Journal of the American College of Cardiology
© 2012 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 59, No. 8, 2012
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.09.071

PRE-CLINICAL RESEARCH

Angiotensin-Converting Enzyme-2 Overexpression Improves Left Ventricular Remodeling and Function in a Rat Model of Diabetic Cardiomyopathy

Bo Dong, MD, PHD,*† Qing Tao Yu, MD,*‡ Hong Yan Dai, MD,* Yan Yan Gao, MD,* Zhao Li Zhou, MD,* Lei Zhang, MD, PHD,* Hong Jiang, PHD,* Fei Gao, MD, PHD,* Shu Ying Li, MD, PHD,* Yue Hui Zhang, MD,* Hong Jun Bian, MD, PHD,* Chun Xi Liu, MD,* Nan Wang, MD,* Hui Xu, MD,* Chun Ming Pan, MD,§ Huai Dong Song, MD, PHD,§ Cheng Zhang, MD, PHD,* Yun Zhang, MD, PHD*

Jinan and Shanghai, China

Objectives The aim of this study was to test the hypothesis that angiotensin (Ang)-converting enzyme-2 (ACE2) overexpression may inhibit myocardial collagen accumulation and improve left ventricular (LV) remodeling and function in diabetic cardiomyopathy.

Background Hyperglycemia activates the renin-Ang system, which promotes the accumulation of extracellular matrix and progression of cardiac remodeling and dysfunction.

Methods Ninety male Wistar rats were divided randomly into treatment (n = 80) and control (n = 10) groups. Diabetes was induced in the treatment group by a single intraperitoneal injection of streptozotocin. Twelve weeks after streptozotocin injection, rats in the treatment group were further divided into adenovirus-ACE2, adenovirus–enhanced green fluorescent protein, losartan, and mock groups (n = 20 each). LV volume; LV systolic and diastolic function; extent of myocardial fibrosis; protein expression levels of ACE2, Ang-converting enzyme, and Ang-(1-7); and matrix metalloproteinase–2 activity were evaluated. Cardiac myocyte and fibroblast culture was performed to assess Ang-II and collagen protein expression before and after ACE2 gene transfection.

Results Four weeks after ACE2 gene transfer, the adenovirus-ACE2 group showed increased ACE2 expression, matrix metalloproteinase–2 activity, and LV ejection fractions and decreased LV volumes, myocardial fibrosis, and ACE, Ang-II, and collagen expression in comparison with the adenovirus–enhanced green fluorescent protein and control groups. ACE2 was superior to losartan in improving LV remodeling and function and reducing collagen expression. The putative mechanisms may involve a shift in balance toward an inhibited fibroblast-myocyte cross-talk for collagen and transforming growth factor–beta production and enhanced collagen degradation by matrix metalloproteinase–2.

Conclusions ACE2 inhibits myocardial collagen accumulation and improves LV remodeling and function in a rat model of diabetic cardiomyopathy. Thus, ACE2 provides a promising approach to the treatment of patients with diabetic cardiomyopathy. (J Am Coll Cardiol 2012;59:739–47) © 2012 by the American College of Cardiology Foundation

The recent discovery of new family members of the renin-angiotensin (Ang) system (RAS), such as Ang-converting enzyme (ACE) 2 (ACE2), Ang-(1-7), and Ang-(1-9), has spurred new research interest in further understanding the relationship between the RAS and cardiovascular disease (1). Recently, we found that ACE2 overexpression stabilized atherosclerotic plaque at a late stage and attenuated the progression of atherosclerotic lesions at an early stage in a rabbit model of atherosclerosis (2,3). Although there is great enthusiasm for treating heart failure via ACE2 overexpression, preliminary results are controversial. ACE2-knockout mice showed severe myocardial contractile dysfunction or no changes in cardiac dimension or function (4), whereas ACE2

See page 748

From *The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health, Qilu Hospital, Shandong University, Jinan, Shandong, China; †Department of Cardiology, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, China; ‡Department of Medicine, People's Hospital of Huai Yin District, Jinan, Shandong, China; and the §Ruijin Hospital, Center of Molecular Medicine, Shanghai Institute of Endocrinology, State Key Laboratory of Medical Genomics, Shanghai Jiaotong University School of Medicine, Shanghai, China. All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received August 9, 2011; accepted September 7, 2011.

JACC Vol. 59, No. 8, 2012
February 21, 2012:739–47

Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ACE2 = angiotensin-converting enzyme 2
Ad-ACE2 = adenovirus–angiotensin-converting enzyme 2
Ad-EGFP = adenovirus–enhanced green fluorescent protein
Ang = angiotensin
ARB = angiotensin receptor blocker
DCM = diabetic cardiomyopathy
ECM = extracellular matrix
ELISA = enzyme-linked immunosorbent assay
HG = high glucose
LV = left ventricular
LVEDD = left ventricular end-diastolic diameter
LVEF = left ventricular ejection fraction
MMP = matrix metalloproteinase
RAS = renin-angiotensin system
TGF = transforming growth factor
TIMP = tissue inhibitor of metalloproteinase

overexpression for 11 weeks in the myocardium of stroke-prone spontaneously hypertensive rats resulted in marked myocardial fibrosis with reduced left ventricular (LV) ejection fraction (LVEF) (5). The mechanisms underlying these discrepancies are unclear and pending further investigation.

Diabetic cardiomyopathy (DCM) is characterized by a variety of morphological changes, including myocyte hypertrophy, myofibril depletion, interstitial fibrosis, and intramyocardial microangiopathy. Among these pathological alterations, myocardial fibrosis is a key feature of the diabetic heart, and accumulation of extracellular matrix (ECM) proteins, particularly in collagen, has been documented. Dysregulation of collagen-degrading matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) was found to play an important role in the pathogenesis of myocardial fibrosis in diabetes (6). Singh et al. (7) found that the intracellular RAS was activated by high glucose (HG), and the activated RAS promoted ECM production in cardiac myocytes and fibroblasts. Westermann et al. (6) reported that Ang type 1 receptor antagonists inhibited inflammation and matrix accumulation and decreased MMP-2 activity in DCM. On the basis of these experiments, it has been proposed that hyperglycemia may activate the RAS, which in turn promotes accumulation of ECM and progression of cardiac remodeling and dysfunction.

ACE2, a homologue of ACE, catalyzes conversion of Ang-II to a vasodilative heptapeptide Ang-(1-7) and conversion of Ang-I to the inactive nonapeptide Ang-(1-9), thereby functioning effectively as an endogenous ACE inhibitor. In the present study, we hypothesized that in the setting of DCM, ACE2 overexpression may inhibit myocardial collagen accumulation and improve LV remodeling and function by down-regulation of Ang-II, up-regulation of Ang-(1-7), and attenuation of ACE expression. A series of in vitro and in vivo experiments were designed and performed to validate this hypothesis.

Methods

See the Online Appendix for details.

Adenoviral vector construction. Murine ACE2 complementary deoxyribonucleic acid was amplified by reverse transcription polymerase chain reaction from the ribonucleic acid of mouse kidney. Recombinant adenoviruses carrying the murine ACE2 (adenovirus-ACE2 [Ad-ACE2]) or a control transgene (adenovirus–enhanced green fluorescent protein [Ad-EGFP]) were prepared with the AdMax system (Microbix Biosystems, Toronto, Ontario, Canada).

Animal model and gene transfer. Ninety male Wistar rats were first divided into treatment (n = 80) and control (n = 10) groups. Rats in the treatment group received a single intraperitoneal injection of streptozotocin to induce a diabetic status. Then rats in the treatment group were again divided into Ad-ACE2, Ad-EGFP, losartan, and mock groups (n = 20 each) (Fig. 1).

ACE2 activity assay. ACE2 fluorescence assay was based on the use of the fluorogenic peptide substrate V (7-Mca-RPPGFSAFK[Dnp]-OH, R&D Systems Inc., Minneapolis, Minnesota). Specific ACE2 activity was expressed as picomoles of substrate converted to the product per unit of time and normalized for protein content (units per milligram of protein).

Histopathology and immunohistochemistry. The extent of myocyte hypertrophy was measured on hematoxylin and eosin–stained sections. ACE2, Ang-(1-7), and collagen I and III were identified with appropriate antibodies. Masson's trichrome staining was performed to display collagen components.

Western blot analysis. The protein expression of ACE2, ACE, TIMP-1, MMP-2, MMP-9, and transforming growth factor (TGF)-β was assayed by Western blot analysis.

Echocardiography. Left ventricular end-diastolic diameter (LVEDD), LV end-systolic diameter, LV fractional shortening, LVEF, mitral peak flow velocities, and the E/A ratio were measured using echocardiography.

Hemodynamic measurement. LV systolic pressure, LV end-diastolic pressure, maximal ascending and descending rates of LV pressure, and heart rate were measured using cardiac catheterization.

Zymography. The activity of MMP-2 was evaluated by zymography.

Real-time reverse transcription polymerase chain reaction. The messenger ribonucleic acid expression of ACE2 was quantitated using reverse transcription polymerase chain reaction.

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed to measure the levels of Ang-II, Ang-(1-7), and soluble collagen I and III proteins.

Co-culture of fibroblasts and myocytes. Cardiac fibroblasts were co-cultured with Ad-ACE2-transfected or non-transfected myocytes or the conditioned media of these cells, and the levels of collagen I and III and TGF-β proteins in the media were determined by ELISA.