More than 1 million Americans experience new or recurrent myocardial infarction (MI) annually with clinical outcomes strongly related to infarct size and the deleterious effects of ventricular remodeling. Standard MI treatments are appropriately directed at early reperfusion, antiplatelet/lipid-lowering therapies, and the administration of β-blockers and angiotensin-converting enzyme inhibitors to help reduce adverse post-MI ventricular remodeling. However, there is strong evidence to suggest that inflammation and the generation of reactive oxygen species, such as the superoxide anion \( \text{O}_2^- \), contribute substantially to myocardial ischemia/reperfusion injury. Thus, there is great interest in developing novel therapies targeting postinfarct inflammatory processes, including oxidative stress.

Attenuating the effects of \( \text{O}_2^- \) is an attractive potential therapy for MI. Not only does \( \text{O}_2^- \) directly damage tissue through the peroxidation of lipids and oxidation of sulfhydryl groups, but it also reacts with NO very rapidly, leading to the formation of another reactive oxidizing agent, peroxynitrite (ONOO\(^-\)), and to decreased levels of NO, an extremely important cardioprotective regulator of cellular function and survival. Extracellular superoxide dismutase (EcSOD), a key enzyme that is secreted into the extracellular space and catalyzes the dismutation of \( \text{O}_2^- \) to hydrogen peroxide and molecular oxygen, has been shown to attenuate ischemia/reperfusion injury by limiting the direct effects of \( \text{O}_2^- \) and ONOO\(^-\) as well as increasing NO bioavailability. To date, administration of antioxidant therapies after MI has been disappointing in patients with intravenous infusion of any of the 3 major superoxide dismutase enzymes, showing mixed results.

Because oxidative stress may last for weeks to months after MI, therapies need to be tailored to exert a sustained, localized antioxidant effect. As a result, parenteral administration of enzymes with relatively short half-lives would be cumbersome. Theoretically, gene targeting techniques that overexpress or inhibit specific genes or translational modifiers that are putatively involved in the development of left ventricular (LV) dysfunction and remodeling may offer a means to provide a continuous endogenous source of antioxidant therapy. However, there have been many obstacles in developing a gene targeting system to treat MI, most notably the identification of appropriate target genes, the development of a vector that is efficient, safe, and localizes to the target cells of interest, and the determination of biological effects.

In this issue of *Circulation: Cardiovascular Imaging*, Konkalmatt et al extend their previous work in gene therapy in a mouse model and overcome many of the challenges of sustained localized release of superoxide dismutase, reflecting the advances in gene therapy delivery during the past 15 years. Using a single intravenous injection of a vector with higher cardiac affinity than previous serotypes, adeno-associated virus serotype 9 (AAV9), in conjunction with a cardiac-specific promoter, cardiac troponin-T, the investigators were able to demonstrate superior gene transduction of cardiac myocytes compared with other AAV serotypes. Interestingly, the comparison of transduction efficiency and time course/tissue distribution of gene expression were evaluated using serial, noninvasive bioluminescence imaging to arrive at the best gene therapy candidate. Postmortem validation demonstrated successful, targeted expression of EcSOD, achieving an 84% transduction rate and a 5.6-fold higher enzymatic activity than sham-treated mice. Importantly, the investigators used separate models of acute MI and post-MI remodeling to show significant protective effects of cardiac-selective overexpression of EcSOD on both infarct size and LV remodeling, as quantified on cardiac magnetic resonance imaging. Using late gadolinium enhancement cardiac magnetic resonance, Konkalmatt et al demonstrated that the infarct size reductions from EcSOD overexpression could be overcome by prolonged ischemia. However, cardiac magnetic resonance enabled the assessment of global changes in LV ejection fraction and LV end-diastolic and LV end-systolic volumes without assumptions of cardiac geometry to show the potential effects of EcSOD overexpression on LV remodeling. As the authors correctly point out, interpretation of the results should be cautionary given the lack of significant improvement in LV ejection fraction, 4-week infarct size data, and late histological studies.

The same investigators previously demonstrated that pretreatment using a direct intramyocardial injection of an AAV9 vector expressing EcSOD under the cardiac troponin-T promoter in adult mice 4 weeks before induction of MI significantly reduced infarct size. The premise of pretreatment weeks before MI is that gene transfer using AAV vectors requires weeks before full expression is achieved in the heart, whereas LV remodeling continues for weeks in
rodents and even longer in larger animals after an ischemic event. However, these investigators have demonstrated that systemic delivery after ischemia/reperfusion may result in sufficient EcSOD expression to exert beneficial effects on LV remodeling as determined by echocardiography 14 and 28 days after infarction compared with sham animals.15 Moreover, enhanced green fluorescent protein expression, which was also regulated by the cardiac troponin-T promoter, was most highly expressed in cardiomyocytes bordering the infarct zone based on cardiac magnetic resonance infarct delineation.12

Obal et al6 demonstrated multiple potential mechanisms of EcSOD action to reduce infarct size after ischemia/reperfusion injury by studying the effects of superoxide scavenging on NO bioavailability in a cardiomyocyte-specific EcSOD transgenic mouse. In this study, a significant attenuation of reactive oxygen species and peroxynitrate production with a concomitant increase in NO was shown. Furthermore, EcSOD was capable of cytosolic localization that affected intracellular signaling transduction and function. The current study by Konkalmatt et al10 found an increased capillary area fraction and a reduction in neutrophil recruitment, which could be additional potential cardioprotective mechanisms of EcSOD overexpression. However, these data need to be interpreted with caution given the small sample sizes studied. Moreover, the long-term effects of prolonged EcSOD overexpression in the heart are unknown because EcSOD may also play a role at the vascular level on endothelial function and blood pressure regulation.13

The importance of gene transfer delivery route has been explored previously.14 Intramyocardial injections enable a higher concentration of gene expression in the heart with minimal tissue redistribution. Intracoronary or cardiac vein administration allows for a more uniform distribution but has a greater propensity to affect other organs. These methods are, of course, also invasive. The use of minimally invasive systemic intravenous delivery method with the potential of EcSOD pretreatment was first performed by Li et al,15 who successfully limited infarct size in a rabbit model using a recombinant adenovirus that overexpressed EcSOD. In this model, EcSOD was produced primarily by the liver, and systemic release to the heart and other organs was achieved using hepatic infusion. The current work by Konkalmatt et al10 demonstrates a significant advance by providing a method of systemic intravenous gene delivery with highly selective myocardial expression by using the cardiac troponin-T promoter. Whether this method will prove equally efficacious in larger animals and humans remains unknown.

Of particular concern is the recent report by Seok et al16 that the time course of gene expression and alterations in response to inflammation in C57BL/6J mice is markedly different from that seen in man. Whereas Seok et al16 explored more traditional inflammatory models, such as endotoxemia, many of the mechanisms of antioxidant therapy for the treatment of MI presumably involves alterations to the inflammatory axis. In addition, the differences between endocardial to epicardial perfusion and collateral formation in the mouse heart relative to man17 suggest that large animal studies would be warranted before seeking AAV9 gene therapy for EcSOD overexpression in patients. Large animal studies could also provide insight into whether EcSOD gene therapy would ultimately be useful in patients at high risk for future MI to limit infarct size or as a treatment delivered acutely after an ischemic event to prevent adverse ventricular remodeling. Alternatively, understanding the role of EcSOD overexpression may lead to enhanced drug therapies rather than gene therapy techniques.

There are also several additional hurdles to clinical translation for AAV9 EcSOD gene therapy. Questions still remain regarding the safety and efficacy of gene therapy. Although recombinant AAV vectors are created from small, non-pathogenic, single-stranded DNA viruses that do not induce human disease, there is a potential risk of oncogenesis and gene mutation because of the random insertion of DNA.18 However, recently published results from the randomized, double-blind, placebo-controlled phase II clinical trial, Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID),19 are promising. In CUPID, the AAV1 vector was administered via a single percutaneous infusion into the dominant coronary artery to enhance myocardial expression of SERCA2a in patients with heart failure. This approach was not associated with adverse events, and several heart failure clinical indices were improved.19 Thus, if AAV9-mediated, cardiac-selective gene expression of EcSOD can be shown to be beneficial in a relevant large animal model, the potential for use of EcSOD overexpression as a means to reduce infarct size or prevent adverse LV remodeling would appear bright.

Disclosures

None.

References


**Key Words:** Editorials ■ gene therapy ■ CT and MRI ■ nitric oxide, endothelium-derived ■ angiogenesis
From Mice to Men: Gene Therapy's Future for Treatment of Myocardial Infarction
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Circ Cardiovasc Imaging. 2013;6:360-362
doi: 10.1161/CIRCIMAGING.113.000465
Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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