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COMMENTARY

The Yin/Yang of superoxide dismutase mimetics: potential cardiovascular therapies?

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Abbreviations: ApoE, apolipoprotein (E); SOD, superoxide dismutase; VSMC, vascular smooth muscle cells

Oxidative stress, defined as an alteration in the balance between the production and removal of reactive oxygen species (ROS), plays a role in many pathological conditions. Superoxide ($O_2 \cdot ^-$) is generated *via* a one electron reduction of O_2, which subsequently can give rise to hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), and the hydroxyl radical (OH^-). These ROS have multiple effects on cardiovascular cells, including induction of inflammatory molecules, inactivation of nitric oxide ($NO \cdot$), and the mediation of vascular smooth muscle cell (VSMC) hypertrophy, hyperplasia, and cell migration. All of these processes are important in cardiovascular pathology and contribute to the vascular changes associated with hypertension, atherosclerosis, and restenosis.

One early indicator of vascular pathologies is endothelial dysfunction, associated with an increase in $O_2 \cdot ^-$ formation resulting in a net decrease in $NO \cdot$ bioavailability. Under normal conditions, removal of $O_2 \cdot ^-$ is regulated by a group of oxidoreductases known as superoxide dismutases (SODs). These enzymes contain Mn, Cu, or Fe at the active site and catalyze the dismutation of $O_2 \cdot ^-$ to O_2 and H_2O_2. In the current study by Jiang *et al.* (2003), the authors test the ability of a nonpeptide mimetic of SOD, M40403, to antagonize $O_2 \cdot ^-$ generated by VSMCs in response to exogenous NAD(P)H and angiotensin II, and to reverse impaired endothelial function in Apolipoprotein (E) (ApoE)-deficient mice, a model of hyperlipidemia and atherosclerosis.

While the role of the SODs in maintaining oxidative balance and its potential therapeutic application has been characterized by use of its native forms, the ability to translate these findings into clinically relevant therapies has faced major pitfalls. These challenges have included solution instability, limited cellular accessibility, immune responses to non-human enzymes, proteolytic digestion, and an inability to penetrate cells or cross the blood–brain barrier. To circumvent these issues, a class of stable, Mn-containing, nonpeptidyl SOD mimetics have been synthesized (Salvemini *et al.*, 1999). These SOD mimetics catalyze dismutation of $O_2 \cdot ^-$ at a rate similar to native MnSOD, and have been shown to distribute widely into organs following intravenous administration while maintaining their chemical structure intact.

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In the cardiovascular system, NAD(P)H oxidases are major generators of ROS in the vessel wall. These oxidases produce ROS both intracellularly and extracellularly. A clear advantage of the SOD mimetic M40403 over native enzymes is molecule size (MW 483 vs 30,000 Da). While the cellular location of ROS production was not specifically addressed in Jiang *et al.'s* (2003) study, in theory, the ability of M40403 to gain access to intracellular and subcellular locations would provide significant advantages over other SOD mimetics both clinically and experimentally. In addition, unlike other SOD mimetics, M40403 is specific for the removal of $O_2 \cdot ^-$ and does not react with other ROS, limiting the effects of nonspecific inhibition of other free radicals. Finally, this mimetic is resistant to nitration by $ONOO^-$, which inactivates native MnSOD enzyme (Salvemini *et al.*, 2001).

A key issue when assessing the therapeutic applications of M40403 is to approach ROS from a holistic perspective. Elevated $O_2 \cdot ^-$ levels contribute significantly to certain vascular changes such as the endothelial dysfunction during hypertension and diabetes, preconditioning during ischemia/reperfusion injury, and inflammation. Previous studies have clearly indicated that treatment with M40403 in several animal models of inflammation prevented edema, macrophage infiltration, and upregulation of inflammatory mediators (Salvemini *et al.*, 1999; 2001). It is crucial to recognize that an increase in SOD activity will facilitate an elevation of H_2O_2, which is known to act as a second messenger, mediate DNA synthesis and matrix remodeling, and facilitate VSMC proliferation, hypertrophy and migration (Griendling *et al.*, 2000). All of these processes contribute to vascular pathology, emphasizing the importance of maintaining a perspective on how SOD mimetics affect the overall balance of ROS.

In the current study, Jiang *et al.* test the role of this potentially important new compound in $O_2 \cdot ^-$ production and endothelial dysfunction in ApoE-deficient mice. These findings support previous studies in which endothelial dysfunction in ApoE-deficient mice was reversed by the use of liposomal SOD (Laursen *et al.*, 2001), SOD mimetics (d'Uscio *et al.*, 2001) or antioxidants (d'Uscio *et al.*, 2003). The advantage of M40403 in restoring endothelium-dependent relaxation may center upon the specificity of this compound for $O_2 \cdot ^-$. Jiang *et al.*'s data indicate that M40403 is effective in inhibiting ROS derived fromNAD(P)H oxidases. To investigate the source of ROS in their experiments, they measured $O_2 \cdot ^-$ production following stimulation with exogenous NAD(P)H, and con-

cluded that this enzyme system was the source of ROS. However, the addition of NAD(P)H to the outside of VSMCs raises the possibility that NAD(P)H is acting as a substrate for a surface-bound enzyme. Since the catalytic site of NAD(P)H oxidases is on the intracellular surface of the membrane, it is possible that other NAD(P)H-dependent enzymes also contribute to the observed changes in ROS. One candidate is xanthine oxidase, which utilizes NAD(P)H, is inhibitable by DPI and generates $O_2 \cdot^-$ in an allopurinol-independent manner. The authors suggest that the availability of substrate is a key factor limiting $O_2 \cdot^-$ production, but given the high intracellular concentrations of NAD(P)H ($\sim$0.1 mM) (Schafer & Buettner, 2001) and the basal oxidase activity in ApoE-deficient mice, alternative interpretations are possible.

This study reports a significant increase in basal $O_2 \cdot^-$ production in ApoE mice (following maintenance of a high-fat diet), indicating that $O_2 \cdot^-$ may be a contributing factor to the vascular alterations that occur during atherosclerosis. The role of the vascular NAD(P)H oxidase in ApoE-deficient mice has been controversial. Initial studies indicated that elimination of either the phagocytic NAD(P)H oxidase (Kirk *et al*., 2000) or disruption of p47phox had no effect on lesion size in ApoE-deficient mice (Hsich *et al*., 2000). Subsequent studies by Barry-Lane *et al*. (2001) demonstrated a significant decrease in $O_2 \cdot^-$ levels and aortic lesion area in ApoE mice lacking the p47phox subunit of the oxidase. Jiang *et al*. (2003) show that treatment with the SOD mimetic M40403 successfully reverses endothelial dysfunction, an important early event in the progression of atherosclerosis, and it will be interesting to investigate its effects on lesion formation in future studies. The outcome of these experiments is difficult to predict, since lesion formation is multifactorial and may involve other ROS including H_2O_2. If H_2O_2 is in fact involved, administration of a SOD mimetic could potentiate lesion formation.

Pathologies associated with increased levels of ROS are complex. As a result of its specificity, this novel compound M40403 can be used experimentally to gain insight into which mechanisms are associated with increased $O_2 \cdot^-$ levels, as opposed to those mediated by other ROS. Eventually this mimetic could be applied clinically to treat diseases where elevated levels of $O_2 \cdot^-$ are the main culprits. At the same time, one must remain cognizant of the overall effect of shifting the redox balance of the system in utilizing these approaches.

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