance in which superoxide is generated, thereby providing insight into the mechanism by which hydralazine may offset this phenomenon.[4]

Indeed, the success of the A-HeFT coupled with the growing understanding of the biochemistry of nitric oxide and superoxide sets the stage for investigations to determine the precise mechanism of action of this drug combination and, just as important, could lead to many other novel therapies aimed at restoring the nitroso–redox balance. To the extent that this therapy ultimately proves to work preferentially in certain demographic subgroups, knowledge of the operative biochemical pathways will facilitate the search for the genetic and environmental determinants of this selective susceptibility.

Supported by a grant (HL-065455) from the National Institutes of Health.

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