Mitigation of myocardial fibrosis by molecular cardiac surgery–mediated gene overexpression

Michael G. Katz, MD, PhD,[a,b] Elizabeth Brandon-Warner, PhD,[c] Anthony S. Fargnoli, PhD,[a,b] Richard D. Williams, BS,[a] Andrew P. Kendle, BS,[a] Roger J. Hajjar, MD,[b] Laura W. Schrum, PhD,[c] and Charles R. Bridges, MD, ScD[a]

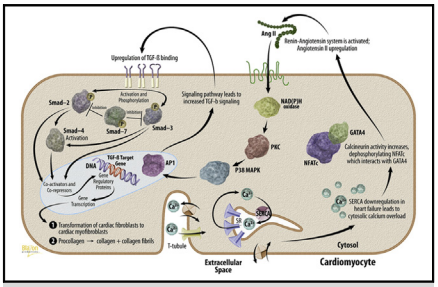
ABSTRACT

Objective: Heart failure is accompanied by up-regulation of transforming growth factor beta signaling, accumulation of collagen and dysregulation of sarcoplasmic reticulum calcium adenosine triphosphatase cardiac isoform 2a (SERCA2a). We examined the fibrotic response in small and large myocardial infarct, and the effect of overexpression of the SERCA2a gene.

Methods: Ischemic cardiomyopathy was induced via creation of large or small infarct in 26 sheep. Animals were divided into 4 groups: small infarct; large infarct with heart failure; gene-treated (large infarct with heart failure followed by adeno-associated viral vector, serotype 1.SERCA2a gene construct transfer by molecular cardiac surgery with recirculating delivery); and control.

Results: Heart failure was significantly less pronounced in the gene-treated and small-infarct groups than in the large-infarct group. Expression of transforming growth factor beta signaling components was significantly higher in the large-infarct group, compared with the small-infarct and gene-treated groups. Both the angiotensin II type 1 receptor and angiotensin II were significantly elevated in the small- and large-infarct groups, whereas gene treatment diminished this effect. Active fibrosis with de novo collagen synthesis was evident in the large-infarct group; the small-infarct and gene-treated groups showed less fibrosis, with a lower ratio of de novo to mature collagen.

Conclusions: The data presented provide evidence that progression of fibrosis is mediated through increased transforming growth factor beta and angiotensin II signaling, which is mitigated by increased SERCA2a gene expression. (J Thorac Cardiovasc Surg 2016;151:1191-200)



Proposed mechanism due to SERCA2a down-regulation through angiotensin II and TGF-β to fibrosis.

Central Message

Gene therapy by molecular cardiac surgery–mediated SERCA2a has a cardioprotective effect, with mitigation of fibrosis.

Perspective

Molecular cardiac surgery with recirculating delivery would offer a unique solution for the clinic and usher in the concept of targeted transvascular gene delivery. We found that SERCA2a gene delivery restricts the progression of fibrosis in our heart failure model. By combining this delivery method with this therapy, SERCA2a could be safely and effectively delivered after myocardial infarction, to prevent cardiac remodeling.

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From the [a]Department of Cardiothoracic Surgery, Sanger Heart and Vascular Institute, Charlotte, NC; [b]Cardiovascular Research Center, Mount Sinai School of Medicine, New York, NY; and [c]Liver Pathobiology Laboratory, Carolinas Medical Center, Charlotte, NC.

This study was supported by National Institutes of Health grant 2-R01 HL083078-08, and grants from the James H. Heineman Foundation.

M.G.K. and E.B.-W. contributed equally to this work.

L.W.S. and C.R.B. are co-senior authors of this article.

Received for publication July 14, 2015; revisions received Nov 11, 2015; accepted for publication Nov 19, 2015; available ahead of print Jan 5, 2016.

Address for reprints: Michael G. Katz, MD, PhD, Sanger Heart and Vascular Institute, 1001 Blythe Blvd, Ste 300, Charlotte, NC 28203 (E-mail: crbridges99@gmail.com or dr.michael.katz@gmail.com).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2015.11.031

Supplemental material is available online.

Myocardial infarction (MI) results in extensive left ventricular remodeling in both infarcted and noninfarcted zones, with subsequent development of fibrosis and heart failure. At the molecular level, the remodeling is accompanied by a reparative deposition of extracellular matrix, designed to maintain cardiac structural integrity. Altering this process presents a significant therapeutic opportunity in the management of heart failure.[1] In contrast to traditional

Abbreviations and Acronyms

AAV1 = adeno-associated viral vector, serotype 1
AT_1R = angiotensin II receptor 1
BZ = border zone
Ca^{2+} = calcium ion
DAB = 3,3′-diaminobenzidine
IZ = infarct zone
MCARD = molecular cardiac surgery with recirculating delivery
MI = myocardial infarction
RZ = remote zone
SERCA2a = sarcoplasmic reticulum calcium adenosine triphosphatase isoform 2a
SMAD = an intracellular signaling protein family
TGFβ = transforming growth factor beta

treatments, gene therapy seems promising because it can alter the genetic structure of myocardial cells and the extracellular matrix.

One important contributor to the development of fibrosis is the transforming growth factor beta (TGFβ1)-SMAD signaling cascade, which stimulates collagen expression and other downstream profibrotic targets and is markedly up-regulated after MI.[2] Additionally, it is a potent regulator of the multiple stages of the cell cycle in the heart and is integral to infarct healing, myocardial hypertrophy, and post-infarction remodeling.[3] The signaling of TGFβ1 can be inhibited with antisense oligonucleotides[4] and neutralizing antibodies,[5] resulting in attenuated left ventricular remodeling and reduced interstitial fibrosis.[6]

Yet, the contribution of TGFβ1-SMAD signaling to the development of cardiac fibrosis as a function of MI extent and zonal proximity to the infarct is unknown.[2] In addition, the issue of whether these pathways can be manipulated with gene therapy is largely unclear and controversial.[6] Moreover, the impact of gene therapy acting on cellular structures to modify the structural integrity of myocytes is a new area of research.

The cardiac extracellular matrix is composed mostly of fibrillar collagen type I (tensile strength) and type III (elasticity and structural integrity).[7] Both types are synthesized by cardiac myofibroblasts wherein a procollagen (a prerequisite of fibrillar collagen) forms in the sarcoplasmic reticulum and is dependent on the function of sarcoplasmic reticulum calcium adenosine triphosphatase2a (SERCA2a). In heart failure, the ratio of type I to type III collagen, as well as the ratio of mature to de novo collagen, is altered.[8] However, the changes in these ratios after ischemic injury, and the kinetics of de novo collagen deposition in border and remote zones of infarcted hearts, are not yet understood.[9]

A hallmark of heart failure is abnormal intracellular calcium ion (Ca^{2+}) handling and down-regulation of SERCA2a. Failing hearts have a dysfunction in excitation-contraction coupling and deficient SERCA2a uptake. We[10] and others[11] have demonstrated that the normalization of SERCA2a expression improves cardiac function in the infarcted heart. Given that SERCA2a closely controls intracellular Ca^{2+}, we hypothesized that the effects of overexpression of SERCA2a are linked with MI-stimulated fibrogenesis.

METHODS

Animals

All animals received humane care in compliance with the requirements of the National Institutes of Health and the local institutional animal care and use committee. Dorsett male sheep (n = 26) weighing 46.1 ± 3.6 kg were used. All animals were divided into 4 groups: group 1 (n = 6) had animals with small MI, without clinical signs of heart failure, after proximal ligation of the first branch of the circumflex artery; group 2 (n = 10) had animals with large MI, with clinical heart failure, after proximal ligation of the first 2 branches of the circumflex artery; group 3 (n = 7) had animals with large MI followed by gene construct (adeno-associated viral vector, serotype 1 [AAV1]. cytomegalovirus.SERCA2a) transfer using MCARD after 4 weeks (large MI/SERCA); and group 4 (n = 3) was control animals, with tissue collected for molecular studies before any procedure was performed. Animals in groups 1 to 3 were killed at 12 weeks. All of the data presented in this article are new; however, we have published some data from subsets of these animals previously.[12,13]

Vector Production

A recombinant single-stranded AAV1 encoding SERCA2a, under the control of the human immediate early cytomegalovirus gene promoter with a splice donor/acceptor sequence was used. A polyadenylation signal from the human globin gene was constructed in a validated preclinical grade system. This process resulted in high-quality super sampling–AAV1. cytomegalovirus. SERCA2a titers, using the Penn Vector Core (University of Pennsylvania, Philadelphia, Pa).

Surgical Procedures and Gene Delivery

For the infarct model, surgical details of the creation of small and large infarcts have been described,[10,12] as have those of the MCARD procedure.[10,14] We briefly describe the main features. The heart was isolated, and closed-loop cardiac circuit flow was initiated. The virus solution consisting of 10^{13} genome copies of super sampling–AAV1.CMV.SERCA2a was injected into the coronary sinus catheter and recirculated. The circuit was flushed to wash out residual vector, and the chest was closed. All animals received postoperative veterinary intensive care unit management.

Analyses and Assessments

Magnetic resonance imaging (Signa LX, GE Healthcare, Chalfont St Giles, United Kingdom) acquisition and analysis of cardiac hemodynamics were conducted for each animal at various time points.[15] More details are described in Appendix 1. For messenger ribonucleic acid analysis, total ribonucleic acid was isolated using TRIzol Reagent (Invitrogen, Carlsbad, Calif), and complementary deoxyribonucleic acid synthesis was performed as previously described.[16] Western blots were done as previously described[12] and normalized to glyceraldyhde-3-phosphate dehydrogenase

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