Role of Natriuretic Peptide Receptor Guanylyl Cyclase-A in Myocardial Infarction Evaluated Using Genetically Engineered Mice

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Abstract—Although plasma levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are elevated early after myocardial infarction (MI), the significance is not fully understood. We therefore investigated the function of natriuretic peptides after induction of MI in knockout (KO) mice lacking the natriuretic peptide receptor guanylyl cyclase-A, the receptor for ANP and BNP. KO and wild-type (WT) mice were subjected to left coronary artery ligation and then followed up for 4 weeks. Irrespective of genotype, almost all deaths occurred within 1 week after induction of MI. KO mice showed significantly higher mortality because of a higher incidence of acute heart failure, which was associated with diminished water and sodium excretion and with higher cardiac levels of mRNAs encoding ANP, BNP, transforming growth factor-β1, and type I collagen. By 4 weeks after infarction, left ventricular remodeling, including myocardial hypertrophy and fibrosis, and impairment of left ventricular systolic function were significantly more severe in KO than WT mice. Notably, the enhanced myocardial fibrosis seen in KO mice was virtually absent in infarcted double-KO mice, lacking guanylyl cyclase-A and angiotensin II type 1a receptors, although there was no improvement in survival and no attenuation of cardiac hypertrophy. Thus, guanylyl cyclase-A activation by endogenous cardiac natriuretic peptides protects against acute heart failure and attenuates chronic cardiac remodeling after MI. These beneficial effects are mediated partly through inhibition of the renin-angiotensin system (RAS), although RAS-independent protective actions of guanylyl cyclase-A are also suggested. (Hypertension. 2005;46:441-447.)

Key Words: receptors, angiotensin ■ coronary artery disease ■ hypertrophy ■ remodeling

Early reperfusion therapy and other recent advances in the treatment of acute myocardial infarction (MI) have substantially reduced mortality and cardiovascular morbidity among MI patients. However, the fact that acute heart failure and chronic left ventricular (LV) remodeling continue to be major determinants of clinical outcome after MI highlights the need for a better understanding of the pathophysiological mechanisms involved in those processes. In that regard, accumulating clinical and experimental evidence indicates that inhibition of the renin-angiotensin system (RAS) and the sympathetic nervous system improves postinfarct survival and mitigates LV remodeling and dysfunction.1-4 Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are produced mainly in the atrial and ventricular myocardium, respectively, in response to volume expansion and pressure overload and counteract the effects of the sympathetic nervous system and RAS by promoting diuresis, natriuresis, and vasodilatation. The actions of both peptides are mediated via the natriuretic peptide receptor guanylyl cyclase-A (GC-A), which is expressed in a variety of tissues, including kidneys, blood vessels, adrenal glands, and heart.5 Plasma levels of natriuretic peptides are elevated in congestive heart failure (CHF) and are frequently used to aid diagnosis of CHF, to assess prognosis, and to tailor therapy.6-9 In addition, exogenous administration of recombinant natriuretic peptides is now being used therapeutically to treat decompensated CHF.10 Plasma natriuretic peptide levels are also elevated early after MI;11 in particular, the level of BNP has been shown to be a good predictor of LV systolic function and a prognostic indicator of long-term survival.12 Although in a previous study, we suggested a role for GC-A in myocardial reperfusion injury and inflammation after ischemia-reperfusion,13 it remains unclear whether activation of the GC-A pathway by endogenous natriuretic peptides has a significant effect on survival or LV remodeling after MI. Therefore, to better
understand the function and significance of the increased natriuretic peptide levels seen after MI, we induced MI by occluding the left coronary artery (LCA) in GC-A knockout (KO) mice and their wild-type (WT) littermates and examined survival, LV structure, LV function, and cardiac gene expression.

Materials and Methods
Experimental Model
All experimental procedures were performed according to Kyoto University standards for animal care. Homozygous GC-A KO mice and their WT littermates were produced from crossing heterozygous mice as described previously, after which male mice were used for experimentation at 8 to 10 weeks of age.

Experimental MI
MI was produced by permanent ligation of the LCA, and sham-operated mice underwent the same operation except for the LCA ligation. Infarct size was calculated and expressed as the ratio of the infarcted circumference divided by total LV circumference, as described previously.

Noninvasive Blood Pressure Measurements
Blood pressures and pulse rates were measured noninvasively in conscious mice using a computerized tail-cuff method (Softron Co, Ltd).

Urine Volume and Sodium Excretion
Animals were kept in individual metabolic cages from the day before surgery until 4 days after surgery. Urine was collected daily, and urine volume and sodium excretion were measured. Data were normalized to body weight (BW).

Cardiac Gene Expression
On day 3 after surgery, hearts were excised and the LVs were snap-frozen in liquid nitrogen. Total RNA was extracted from LVs, and expression of mRNAs was evaluated using quantitative RT-PCR analysis with gene-specific primers and probes in an ABI PRISM 7700 Sequence Detector (Applied Biosystems). Expression of the RNA in question was normalized to that of the corresponding GAPDH mRNA.

Echocardiography Examination
After anesthetizing mice by intraperitoneal administration of a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), percent fractional shortening (%FS), and LV posterior wall (PW) thickness were calculated before (baseline) and 4 weeks after induction of MI using an echocardiographic system (Toshiba Power Vision 8000) equipped with a 12-MHz imaging transducer.

Histological Analysis
To determine the degree of collagen fiber accumulation, we randomly selected 20 fields in 3 separate sections of formalin-fixed ventricles and calculated the ratio of the van Gieson–stained fibrotic area to the total myocardial area using image analysis software (KS400 image system; Zeiss).

Double-KO Mice Lacking GC-A and Angiotensin II Type 1a Receptors
Double-KO (DKO) mice lacking GC-A and angiotensin II type 1a (AT1a) receptors were generated from heterozygous mice after crossing of a single GC-A KO mouse and an AT1a KO mice.

Table 1. Baseline Characteristics and Causes of Death After MI

<table>
<thead>
<tr>
<th>Variables</th>
<th>WT (n=37)</th>
<th>GC-A KO (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>9.6±0.2</td>
<td>9.2±0.1</td>
</tr>
<tr>
<td>BW (g)</td>
<td>25.3±0.4</td>
<td>25.7±0.5</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>98.9±1.5</td>
<td>128.3±1.5*</td>
</tr>
<tr>
<td>PR (bpm)</td>
<td>569.9±13.9</td>
<td>553.6±10.4</td>
</tr>
<tr>
<td>Causes of death, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart failure</td>
<td>2 (5.4%)</td>
<td>18 (54.5%)*</td>
</tr>
<tr>
<td>LV rupture</td>
<td>8 (21.6%)</td>
<td>5 (15.2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2.7%)</td>
<td>1 (3.0%)</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; PR, pulse rate. Values are mean±SEM; *P<0.05 vs WT mice.

Hydralazine Administration
The blood pressures of GC-A KO mice were reduced to a level comparable to those seen in WT mice by orally administering hydralazine (50 mg/L of drinking water). Hydralazine was started 1 week before MI and continued until death, 4 weeks after MI.

Statistical Analysis
All data are expressed as means±SEM. Analysis of survival after MI was performed using the Kaplan–Meier method with the log-rank test. Data were analyzed by 1-factor ANOVA. If a statistically significant effect was found, a post hoc Newman–Keuls test was performed to isolate the differences between groups. Values of P<0.05 were considered significant.

Results
Survival After MI
The baseline characteristics of the KO and WT genotypes are shown in Table 1. There were no differences with respect to age, BW, or pulse rate between the 2 groups, although blood pressure was significantly higher in KO mice, as reported previously. Postoperative survival was monitored for 4 weeks (Figure 1). Irrespective of genotype, all deaths but 1 occurred within 1 week after induction of MI; 1 WT mouse died of heart failure during the second week after MI.

Figure 1. Kaplan–Meier analysis of survival after MI among WT (n=37) and GC-A KO mice (n=33). GC-A KO mice showed a significantly higher 4-week mortality rate than WT mice (P<0.0005).
died on day 14 after MI. Despite the fact that infarct sizes were similar in the 2 groups (KO 43.7±1.2% versus WT 46.8±1.8%), the survival rate was significantly (*P<0.0005) lower among KO mice (27.3%; 9 of 33) than among WT mice (70.3%; 26 of 37).

Causes of Death
Based on postmortem findings, the causes of death were classified into 3 groups: heart failure, LV rupture, or unknown causes (Table 1). Heart failure was diagnosed from pulmonary congestion with increased lung weight, and LV rupture from the large amount of blood observed filling the chest cavity. The incidence of heart failure was significantly higher among KO mice than WT mice, although there were no significant differences between the 2 groups in the incidences of LV rupture or death by unknown causes.

Natriuretic and Diuretic Responses During Early Phase After MI
As shown in Figure 2A, there was no difference in urine volume between the 2 genotypes before surgery, and urine volume was markedly lower in both groups on day 1 after MI. Thereafter, volume increased gradually in both genotypes, but less so in KO mice. As a consequence, KO mice were producing significantly less urine than WT mice on days 3 and 4 after MI.

When we compared the renal responses of infarcted and sham-operated mice (Figure 2B and 2C), we found that the total 4-day urine volume and sodium excretion after the sham operation were similar for both genotypes. Three days after MI, both genotypes showed significantly upregulated expression of TGF-β1 and type I collagen mRNA, but the postinfarction levels were significantly higher in KO than WT mice.

Cardiac Gene Expression During Early Phase After MI
On day 3 after sham operation, ventricular levels of ANP and BNP mRNA (Figure 3A and 3B) were higher in KO than WT hearts, probably because of basal LV hypertrophy in the former. In response to MI, both genotypes showed significant upregulation of ANP and BNP mRNA, but the postinfarction levels were still significantly higher in KO mice.

The cardiac expression of the mRNAs for transforming growth factor-β1 (TGF-β1) and type I collagen (Figure 3C and 3D) were similar in sham-operated WT and KO mice. Three days after MI, both genotypes showed significantly upregulated expression of TGF-β1 and type I collagen mRNA, but the postinfarction levels were significantly higher in KO than WT mice.

Echocardiographic Findings During Late Phase After MI
To evaluate chronic LV remodeling, echocardiographic examination of the infarcted mice was performed before (baseline) and 4 weeks after induction of MI (Table 2). Baseline measurements showed KO mice to have greater LVESD, LVEDD, and PW thickness than WT mice, but %FS was similar, as reported previously.17 Four weeks after MI, both genotypes showed significant chamber enlargement and impaired LV contractility. Although no significant difference in the absolute increase in LVEDD was observed, the absolute decrease in %FS and the absolute increases in LVESD and PW thickness were significantly greater in KO than in WT mice.

Changes in Heart Weights During Late Phase After MI
Among sham-operated animals, KO mice had larger heart weight-to-BW (HW/BW) ratios, which reflected basal myo-
cardiac hypertrophy (WT 5.44 ± 0.14 mg/g versus KO 7.94 ± 0.20 mg/g; P < 0.001). Four weeks after MI, the HW/BW ratios were higher than in sham-operated mice, irrespective of genotype, but the effect was more pronounced in KO (Sham 7.94 ± 0.20 mg/g versus MI 10.12 ± 0.20 mg/g; 27% increase; P < 0.0001) than WT (Sham 5.44 ± 0.14 mg/g versus MI 6.29 ± 0.14 mg/g; 16% increase; P < 0.005) mice.

Myocardial Fibrosis During Late Phase After MI

As shown in Figure 4A, sections of ventricle from sham-operated KO mice showed significantly (P < 0.01) more myocardial collagen accumulation than those from WT mice. Four weeks after MI, the collagen volume fraction in the noninfarcted septa was significantly increased in KO mice (P < 0.0001) but not in WT mice (P = 0.6). This marked difference in the degree of interstitial fibrosis in the noninfarcted septa from KO and WT mice can be seen in Figure 4B.

Effects of Genetic Disruption of AT1a Receptors in KO Mice

In an additional experiment, we induced MI in 8- to 10-week-old male DKO mice lacking GC-A and AT1a receptors. Although basal systolic blood pressures were significantly lower in DKO than KO mice (DKO 105.8 ± 2.6 mm Hg; P < 0.0001 versus KO mice), the high early mortality rate seen in the latter was not significantly improved in the former (Figure 5); and, as in KO mice, most of the deaths were attributable to acute heart failure (68.2%). Four weeks after MI, HW/BW ratios in DKO mice were 26% higher than in sham-operated animals (Sham 7.06 ± 0.20 mg/g versus MI 8.88 ± 0.42 mg/g; P < 0.001), which is also similar to the response seen in KO mice. In contrast, there was no significant difference in the collagen volume fraction in the noninfarcted septa from infarcted and sham-operated DKO hearts (Figure 4A); indeed, the marked interstitial fibrosis seen in the noninfarcted septa from KO hearts was virtually absent in DKO hearts (Figure 4B).

Effects of Hydralazine Administration in KO Mice

To evaluate the involvement of blood pressure difference between WT and KO mice, we orally administered hydralazine to KO mice from 1 week before MI until 4 weeks after MI. Although systolic blood pressure was significantly reduced in hydralazine-treated KO mice (103.6 ± 1.2 mm Hg; P < 0.0001 versus nontreated KO mice), the high early mortality rate was not significantly improved (Figure 5), and histological analysis showed there to be no significant attenuation of the interstitial fibrosis in the noninfarcted septum 4 weeks after MI (Figure 4).
Discussion

Although plasma ANP and BNP levels are known to be elevated early after MI,\(^1\) their function in that context has been unclear. The present study shows that disrupting GC-A in mice results in a higher incidence of acute heart failure leading to increased early mortality and, later, in exaggerated LV dysfunction and remodeling. Thus, natriuretic peptides appear to exert beneficial effects during the early and late stages after MI.

During the 4-week study period, almost all deaths among both genotypes occurred within the first week after MI and were the result of heart failure or LV rupture. This survival pattern suggests that the rapid worsening of hemodynamics and mechanical stress after MI is, for the most part, compensated by the end of 1 week and stable for ≥3 weeks thereafter. In contrast to our previous study performed using an ischemia-reperfusion model,\(^1\) infarct sizes were similar among the 2 genotypes, which is likely because permanent coronary occlusion changes the entire area at risk into infarction. This enabled us to compare the mortality rates and chronic LV remodeling resulting from similar insults in the 2 groups. Despite similar infarct sizes, the 1-week mortality rate was markedly higher in KO than WT mice. Postmortem findings indicate that the higher 1-week mortality rate in KO mice is attributable to a higher incidence of acute heart failure, which is consistent with the higher levels of cardiac expression of ANP and BNP mRNA observed in KO mice early after MI. Together, these results indicate that KO mice have a diminished capacity to compensate for acute heart failure after MI.

KO mice excreted less water and sodium than WT mice after MI, despite similar excretion during the early phase after the sham operation. Previous studies have shown that the infusion of 0.9% NaCl containing 4% albumin (isooncotic solution) results in impaired diuretic and natriuretic responses in GC-A KO mice, whereas the infusion of physiological saline (isotonic solution) leads to normal renal responses.\(^1\) Because CHF could cause isooncotic volume expansion, the impaired renal response after MI in GC-A KO mice is consistent with the idea that GC-A plays an important role in diuresis and natriuresis under such pathological conditions as heart failure, although it is not essential under basal conditions.

Natriuretic peptides are known to act as negative regulators of renin-angiotensin-aldosterone system. Indeed, we showed previously that cardiac responsiveness to angiotensin II was significantly enhanced in the absence of GC-A,\(^1\) and a previous study reported that natriuretic peptides reduced gene expression of aldosterone synthase in cultured neonatal rat cardiocytes.\(^1\) In addition, there is clinical and experimental evidence that suppression of RAS activity reduces postinfarction mortality.\(^1\) Therefore, we hypothesized that the higher mortality in KO mice was associated with greater activation...
of RAS resulting from the lack of GC-A-mediated inhibition. To test that hypothesis, we compared postinfarction survival rates in GC-A KO and DKO mice, which lack GC-A and AT1a receptors. However, surprisingly, the 4-week survival rate for DKO mice was no better than that for KO mice, despite their significantly lower basal blood pressures (to a level similar to WT mice), and again most deaths were attributable to acute heart failure. Apparently, the protective role against heart failure played by GC-A early after MI is not mediated by reduction of blood pressure or inhibition of RAS.

GC-A attenuates chronic cardiac remodeling in a pressure-overload model (transverse aortic constriction) and in a chronic hypoxia model. The present study demonstrates that GC-A also attenuates cardiac hypertrophy and fibrosis and impaired LV contractility during the chronic phase after MI. Because previous studies have shown that inhibition of RAS diminishes LV remodeling after MI, we were also interested in comparing LV remodeling in DKO and GC-A KO mice. Notably, the patterns of development of myocardial fibrosis and cardiac hypertrophy differed in the 2 genotypes (ie, the augmented fibrotic response was virtually abolished in DKO mice, although there was no attenuation of the enhanced hypertrophic response). Because previous reports have shown that natriuretic peptides exert a direct local antihypertrophic effect in vivo and in vitro, it is suggested that a direct action mediated via GC-A expressed on cardiac myocytes may be important after MI. We also evaluated the fibrotic response in hydralazine-treated GC-A KO mice, for which blood pressures were similar to those of DKO mice. However, hydralazine did not significantly affect the fibrotic response in KO mice, indicating the antifibrotic effect of GC-A is mediated mainly through inhibition of RAS, including aldosterone, not through reduction of blood pressure.

A recent clinical trial demonstrated that intravenous infusion of nesiritide (synthetic human BNP) improves the hemodynamic function and clinical status of patients with decompensated CHF. Although patients with recent MIs were excluded from that trial, the present study suggests that infusion of BNP could also have beneficial effects in patients with heart failure early after MI. In addition, a study of 60 Japanese patients with anterior MI showed that infusion of carperitide (recombinant ANP) suppressed LV remodeling evaluated 1 month after MI better than nitroglycerin did. Certainly, the lack of an oral form makes the use of natriuretic peptides somewhat impractical for long-term treatment; nevertheless, therapeutic strategies aimed at increasing levels of endogenous natriuretic peptides might be beneficial for patients after MI and deserve further investigation.

Functional deletion of the 5′-flanking region of the GC-A gene reduces transcriptional activity and is associated with essential hypertension and LV hypertrophy. This means that individuals with congenital or acquired GC-A deficiencies would likely be at higher risk of death and exaggerated LV remodeling after MI. Perhaps genotyping the GC-A locus would be a useful approach to identifying these patients, thereby enabling early preventative measures to be taken.

Perspectives
Activation of GC-A by endogenous natriuretic peptides after MI prevents acute heart failure and attenuates chronic LV remodeling. Although these beneficial effects are mediated partly through inhibition of RAS activity, RAS-independent protective actions of GC-A are also suggested. The results of this study are suggestive of the potential for using exogenous ANP or BNP to improve short- and long-term outcomes among MI patients.

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References


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