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EDITORIAL COMMENT

An ACE for My Sweet Heart*

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Diabetes is extremely common, and its prevalence is rising throughout the world. Associations between diabetes and cardiovascular disease are well established, although the mechanisms involved are incompletely understood. Diabetes has been shown to be an important risk factor for heart failure with either preserved or reduced ejection fraction, and there is evidence that the extent of glucose elevation relates directly to adverse outcomes in heart failure patients (reviewed by Murarka and Movahed [1]).

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Activation of the renin-angiotensin (Ang) system (RAS) is involved in the development and progression of heart failure. Ang II, the main effector molecule of this system, causes both vasoconstriction and salt and water retention. It also stimulates cardiomyocyte hypertrophy and enhanced cardiac fibroblast production of the extracellular matrix and secondary growth factors that further promote cardiac remodeling (2–4). The importance of the RAS in the pathogenesis of heart failure has been confirmed by large-scale clinical trials in human patients showing that targeting Ang II by either preventing its formation or blocking its interaction with the Ang type 1 (AT_1) receptor inhibits maladaptive cardiac remodeling and substantially improves clinical outcomes (5–7).

Over time, it has become apparent that the RAS is much more complex than was originally envisioned. Although the classic RAS, in which circulating angiotensinogen released from the liver is sequentially degraded by renin activity and Ang-converting enzyme (ACE) to form Ang II, plays a major role in maintaining cardiovascular homeostasis and the pathogenesis of cardiovascular disease, other components and pathways have been identified. These include alterative enzymes (e.g., chymase) for converting Ang I to Ang II, additional Ang II receptors (e.g., Ang type 2) and the existence of a parallel, independently regulated tissue-based RAS (4). Although of great theoretical interest, the actual importance of many of these pathways (other than the tissue-based RAS) in the pathogenesis of human cardiovascular disease remains largely unproven. Thus, enthusiasm for developing strategies targeting alternative RAS pathways with the hope that they could improve outcomes beyond that seen with existing therapies has been restrained.

One of the more interesting recently identified alternative pathways of the RAS involves a homologue of ACE that has been termed ACE2. This enzyme has been identified in many organs throughout the body, including liver, kidney, brain, testes, blood vessels, and heart (8,9). The preferred substrate appears to be Ang II, from which it cleaves the C-terminal amino acid to form Ang-(1-7), a heptapeptide that has vasodilatory and antigrowth properties. By its ability to reduce Ang II while increasing Ang-(1-7) levels, ACE2 has effects that would appear to help protect the cardiovascular system in settings in which the RAS is activated. In support of this is evidence that overexpression of ACE2 has generally been found to have favorable effects on cardiac structure and function (10–12). Germline ablation of ACE2 has been associated with adverse effects on cardiac function, but these results have not been consistent, because they occurred spontaneously in some situations but required pathological intervention in others (13–16). In an experimental model of post–myocardial infarction remodeling, inhibition of ACE2 enzyme activity had (mostly) unfavorable effects on cardiac structure and function (17). These findings suggest that strategies designed to enhance ACE2 activity could be cardioprotective in cardiovascular diseases in which the RAS is activated.

In this issue of the *Journal*, Dong et al. (18) present the results of a series of experiments designed to assess the effects of ACE2 overexpression in an experimental model of diabetic cardiomyopathy. They generated a rat model of diabetes by administering streptozotocin, and they confirmed previous reports that the diabetic rats developed cardiac dilatation as well as systolic and diastolic dysfunction. A group of these diabetic rats was administered direct intramyocardial injection of an adenoviral vector containing complementary deoxyribonucleic acid for the murine ACE2 gene. Compared with appropriate control groups, the treated rats had evidence of increased ACE2 enzyme activity, reduced Ang II levels, and increased Ang-(1-7) levels. They further demonstrated that ACE2 gene therapy was associated with protection against maladaptive cardiac remodeling, a reduction in intracardiac collagen accumulation and protection against systolic and diastolic dysfunction.

The possible mechanisms by which ACE2 overexpression exerted its cardioprotective effects in this experimental model were also investigated. In a series of in vitro experiments, the investigators showed that exposing cultured neonatal rat cardiac fibroblasts and myocytes to media containing increased glucose concentration increased Ang II

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levels in the cells. After adenoviral transduction to increase ACE2 expression, reduced Ang II and increased Ang-(1-7) levels were detected. Moreover, expression of collagen I, collagen III, and transforming growth factor–beta and ^{3}H-proline incorporation (as an indicator of collagen production) were suppressed by ACE2 treatment in the cultured fibroblasts. These antifibrotic effects were inhibited by A779, a compound that acts as an Ang-(1-7) receptor blocker, suggesting that they were mediated by the heptapeptide rather than by ACE2 effects on other pathways. These findings are consistent with a previous study demonstrating that Ang-(1-7) inhibits Ang II–stimulated production of collagen and secondary growth factors in adult rat cardiac fibroblasts (19).

The investigators also present evidence that the antifibrotic and other favorable cardiac effects of ACE2 exceed those of the AT_1 receptor blocker losartan. Potential explanations for these findings include increased Ang-(1-7) generation by ACE2 or the effects of the enzyme on other peptides (e.g., apelin) that might favorably affect cardiac structure and function. The possibility that overexpression of ACE2 is more effective than AT_1 receptor blockade by virtue of its ability to protect against glucose-induced increases in intracellular Ang II levels, however, seems unlikely, because ACE2 is a type I ectoenzyme anchored to the cell membrane with its catalytic domain(s) in the extracellular space. Thus, like an AT_1 receptor blocker, ACE2's effects are most likely related to events occurring on the cell surface or surrounding milieu.

Although the precise mechanism(s) for the protective effect of ACE2 that are reported are uncertain, these findings raise the intriguing possibility that therapeutic strategies designed to increase cardiac ACE2 activity could help protect patients from some of the adverse effects of diabetes on the heart. Because increased Ang II levels are associated with many of the pathologic effects in this experimental model of diabetes, the results also imply that increasing ACE2 might be helpful in other cardiovascular diseases in which RAS activation is involved. The activity of ACE2 in the heart could be enhanced in several ways, including increased expression or modified post-translational processing of the enzyme. There is evidence that AT_1 receptor blockers increase ACE2 expression in some experimental models, and it is possible that this property may contribute to their beneficial effects. Discovery of novel compounds with more robust effects on ACE2 expression would be helpful in determining if this strategy could be effective in treating human cardiovascular disease. Overexpression of ACE2 throughout the body, however, could have systemic effects, including a reduction in blood pressure, which may limit this approach. Reasonably selective cardiac expression of candidate genes appears to be possible by means of gene transfer therapy using viral vectors that contain cardiac specific promoters and avidly transduce cardiomyocytes (20). This approach has been successfully used to deliver complementary deoxyribonucleic acid from relevant genes to increase expression of key proteins that are down-regulated in the failing heart. Preliminary results from a study in which an adeno-associated viral vector carrying recombinant human complementary deoxyribonucleic acid for the sarco/endoplasmic reticulum calcium ATPase-2 gene was administered by direct coronary artery infusion to patients with advanced heart failure are encouraging (21). Targeting cardiac fibroblasts with viral vectors, however, is problematic, because there appears to be considerably less uptake of viral particles by these cells compared with that seen in cardiomyocytes that occupy a much greater volume of the myocardium. Evidence of cross-talk between cardiac cells demonstrated in this and previous studies suggest that low uptake of viral particles in fibroblasts may not be limiting. As shown in the present study, overexpression of ACE2 in cardiomyocytes appears to be able to influence cardiac fibroblast activities through release of secondary signaling molecules. Further investigation of this approach seems warranted at this time.

Another possible approach to altering local ACE2 effects in the heart would be to modify post-translational events involved in regulating the enzyme's availability. Because ACE2 is a type I ectoenzyme anchored to the cell surface, it is vulnerable to cleavage and release into the interstitial space, from which it can then enter into the circulation. There are reports of elevated levels of ACE2 in patients with heart failure, although the significance of this finding is uncertain. Modification of ACE2 that is designed to inhibit cleavage from the cell surface is another potential strategy that might be able to enhance local ACE2 within microenvironments of the heart. This is a more difficult prospect than might be imagined, however, because ACE2 can be cleaved at multiple sites, and more than 1 enzyme appears to be capable of accomplishing this (22). Nonetheless, studies designed to assess the effects of retaining cell-surface ACE2 on local concentrations of Ang peptides in the extracellular milieu would be of interest.

Overall, the findings of Dong et al. (18) add to the growing body of results indicating that increased ACE2 activity in the heart may be a useful strategy for preventing or treating cardiovascular diseases, particularly those in which RAS activation is involved. Although there are existing therapeutic approaches targeting either ACE or the AT_1 receptor, it is becoming increasingly clear that the complexity of the RAS may provide an avenue for additional therapies that could further improve outcomes.

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REFERENCES

1. Murarka S, Movahed MR. Diabetic cardiomyopathy. J Card Fail 2010;16:971–9.

2. Villarreal FJ, Kim NN, Ungab GD, Printz MP, Dillmann WH. Identification of functional angiotensin II receptors on rat cardiac fibroblasts. Circulation 1993;88:2849–61.
3. Gray MO, Long CS, Kalinyak JE, Li HT, Karliner JS. Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-beta 1 and endothelin-1 from fibroblasts. Cardiovasc Res 1998;40:352–63.
4. Dostal DE, Baker KM. The cardiac renin-angiotensin system: conceptual, or a regulator of cardiac function? Circ Res 1999;85:643–50.
5. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med 1991;325:293–302.
6. Greenberg B, Quinones MA, Koilpillai C, et al. Effects of long-term enalapril therapy on cardiac structure and function in patients with left ventricular dysfunction. Results of the SOLVD echocardiography substudy. Circulation 1995;91:2573–81.
7. Granger CB, McMurray JJ, Yusuf S, et al. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. Lancet 2003;362:772–6.
8. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 2000;275:33238–43.
9. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ Res 2000;87:E1–9.
10. Huentelman MJ, Grobe JL, Vazquez J, et al. Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats. Exp Physiol 2005;90:783–90.
11. Diez-Freire C, Vazquez J, Correa de Adjounian MF, et al. ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR. Physiol Genomics 2006;27:12–9.
12. Der Sarkissian S, Grobe JL, Yuan L, et al. Cardiac overexpression of angiotensin converting enzyme 2 protects the heart from ischemia-induced pathophysiology. Hypertension 2008;51:712–8.
13. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002;417:822–8.
14. Yamamoto K, Ohishi M, Katsuya T, et al. Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin II. Hypertension 2006;47:718–26.
15. Oudit GY, Kassiri Z, Patel MP, et al. Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. Cardiovasc Res 2007;75:29–39.
16. Kassiri Z, Zhong J, Guo D, et al. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. Circ Heart Fail 2009;2:446–55.
17. Kim MA, Yang D, Kida K, et al. Effects of ACE2 inhibition in the post-myocardial infarction heart. J Card Fail 2010;16:777–85.
18. Dong B, Yu QT, Dai HY, et al. Angiotensin-converting enzyme-2 overexpression improves left ventricular remodeling and function in a rat model of diabetic cardiomyopathy. J Am Coll Cardiol 2012;59:739–47.
19. Iwata M, Cowling RT, Gurantz D, et al. Angiotensin-(1-7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. Am J Physiol Heart Circ Physiol 2005;289:H2356–63.
20. Prasad KM, Xu Y, Yang Z, Acton ST, French BA. Robust cardiomyocyte-specific gene expression following systemic injection of AAV: in vivo gene delivery follows a Poisson distribution. Gene Ther 2011;18:43–52.
21. Jessup M, Greenberg B, Mancini D, et al., for the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) Investigators. Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. Circulation 2011;124:304–13.
22. Iwata M, Silva Enciso JE, Greenberg BH. Selective and specific regulation of ectodomain shedding of angiotensin-converting enzyme 2 by tumor necrosis factor alpha-converting enzyme. Am J Physiol Cell Physiol 2009;297:C1318–29.

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