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Effects of ACE2 Inhibition in the Post-Myocardial Infarction Heart

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Abstract

There is evidence that angiotensin-converting enzyme 2 (ACE2) is cardioprotective. To assess this in the post-myocardial infarction (MI) heart, we treated adult male Sprague-Dawley rats with either placebo (PL) or C16, a selective ACE2 inhibitor, following permanent coronary artery ligation or sham operation.

Coronary artery ligation resulting in MI between 25–50% of the left ventricular (LV) circumference caused substantial cardiac remodeling. Daily C16 administration from post-operative days 2 to 28 at a dose that inhibited myocardial ACE2 activity was associated with a significant increase in MI size and reduction in LV % fractional shortening. Treatment with C16 did not significantly affect post-MI increases in LV end-diastolic dimension but did inhibit increases in wall thickness and fibrosis in non-infarcted LV. On post-operative day 7, C16 had no significant effect on the increased level of apoptosis in the infarct and border zones nor did it significantly affect capillary density surrounding the MI. It did, however, significantly reduce the number of c-kit⁺ cells in the border region.

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These findings support the notion that ACE2 exerts cardioprotective effects by preserving jeopardized cardiomyocytes in the border zone. The reduction in hypertrophy and fibrosis with C16, however, suggests that ACE2 activity has diverse effects on post-MI remodeling.

Keywords

Angiotensin-converting enzyme 2 (ACE2); C16; renin-angiotensin system; c-kit⁺ stem cells; cardiac remodeling

Introduction

The renin-angiotensin system (RAS) plays a major role in the pathogenesis of heart failure^{1–4}. Cardiac remodeling initiated by increased wall stress or injury to the myocardium is recognized as being fundamental to the progressive deterioration of cardiac function that occurs in the failing heart. Angiotensin (Ang) II, mostly through its Type 1 (AT₁) receptor, promotes maladaptive remodeling by stimulating cardiomyocyte hypertrophy and activating cardiac fibroblasts^{5–8}. Therapeutic strategies that block RAS activation using either angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) favorably affect remodeling and reduce morbidity and mortality in post-myocardial infarction (MI) and heart failure patients^{9–11}.

There is evidence that angiotensin-converting enzyme 2 (ACE2), a homologue of ACE, regulates the RAS in both health and disease^{12–15}. This enzyme, which is impervious to the effects of classical ACE inhibitors, is expressed in organs throughout the body including the heart, kidneys and blood vessels^{13, 15, 16}. The preferred substrate of ACE2 appears to be Ang II from which it cleaves the carboxy-terminal amino acid to generate the heptapeptide Ang-(1-7)^{17, 18}. As opposed to Ang II which has vasoconstricting and growth promoting properties, Ang-(1-7) has been shown to cause vasodilation and to inhibit cardiomyocyte hypertrophy as well as cardiac fibroblast proline incorporation and expression of secondary growth factors^{19–23}. Targeted disruption of ACE2 has been reported to result in severe impairment in myocardial contractility over time in male mice¹⁴. Evidence that this phenotype can be rescued by the genetic ablation of ACE suggests that ACE2 regulates the cardiovascular RAS by opposing the effects of ACE. However, the persistent absence of ACE2 throughout development may have influenced these findings and its genetic ablation has not been uniformly found to adversely affect cardiac function²⁴. Thus, the potential role of ACE2 in cardiac remodeling is not yet fully defined. Herein, we report that administration of C16, a compound that selectively inhibits ACE2 activity, adversely affects MI size and cardiac function in rats following coronary artery ligation and provide evidence supporting the notion that ACE2 activity may help preserve viable but jeopardized cardiomyocytes in the infarct border zone, possibly by increasing the number of ckit⁺ cells in this region. However, evidence that C16 administration inhibits hypertrophy and fibrosis of non-infarcted myocardium and that it reduces abnormalities in diastolic relaxation raise the possibility that ACE2 activity may also have some adverse effects on post-MI cardiac remodeling.

Methods and Materials

Study Design

Coronary artery ligation or sham operation was performed in male Sprague-Dawley rats weighing 225–250g. On day 2, rats that had undergone coronary artery ligation had echocardiographic evaluation to confirm the presence of akinesis extending over 25% to 50% of the left ventricular (LV) circumference. Although echocardiographic assessment of akinesis does not exactly represent MI size at this time, a pilot study performed 2 days post-MI in 11

rats demonstrated good correlation with triphenyltetrazolium chloride (TTC) staining of myocardium ($r=0.86$). Rats fulfilling infarct size criteria and sham operated rats were then randomized to receive either C16 or distilled water, both of which were administered by daily intraperitoneal injection. On post-operative day 28, a second echocardiogram was performed in all experimental rats, followed by left heart catheterization, sacrifice and removal of the heart for evaluation. To further investigate mechanisms that could account for the effects of C16 on cardiac structure and function, additional rats selected using the same echocardiographic criteria and treated in the same manner underwent final study and harvesting of cardiac tissue 7 days post-MI.

Generation of MI by coronary artery ligation

Coronary artery ligation, performed as described previously^{25, 26} using a protocol approved by the University of California, San Diego Animal Care Committee, is described with the supplemental information.

Administration of Compound 16 (C16) after surgery

Compound 16 (C16), a selective non-peptiditic ACE2 inhibitor²⁷, was obtained as a generous gift from Millenium Pharmaceuticals Inc. (Cambridge, MA). On day 2 post-MI rats with qualifying echocardiograms were randomized to receive either 25mg/kg/d of C16 (as a solution of 42mg/ml in distilled water) or an equal volume of distilled water by intraperitoneal injection. This dose was selected based on preliminary results demonstrating good efficacy in inhibiting ACE2 activity in rats. Drug administration continued daily from day 2 until study termination (i.e. at either 7 or 28 days). Sham operated rats were treated identically with injection of active drug or distilled water.

Methods used for echocardiography, left heart catheterization, gross morphometric and histologic studies, detection of apoptosis, quantification of c-kit⁺ cells and capillary density in the myocardium and measurement of ACE2 activity are described in the supplemental information

Statistical analysis

Data were presented as mean $\pm$ SEM or mean $\pm$ SD. Statistical analysis among groups was performed using Mann-Whitney and one-way ANOVA followed by Bonferroni's *t*-test. A probability value of $p<0.05$ was considered statistically significant.

Results

I. Effects of ACE2 inhibition between post-operative days 2 and 28 on cardiac structure and function

A. Echocardiographic measurements (Table 1)—On day 2 post-MI rats with echocardiographic evidence of akinesis involving 25–50% of the LV circumference as well as sham operated rats were randomized to receive either C16 or placebo (PL) on a daily basis through day 28. As shown in Table 1, the study groups that had undergone coronary artery ligation had comparable MI size and % FS as well as LV dimensions and wall thickness at the time of randomization.

On day 28 LV end-diastolic and end-systolic dimensions were greater in the MI group than in sham operated controls. Fractional shortening was lower in both MI groups than in their respective sham operated controls. The % FS did not change significantly in the placebo treated group between days 2 and 28 while it was significantly reduced in the C16 treated group over this time period. Despite being similar at day 2, % FS was significantly lower in C16 treated rats than in the PL group at day 28. Change in % FS occurring between 2–28 days in the

experimental groups are depicted in Figure 1. End-diastolic dimension was comparable between the C16 and PL treated post-MI rats at this time point. While end-systolic dimension tended to be larger in rats treated with C16 compared to PL, the difference was not significant. The LV posterior wall thickness increased only in the PL treated post-MI rats and at day 28 it was significantly thicker than in the MI group receiving C16. A similar trend was seen post-MI in the interventricular septum which tended to thicken in the PL but not C16 treated rats. MI size, which had been comparable between the 2 groups of infarcted rats on day 2, was significantly larger by 20% in the C16 than in the placebo group at day 28. As shown in Figure 1, this was due to a slight reduction in MI size in the PL group compared to a significant increase in MI size in rats that had received C16.

In sham operated rats LV end-diastolic dimension was significantly greater and LV end-systolic dimension tended to be greater in the group treated with C16 compared to the PL treated group at 28 days. Both wall thickness and % FS were similar in the sham operated groups at this time point.

B. Hemodynamic variables (Table 2)—On day 28 rats with an MI demonstrated increased LV end-diastolic pressure (LVEDP) compared to sham operated rats. Left ventricular pulse pressure and mean arterial pressures tended to be lower with MI and these values were significantly reduced in the MI group that had received C16 compared to its sham operated control group. There were no significant differences in any of these variables between PL and C16 treated rats in either the sham or MI groups. Tau was increased post-MI only in the PL treated rats and it was significantly greater at this time point than in the C16 treated post-MI group.

C. Morphometric and histologic variables (Table 3)—Body weight (BW) on post-operative day 28 did not differ significantly between MI groups and their respective controls or according to C16 treatment. While the LV/BW ratio did not differ according to surgical treatment or administration of C16, RV/BW ratio tended to be higher in post-MI rats but this difference was significant only in the PL group. Differences between post-MI treatment groups were not, however, significant.

On histologic evaluation interventricular septal width tended to be less in the post-MI groups than their respective sham operated controls but this difference was significant only in the group treated with C16. For both control and C16 treated rats the total LV circumference was significantly larger in the MI groups than in the sham operated groups and C16 did not significantly alter LV circumference in the sham operated or in the infarcted rats. Scar length was 21% larger in the C16 compared to placebo treated rats (9.8mm vs 8.1 mm) and scar length compared to the LV circumference was 23% relatively greater in the C16 treated group than in the placebo group (45.8% vs 37.3%). Although histologic evaluation of scar size failed to demonstrate significant differences between the treatment groups, it did provide information that the magnitude of difference in scar size between the groups was similar to the significant 20% larger scar size measured by echocardiography at day 28 in the C16 treated rats compared to the placebo controls.

As depicted in Table 4, myocyte cross-sectional area (MCA) was significantly larger in the post-MI groups than in their corresponding control groups but the modest difference between C16 and PL treated rats were not significant. The % collagen volume of the non-infarcted septum tended to increase in infarcted rats but this achieved significance only in the PL group and on day 28 post-MI the amount of collagen was significantly less in the C16 group than in the PL group.

D. ACE2 activity—As shown in Figure 2, rats treated with C16 from days 2 through 28 had significantly less ACE2 activity in the infarct, border and non-infarcted zones of the LV compared to rats treated with PL.

II. Effects of ACE2 inhibition between post-operative days 2 and 7 on apoptosis, capillary density and c-kit⁺ cells in the LV

On both days 2 and 7 LV end-diastolic and end-systolic dimensions were significantly greater and % FS was significantly less in rats that had undergone coronary artery ligation than in the respective sham operated controls but there was no significant differences in any of these variables either before or after C16 treatment (data not shown).

Although C16 administration did not affect infarct size or cardiac size and function measured at 7 days, we reasoned that cellular changes that could influence scar size and LV function at a later time point were likely to be manifest. To determine if on-going cardiomyocyte death by apoptosis was involved, TUNEL staining was performed on LV sections from C16 and PL treated rats and the % of apoptotic cells in the infarct, border and non-infarcted LV zones was determined. As summarized in Table 5, infarcted rats had significantly more apoptotic cells in the infarct and border zones than in the non-infarcted septal region of the LV and in the free wall of sham operated rats. Apoptosis, however, was not significantly affected in the infarcted or sham operated rats by C16 treatment. As shown in Figure 3, although there was a slight trend towards a reduction in capillary density in the border zone surrounding the MI with C16 the difference between treatment groups was not significantly different.

Accumulation of stem cells occurs in the post-MI heart and their presence could influence both scar size and LV function. Consequently, we assessed the presence of c-kit⁺ cells in the LV of infarcted and sham operated rats by means of immunohistochemistry. We found that c-kit⁺ cells were rare in the LV free wall of sham operated rats and in the non-infarcted septum of rats that had undergone coronary ligation (data not shown). As shown in Figure 4 and Supplemental Figure 1, c-kit⁺ cells were more abundant in the infarct zone and while the number tended to be less in the C16 treated rats, the difference was not significant. These cells were most commonly found in the border zone surrounding the infarct. In this region the number of c-kit⁺ cells was significantly less in rats that had received C16 than in those treated with placebo.

Discussion

Involvement of the RAS in post-MI cardiac remodeling has been convincingly demonstrated in numerous experimental studies and in well-designed, large-scale clinical trials^{9, 11, 31–35}. The discovery of ACE2, a homologue of ACE which degrades Ang II to form Ang-(1-7), a peptide reported to have vasodilatory and anti-growth properties^{20, 22, 36}, suggests that an alternative pathway of the RAS involving this enzyme might be cardioprotective^{12–15}. Our results using C16 in rats following coronary artery ligation support this possibility. Administration of C16 from days 2 to 28 post-MI reduced ACE2 activity and was associated with a significant increase in infarct size and reduction in LV contractile function. However, treatment with C16 also resulted in less myocardial hypertrophy and fibrosis and prevented an increase in tau suggesting varied cardiac effects of ACE2, some of which might be detrimental to the post-MI heart.

In this study significant differences in the change in MI size between days 2 to 28 days were seen between C16 treated and placebo treated rats. Although extent of akinesis was comparable in the groups prior to randomization, C16 treated rats increased infarct size while there was a small reduction in placebo treated rats at 28 days. Overall, infarct size at 28 days was 20% greater by echo in the C16 treated group compared to the placebo group while scar length was

21% greater by histology. We believe that the concordance in direction and magnitude of difference in infarct size between these 2 independent techniques provides additional confirmation that C16 was associated with an increase in infarct size. This was accompanied by differences in the change in % FS, which decreased significantly more in the C16 than in the PL treated group. Infarct size was determined by echocardiographic assessment of akinesis. While useful in determining MI size for inclusion into the study on day 2 post-coronary artery ligation, the extent of akinesis at this time is not a precise indicator of infarct size since it cannot distinguish devitalized from potentially viable but stunned or hibernating myocardium. The possibility that there was recovery of hibernating myocardium in the border zone is supported by a trend towards a reduction in infarct size in the placebo group. The finding that the opposite occurred with C16 supports the notion that this compound affected local conditions and/or factors that are important in determining the viability of jeopardized myocytes in this region. The adverse effects of C16 do not appear to be mediated by hemodynamic changes since there was no significant effect on LV filling pressure and the modest reduction in LV and aortic pressures would have been expected to relieve local ischemia and improve contractile function by reducing LV afterload. Thus, these findings favor the notion that ACE2 activity favorably affects viability of hibernating cardiomyocytes in the infarct border zone and that inhibition of this activity increases cardiomyocyte death over time. Caution is warranted in interpreting these results, however, since the size of the induced infarct varies according to how high up the left coronary artery is ligated and the inherent imprecision in the measures of infarct size that were employed in this study.

To gain further insights into ACE2 effects in the post-MI heart we evaluated C16 treatment in rats 7 days post-MI. Although MI size and % FS were comparable between the study groups we reasoned that the effects of C16 on factors which could determine the fate of jeopardized myocytes would likely be detectable. As expected³⁷, we found increased apoptosis in the infarct and border zones of rats that had undergone coronary artery ligation (compared to either the non-infarcted septal region or the LV free wall of non-infarcted rats) and increased capillary density in the border zone. There were, however, no significant effects of C16 suggesting that neither differences in apoptosis nor in neo-vascularization accounted for the effects on MI size and % FS. It is possible that by altering the ratio between the angiotensin peptides in the coronary vasculature ACE2 inhibition disturbed the balance between Ang II mediated vasoconstriction and Ang-(1-7) mediated vasodilation¹⁹ and that the resulting decrease in coronary perfusion accounted for the increase in infarct size. Further studies to assess the effects and consequences of ACE2 inhibition on coronary perfusion in the post-MI heart would be needed to determine if this is the case.

Stem cells are seen post-MI in the border zone surrounding the infarct³⁸. They are believed to influence remodeling either by transformation into cardiomyocytes to replace ones that have been destroyed or through release of factors that favorably influence the function and/or viability of neighboring cells³⁸⁻⁴⁰. We found an increase in c-kit⁺ cells in the border zone of rats treated with placebo but that C16 treatment significantly reduced their number. Given the relatively small number of c-kit⁺ cells that were detected, it seems unlikely that differences in MI size and % FS seen with C16 were due to a lower number of replacement myocytes originating from these cells. While it is possible that a paracrine function of these c-kit⁺ cells improved the function and/or survival of hibernating cardiomyocytes in their vicinity, the significance of their presence in the border zone and the impact that they had on our results remains uncertain.

Post-MI remodeling was seen in both C16 and PL treated rats. Although LV end-diastolic dimensions were not significantly different between these groups 28 days post-MI, trends towards an increase in end-systolic dimension in the C16 treated group accounted for the differences in % FS. Echocardiography demonstrated an increase in LV septal and posterior

wall thickness in the PL but not in the C16 treated group between 2 and 28 days post-MI. By histologic examination, septal thickness was significantly less in C16 treated but not placebo treated rats post-MI compared to their respective sham operated controls. These findings from studies using complementary but independent methods suggest that post-MI LV hypertrophy was reduced by C16. A similar effect of C16 on LV to body weight ratio was not seen but as we have noted in the past this variable which is affected by multiple factors (e.g. replacement of myocytes by scar tissue, edema within the ventricular wall and cardiomyocyte hypertrophy) is insensitive for detecting LV hypertrophy at this relatively early time point²⁵. We also observed that cardiac fibrosis was increased in the non-infarcted septum in placebo but not C16 treated rats and that *tau*, a measure of LV lusotropic properties was increased post-MI only in the placebo group. The mechanism(s) responsible for these favorable effects of C16 on post-MI cardiac structure and diastolic function are uncertain since inhibition of ACE2 activity would be expected to promote hypertrophy and fibrosis by increasing Ang II and reducing Ang-(1-7) concentrations in the post-MI heart.^{18, 41} The reason why *tau* was preserved in the C16 treated group is also not certain but might have been related to the lesser amount of LV hypertrophy²⁵. Stimuli for remodeling, however, are diverse and structural changes within the heart develop as the net effect of numerous factors. It is possible that the effects of C16 on post-MI LV hypertrophy and fibrosis were also influenced by alterations in loading conditions. There was a reduction in arterial pressure in the C16 treated post-MI rats compared to their placebo controls and this would contribute to a reduction in LV wall stress, which is a major factor in promoting hypertrophy and fibrosis. It is important to recognize, however, that in addition to the changes in pressure there was also a trend towards increased LV end-systolic dimension, a factor that would result in an increase in wall stress. Lower systolic pressures with C16 treatment were likely caused by greater impairment in contractility due to an increase in infarct size with C16 since the effects of this agent on the angiotensin peptides would have been expected to increase rather than decrease arterial pressure⁴². It is known that ACE2 degrades substrates other than Ang II^{13, 17} and these properties of the drug could have contributed to the observed structural changes in the LV. It is also possible that some properties of ACE2 activity could promote MI hypertrophy and fibrosis and, thus, ACE2 inhibition had some favorable effects on the post-MI remodeling process. Support for this notion comes from a recent study showing that overexpression of ACE2 in cultured myocytes increased cell volume while overexpression *in vivo* in stroke-prone spontaneously hypertensive rats resulted in a substantial increase in myocardial fibrosis⁴³.

In a previous study male ACE2 knockout mice developed cardiac dilation and impaired myocardial contractility over time¹⁴. Other investigators, however, were unable to duplicate the effects of genetic ablation of ACE2, perhaps due to differences in the genetic background of the mice or other unidentified factors²⁴. Nonetheless, even in mice in which the deletion of ACE2 does not lead to a cardiac phenotype under normal circumstances the imposition of pressure overload accelerates the development of cardiac dysfunction, suggesting that the function of ACE2 takes on increased importance in settings when the heart is stressed⁴⁴. In a rat coronary ligation model in which gene transfer using a lentiviral vector was used to overexpress ACE2 there was a 60% reduction in delayed contrast-enhanced gadolinium enhancement suggesting early ischemic protection of myocardium at 24 hours post-MI⁴⁵. Moreover, this was associated with a favorable effect on cardiac structure and function after 6 weeks. Although it has been reported that the intracardiac delivery of ACE2 by lentiviral vector protected rats from Ang II induced cardiac hypertrophy and fibrosis⁴⁶, other investigators have found that ACE2 overexpression is associated with cardiomyocyte hypertrophy and fibrosis in other settings⁴³.

There is evidence that ACE2 is upregulated post-MI in both a rat model and in human patients and in the failing heart^{41, 47, 48}. An increase in plasma ACE2 activity has also been reported in heart failure patients⁴⁹. These findings are consistent with the possibility that ACE2 may

play an important role in the post-MI heart. Angiotensin II is believed to be the preferred substrate of ACE2 and the enzyme cleaves the terminal amino acid from the octapeptide to form Ang-(1-7)¹⁷. Increases in Ang II levels in the post-MI heart are believed to promote maladaptive cardiac remodeling^{4, 8} while Ang-(1-7) has been shown to inhibit cardiomyocyte hypertrophy and to inhibit collagen production^{21, 50, 51}. Evidence that direct administration of Ang-(1-7) to rats following an MI favorably affects cardiac function supports this possibility⁵². Inhibition of ACE2 activity would then be expected to alter the balance between these angiotensin peptides in a manner that would adversely affect cardiac structure and function. Our results that infarct size is increased and % FS is reduced with C16 administrations suggest that ACE2 has cardioprotective effects related to preservation of jeopardized cardiomyocytes in the border zone. However, the reduced fibrosis and LV hypertrophy and preservation of tau in the post-MI hearts of rats treated with C16 raises the question of whether under some conditions ACE2 might adversely affect the heart. Further clarification of this issue is needed in order to understand the potential drawbacks of therapeutic strategies designed to alter ACE2 expression or function in the post-MI heart.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Changes in % fractional shortening and MI size between days 2 and 28 post-MI
 Changes were calculated as the 28 day measure subtracted from the 2 day measure. There was a reduction in fractional shortening accompanied by an increase in infarct size in C16 treated rats. These changes were significantly different in the C16 rats than in the placebo treated rats that had undergone coronary artery ligation. Abbreviations: Δ , change; FS, fractional shortening; MI, myocardial infarction; PL, placebo. Values are for mean $\pm$ S.E.
 * $p < 0.05$

Figure 2. ACE2 activity in the post-MI on day 28 post-MI

Rats had been treated with C16 between days 2 and 28 when they were sacrificed. ACE2 activity was significantly greater in the infarct zone than in the non-infarcted interventricular septum in both placebo and C16 treated rats and it was greater in the border zone than in the non-infarcted zone in the placebo group. In each of these zones, however, ACE2 activity was significantly lower in rats that had been given C16 than in rats that had received placebo. N=8 for both C16 and PL treated rats. Abbreviations: ACE2, angiotensin converting enzyme 2; MI, myocardial infarction; N, non-infarcted septum, B, border zone; I, infarct zone. Values are for mean $\pm$ S.E.

** P<0.01; * P<0.05 for comparisons between placebo and C16 groups in the same zone # P<0.05, ## P<0.01, ### P<0.001 for comparisons between zones in rats receiving the same treatment.

Figure 3. Capillary density in the border zone

There was no significant difference between rats that received C16 and those that received placebo. Abbreviations: MI, myocardial infarction.

Figure 4. Results of immunohistochemistry for c-kit⁺ cells at day 7 post-MI

Rats treated with C16 had significantly fewer c-Kit⁺ cells in the border zone compared to rats that received placebo. The number of c-kit⁺ cells from 12 different fields in the infarct, border and non-infarcted zones from each heart were counted by a single observer blind to the treatment regimen. Few c-Kit⁺ cells were seen in either the non-infarcted septum of rats that had undergone coronary artery ligation or the LV free wall of sham operated rats of either C16 or placebo treated rats. Abbreviations: PL, placebo; B, border zone; I, infarct zone.

Table 1

Effects of ACE2 Inhibition on Echocardiographic Measurements at 28 Days

	LVDD (mm)		LVDs (mm)		LVPW (mm)		IVS (mm)		FS (%)		MI Size (%)	
	Day 2	Day 28	Day 2	Day 28	Day 2	Day 28	Day 2	Day 28	Day 2	Day 28	Day 2	Day 28
Sham+PL (n=8)	—	6.7 ± 0.4	—	3.9 ± 0.3	—	1.2 ± 0.1	—	1.2 ± 0.1	—	42.2 ± 2.7	—	—
Sham+C16 (n=8)	—	7.9 ± 0.5*	—	4.6 ± 0.5	—	1.2 ± 0.3	—	1.2 ± 0.1	—	42.0 ± 3.3	—	—
MI+PL (n=7)	6.7 ± 0.7	9.0 ± 0.6 ⁺ ,*	4.6 ± 0.9	6.3 ± 0.8 ⁺ ,*	1.3 ± 0.1	1.5 ± 0.1 ⁺	1.2 ± 0.1	1.4 ± 0.1 ⁺	31.7 ± 9.0	30.1 ± 6.4*	41.7 ± 6.7	38.6 ± 10.2
MI+C16 (n=9)	6.9 ± 0.6	9.2 ± 0.9 ⁺ ,#	4.8 ± 0.6	7.3 ± 0.9 ⁺ ,#	1.3 ± 0.1	1.2 ± 0.1 ^Δ	1.3 ± 0.1	1.2 ± 0.2	31.3 ± 5.2	20.6 ± 7.0 ⁺ ,# ^Δ	38.2 ± 5.5	46.5 ± 5.8 ⁺ , ^Δ

Values are mean ± standard deviation

Abbreviations: ACE2, angiotensin converting enzyme 2; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; LVPW, left ventricular posterior wall; IVS, interventricular septum; FS, fractional shortening; MI, myocardial infarction.

⁺ P<0.05 compared to day 2

* P<0.01 compared to sham at day 28

P<0.01 compared to sham + C16 at day 28

^Δ P<0.01 compared to MI at day 28

Table 2

Effects of ACE2 Inhibition on Hemodynamic Variables at 28 Days

	LVEDP (mmHg)	LVPP (mmHg)	MAP (mmHg)	TAU (msec)
Sham +PL (n=7)	4.5 ± 1.7	117.4 ± 12.1	101.4 ± 9.6	15.6 ± 2.1
Sham + C16 (n=8)	5.8 ± 1.6	122.1 ± 19.9	106.7 ± 15.7	16.0 ± 2.4
MI +PL (n=7)	11.5 ± 5.9 [*]	109.2 ± 13.0	97.1 ± 10.1	22.3 ± 5.8 ^{*,Δ}
MI + C16 (n=9)	10.8 ± 3.7 [#]	100.5 ± 9.6 [#]	89.7 ± 9.3 [#]	18.0 ± 3.3

Values are mean ± standard deviation

Abbreviations: ACE2, angiotensin converting enzyme 2; LVEDP, left ventricular end-diastolic pressure; LVPP, left ventricular pulse pressure; MAP, mean arterial pressure, MI, myocardial infarction

^{*} P<0.05 compared to sham[#] P<0.05 compared to sham+C16^Δ P<0.05 compared to MI+C16

Table 3

Effects of ACE2 Inhibition on LV Morphometric and Histologic Variables at 28 Days

	BW (g)	LV/BW (mg/g)	RV/BW (mg/g)	Septal Thickness (mm)	Scar Length (mm)	Total Circ (mm)	% MI
Sham + PL (n=8)	343 ± 33	2.3 ± 0.1	0.6 ± 0.1	2.0 ± 0.1	-	18.1 ± 1.0	-
Sham + C16 (n=8)	354 ± 23	2.4 ± 0.2	0.7 ± 0.1	2.0 ± 0.1	-	18.1 ± 1.1	-
MI + PL (n=7)	350 ± 15	2.4 ± 0.2	0.9 ± 0.2*	1.9 ± 0.3	8.1 ± 2.3	21.5 ± 3.0*	37.3 ± 6.1
MI + C16 (n=9)	340 ± 26	2.6 ± 0.4	0.9 ± 0.3	1.7 ± 0.3#	9.8 ± 1.9	21.4 ± 2.5#	45.8 ± 8.3

Values are mean ± standard deviation

Abbreviations: ACE2, angiotensin converting enzyme 2; LV, left ventricular; BW, body weight; LV/BW, left ventricular weight; RV, right ventricular weight; total circ, total left ventricular circumference; % MI, scar length expressed as % of LV circumference myocardial infarction. PL, placebo

* P<0.05 compared to sham

P<0.05 compared to sham + C16

Table 4

Effects of ACE2 Inhibition on Myocyte Hypertrophy and Fibrosis at 28 days

	MCA (μm)	Collagen (%)
Sham + PL (n=8)	348 $\pm$ 36	1.8 $\pm$ 0.2
Sham + C16 (n=8)	344 $\pm$ 19	1.9 $\pm$ 0.1
MI + PL (n=7)	415 $\pm$ 57	2.7 $\pm$ 0.3 *
MI + C16 (n=9)	399 $\pm$ 58	2.1 $\pm$ 0.5 #

Values are mean $\pm$ standard deviation

Abbreviations: MCA, myocyte cross sectional area; MI, myocardial infarction.

*
P<0.05 vs Sham#
P<0.05 vs MI

Table 5

Apoptotic Rate in the LV of Rats at Day 7 post-MI or Sham Operation

	Infarct Zone (%)	LV Free Wall (%)	Border Zone (%)	IV Septum (%)
Sham +PL (n=9)	-	0.14 ± 0.05	-	-
Sham + C16 (n=7)	-	0.19 ± 0.08	-	-
MI +PL (n=9)	5.99 ± 2.63	-	2.23 ± 1.46	0.21 ± 0.10
MI + C16 (n=11)	5.51 ± 1.84	-	2.28 ± 1.07	0.19 ± 0.06
	<i>p</i> = 0.65	<i>p</i> = 0.18	<i>p</i> = 0.92	<i>p</i> = 0.60

Abbreviations: LV, left ventricular; MI, myocardial infarction; IV, interventricular

All values are mean ± standard deviation. T-tests were performed on column data to generate the indicated p values.