Cardiac Overexpression of Angiotensin Converting Enzyme 2 Protects the Heart From Ischemia-Induced Pathophysiology

Shant Der Sarkissian, Justin L. Grobe, Lihui Yuan, Dhruv R. Narielwala, Glenn A. Walter, Michael J. Katovich, Mohan K. Raizada

Abstract—Angiotensin converting enzyme 2 (ACE2) has been linked to cardiac dysfunction and hypertension-induced cardiac pathophysiology. In this study, we used a gene overexpression approach to investigate the role of ACE2 in cardiac function and remodeling after myocardial infarction. Rats received an intracardiac injection of $4.5 \times 10^8$ lentivirus containing ACE2 cDNA, followed by permanent coronary artery ligation (CAL) of the left anterior descending artery. At 24 hours and 6 weeks after surgery, cardiac functions, viability, and pathophysiology were assessed by MRI and by histological analysis. At 24 hours post-CAL, left ventricular (LV) anterior wall motion was stunted to the same extent in control CAL and lenti-ACE2–treated CAL rats. However, lenti-ACE2–treated CAL rats showed a 60% reduction in delayed contrast-enhanced LV volume after gadodiamide injection, indicating early ischemic protection of myocardium by ACE2. At 6 weeks after CAL, lenti-ACE2 rats demonstrated a complete rescue of cardiac output, a 41% rescue of ejection fraction, a 44% rescue in contractility, a 37% rescue in motion, and a 53% rescue in LV anterior (infarcted) wall thinning compared with control CAL rats. No changes were observed in the LV posterior (noninfarcted) wall other than an 81% rescue in motion produced by ACE2 in CAL rats. Finally, infarct size measured by 2,3,5-triphenyl-tetrazolium chloride staining was not significantly different between the ligated groups. These observations demonstrate that cardiac overexpression of ACE2 exerts protective influence on the heart during myocardial infarction by preserving cardiac functions, LV wall motion and contractility, and by attenuating LV wall thinning. (Hypertension. 2008;51:712-718.)

Key Words: ACE2 • heart disease • myocardial infarction • lentiviral vector • gene therapy

Overactivity of the renin-angiotensin system leading to increased levels of angiotensin (Ang) II is associated with cardiovascular disease, including hypertension, stroke, myocardial infarction (MI), and heart failure (HF). Therefore, inhibitors of Ang II formation or its actions are potent pharmacotherapy for cardiovascular disease. Mounting evidence suggest that Ang converting enzyme 2 (ACE2), the newest member of the renin-Ang system, plays a critical role in cardiovascular homeostasis because it acts with high catalytic efficiency on different cardiovascular-relevant peptides and, mainly, because it balances the vasoactive and growth-promoting actions of Ang II with opposing vasodilatory effects of Ang(1-7).1,2 In other words, ACE2 counter-regulates ACE activity by reducing Ang II bioavailability and increasing Ang(1-7) formation.

Evidence for the critical role of ACE2 in cardiovascular homeostasis includes the following. First, ACE2-deficient mice exhibit a dysfunctional cardiac phenotype characterized by significant reduction in aortic and ventricular pressures and severe decrease in cardiac contractility.3 A similar phenotype is observed in humans and experimental models of ischemic heart disease. Also, local increase of AngII in the heart of ACE2-deficient mice accelerates pressure overload–induced cardiac dysfunction (ie, reduction in contractility and increase in hypertrophy and dilation).4 Second, ACE2 expression is increased in the myocardium after coronary artery ligation (CAL),5,6 in addition to an increase in activity in failing human hearts.7–9 Third, the major product of ACE2, Ang(1-7), produces beneficial outcomes on cardiac function (ie, coronary perfusion, endothelial function, and contractility)10,11 and attenuates development of HF postischemia.11,12 Fourth, overexpression of ACE2 protects the heart from hypertension-induced cardiac pathophysiology (ie, hypertrophy and fibrosis)12–14 and inhibits hypoxia-induced collagen production by cardiac fibroblasts.15 Finally, Ang pathway inhibitors used in the treatment of MI increase ACE2 gene expression and attenuate ACE2 gene downregulation in the myocardium after CAL.6,16,17 Taken together, these observa-
tions led us to hypothesize that preemptive cardiac overexpression of ACE2 would exert a protective influence on the heart during prolonged ischemia. Our hypothesis was investigated using lentiviral gene transfer of ACE2 in the heart of Sprague-Dawley rats in the prevention of CAL-induced cardiac pathophysiology.

Materials and Methods
Characterization of ACE2 in Lentiviral Vector
Murine ACE2 cDNA was cloned in lentiviral vector (lenti-ACE2) essentially as described elsewhere,18,19 and production and titration of lentiviral particles were carried out as described previously.20,21 Lentiviral vector containing no insert was used as control, whereas a vector containing placental alkaline phosphatase reporter gene was used to characterize in vivo transduction efficiency. Both efficacy of lenti-ACE2 construct and transduction efficiency were assessed according to pre-established methods.14,18

Animal Studies and Myocardial Ischemia Model
All of the animal protocols were approved by the institutional animal care and use committee and conducted according to National Institutes of Health guidelines. Five-day- old male Sprague-Dawley rats received a single intravenous injection of 4.5 x 10^9 transfection units of lenti-ACE2 or lentiviral vector containing no insert in 40 μL of colony stimulating factor, as described previously.20,22 This method of gene transfer by our lentiviral vector provides a 100% animal survival rate and has been established to produce efficient and long-term transduction of the heart.20 After viral administration, animals were returned to their mothers until weaning. Rats were given ad libitum access to food and water throughout the study.

At 8 weeks of age, rats were separated into 4 experimental groups (control sham, control CAL, ACE2 sham, and ACE2 CAL; n=8 per group) and subjected to either CAL or mock surgery. Rats were anesthetized with 2.5% to 3.0% isoflurane (Abbott Laboratories) in 1 L/min of oxygen, placed on a heating pad, intubated, and monitored using the Avance Console and Paravision software (PV3.02, Bruker BioSpin). Anesthesia was maintained in 1 L/min of oxygen and monitored using the Small Animal Instrument monitoring and gating system for respiration rate and cardiac triggering. Dorsal and sagittal images were acquired using a cardiac-gated gradient echo cine magentic sequence. Based on these images, 8 short-axis views of 2-mm thickness and 8 to 10 frames were prescribed from apex to base, as described previously.15,23 Toperform viability analysis with a contrast agent, gadodiamide (Omniscan [0.5 mmol/L per kilogram], Amersham Health), was injected via the tail vein 10 minutes before imaging. Gadolinium chelates, such as gadodiamide, are commonly used MRI contrast agents that result in delayed contrast enhancement (DCE) of injured portions of the myocardium and areas at risk for infarct.

Histological Analysis
Infarct size was assessed by incubating thick tissue sections in 1% 2,3,5-triphenyl-tetrazolium chloride (Sigma Chemical Co) solution in PBS (PH 7.4) for 20 minutes at 37°C and postfixing overnight in 4% parafomaldehyde. Sections were then photographed on both sides and analyzed using an automated threshold function in ImageJ software (http://rsb.info.nih.gov/ij). Infarct areas on both sides of each section were averaged, and the total infarct size of the heart was calculated as a percentage of the total myocardial area.

ACE2 immunohistochemistry was carried out by incubating aceton-fixed frozen sections with a 1:75 dilution of polyclonal ACE2 antibody (Santa Cruz Biotech) for 1 hour at room temperature according to the manufacturer’s protocol. As a second step, sections were incubated with a 1:500 dilution of secondary anti-rabbit IgG fluorescein isothiocyanate conjugate antibody (Sigma Chemical Co) overnight at 4°C. Sections were then mounted with a cover glass and Vectashield mounting medium (Vector Laboratories) and photographed using a Zeiss Axiosplan fluorescence microscope equipped with a sensor placement optimization tool with the digital camera.

Capillary density was determined by using a standard protocol by incubating ventricular sections with rhodamine-labeled Griffonia simplicifolia agglutinin I (lectin GS1, [3 μg/mL], Vector Laboratories) for 45 minutes at room temperature. Six randomly selected fields from the epicardial and endocardial regions of the viable myocardium were photographed, and capillary density was evaluated by counting individual endothelial cell profiles. An average of 549 endothelial cell profiles was counted for each rat, and results were expressed as the number of capillaries per millimeter squared. Next, collagen density was evaluated in Masson’s Trichrome–stained sections. Six randomly selected fields in the viable myocardium were photographed and analyzed in Image J software using a color deconvolution plug-in and threshold function to estimate the percentage of the LV viable myocardium occupied by collagen. Finally, cardiomyocyte cross-sectional area was evaluated in ImageJ by averaging values obtained from 70 transversely oriented myocytes chosen randomly from epicardial and endocardial regions of viable LV myocardium.
Representative MRI of heart slices from control CAL and lenti-ACE2 CAL rats are shown in Figure 2A. Quantitative analysis of DCE in the LV in relation to the total myocardial area at 24 hours post-CAL is represented in Figure 2B. In control rats, CAL resulted in an 11.2-fold increase in DCE in the LV (control sham: 0.68 ± 0.17% versus control CAL: 7.64 ± 1.95; P = 0.0002), whereas in lenti-ACE2 rats, CAL resulted in a 4.5-fold increase in DCE in the LV (ACE2 sham: 1.12 ± 0.43% versus ACE2 CAL: 5.02 ± 0.82%; P = 0.0482). For CAL rats normalized over their respective controls, lenti-ACE2 treatment significantly reduced DCE in the LV by 60% compared with controls (4.50 ± 0.77 versus 11.21 ± 2.86, respectively; P = 0.037). Thus, lenti-ACE2 provides an early ischemic protection of the myocardium.

**MRI Acquired Functional Results**

MRI was also used to determine the effects of lenti-ACE2 on functional parameters such as CO, EF, LV contractility, and motion at 24 hours and 6 weeks post-CAL (Table 1). At 24 hours, CAL similarly decreased LV angular wall motion in the anterior (infarcted) wall in ACE2-treated and control rats (ACE2 CAL: 0.80 ± 0.13 mm, control CAL: 0.79 ± 0.11 mm versus control sham: 1.22 ± 0.04 mm; P < 0.0083). However, at 6 weeks, reduced wall motion was rescued in lenti-ACE2 CAL rats (1.03 ± 0.14 mm) but remained impaired in control CAL rats (0.84 ± 0.12 mm versus control sham: 1.35 ± 0.09; P = 0.0029). At 6 weeks, control CAL rats exhibited an 18% decrease in CO (107 ± 6 mL/min versus control sham: 130 ± 6 mL/min; P = 0.012), a 24% decrease in EF (53 ± 3% versus control sham: 70 ± 1%; P < 0.0001), and a 71% decrease in LV anterior wall contractility (14 ± 4% versus control sham: 48 ± 7%; P = 0.0002; Table 1). Lenti-ACE2 treatment in CAL rats rescued cardiac dysfunction by a complete recovery in CO (133 ± 5 mL/min), a 41% rescue in EF (60 ± 3%), and a 44% rescue in LV anterior wall contractility (29 ± 6%). Finally, although LV contractility of the posterior (noninfarcted) wall was not altered in CAL rats, motion of the posterior wall was reduced by 22% (control CAL:
Effect of lenti-ACE2 on left ventricular wall thickness after CAL. A, Representation of the LV at 6 weeks post-CAL for lenti-ACE2–treated and control animals using CAAS MRV software. The inner to outer concentric circles each represent MRI-acquired LV slices of 2-mm thickness spanning from the apex to the base of the heart. The darker shaded segments are thinner compared with the lighter segments. B, LV anterior (infarcted) wall thickness data for the 24 hour and 6 week post-CAL MRI acquisitions. Data are analyzed based on a 17-segment model. *Significantly different (P<0.0083) from control sham, ACE2 sham, and ACE2 CAL.

Table 1. Cardiac Functional Data for ACE2 Treated and Untreated Sprague-Dawley Rats Obtained by MRI at 24 Hours and 6 Weeks Postsurgery

<table>
<thead>
<tr>
<th>Cardiac Hemodynamics</th>
<th>24 Hours</th>
<th>6 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Control Sham CAL</td>
<td>Control Sham CAL</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>69±1</td>
<td>58±5</td>
</tr>
<tr>
<td>LV contractility, radial thickening, %</td>
<td>32±2</td>
<td>21±6</td>
</tr>
<tr>
<td>Anterior wall</td>
<td>1.22±0.04</td>
<td>0.80±0.13†</td>
</tr>
<tr>
<td>Posterior wall</td>
<td>1.53±0.08</td>
<td>1.21±0.12</td>
</tr>
<tr>
<td>LV motion, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior wall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior wall</td>
<td></td>
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</tbody>
</table>

*Versus control sham or †ACE2 sham, respectively, when P<0.0083.
†ACE2 CAL vs control CAL, when P<0.05.

1.28±0.07 mm versus control sham: 1.65±0.10 mm; (P=0.006), and this effect was rescued in lenti-ACE2–treated CAL rats (1.58±0.07 mm). These results demonstrate that lenti-ACE2 induces protective effects on cardiac functional parameters in MI.

MRI-Acquired Structural Results
Cardiac structural data such as LV wall thickness were also acquired through MRI and analyzed using CAAS MRV software. Figure 3A shows a representation of the LV at 6 weeks after CAL for lenti-ACE2–treated and control animals. The inner to outer concentric circles each represent an MRI-acquired 2-mm-thick LV section spanning from the apex to the base of the heart. The dark shaded segments are thinner compared with the light color segments. The data were analyzed based on a 17-segment model, and LV wall thickness results for the anterior (infarcted) portion of the LV are represented in Figure 3B. At 24 hours post-CAL, LV wall thickness remained unchanged in lenti-ACE2 and control rats. However, at 6 weeks, CAL produced a 31% decrease in LV wall thickness of the anterior (infarcted) wall in control rats (1.20±0.10 mm versus control sham: 1.73±0.09 mm; P=0.0001). Lenti-ACE2 provided a 53% rescue of LV anterior wall thinning (1.48±0.07 mm) demonstrating the protective influence of ACE2 on cardiac structural parameters after MI. Finally, neither CAL nor ACE2 treatment modulates LV posterior (noninfarcted) wall thickness at 24 hours and 6 weeks after surgery (not shown).

Molecular and Histological Results
After the final set of MRIs, rats were euthanized, and hearts were removed and separated into 5 sections of equal thickness perpendicular to the long axis. The basal section was used to quantify ACE2 mRNA, and the remaining sections were used for histological analysis. Table 2 shows a 4.2-fold increase in endogenous ACE2 mRNA and an 12.2-fold increase in total ACE2 mRNA in ACE2-treated CAL rats (3.44±1.57, P=0.041, and 9.91±3.26, P=0.0043, respectively, versus control sham: 0.81±0.27). In addition, increased expression of total ACE2 in CAL animals treated...
with lenti-ACE2 was further confirmed by immunohistochemistry. ACE2-treated animals had a marked increase in immunostaining throughout the myocardium, however, retaining a larger proportion of staining at the border of the infarct zone as seen in Figure 4.

Histological methods were also used to assess the effect of lenti-ACE2 treatment on cardiac structure after CAL. First, 2,3,5-triphenyl-tetrazolium chloride staining revealed a trend toward a decrease in infarct area in lenti-ACE2-treated versus control rats 6 weeks after CAL (19.8 ± 3.1% versus 25.0 ± 2.4%, respectively); however, it did not reach a statistically significant level. Histological analysis of the LV viable myocardium revealed no significant change in capillary density (control sham: 2189 ± 79 capillaries per millimeter squared; control CAL: 2084 ± 42 capillaries per millimeter squared; ACE2 CAL: 2241 ± 40 capillaries per millimeter squared; ACE2 sham: 2331 ± 135 capillaries per millimeter squared; P value not significant), cardiomyocyte cross-sectional area (control sham: 527 ± 45 μm²; control CAL: 628 ± 55 μm²; ACE2 CAL: 607 ± 27 μm²; ACE2 sham: 541 ± 74 μm²; P value not significant), and collagen density (control sham: 23.1 ± 3.6%; control CAL: 25.3 ± 2.6%; ACE2 CAL: 17.2 ± 2.5%; ACE2 sham: 22.9 ± 2.0%; P value not significant) that could entirely account for the improved functional and structural parameters in ACE2-overexpressing rats at 6 weeks after CAL.

**Discussion**

The most significant observation of our study is that long-term cardiac overexpression of ACE2 provides protection against ischemia-induced cardiac dysfunction in a permanent ligation model. We demonstrate for the first time that lenti-viral vector-mediated cardiac ACE2 gene overexpression attenuates early myocardial tissue damage as seen by the DCE MRI technique, preserves LV wall motion and contractility, and attenuates LV wall thinning and cardiac dysfunction (ie, CO and EF) at 6 weeks after CAL.

Recent studies have shown that ACE2 gene knockdown results in severe cardiac dysfunction (ie, reduced contractility, increased hypertrophy, and dilation), whereas cardiac dysfunction in the form of HF or because of CAL-induced MI produces an increase of ACE2 gene expression in the myocardium. In addition, ACE inhibitors and AngII type 1 receptor antagonists, which have proven beneficial for the treatment of MI and HF, increase ACE2 gene expression, attenuate ACE2 gene downregulation, and normalize AngII type 1 receptor expression in the myocardium post-MI. Taken together, these observations suggest that the increase of ACE2 expression in the myocardium is because of a compensatory mechanism to cardiac dysfunction and also is responsible, at least in part, to the cardioprotective and beneficial outcomes observed with the use of Ang pathway inhibitors during treatment of MI and HF. Our study is relevant in this regard because, for the first time, it provides direct evidence that overexpression of ACE2 exerts a long-term protective influence on the heart after MI-induced cardiac dysfunction.

The impressive beneficial outcome on cardiac dysfunction post-CAL–induced MI by ACE2 gene transfer is likely to be a result of the multifunctional nature of this enzyme. First, ACE2 catalyzes the conversion of Ang I to Ang (1-9) and Ang II to Ang(1-7). This results in an increase in Ang(1-7) and a decrease in AngII. Therefore, the early reduction of DCE myocardial volume observed in ACE2-overexpressing rats, which is indicative of reduced tissue damage and/or increased tissue perfusion, may be because of a decrease in AngII-mediated local effects (ie, apoptosis or vasoconstriction) coupled with an increase of vasodilatory effects mediated by Ang(1-7). Indeed, studies have shown that Ang(1-7) formed within the heart has beneficial effects on coronary perfusion, endothelial function, cardiac contractility, and rhythm and that infusion of Ang(1-7) attenuates the development of HF post-MI. Moreover, the potential beneficial effects because of increased tissue perfusion may likely be caused by increased vasodilation instead of angiogenesis, because capillary density was not significantly increased in ACE2-overexpressing rats at 14 weeks of age.

Table 2. Endogenous, Exogenous, and Total ACE2 mRNA in ACE2-Treated and Untreated Sprague-Dawley Rat Hearts Obtained by Real-Time PCR at 6 Weeks Postsurgery

<table>
<thead>
<tr>
<th>ACE2 mRNA</th>
<th>Control</th>
<th>CAL</th>
<th>ACE2</th>
<th>Control</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (endogenous)</td>
<td>0.81 ± 0.27</td>
<td>0.86 ± 0.07</td>
<td>3.44 ± 1.57**‡‡</td>
<td>0.70 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Mouse (exogenous)</td>
<td>NA</td>
<td>NA</td>
<td>6.47 ± 2.87</td>
<td>4.68 ± 3.49</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.81 ± 0.27</td>
<td>0.86 ± 0.07</td>
<td>9.91 ± 3.26‡‡</td>
<td>5.38 ± 3.47</td>
<td></td>
</tr>
</tbody>
</table>

*Versus control sham when P < 0.0003 or **vs control sham when P < 0.05.
‡ACE2 CAL vs control CAL when P < 0.0003 or ‡‡ACE2 CAL vs control CAL when P < 0.05.

Figure 4. Representative images of ACE2 immunocytochemistry in frozen sections. A, Control sham animals have a basal expression of ACE2 throughout the myocardium. B, Rats receiving lenti-ACE2 treatment have a marked increase of ACE2 immunostaining throughout the myocardium, retaining, however, a larger increase of ACE2 immunostaining at the border of the infarct zone.

Discussion The most significant observation of our study is that long-term cardiac overexpression of ACE2 provides protection against ischemia-induced cardiac dysfunction in a permanent ligation model. We demonstrate for the first time that lenti-viral vector-mediated cardiac ACE2 gene overexpression attenuates early myocardial tissue damage as seen by the DCE MRI technique, preserves LV wall motion and contractility, and attenuates LV wall thinning and cardiac dysfunction (ie, CO and EF) at 6 weeks after CAL.

Recent studies have shown that ACE2 gene knockdown results in severe cardiac dysfunction (ie, reduced contractility, increased hypertrophy, and dilation), whereas cardiac dysfunction in the form of HF or because of CAL-induced MI produces an increase of ACE2 gene expression in the myocardium. In addition, ACE inhibitors and AngII type 1 receptor antagonists, which have proven beneficial for the treatment of MI and HF, increase ACE2 gene expression, attenuate ACE2 gene downregulation, and normalize AngII type 1 receptor expression in the myocardium post-MI. Taken together, these observations suggest that the increase of ACE2 expression in the myocardium is because of a compensatory mechanism to cardiac dysfunction and also is responsible, at least in part, to the cardioprotective and beneficial outcomes observed with the use of Ang pathway inhibitors during treatment of MI and HF. Our study is relevant in this regard because, for the first time, it provides direct evidence that overexpression of ACE2 exerts a long-term protective influence on the heart after MI-induced cardiac dysfunction.

The impressive beneficial outcome on cardiac dysfunction post-CAL–induced MI by ACE2 gene transfer is likely to be a result of the multifunctional nature of this enzyme. First, ACE2 catalyzes the conversion of Ang I to Ang (1-9) and Ang II to Ang(1-7). This results in an increase in Ang(1-7) and a decrease in AngII. Therefore, the early reduction of DCE myocardial volume observed in ACE2-overexpressing rats, which is indicative of reduced tissue damage and/or increased tissue perfusion, may be because of a decrease in AngII-mediated local effects (ie, apoptosis or vasoconstriction) coupled with an increase of vasodilatory effects mediated by Ang(1-7). Indeed, studies have shown that Ang(1-7) formed within the heart has beneficial effects on coronary perfusion, endothelial function, cardiac contractility, and rhythm and that infusion of Ang(1-7) attenuates the development of HF post-MI. Moreover, the potential beneficial effects because of increased tissue perfusion may likely be caused by increased vasodilation instead of angiogenesis, because capillary density was not significantly increased in ACE2-overexpressing rats at 14 weeks of age. In
addition to mechanisms involved in tissue protection, ACE2 may be implicated in early myocardial tissue repair. In fact, in a recent study, we demonstrated that ACE2 overexpression inhibits hypoxia-induced collagen production by cardiac fibroblasts, which may reduce infarct scar size and improve ventricular distensibility and function. In the present study, ACE2 treatment attenuated collagen content only in the viable myocardium of CAL rats; however, it did not reach statistical significance at 6 weeks post-MI. Interestingly, ACE2 overexpression did not modulate collagen content in sham rats, an observation supported with previous results obtained from our group demonstrating that ACE2 overexpression does not influence collagen content in basal conditions. Finally, because ACE2 acts with high catalytic efficiency on different cardiovascular-relevant peptides, such as apelin, neurotensin, kinestatin, des-Arg bradykinin, and dynorphin A, in an in vitro situation, it is also conceivable that beneficial cardioprotective effects of ACE2 may also be observed through such pathways. However, in spite of the fact that these peptides produce significant effects on cardiac functions, in vivo implications of the effects of ACE2 on these peptides remain to be validated.

In addition to the potential implication of multiple pathways involved in the cardioprotective effects of ACE2, adding another level of intricacy is the use of the lentiviral vector to deliver the transgene. Albeit a crucial requirement because of its capacity to efficiently transduce dividing, as well as quiescent cells, after intracardiac injection, in addition to allowing for long-term expression of the therapeutic transgene, it is these same important qualities that complicate identifying cell-type contributions to the observed beneficial outcomes. Although our lentiviral gene delivery protocol primarily transduces cardiac myocytes, the use of cell type–selective promoters (ie, myosin light chain 2v isoform) would be helpful to determine the mechanisms and cell type contributions by which the protective effects of ACE2 are achieved.

In our study we observed an increase of ACE2 immunoreactivity in ligated versus sham-operated rat hearts, particularly at the border of the infarct zone. This is in agreement with the observation of Averill et al, showing the presence of Ang1-7 in high concentration in the myocardium surrounding the infarct zone. However, contrary to immunocytochemistry, real-time PCR failed to show an increase in endogenous ACE2 mRNA in control CAL rats. This discrepancy between immunocytochemistry and mRNA data could be because of the fact that tissue homogenates containing different proportions of both normal and ischemia-affected myocardium were used for mRNA isolation. It is also a possibility that transcriptional rates of ACE2 are not affected by CAL in its late phase. In fact, this has been observed in rat hearts at 4 and 8 weeks post-MI. Of interest, however, is the significant increase in endogenous ACE2 mRNA in CAL rats having received exogenous ACE2 transgene. In these animals, ACE2 overexpression may be permissive for the increase of endogenous ACE2 at 6 weeks post-MI. Indeed, this observation is supported by studies showing that AngII type 1 receptor blockers increase ACE2 mRNA, and ACE inhibitors prevent ACE2 mRNA downregulation at 4 weeks and 8 weeks post-MI, respectively.

For the first time, our study provides evidence that cardiac overexpression of ACE2 protects the heart from ischemia-induced pathophysiology. We believe that ACE2 offers a valuable gene target in myocardial protection because of the capacity of this enzyme to establish renin-Ang II homeostasis during dysregulation, as well as because of its multifunctional profile on different cardiovascular-relevant systems. Demonstrating the cardioprotective role of ACE2, our study establishes the proof of concept for the design of novel treatments modulating cardiac ACE2 activity for the treatment of ischemic heart disease.

**Perspectives**

In this study, we report that ACE2 gene overexpression preserves LV wall motion and contractility and attenuates LV wall thinning and cardiac dysfunction (ie, CO and EF) with minimal effect on infarct size at 6 weeks post-CAL. Although this further attests to the impressive functional effect of ACE2 gene overexpression in this model, it would be of interest to evaluate the effect of ACE2 on infarct size in relation to the area at risk in an ischemia/reperfusion injury model. Furthermore, because ACE2 deficiency induces cardiac dysfunction similar to cardiac stunning, whereas its overexpression preserves cardiac contractility and ventricular function in CAL-induced MI model as we report, ACE2 therapy may be beneficial in clinical cases of permanent coronary occlusion. It would also be a logical next step to evaluate in this paradigm the hypoxia response element promoter–driven ACE2 vector system and ACE2-transduced stem cell transplants in the infarcted myocardium. Finally, our study provides evidence of proof of concept for gene therapy with the use of a lentiviral vector in animals. However, its use in humans must await the development of safe viral vectors.

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**Disclosures**

None.

**References**


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