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EDITORIAL COMMENTARY

Returning to the bench with adeno-associated virus 1 *SERCA2a* gene therapy

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The basic science of postinfarct remodeling may not be the most familiar topic for readers of the *Journal*. Yet the processes that lead to pathologic changes in the form and function of the left ventricle after a transmural myocardial infarction (MI) are fertile areas for research, because current medical and surgical therapies do not avert the process. After a transmural MI, a full-thickness area of myocardium is replaced with fibrotic scar. Regions of the myocardium that border the infarct zone (the “border zone” myocardium) are subjected to stresses that may lead to additional myocyte loss, thereby expanding the zone of injury. With time, a maladaptive process of remodeling can ensue, with ventricular dilatation, increased sphericity, and worsening ejection fraction, leading in some cases to acute and chronic systolic heart failure. Mitral valve regurgitation, secondary to annular dilatation or papillary muscle scarring and posterior leaflet tethering, further contributes to the adverse remodeling process by introducing volume overload and pulmonary hypertension.

Revascularization is the standard approach for acute ST-elevation MI. Early percutaneous coronary intervention and thrombolytic agents have supplanted surgical revascularization for most of these cases. For missed ST-elevation MIs, the role of revascularization is controversial, although

late revascularization may protect the border zone myocardium. Other surgical therapies, including mitral repair or replacement for ischemic mitral regurgitation and surgical ventricular restoration for dilated ischemic cardiomyopathy, are treatment options for advanced stages of postinfarct remodeling; in randomized, controlled trials, however, neither has resulted in improved long-term survival.^{1,2}

Experimental models of MI have drawn attention to the molecular and cellular pathophysiology of postinfarct remodeling and have attempted to alter or ameliorate the process. Small animal models (primarily in the mouse and rat) have evaluated the impact of genetic and pharmacologic interventions on remodeling after transmural MI from ligation of the left anterior descending coronary artery. Gene-based

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Central Message

After CUPID 2, investigators take AAV1 *SERCA2a* gene therapy back to the bench with an experimental model of ischemic cardiomyopathy.

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interventions have focused on processes such as apoptosis, thought to be the effector of myocyte loss outside the infarct zone (in the border zone and remote myocardium), and fibrosis, which is mediated by transforming growth factor β signaling. Modulation of gene expression has been achieved in transgenic and knockout animals, as well as with targeted gene therapy with adenoviral vectors. Recently, a variety of microRNAs, which are noncoding small RNAs that bind to target messenger RNA and inhibit translation, have been implicated in postinfarct remodeling as well. Regenerative strategies, including stem cell therapies, have also been used to modulate the process.

In this issue of the *Journal*, Katz and colleagues³ evaluate the effect of sarcoplasmic reticulum calcium adenosine triphosphatase 2a (SERCA2a) overexpression on myocardial fibrosis in an ovine model of ischemic cardiomyopathy. SERCA2a is involved in calcium handling, and the same group^{4,5} previously demonstrated that normalization of *SERCA2a* gene expression after MI resulted in improved cardiac function. They hypothesize that this is secondary to mitigation of myocardial fibrosis, and in their study in this issue, this is evaluated in a model of small and large MI that is created by proximal ligation of 1 (small) or 2 (large) branches of the circumflex coronary artery. Local delivery of *SERCA2a* gene construct is achieved 4 weeks postinfarct with an adeno-associated virus 1 (AAV1) vector that is delivered with a technique called “molecular cardiac surgery with recirculating delivery” [MCARD].⁶ The MCARD technique involves local delivery of genetically engineered viral vector into the coronary sinus and results in upregulation of SERCA2a protein expression, primarily in the border zone myocardium. At 12 weeks postinfarct, myocardial fibrosis and function were assessed with magnetic resonance imaging, and tissues were collected for histologic and molecular analyses.

Katz and colleagues³ found less adverse postinfarct remodeling in the large MI group with *SERCA2a* overexpression (large MI–*SERCA*) than in the large MI–only group—despite the fact that their infarct sizes were comparable ($20.4\% \pm 2.6\%$ vs $19.8\% \pm 2.4\%$).³ Although the study is lacking a null vector large MI control group, Katz and colleagues³ offer a plausible argument that the effect is not due simply to transfection with the viral vector. At a molecular level, they demonstrate that transforming growth factor β signaling, which is upregulated postinfarct (most markedly in the infarct zone, but also in the border zone and remote myocardium) is reduced in the large MI–*SERCA* animal group. In addition, angiotensin II plasma levels and myocardial angiotensin 1 receptor expression are significantly lower in the large MI–*SERCA* group than in the large MI–only group. Both transforming growth factor β and angiotensin II signaling are involved in fibrogenesis in the heart, so blocking these mediators of fibrosis may limit adverse remodeling and infarct expansion. At a histologic

level, Katz and colleagues³ find less de novo collagen synthesis in the large MI–*SERCA* and small MI groups relative to the large MI–only group. Ultrastructurally, the morphology of the border zone myocytes was largely preserved in the large MI–*SERCA* and small MI samples, as opposed to the large MI–only group, in which marked sarcomere fragmentation, myofibril disorganization, mitochondrial damage, and disrupted Z-discs were noted.

Translating the basic science of experimental postinfarct remodeling to human therapies remains challenging. Mechanistic studies point to potential molecular targets; however, the optimal effectors for modulating, inhibiting, or upregulating these targets are uncertain. In the study of Katz and colleagues³ in this issue, a viral vector gene therapy approach was selected; however, there are limitations to this strategy. For example, the expression of the viral vector plateaus at 4 to 6 weeks postinfection, offering only transient modulation of gene expression. Moreover, one might ask, what is the optimal timing for therapy postinfarct? Katz and colleagues³ chose 4 weeks postinfarct, but would earlier treatment be more protective? The MCARD technique enables targeted delivery of the vector to myocardial cells; however, the impact of low-level transfection of nontarget tissues is unknown. Finally, the short- and long-term safety profile of viral transfection in human beings requires further study.

The long-term effects of AAV1 *SERCA2a* gene transfer in patients with advanced heart failure was recently reported.^{7,8} The Calcium Up-Regulation by Percutaneous Administration of Gene Therapy In Cardiac Disease (CUPID) trial was a phase 2 trial that evaluated the clinical effects of AAV1-mediated *SERCA2a* gene therapy in 39 patients with New York Heart Association functional class III or IV heart failure symptoms. Three dose regimens of AAV1 *SERCA2a* (low, mid, or high) or placebo were administered through a single intracoronary arterial injection. Patients with neutralizing antibodies to the virus (a finding in nearly half of patients screened) were excluded. Dose-related reductions in cardiovascular events and mortality were noted through the 3-year follow-up period. In addition, long-term expression of transgene was noted in high-dose patients. The follow-up trial, CUPID 2, was a multicenter, randomized, controlled trial sponsored by Celladon Corporation (the manufacturer of Mydicar, the AAV1 *SERCA2a* construct used in the trials) that enrolled 250 patients with heart failure with reduced ejection fraction, comparing outcomes of a single intracoronary treatment with high-dose AAV1 *SERCA2a* versus placebo.⁹ Unfortunately, the trial failed to reach both its primary end point (time to recurrent heart failure hospitalization) and its secondary end point (time to death). These results temper enthusiasm for AAV1 *SERCA2a* gene therapy in clinical heart failure and suggest that the therapy is not ready for clinical translation. In fact, Celladon reports on its website that it has currently stopped research and development for the Mydicar product.¹⁰

Will MCARD be the answer to *SERCA2a* gene therapy? The technique may result in better transgene distribution and transduction, and Katz and colleagues have demonstrated its reproducibility.⁶ That said, Katz and colleagues³ are clear that the success of the technique depends on concurrent use of cardiopulmonary bypass, which may be good news for cardiac surgeons but less good news for patients and referring cardiologists.

Despite recent controversies related to *SERCA2a* gene therapy in human beings, the study of Katz and colleagues³ adds to our arsenal of understanding of postinfarct remodeling, which was clearly not a focus of the CUPID trials. Future strategies to limit maladaptive remodeling, whether gene therapy, cell-based therapy, or pharmacologic, will build on this basic science foundation and ultimately result in translatable treatments for a prevalent human disease. Will the cardiac specialist of the future find MCARD with *SERCA2a* constructs in their armamentarium? As with all innovations, the test of time still lies ahead.

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