Journal of the American Heart Association

ORIGINAL RESEARCH

Recombinant Soluble Corin Improves Cardiac Function in Mouse Models of Heart Failure

Yayan Niu, MS; Shengnan Zhang, MS; Xiabing Gu, BS; Tiantian Zhou, PhD; Feng Li, MS; Meng Liu, PhD; Qingyu Wu, MD, PhD; Ningzheng Dong, MD, PhD

BACKGROUND: Corin is a transmembrane protease that activates ANP and BNP (atrial and B-type natriuretic peptides). Impaired corin expression and function are associated with heart failure. In this study, we characterized a soluble form of corin (sCorin) and examined its effects on cardiac morphology and function in mouse heart failure models.

METHODS AND RESULTS: sCorin, consisting of the full-length extracellular fragment of human corin with an engineered activation site, was expressed in Chinese hamster ovary cells, purified from the conditioned medium with affinity chromatography, and characterized in pro-ANP processing assays in vitro and pharmacokinetic studies in mice. Effects of sCorin on mouse models of heart failure induced by left coronary artery ligation and transverse aortic constriction were assessed by ELISA analysis of plasma markers, histologic examination, and echocardiography. We showed that purified and activated sCorin converted pro-ANP to ANP that stimulated cGMP production in cultured cells. In mice, intravenously and intraperitoneally administered sCorin had plasma half-lives of 3.5±0.1 and 8.3±0.3 hour, respectively. In the mouse heart failure models, intraperitoneal injection of sCorin increased plasma ANP, BNP, and cGMP levels; lowered plasma levels of NT-proANP (N-terminal-pro-ANP), angiotensin II, and aldosterone; reduced cardiac hypertrophy and fibrosis; and improved cardiac function.

CONCLUSIONS: We show that sCorin treatment enhanced natriuretic peptide processing and activity, suppressed the renin-angiotensin-aldosterone system, and improved cardiac morphology and function in mice with failing hearts.

Key Words: cardiac function ■ cardiac hypertrophy ■ corin ■ heart failure ■ mouse models

ANP and BNP (atrial and B-type natriuretic peptides, respectively) are key hormones of the cardiac endocrine mechanism, which increases vasodilation, natriuresis, and diuresis to regulate cardiovascular homeostasis.[1–3] ANP-mediated signaling also serves as an antihypertrophic and anti-inflammatory mechanism in the heart.[4–7] Like most peptide hormones, the natriuretic peptides are synthesized in precursor forms, that is, pro-ANP and pro-BNP, which are converted to active ANP and BNP by proteolytic processing. In patients with heart failure (HF), circulating pro-ANP and pro-BNP levels are highly elevated, suggesting an underlying rate-limiting step in natriuretic peptide processing in failing hearts.[8–10]

Corin is a transmembrane protease that processes the natriuretic peptides, particularly pro-ANP, in the heart.[11,12] In mice, *Corin* gene deletion or rearrangement prevents pro-ANP processing, resulting in cardiac hypertrophy and declined cardiac function in an age-dependent manner.[13–15] Pregnant corin-deficient mice also exhibit gestational hypertension and cardiac hypertrophy, resembling peripartum cardiomyopathy in patients.[16–19] In patients with HF, reduced corin expression and activity are associated with impaired natriuretic peptide processing and

Correspondence to: Ningzheng Dong, MD, PhD, Cyrus Tang Hematology Center, Soochow University, 199 Ren Ai Road, Suzhou 215123, China. E-mail: ningzhengdong@suda.edu.cn and Qingyu Wu, MD, PhD, Cardiovascular & Metabolic Sciences, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195. E-mail: qywu88@yahoo.com

Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.019961

For Sources of Funding and Disclosures, see page 11.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- This study shows that a recombinant soluble form of corin activates ANP (atrial natriuretic peptide) in vitro and has desired pharmacokinetic properties in mice.
- When administered intraperitoneally in mice, the soluble corin enhances natriuretic peptide activity in plasma, reduces cardiac hypertrophy and fibrosis, and improves cardiac function in 2 models of heart failure.

What Are the Clinical Implications?

- Corin is a serine protease that activates natriuretic peptides in the heart.
- High levels of unprocessed natriuretic peptides in patients with heart failure indicate that corin activity is a rate-limiting factor in failing hearts.
- Our results suggest that recombinant forms of corin may be used as therapeutic agents to increase natriuretic peptide activity and improve cardiac function in patients with heart failure.

Nonstandard Abbreviations and Acronyms

BW	body weight
sCorin	soluble corin
TAC	transverse aortic constriction
TL	tibia length

worsening cardiac function.[20–22] Consistently, low levels of circulating corin have been reported in patients with HF with poor clinical outcomes.[23–26] These data indicate that reduced corin expression and activity are part of the pathological mechanism in HF.

Recombinant natriuretic peptides are used in patients with HF to reduce body fluid retention, lower blood pressure, and ameliorate cardiac function.[1,27] As shown in a recent study, the vasodilatory and natriuretic/diuretic activities of ANP are mediated by distinct residues.[28] In principle, increased corin expression and activity in failing hearts could enhance natriuretic peptide production in vivo. In agreement with this hypothesis, transgenic overexpression of corin in the heart enhanced cardiac function and prolonged survival in mouse models of cardiomyopathy.[29,30] These results suggest that corin-based approaches may be considered as a strategy to treat HF.

Corin is a type II transmembrane protein. The N-terminal cytoplasmic fragment and the transmembrane domain are unnecessary for natriuretic peptide processing.[31] In this study, we expressed a soluble form of corin consisting of the entire extracellular region. We show that administration of the soluble corin (sCorin) reduced cardiac hypertrophy and improved cardiac function in mouse models of HF induced by left coronary artery ligation and transverse aortic constriction (TAC).

METHODS

The authors declare that all supporting data are presented in the article and the supplementary materials. The raw data that support the findings of this study are available from the corresponding authors upon reasonable request.

Expression Vector

To express a soluble form of human corin, a cDNA fragment (nucleotides 463–3219 of corin cDNA[32]) was amplified by polymerase chain reaction and inserted into the vector pMH3 (Addgene). The resulting plasmid encodes a soluble corin consisting of an Igκ signal peptide and a 919-amino-acid extracellular fragment of corin (residues 124–1042) with C-terminal V5 and His tags. Site-directed mutagenesis was performed to replace the RMNKR (residues 797–801) sequence at the corin activation site with a sequence (DDDDK) cleavable by enteropeptidase (also called enterokinase) (Figure 1A).

Expression of Soluble Corin

Chinese hamster ovary–derived CHO-K1 cells (NingBoMingZhou Tech, China)[33] were cultured in DMEM/F12 (Corning, Corning, NY) medium with 10% FBS (Gemini Bio Products, West Sacramento, CA) at 37°C in a humidified incubator with 5% CO_2. The plasmid expressing sCorin was transfected into the cells using PolyJet reagents (SignaGen Laboratories, Gaithersburg, MD). Stable clones were selected in DMEM/F12 medium containing 10% FBS and G418 (1 mg/mL, Life Technologies, Carlsbad, CA) and screened by western blotting using an anti-V5 antibody (1:5000 dilution; Invitrogen,) for corin expression. Positive clones were expanded in serum-free OPTI-MEMI medium (Life Technologies) with 1 mg/mL G418.

Purification of Soluble Corin

The conditioned medium containing sCorin was centrifuged (233*g*, 5 minutes), filtered through a 0.22-μm membrane (Millipore, Billerica, MA), and loaded onto a 5-mL Ni-Sepharose column (HisTrap HP, GE Healthcare, Chicago, IL) equilibrated with 20 mmol/L Tris-HCl, 300 mmol/L NaCl, and 5 mmol/L imidazole

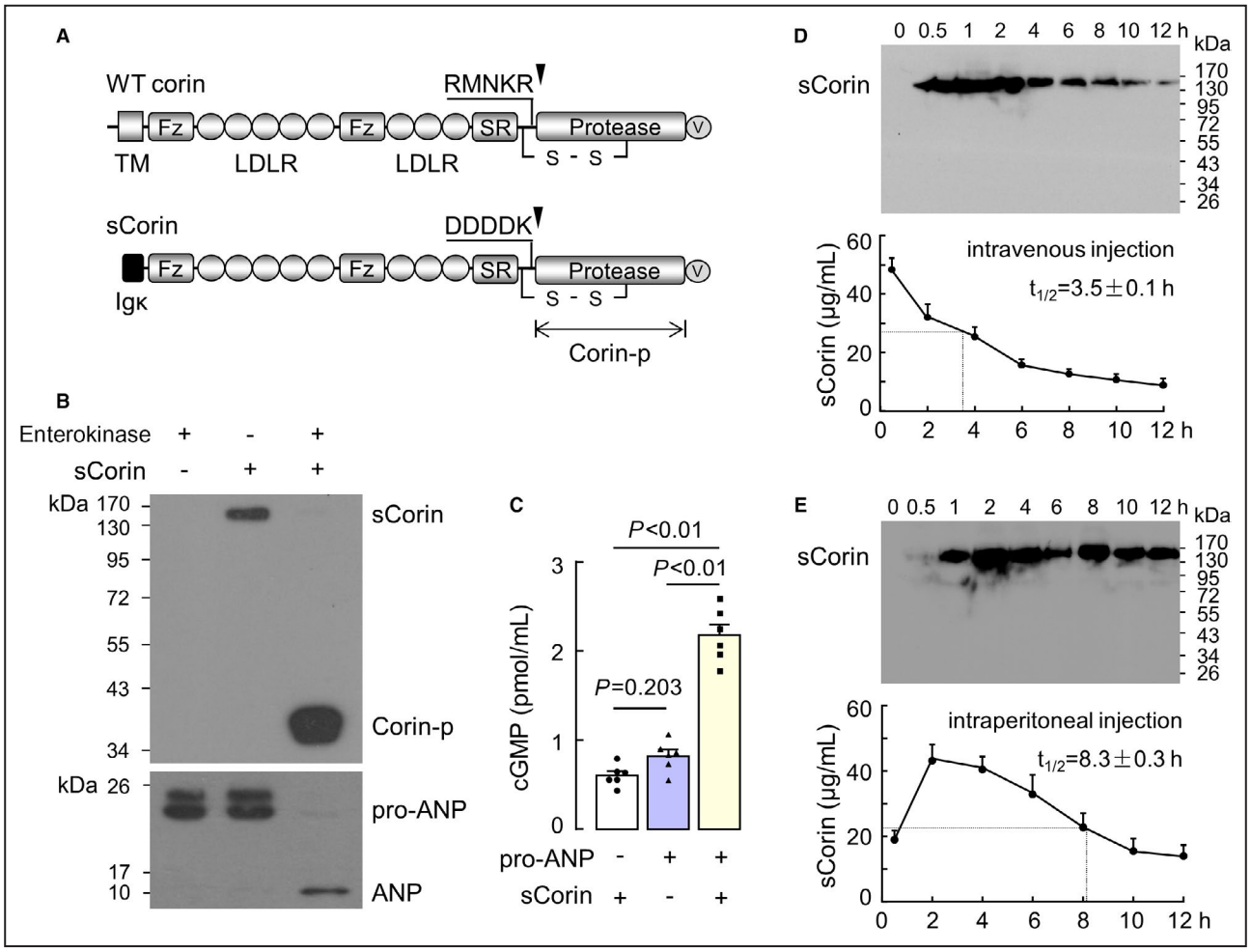


Figure 1. Characterization of soluble corin (sCorin) and pharmacokinetic studies in mice.
A, Corin protein domains are illustrated. The RMNKR sequence at the activation cleavage site (arrowhead) is shown. A disulfide bond (s-s) linking the propeptide region and the protease domain is indicated. sCorin consists of an Igκ signal peptide and the extracellular fragment of corin, in which the RMNKR sequence is replaced by DDDDK that is cleavable by enterokinase. **B**, Enterokinase-activated sCorin (top panel) converted pro-ANP to ANP (lower panel), as indicated by western blotting under reducing conditions. **C**, Soluble corin activated ANP-stimulated cGMP production in cell-based assays. *P* values were analyzed by 1-way ANOVA and Tukey's post hoc analysis. **D** and **E**, Soluble corin was injected intravenously (**D**) or intraperitoneally (**E**) in mice (n=7 per group). Plasma samples were collected over time. Soluble corin was analyzed by western blotting under nonreducing conditions (top panels) and ELISA (lower panels) to calculate plasma half-lives. ANP indicates atrial natriuretic peptide; Fz, frizzled; LDLR, LDL receptor; SR, scavenger receptor; TM, transmembrane; and V, V5 tag.

(pH 8.0). After washing with 40 mL of a gradient (5%–60%) solution (Buffer B) (20 mmol/L Tris-HCl, 300 mmol/L NaCl, and 250 mmol/L imidazole, pH 8.0), proteins were eluted with Buffer B and dialyzed again with PBS. Fractions containing sCorin were verified by western blotting with the anti-V5 antibody. Purified protein was quantified with a Bradford assay (Thermo Fisher Scientific, Waltham, MA) and stored at –80°C until use.

Activation of Soluble Corin by Enterokinase and Western Blotting

Purified sCorin (2.5 μg) was incubated with recombinant enterokinase (1–15 U/mL) (BBI Life Sciences, Shanghai, China) in 100 mmol/L Tris-HCl, pH 7.5, and 10 mmol/L $CaCl_2$ at 25°C for 2 hours. Enterokinase was removed from the solution using EKapture beads (Novagen, Madison, WI) and centrifugation (233*g*, 10 minutes). Enterokinase-treated sCorin was mixed with a Laemmli sample buffer (Bio-Rad Laboratories, Hercules, CA) with (reducing) or without (nonreducing) β-mercaptoethanol (2.5% v/v) and analyzed by SDS-PAGE and western blotting using a horseradish peroxidase–conjugated anti-V5 antibody (1:5000 dilution; Invitrogen). After 2 hours at 37°C, the blot was washed and incubated with a solution containing an enhanced chemiluminescent substrate (NcmECL Ultra; NCM Biotech, Newport, RI) at room temperature for 1 minute. The blot was exposed to

a chemiluminescent imager (Imager 600; Amersham Biosciences, Amersham, UK).

Pro-ANP Processing by Soluble Corin

HEK293 cells (ATCC, CRL-1573, authenticated by short tandem repeat profiling) were cultured in DMEM (Corning) with 10% FBS. A plasmid expressing human pro-ANP with a C-terminal V5 tag[31] was transfected into the cells using PolyJet reagents, as described above. To examine the activity of sCorin, human pro-ANP in the conditioned medium from the transfected HEK293 cells was incubated with purified sCorin without or with enterokinase treatment. sCorin activation and pro-ANP to ANP conversion were analyzed by western blotting. To verify the activity of sCorin–activated ANP, pro-ANP without or with sCorin treatment was added to baby hamster kidney cells (NingBoMingZhou Tech, China) cultured in 96-well plates with MEM medium (Hyclone Laboratories, Logan, UT) and 10% FBS. After 30 minutes at 37°C, the cells were lysed with 1% (v/v) Nonidet P-40 in a solution with 5% (v/v) glycerol, 25 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, and 2% (v/v) a protease inhibitor mixture (Thermo Fisher Scientific). Levels of cGMP in cell lysates were examined by ELISA (Enzo Life Sciences, Farmingdale, NY).

Pharmacokinetic Studies in Mice

Experiments in mice were approved by the Animal Use and Care Committee of Soochow University and conducted in accordance with the approved protocol (201603A181) and the National Institutes of Health guidelines for the ethical treatment and handling of animals in research. The mice were housed in ventilated cages with free access to food and water at a temperature- and humidity-controlled pathogen-free facility with 12/12-hour light-dark cycles. Wild-type C57BL/6 mice (10- to 12-week-old males) (n=7 per group) were used. Purified and enterokinase-activated sCorin (3 mg/kg, as suggested in a previous study[14]) or equal volume of vehicle was injected intravenously or intraperitoneally in mice randomized in unblinded groups. At different times, orbital sinus blood was collected in tubes with EDTA and centrifuged at 2095*g* for 10 minutes at room temperature. Levels of plasma sCorin were determined by western blotting and ELISA (R&D Systems, Minneapolis, MN).

Mouse Models of HF

Male C57BL/6 mice (10- to 12-week-old) (n=9–10 per group) were anesthetized with 1.5% (v/v) isoflurane in oxygen with a flow rate of 0.3 L/min. An acute myocardial infarction (MI) model was performed, in which the left descending coronary artery was closed permanently with a 6-0 silk suture.[34] In sham controls, similar surgical procedures were done without the coronary artery ligation. In another model, in which HF was induced by TAC, the aortic arch was constricted between the brachiocephalic trunk and the left carotid artery with a 7-0 silk suture and a 27-gauge needle.[35] In sham controls, the aorta was exposed surgically without the suture constriction. Mice were given 0.1 mg/kg buprenorphine for postsurgery analgesia every 12 hours as needed up to 48 hours. Mice that died or without a reduction of the left ventricular ejection fraction below 50% 1 week after the surgery in the MI model were excluded from further study. For sCorin treatment, purified and enterokinase-activated sCorin (3 mg/kg) or an equal volume of vehicle was injected (intraperitoneally daily for 3–7 weeks), starting at 1 week after surgery. To assess cardiac function, mice were anesthetized with 1.5% (v/v) isoflurane in oxygen. Transthoracic echocardiogram (Vevo 2100 with a 30-MHz probe; VisualSonics, Toronto, Canada) was performed before and weekly after the surgery (up to 8 weeks).

Histologic Analysis

Mice were euthanized by exsanguination following isoflurane inhalation (5% in oxygen, 0.3 L/min). Hearts and lungs were quickly isolated, weighed, fixed with 4% (v/v) paraformaldehyde, and embedded in paraffin. Sections (4 μm in thickness) were prepared for histologic analysis, as described previously.[36] For the MI model, transverse heart sections were made from the ligation site to the apex at 200-μm intervals. The sections were stained alternately with hematoxylin and eosin (general morphology), triphenyl tetrazolium chloride (tissue viability), Masson's trichrome (fibrosis), and Prussian blue (iron-containing macrophages). To calculate fibrotic areas, the ratio of collagen-positive area in Masson's trichrome staining versus the total left ventricular (LV) section area was analyzed using Image-Pro-Plus (V6.0; Media Cybernetics, Rockville, MD).[37] To measure cardiomyocyte size, 5 sections per heart were stained with rhodamine-conjugated wheat germ agglutinin (Vector Laboratories, Burlingame, CA). Five randomly selected fields per section were analyzed in a blinded manner. Short axis diameters of at least 200 intact myocytes were measured at the nucleus plane with Image-Pro-Plus software.[16]

Measurements of Plasma Factors and cGMP in Tissues

At 4 weeks after MI or 8 weeks after TAC surgery, blood and LV tissues were collected. ELISA kits were used to measure plasma levels of ANP (E-EL-M0166c; Elabscience, Houston, TX), BNP (E-EL-M0204c; Elabscience), N-terminal (NT)-pro-ANP (SEA484Mu; Cloud-Clone Corporation, Houston, TX), cGMP

(ADI-900-013; Enzo Life Sciences), angiotensin II (EIAM-ANGII-1; RayBiotech, Norcross, GA) and aldosterone (E-EL-0070c; Elabscience). In NT-pro-ANP ELISA, the polyclonal antibody was against a pro-ANP fragment (Asn25-Arg122), overlapping with NT-pro-ANP and pro-ANP moieties. To measure cGMP levels in heart tissues, LV samples were weighed and homogenized in the lysis buffer described above. cGMP levels in tissue homogenates were measured by ELISA. All ELISA procedures were done according to manufacturers' protocols.

Statistical Analysis

Data were analyzed using Prism 8.0 (Graphpad Software, La Jolla, CA). Quantitative data are presented in mean±SEM. Comparisons between 2 groups were done using Student's *t* test. Data from 3 or more groups were analyzed with one-way ANOVA followed by Tukey's post hoc analysis. *P* values < 0.05 were considered as statistically significant.

RESULTS

Expression, Purification, and Activation of Soluble Corin

Corin is a multidomain protease (Figure 1A). The N-terminal cytoplasmic tail is followed with a transmembrane domain and an extracellular region that includes 2 frizzled domains, 8 low-density lipoprotein receptor repeats, a scavenger receptor domain, and a serine protease domain. Corin is synthesized as a zymogen, which is activated on the cell surface by proprotein convertase subtilisin/kexin 6 at a conserved site (Figure 1A).[38] Previously, a soluble form of corin, consisting of an Igκ signal peptide and the entire extracellular region of corin (Figure 1B), was found active in cell-based pro-ANP processing studies.[31] The proprotein convertase subtilisin/kexin 6 cleavage site could be replaced by an enterokinase cleavage site (Figure 1B),[31] allowing better controlled activation of the sCorin before it was administered in vivo.

We expressed sCorin with the enterokinase activation site in CHO-K1 cells and purified it from the conditioned medium by affinity chromatography. In SDS-PAGE followed by Coomassie blue staining and western blotting, sCorin appeared as a single band of ~150 kDa (Figure S1A). Purified sCorin was activated by recombinant enterokinase in a dose-dependent manner, as shown by western blotting under reducing and nonreducing conditions (Figure S1B). The enterokinase-activated sCorin converted human pro-ANP to ANP, as indicated by western blotting (Figure 1B). The sCorin-generated ANP exhibited the activity in stimulating cGMP generation in a cell-based assay (Figure 1C).

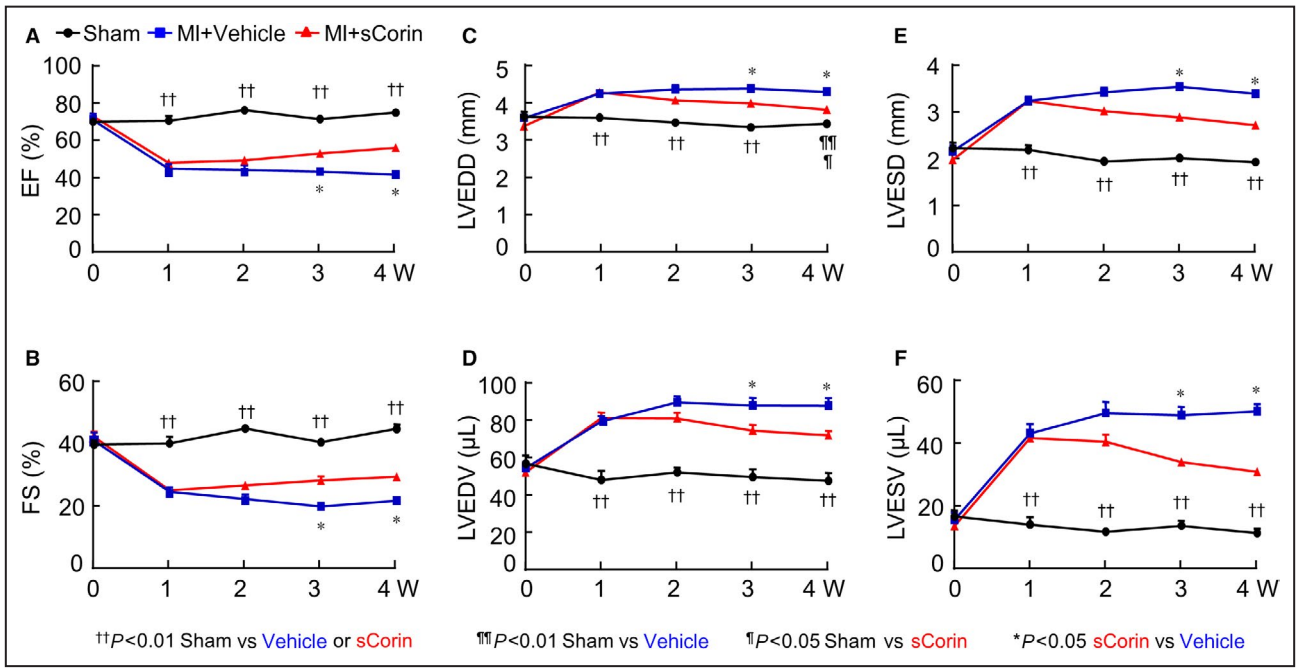


Figure 2. Left ventricular (LV) function in sham-operated mice and mice with myocardial infarction (MI) that were treated with vehicle or soluble corin (sCorin).
Echocardiography was done before (0 week) and after (1–4 weeks) the surgery to examine ejection fraction (EF) (**A**), fractional shortening (FS) (**B**), LV end-diastolic dimension (LVEDD) (**C**) and volume (LVEDV) (**D**), and LV end-systolic dimension (LVESD) (**E**) and volume (LVESV) (**F**). Data are mean± SEM; n=9-10 per group. *P* values among 3 groups at the same time point were analyzed by 1-way ANOVA and Tukey's post hoc analysis.

Pharmacokinetic Studies of Soluble Corin

We next did pharmacokinetic studies in mice. After intravenous injection, sCorin was detected by western blotting and ELISA in plasma samples collected up to 12 hour with a calculated half-life of 3.5±0.1 hour (Figure 1D). In parallel experiments, in which sCorin was injected intraperitoneally, plasma sCorin levels peaked at ~2 hour and decreased gradually over time. The calculated plasma half-life was 8.3±0.3 hour (Figure 1E). These results indicate that sCorin can be administered either intravenously or intraperitoneally to achieve detectable plasma levels in mice.

Soluble Corin Improves Cardiac Function in Mice With HF Induced by MI

We tested a mouse HF model induced by left coronary artery ligation and acute MI. Cardiac function was assessed by echocardiography before and after the surgery (Figure S2A and S2B). Compared with mice in the sham group, mice of the MI group had decreased ejection fraction (68.4±1.6 versus 38.4±4.0%) and fractional shortening (37.4±1.2 versus 18.6±2.2%), increased left ventricular end-diastolic dimension (3.5±0.1 versus 4.3±0.1 mm) and volume (52.1±3.8 versus 81.5±4.0 μL), and increased LV end-systolic dimension (2.2±0.1 versus 3.5±0.1 mm) and volume (16.7±1.8 versus 49.6±2.3 μL) (Figure S2C through S2H). In histologic analysis, cardiac sections from the mice with MI had less viable myocytes and more scar tissues and fibrosis, as indicated by Masson's trichrome and triphenyl tetrazolium chloride staining (Figure S3A and S3B). Prussian blue staining revealed more iron-containing macrophages in lung sections from mice with MI (Figure S3C and S3D). These results are consistent with reported findings in this common mouse model of HF.[37]

To examine the efficacy of sCorin, we injected purified and enterokinase-activated sCorin or vehicle in mice that had MI (intraperitoneally daily for 3 weeks, starting at 1 week after surgery) (Figure S4). As expected, mice with MI had reduced cardiac function, compared with that in sham controls (Figure 2A through 2F). Within the MI group, mice receiving sCorin had better cardiac function compared with that in vehicle-treated mice, as indicated by

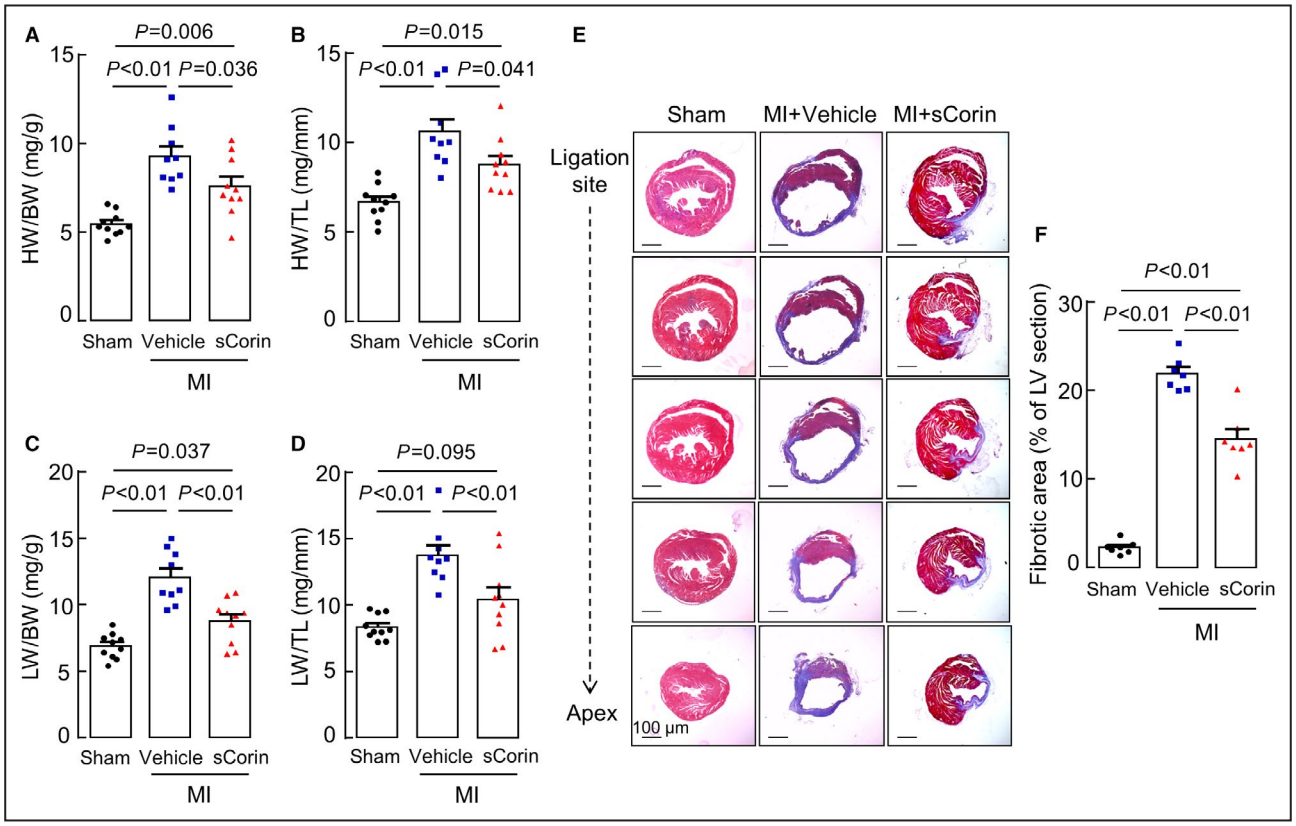


Figure 3. Analysis of heart and lung tissues in sham-operated mice and mice with MI that were treated with vehicle or soluble corin (sCorin).

A and **B**, Ratios of heart weight (HW) were normalized to body weight (BW) (**A**) or tibia length (TL) (**B**). **C** and **D**, Ratios of lung weight (LW) were normalized to BW (**C**) or TL (**D**). Data are mean±SEM. *P* values were analyzed by 1-way ANOVA and Tukey's post hoc analysis. **E**, Serial heart sections from the left coronary artery ligation site toward the apex were stained with Masson's trichrome. Scale bars: 100 μm. Scar areas (blue) were quantified by Image-Pro-Plus software. Quantitative data (mean±SEM) are shown in (**F**). *P* values were analyzed by 1-way ANOVA and Tukey's post hoc analysis.

increased ejection fraction and fractional shortening and decreased LV end-diastolic dimension, LV end-diastolic volume, LV end-systolic dimension, and LV end-systolic volume, starting at 2 weeks after sCorin injection (Figure 2A through 2F).

At 4 weeks after surgery, we analyzed hearts and lungs from the mice. Compared with those in the sham group, mice in the MI group had increased cardiac hypertrophy and pulmonary congestion, as indicated by increased ratios of heart weight and lung weight versus body weight (BW) or tibia length (TL), respectively (Figure 3A through 3D). Within the MI group, heart weight/BW or heart weight/TL and lung weight/BW or lung weight/TL ratios were lower in sCorin–treated mice than vehicle-treated mice (Figure 3A through 3D). In Masson's trichrome-stained serial heart sections, scar tissues and fibrosis were less in sCorin-treated mice than vehicle-treated mice (Figure 3E and 3F).

In plasma samples collected at 4 weeks after surgery (Figure 4A through 4F), decreased levels of ANP and cGMP and increased levels of NT-pro-ANP, angiotensin II, and aldosterone were found in the MI group, compared with those in the sham group. Levels of plasma BNP were similar between the sham group and the vehicle-treated MI group (Figure 4B). Within the MI group, sCorin–treated mice had higher levels of plasma ANP, BNP, and cGMP (Figure 4A through 4C) and lower levels of plasma NT-pro-ANP, angiotensin, and aldosterone (Figure 4D through 4F), compared with those in vehicle-treated mice. In LV tissues collected at 4 weeks after surgery, cGMP levels were higher in sCorin–treated mice than vehicle-treated mice (Figure 4G). These results indicate that sCorin treatment enhanced natriuretic peptide processing and activity, inhibited the renin-angiotensin-aldosterone system, and improved cardiac function in the mouse model of HF induced by acute MI.

Soluble Corin Improves Cardiac Function in Mice With HF Induced by TAC

To verify our findings, we tested another mouse HF model induced by TAC. As reported, TAC reduced cardiac function (Figure S5) and caused cardiac hypertrophy (Figure S6), as shown by echocardiography and histologic analysis. We treated mice with peritoneal injection of enterokinase-activated sCorin

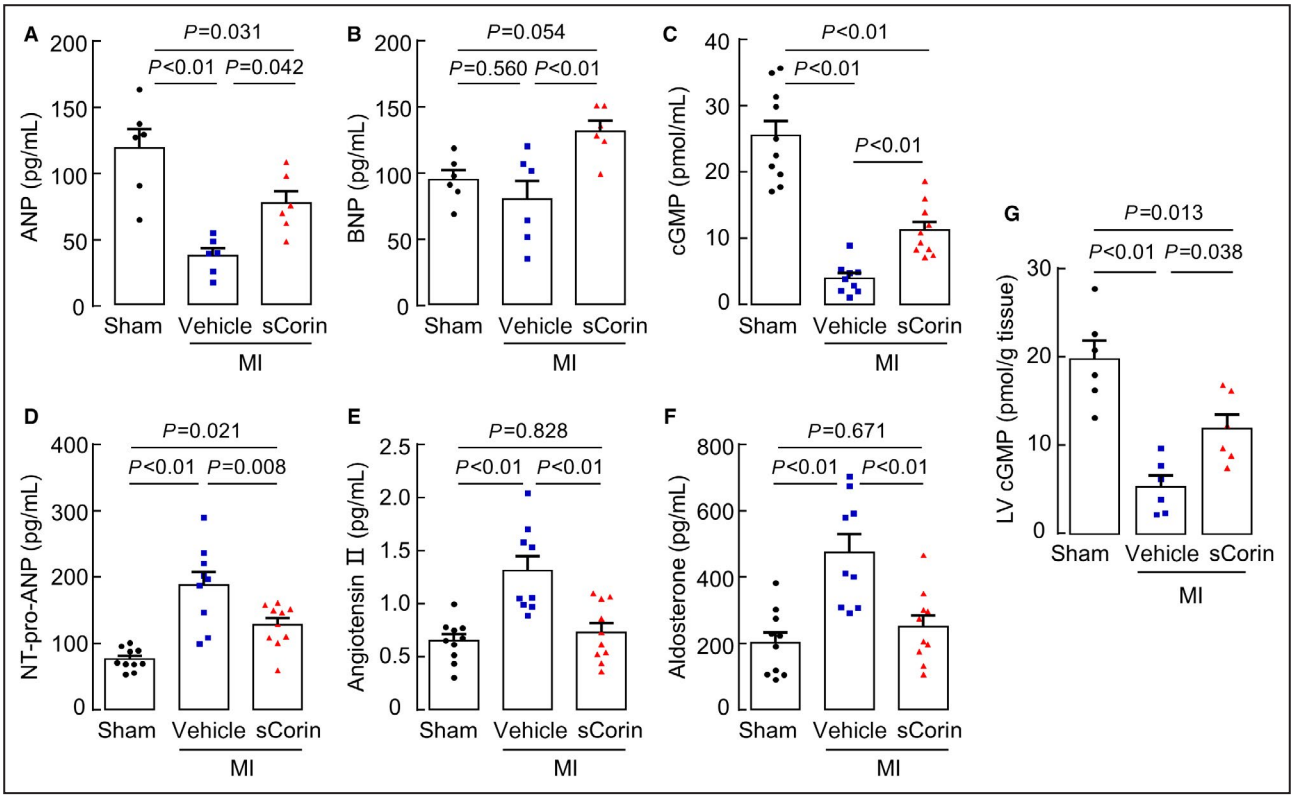


Figure 4. Analysis of plasma factors and left ventricular (LV) cGMP levels in sham-operated mice and mice with myocardial infarction (MI) treated with vehicle or soluble corin (sCorin).
Plasma samples were collected at 4 weeks after surgery. Levels of ANP (**A**), BNP (**B**), cGMP (**C**), NT-pro-ANP (**D**), angiotensin II (**E**), and aldosterone (**F**) in plasma samples and cGMP levels in LV tissues (**G**) were analyzed by ELISA. Data are mean±SEM. *P* values were analyzed by one-way ANOVA and Tukey's post hoc analysis. ANP indicates atrial natriuretic peptide; BNP, B-type natriuretic peptide; and NT-pro-ANP, N-terminal proatrial natriuretic peptide.

or vehicle (intraperitoneally daily for 7 weeks, starting at 1 week after surgery) (Figure S7). Compared with vehicle-treated mice, sCorin–treated mice had improved cardiac function, as indicated by increased ejection fraction and fractional shortening, and decreased LV end-diastolic dimension, LV end-diastolic volume, LV end-systolic dimension, and LV end-systolic volume, starting at 5 weeks after sCorin injection (Figure 5A through 5F).

At 8 weeks after surgery, sCorin-treated mice were found to have smaller hearts (Figure 6A and Figure S8A) and reduced heart weight that was normalized to BW or TL (Figure 6B and 6C), compared with those in vehicle-treated mice. Unlike in the MI model, pulmonary congestion was less severe in the TAC model. Compared with vehicle-treated mice, sCorin–treated mice had reduced lung weight/BW and lung weight/TL ratios, although the difference was not statistically significant (Figure S8B and S8C).

In hematoxylin and eosin–, wheat germ agglutinin–, and Masson's trichrome–stained heart sections prepared at 8 weeks after surgery, increased cardiomyocyte diameters and fibrosis were found in mice with TAC, compared with the sham controls (Figure 6D through 6F). Within the TAC group, myocyte hypertrophy and cardiac fibrosis were less severe in sCorin–treated mice, compared with those in vehicle-treated mice (Figure 6D through 6F).

Compared with vehicle treatment, sCorin treatment increased ANP, BNP, and cGMP levels (Figure 7A through 7C) and decreased plasma NT-pro-ANP, angiotensin II, and aldosterone levels (Figure 7D through 7F) in plasma samples collected at 8 weeks after TAC. Levels of cGMP in LV tissues collected at 8 weeks after TAC were also higher in sCorin–treated mice than vehicle-treated mice (Figure 7G). These results are consistent with findings in the MI model, indicating the therapeutic efficacy of sCorin in mouse models of HF.

DISCUSSION

In this study, we tested the hypothesis of using recombinant corin to enhance natriuretic peptide activity as a therapeutic strategy to improve cardiac morphology and function in HF. We expressed and purified sCorin, consisting of the extracellular region of corin and an engineered enterokinase activation site. We showed that enterokinase-activated sCorin converted pro-ANP to biologically active ANP, which in turn stimulated cGMP production in cultured cells. Importantly, we found that sCorin can be administered either intravenously or intraperitoneally to achieve detectable plasma levels in mice. The observed plasma half-lives of sCorin, administered intravenously and intraperitoneally, were >3 hours and >8 hours, respectively, much longer than those of recombinant ANP and BNP (<15 minutes).[39]

Under physiological conditions, protease activities are tightly regulated to prevent undesired consequences. For example, tissue-type plasminogen

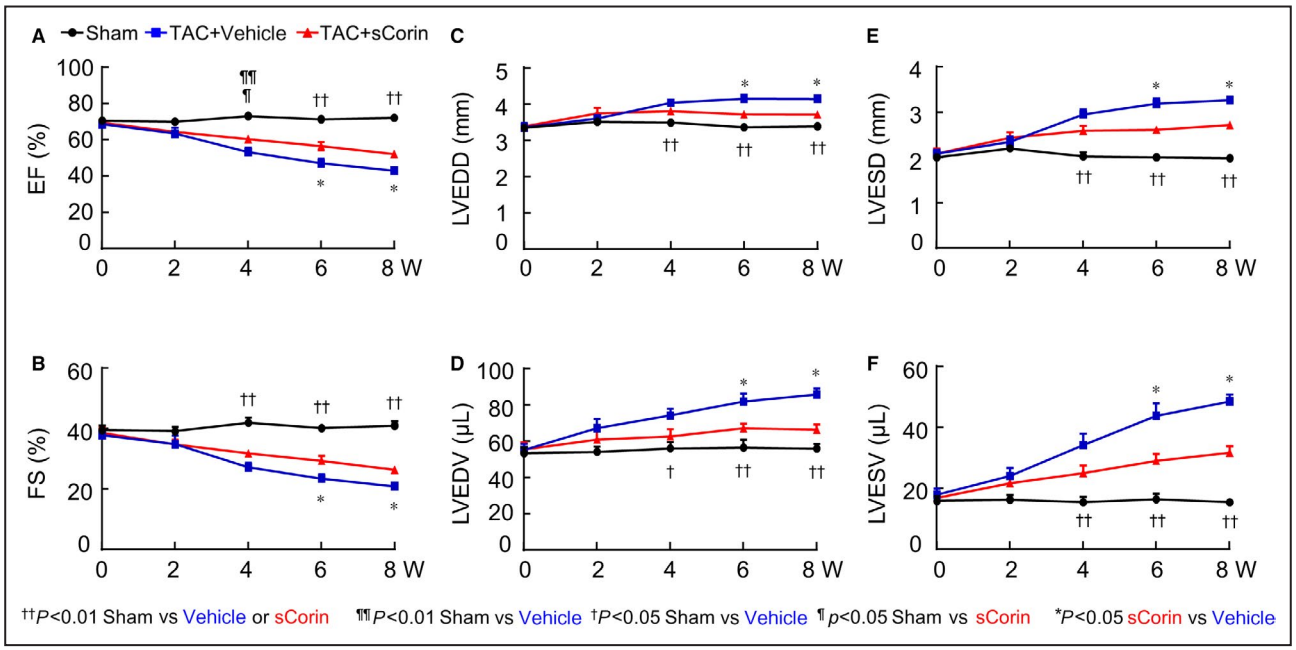


Figure 5. Left ventricular (LV) function in sham-operated mice and mice with transverse aortic constriction (TAC) that were treated with vehicle or soluble corin (sCorin).
Echocardiography was done before (0 week) and after (2–8 weeks) the surgery to examine ejection fraction (EF) (**A**), fractional shortening (FS) (**B**), LV end-diastolic dimension (LVEDD) (**C**) and volume (LVEDV) (**D**), and LV end-systolic dimension (LVESD) (**E**) and volume (LVESV) (**F**). Data are mean±SEM; n=9 per group. *P* values among 3 groups at the same time point were analyzed by 1-way ANOVA and Tukey's post hoc analysis.

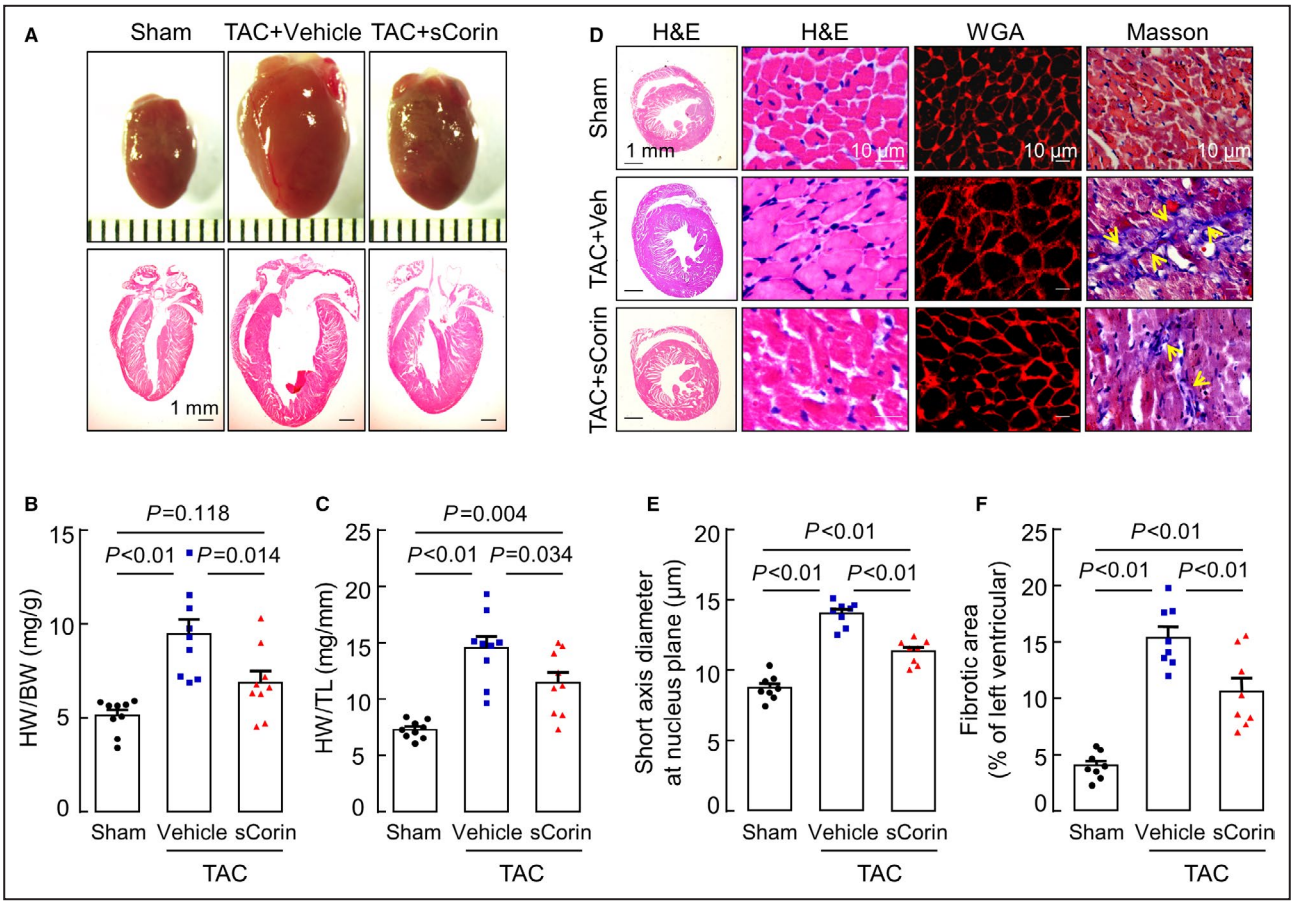


Figure 6. Analysis of heart tissues in sham-operated mice and mice with transverse aortic constriction (TAC) treated with vehicle or soluble corin (sCorin).
A, Representative heart images (top panels) and hematoxylin and eosin (H&E)-stained sections (lower panels) were from mice at 8 weeks after surgery. Scale bars: 1 mm. **B** and **C**, Ratios of heart weight (HW) to body weight (BW) (**B**) or tibia length (TL) (**C**) were calculated. Data are mean±SEM. *P* values were analyzed by one-way ANOVA and Tukey's post hoc analysis. **D**, Representative heart sections stained by hematoxylin and eosin, wheat germ agglutinin (WGA), and Masson's trichrome. Scale bars are indicated. **E**, Short-axis cardiomyocyte diameters at the nucleus plane were measured. Data are mean±SEM from 200 individual cardiomyocytes in >3 randomly selected section from each mouse. n=8 per group. *P* values were analyzed by 1-way ANOVA and Tukey's post hoc analysis. **F**, Areas of fibrosis (blue in Masson's trichrome-stained sections) were quantified by Image-Pro-Plus software. Data are mean±SEM. n=8 per group. *P* values were analyzed by one-way ANOVA and Tukey's post hoc analysis.

activator activity is inhibited by plasminogen activator inhibitor-1 in plasma. Tissue-type plasminogen activator is used as a thrombolytic agent to treat acute MI and stroke.[40] To achieve better thrombolytic efficacy in patients, a mutant tissue-type plasminogen activator has been engineered to be more resistant to plasminogen activator inhibitor-1 inhibition and hence a longer half-life in circulation.[41] To date, no physiological corin inhibitors have been found. Corin-mediated pro-ANP processing occurs in the presence of human plasma.[31] Such a plasma-resistant characteristic suggests that recombinant forms of corin likely remain active in circulation.

The natriuretic peptide function is mediated by the receptor-dependent stimulation of intracellular cGMP production.[2] In healthy individuals and patients with HF, intravenous infusion of ANP or BNP increased plasma cGMP levels.[42,43] In a canine model, intravenous bolus injection of pro-ANP also increased plasma cGMP levels,[44] suggesting that exogenous pro-ANP may be converted to ANP in vivo. Consistently, we found higher plasma ANP, BNP, and cGMP levels in sCorin–treated mice than vehicle-treated mice. We also observed lower levels of NT-pro-ANP in sCorin–treated mice. The antibody in the NT-pro-ANP ELISA kit recognizes both NT-pro-ANP and pro-ANP moieties. It is unclear if the results actually reflect lower plasma pro-ANP levels in sCorin–treated mice. ANP is known to antagonize the renin-angiotensin-aldosterone system.[1,3] We detected reduced plasma levels of angiotensin II and aldosterone in sCorin–treated mice. Moreover, sCorin-treated mice had higher cGMP levels in LV tissues. These data indicate that sCorin is functional in

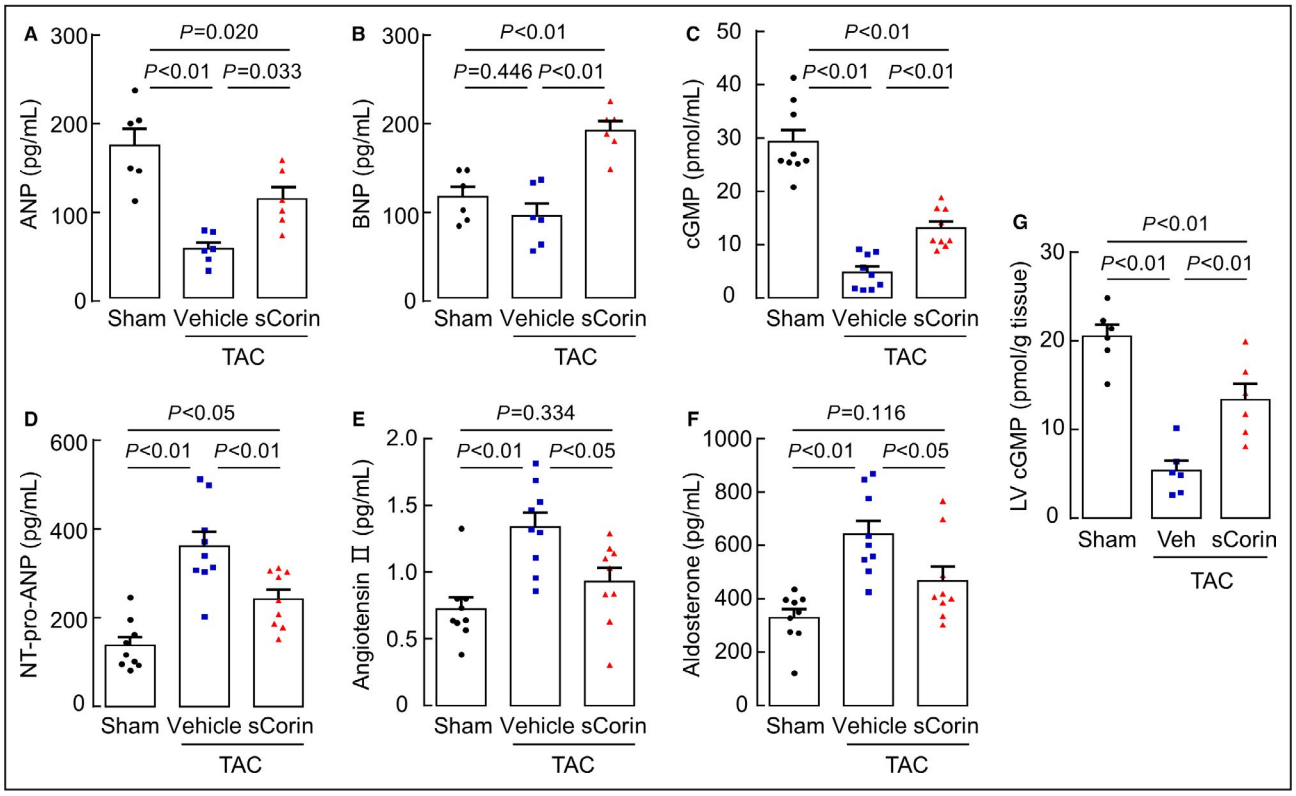


Figure 7. Analysis of plasma factors and left ventricular (LV) cGMP levels in sham-operated mice and mice with transverse aortic constriction (TAC) treated with vehicle or soluble corin (sCorin). Plasma samples were collected at 8 weeks after surgery.

Levels of ANP (**A**), BNP (**B**), cGMP (**C**), NT-pro-ANP (**D**), angiotensin II (**E**), and aldosterone (**F**) in plasma samples and cGMP levels in LV tissues (**G**) were analyzed by ELISA. Data are mean±SEM. *P* values were analyzed by 1-way ANOVA and Tukey's post hoc analysis. ANP indicates atrial natriuretic peptide; BNP, B-type natriuretic peptide; NT-pro-ANP, N-terminal proatrial natriuretic peptide; and Veh, vehicle.

vivo in promoting natriuretic peptide processing and activity, which enhances natriuretic peptide receptor-A signaling and suppresses the renin-angiotensin-aldosterone system. Importantly, we showed that sCorin treatment improved cardiac morphology and function in 2 independent mouse models of HF.

In addition to its systemic function in lowering blood volume and pressure, which reduces cardiac hypertrophy indirectly, ANP also has a direct antihypertrophic function in the heart.[5–7] The molecular mechanism underlying the direct antihypertrophic function of ANP is not fully elucidated. Recently, corin overexpression was shown to inhibit oxidative stress–induced apoptosis in cultured cardiomyocytes via a mechanism involving PI3K/AKT and NF-κB signaling pathways.[45] In transgenic mouse models, corin overexpression in the heart reduced cardiomyocyte death and the mortality caused by cardiomyopathy.[29,30] These data indicate a local corin-ANP signaling mechanism important for cardiomyocyte homeostasis and survival. Consistently, sCorin-treated mice had reduced cardiac hypertrophy and fibrosis, compared with those in vehicle-treated mice, indicating a beneficial effect of sCorin on cardiac morphology and function in the mouse HF models caused by acute MI and pressure overload.

The primary function of proteases is to cleave peptide bonds. Recently, it was reported that overexpression of a catalytically inactive corin mutant ameliorated cardiac function in mice with dilated cardiomyopathy.[46] This finding is intriguing, although the underlying biochemical basis remains unclear. In recent years, noncatalytic activities have been reported in other cell membrane–bound serine proteases, including prostasin[47,48] and matriptase-2,[49] which are crucial in epithelial function and iron metabolism, respectively.[50–52] Within the natriuretic peptide family, corin activates pro-ANP and pro-BNP, but not pro-C-type natriuretic peptide.[53] It is possible that corin has other noncatalytic function(s) that are yet to be discovered. Future studies are required to determine if a catalytically inactive form of soluble corin is efficacious in animal models of HF.

Strengths of this study include in vitro and pharmacokinetic characterization of sCorin and demonstration of beneficial effects of sCorin on natriuretic peptide activity and cardiac morphology and function in 2 independent mouse models of HF. Our experiments involve

multiple complementary approaches, including biochemical, cellular, histologic, and echocardiographic analyses. It should be pointed out that our study is mostly translational but not mechanism oriented. Future studies are required to understand the local versus systemic and the catalytic versus noncatalytic activities of corin in improving the function of failing hearts.

In conclusion, corin is a key protease in the natriuretic peptide system. Here, we show that a soluble form of recombinant corin was biologically active and had desired in vivo pharmacokinetic profiles. In 2 mouse models of HF, intraperitoneally administered sCorin enhanced natriuretic peptide activity, inhibited the renin-angiotensin-aldosterone system, reduced cardiac hypertrophy and fibrosis, and improved cardiac function. These results suggest that recombinant corin–based strategies may be used to develop new agents to treat HF.

ARTICLE INFORMATION

Received October 27, 2020; accepted February 8, 2021.

Affiliations

From the Cyrus Tang Hematology Center, Collaborative Innovation Center of Hematology, State Key Laboratory of Radiation Medicine and Prevention, The First Affiliated Hospital, Medical College, Soochow University, Suzhou, China (Y.N., S.Z., X.G., T.Z., F.L., M.L., Q.W., N.D.); MOH Key Laboratory of Thrombosis and Hemostasis, Jiangsu Institute of Hematology, Soochow University, Suzhou, China (Y.N., S.Z., X.G., F.L., N.D.); and Cardiovascular & Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, OH (Q.W.).

Acknowledgments

The authors thank Dr Zegeng Wang for helping with the MI model and echocardiography in mice.

Sources of Funding

This work was supported in part by grants from the National Natural Science Foundation of China (81873840, 81570457 to Dr Dong and 31500636 to Dr Zhou) and the Priority Academic Program Development of Jiangsu Higher Education Institutes (to Soochow University).

Disclosures

Dr Wu is an inventor on several corin-related patents that are owned by Bayer Healthcare. Dr Wu does not own Bayer stock and has not and will not receive any royalties from those patents. The remaining authors have no disclosures to report.

Supplementary Material

Figures S1–S8

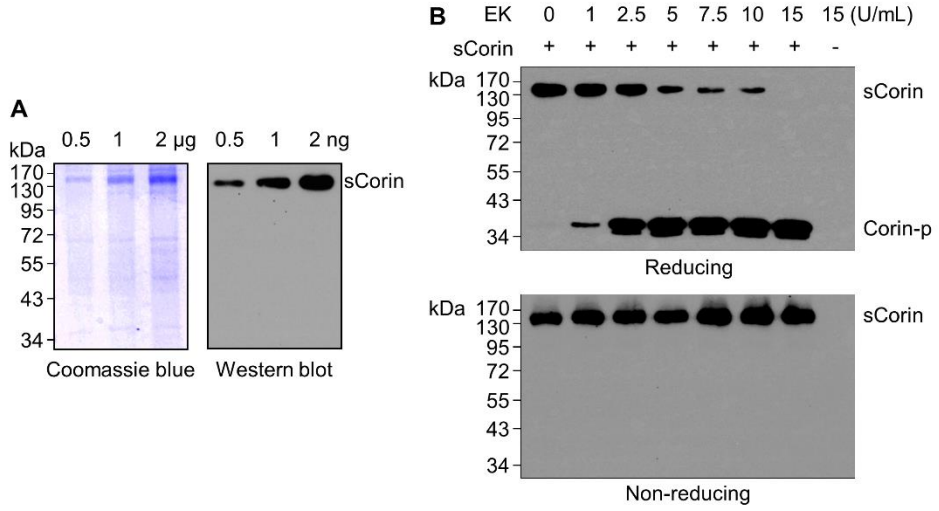
REFERENCES

1. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. Cardiac natriuretic peptides. *Nat Rev Cardiol*. 2020;17:698–717. DOI: 10.1038/s41569-020-0381-0.
2. Kuhn M. Molecular Physiology of membrane guanylyl cyclase receptors. *Physiol Rev*. 2016;96:751–804. DOI: 10.1152/physrev.00022.2015.
3. Song W, Wang H, Wu Q. Atrial natriuretic peptide in cardiovascular biology and disease (NPPA). *Gene*. 2015;569:1–6. DOI: 10.1016/j.gene.2015.06.029.
4. Chen W, Spitzl A, Mathes D, Nikolaev VO, Werner F, Weirather J, Špiranec K, Röck K, Fischer JW, Kämmerer U, et al. Endothelial actions of ANP enhance myocardial inflammatory infiltration in the early phase after acute infarction. *Circ Res*. 2016;119:237–248. DOI: 10.1161/CIRCRESAHA.115.307196.
5. Holtwick R, van Eickels M, Skryabin BV, Baba HA, Bubikat A, Begrow F, Schneider MD, Garbers DL, Kuhn M. Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-A. *J Clin Invest*. 2003;111:1399–1407. DOI: 10.1172/JCI17061.
6. Kinoshita H, Kuwahara K, Nishida M, Jian Z, Rong X, Kiyonaka S, Kuwabara Y, Kurose H, Inoue R, Mori Y, et al. Inhibition of TRPC6 channel activity contributes to the antihypertrophic effects of natriuretic peptides-guanylyl cyclase-a signaling in the heart. *Circ Res*. 2010;106:1849–1860. DOI: 10.1161/CIRCRESAHA.109.208314.
7. Knowles JW, Esposito G, Mao L, Hagaman JR, Fox JE, Smithies O, Rockman HA, Maeda N. Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor A-deficient mice. *J Clin Invest*. 2001;107:975–984. DOI: 10.1172/JCI11273.
8. Davidovski FS, Goetze JP. ProANP and proBNP in plasma as biomarkers of heart failure. *Biomark Med*. 2019;13:1129–1135. DOI: 10.2217/bmm-2019-0158.
9. Dries DL. Process matters: emerging concepts underlying impaired natriuretic peptide system function in heart failure. *Circ Heart Fail*. 2011;4:107–110. DOI: 10.1161/CIRCHEARTFAILURE.111.960948.
10. Xu-Cai YO, Wu Q. Molecular forms of natriuretic peptides in heart failure and their implications. *Heart*. 2010;96:419–424. DOI: 10.1136/hrt.2008.164145.
11. Dong N, Niu Y, Chen Y, Sun S, Wu Q. Function and regulation of corin in physiology and disease. *Biochem Soc Trans*. 2020;48:1905–1916. DOI: 10.1042/BST20190760.
12. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proc Natl Acad Sci USA*. 2000;97:8525–8529. DOI: 10.1073/pnas.150149097.
13. Buckley CL, Stokes AJ. Corin-deficient W-sh mice poorly tolerate increased cardiac afterload. *Regul Pept*. 2011;172:44–50. DOI: 10.1016/j.regpep.2011.08.006.
14. Chan JC, Knudson O, Wu F, Morser J, Dole WP, Wu Q. Hypertension in mice lacking the proatrial natriuretic peptide convertase corin. *Proc Natl Acad Sci USA*. 2005;102:785–790. DOI: 10.1073/pnas.0407234102.
15. Wang W, Cui Y, Shen J, Jiang J, Chen S, Peng J, Wu Q. Salt-sensitive hypertension and cardiac hypertrophy in transgenic mice expressing a corin variant identified in blacks. *Hypertension*. 2012;60:1352–1358. DOI: 10.1161/HYPERTENSIONAHA.112.201244.
16. Baird RC, Li S, Wang H, Naga Prasad SV, Majdalany D, Perni U, Wu Q. Pregnancy-associated cardiac hypertrophy in corin-deficient mice: observations in a transgenic model of preeclampsia. *Can J Cardiol*. 2019;35:68–76. DOI: 10.1016/j.cjca.2018.11.001.
17. Cui Y, Wang W, Dong N, Lou J, Srinivasan DK, Cheng W, Huang X, Liu M, Fang C, Peng J, et al. Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy. *Nature*. 2012;484:246–250. DOI: 10.1038/nature10897.
18. Vaiman D. At the core of preeclampsia genetics: key insights into the neurohormonal contribution to hypertensive diseases of pregnancy and their complications. *Can J Cardiol*. 2019;35:19–22. DOI: 10.1016/j.cjca.2018.11.026.
19. Wang C, Wang Z, He M, Zhou T, Niu Y, Sun S, Li H, Zhang C, Zhang S, Liu M, et al. Krüppel-like factor 17 upregulates uterine corin expression and promotes spiral artery remodeling in pregnancy. *Proc Natl Acad Sci USA*. 2020;117:19425–19434. DOI: 10.1073/pnas.2003913117.
20. Rame JE, Tam SW, McNamara D, Worcel M, Sabolinski ML, Wu AH, Dries DL. Dysfunctional Corin i555(p568) allele is associated with impaired brain natriuretic peptide processing and adverse outcomes in blacks with systolic heart failure: results from the Genetic Risk Assessment in Heart Failure substudy. *Circ Heart Fail*. 2009;2:541–548. DOI: 10.1161/CIRCHEARTFAILURE.109.866822.
21. Verstreken S, Delrue L, Goethals M, Bartunek J, Vanderheyden M. Natriuretic peptide processing in patients with and without left ventricular dysfunction. *Int Heart J*. 2019;60:115–120. DOI: 10.1536/ihj.18-012.
22. Zhao J, Lv T, Quan J, Zhao W, Song J, Li Z, Lei H, Huang W, Ran L. Identification of target genes in cardiomyopathy with fibrosis and cardiac remodeling. *J Biomed Sci*. 2018;25:63. DOI: 10.1186/s12929-018-0459-8.

23. Dong N, Chen S, Yang J, He L, Liu P, Zheng D, Li L, Zhou Y, Ruan C, Plow E, et al. Plasma soluble corin in patients with heart failure. *Circ Heart Fail*. 2010;3:207–211. DOI: 10.1161/CIRCHEARTFAILURE.109.903849.
24. Ibebuogu UN, Gladysheva IP, Houng AK, Reed GL. Decompensated heart failure is associated with reduced corin levels and decreased cleavage of pro-atrial natriuretic peptide. *Circ Heart Fail*. 2011;4:114–120. DOI: 10.1161/CIRCHEARTFAILURE.109.895581.
25. Yu R, Han X, Zhang X, Wang Y, Wang T. Circulating soluble corin as a potential biomarker for cardiovascular diseases: a translational review. *Clin Chim Acta*. 2018;485:106–112. DOI: 10.1016/j.cca.2018.06.036.
26. Yu Z, Lu X, Xu W, Jin M, Tao Y, Zhou X. Serum corin is associated with the risk of chronic heart failure. *Oncotarget*. 2017;8:100353–100357. DOI: 10.18632/oncotarget.22227.
27. Vinnakota S, Chen HH. The importance of natriuretic peptides in cardiometabolic diseases. *J Endocr Soc*. 2020;4:bvaa052. DOI: 10.1210/jendso/bvaa052.
28. Rademaker MT, Scott NJA, Koh CY, Kini RM, Richards AM. Natriuretic peptide analogues with distinct vasodilatory or renal activity: integrated effects in health and experimental heart failure. *Cardiovasc Res*. 2021;117:508–519. DOI: 10.1093/cvr/cvaa052.
29. Gladysheva IP, Wang D, McNamee RA, Houng AK, Mohamad AA, Fan TM, Reed GL. Corin overexpression improves cardiac function, heart failure, and survival in mice with dilated cardiomyopathy. *Hypertension*. 2013;61:327–332. DOI: 10.1161/HYPERTENSIONAHA.112.193631.
30. Sullivan RD, Houng AK, Gladysheva IP, Fan TM, Tripathi R, Reed GL, Wang D. Corin overexpression reduces myocardial infarct size and modulates cardiomyocyte apoptotic cell death. *Int J Mol Sci*. 2020;21:3456. 10.3390/ijms21103456.
31. Knappe S, Wu F, Masikat MR, Morser J, Wu Q. Functional analysis of the transmembrane domain and activation cleavage of human corin: design and characterization of a soluble corin. *J Biol Chem*. 2003;278:52363–52370. DOI: 10.1074/jbc.M309991200.
32. Yan W, Sheng N, Seto M, Morser J, Wu Q. Corin, a mosaic transmembrane serine protease encoded by a novel cDNA from human heart. *J Biol Chem*. 1999;274:14926–14935. DOI: 10.1074/jbc.274.21.14926.
33. Liu H, Wang X, Shi S, Chen Y, Han W. Efficient production of FAM19A4, a novel potential cytokine, in a stable optimized CHO-S cell system. *Protein Expr Purif*. 2015;113:1–7. DOI: 10.1016/j.pep.2015.05.004.
34. Gao E, Lei YH, Shang X, Huang ZM, Zuo L, Boucher M, Fan Q, Chuprun JK, Ma XL, Koch WJ. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. *Circ Res*. 2010;107:1445–1453. DOI: 10.1161/CIRCRESAHA.110.223925.
35. Hu P, Zhang D, Swenson L, Chakrabarti G, Abel ED, Litwin SE. Minimally invasive aortic banding in mice: effects of altered cardiomyocyte insulin signaling during pressure overload. *Am J Physiol Heart Circ Physiol*. 2003;285:H1261–H1269. DOI: 10.1152/ajpheart.00108.2003.
36. Dong L, Wang H, Dong N, Zhang C, Xue B, Wu Q. Localization of corin and atrial natriuretic peptide expression in human renal segments. *Clin Sci (Lond)*. 2016;130:1655–1664. DOI: 10.1042/CS20160398.
37. Nascimento DS, Valente M, Esteves T, de Pina MF, Guedes JG, Freire A, Quelhas P, Pinto-do-Ó P. MIQuant–semi-automation of infarct size assessment in models of cardiac ischemic injury. *PLoS One*. 2011;6:e25045. 10.1371/journal.pone.0025045.
38. Chen S, Cao P, Dong N, Peng J, Zhang C, Wang H, Zhou T, Yang J, Zhang Y, Martelli EE, et al. PCSK6-mediated corin activation is essential for normal blood pressure. *Nat Med*. 2015;21:1048–1053. DOI: 10.1038/nm.3920.
39. Kimura K, Yamaguchi Y, Horii M, Kawata H, Yamamoto H, Uemura S, Saito Y. ANP is cleared much faster than BNP in patients with congestive heart failure. *Eur J Clin Pharmacol*. 2007;63:699–702. DOI: 10.1007/s00228-007-0309-1.
40. Loscalzo J, Braunwald E. Tissue plasminogen activator. *N Engl J Med*. 1988;319:925–931. DOI: 10.1056/NEJM198810063191407.
41. Keyt BA, Paoni NF, Refino CJ, Berleau L, Nguyen H, Chow A, Lai J, Peña L, Pater C, Ogez J, et al. A faster-acting and more potent form of tissue plasminogen activator. *Proc Natl Acad Sci USA*. 1994;91:3670–3674. DOI: 10.1073/pnas.91.9.3670.
42. Jensen KT, Eiskjaer H, Carstens J, Pedersen EB. Renal effects of brain natriuretic peptide in patients with congestive heart failure. *Clin Sci (Lond)*. 1999;96:5–15. DOI: 10.1042/cs0960005.
43. Ozawa T, Shinke T, Shite J, Takaoka H, Inoue N, Matsumoto H, Watanabe S, Yoshikawa R, Otake H, Matsumoto D, et al. Effects of human atrial natriuretic peptide on myocardial performance and energetics in heart failure due to previous myocardial infarction. *J Cardiol*. 2015;66:232–238. DOI: 10.1016/j.jjcc.2014.12.020.
44. Ichiki T, Huntley BK, Sangaralingham SJ, Burnett JC Jr. Pro-atrial natriuretic peptide: a novel guanylyl cyclase-a receptor activator that goes beyond atrial and B-type natriuretic peptides. *JACC Heart Fail*. 2015;3:715–723. DOI: 10.1016/j.jchf.2015.03.015.
45. Li Y, Xia J, Jiang N, Xian Y, Ju H, Wei Y, Zhang X. Corin protects H(2)O(2)-induced apoptosis through PI3K/AKT and NF-κB pathway in cardiomyocytes. *Biomed Pharmacother*. 2018;97:594–599. DOI: 10.1016/j.biopha.2017.10.090.
46. Tripathi R, Sullivan RD, Fan TM, Houng AK, Mehta RM, Reed GL, Gladysheva IP. Cardiac-specific overexpression of catalytically inactive corin reduces edema, contractile dysfunction, and death in mice with dilated cardiomyopathy. *Int J Mol Sci*. 2019;21:203. 10.3390/ijms21010203.
47. Friis S, Uzzun Sales K, Godiksen S, Peters DE, Lin CY, Vogel LK, Bugge TH. A matriptase-prostasin reciprocal zymogen activation complex with unique features: prostasin as a non-enzymatic co-factor for matriptase activation. *J Biol Chem*. 2013;288:19028–19039. DOI: 10.1074/jbc.M113.469932.
48. Szabo R, Lantsman T, Peters DE, Bugge TH. Delineation of proteolytic and non-proteolytic functions of the membrane-anchored serine protease prostasin. *Development*. 2016;143:2818–2828. DOI: 10.1242/dev.137968.
49. Enns CA, Jue S, Zhang AS. The ectodomain of matriptase-2 plays an important nonproteolytic role in suppressing hepcidin expression in mice. *Blood*. 2020;136:989–1001. DOI: 10.1182/blood.2020005222.
50. Cui Y, Wu Q, Zhou Y. Iron-refractory iron deficiency anemia: new molecular mechanisms. *Kidney Int*. 2009;76:1137–1141. DOI: 10.1038/ki.2009.357.
51. Miller GS, List K. The matriptase-prostasin proteolytic cascade in epithelial development and pathology. *Cell Tissue Res*. 2013;351:245–253. DOI: 10.1007/s00441-012-1348-1.
52. Szabo R, Bugge TH. Membrane-anchored serine proteases as regulators of epithelial function. *Biochem Soc Trans*. 2020;48:517–528. DOI: 10.1042/BST20190675.
53. Wu C, Wu F, Pan J, Morser J, Wu Q. Furin-mediated processing of pro-C-type natriuretic peptide. *J Biol Chem*. 2003;278:25847–25852. DOI: 10.1074/jbc.M301223200.

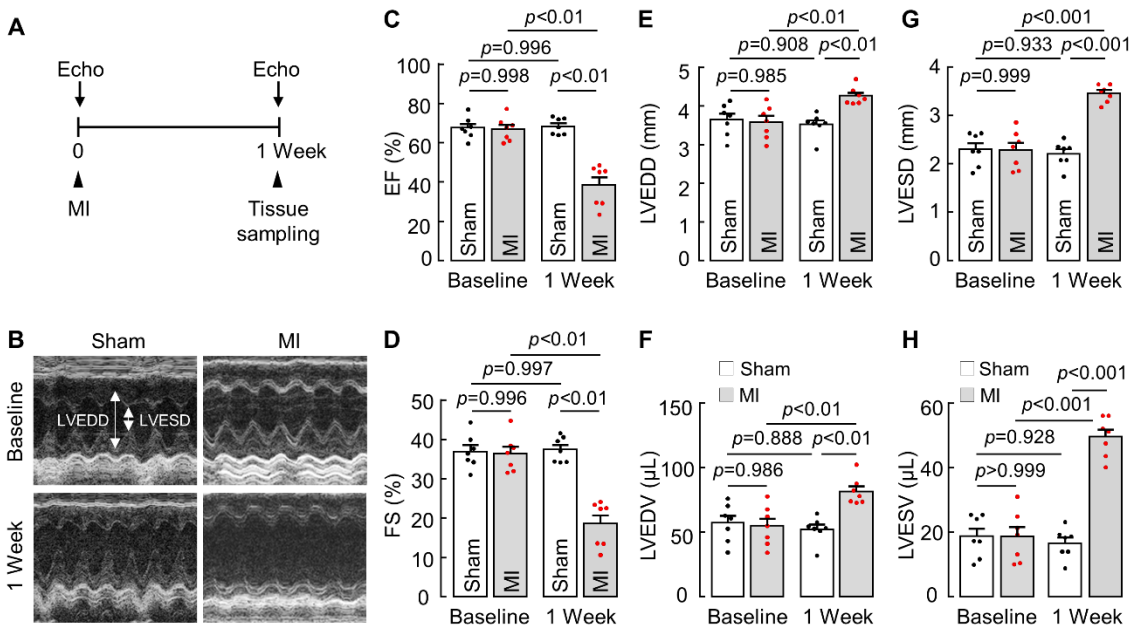
SUPPLEMENTAL MATERIAL

Figure S1. Analysis of purified and enterokinase (EK)-activated sCorin.



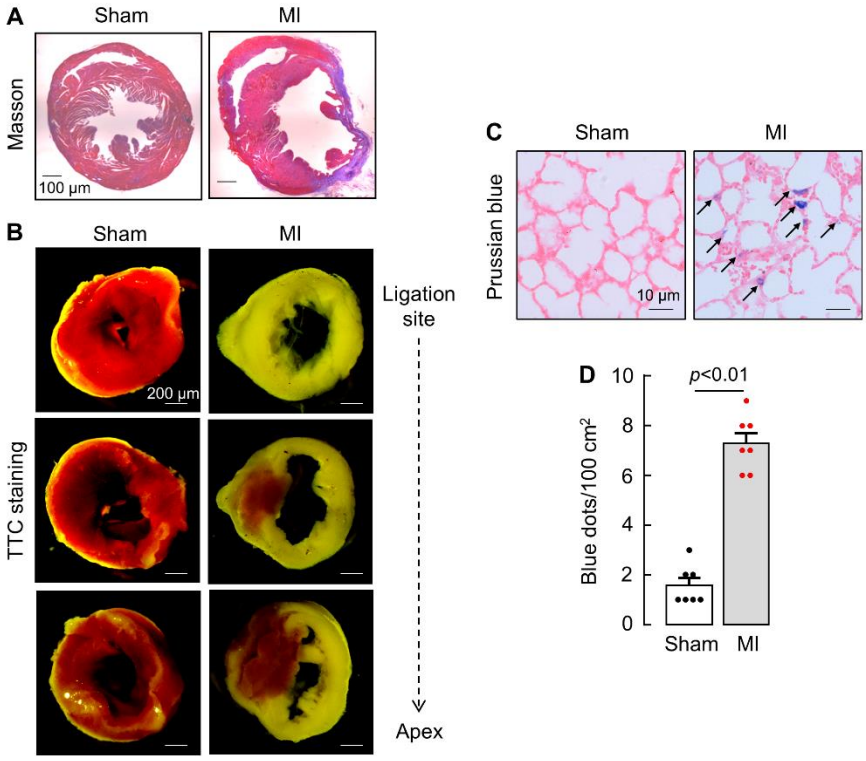
A, sCorin was expressed in CHO-K1 cells, purified by affinity chromatography, and analyzed by SDS-PAGE followed by Coomassie blue staining (left panel) and western blotting using an anti-V5 antibody (right panel). **B**, sCorin treated with increasing concentrations of EK was analyzed by western blotting under reducing (top panel) and non-reducing (lower panel) conditions. The corin protease domain fragment (corin-p) after activation cleavage is indicated. Data are representative at least three experiments.

Figure S2. Myocardial infarction (MI)-induced HF model in mice.



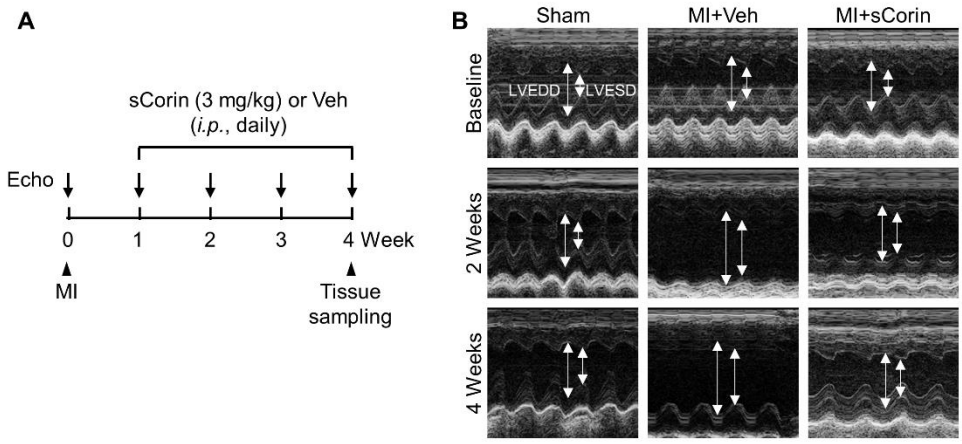
Left coronary artery ligation or sham operation was performed in male C57BL/6 mice (10-12-week old) (n = 7 per group). Echocardiography was used to assess cardiac function before (0 w) and one week post-surgery, as illustrated in (**A**). Representative echocardiographic images are shown in (**B**). Values of ejection fraction (EF) (**C**), fractional shortening (FS) (**D**), LV end diastolic dimension (LVEDD) (**E**) and volume (LVEDV) (**F**), and LV end systolic dimension (LVESD) (**G**) and volume (LVESV) (**H**) are shown. Data are mean ± SEM. *P* values were analyzed by one-way ANOVA and Tukey's post hoc analysis.

Figure S3. Analysis of heart and lung tissues in sham-operated mice and mice with MI caused by left coronary artery ligation.



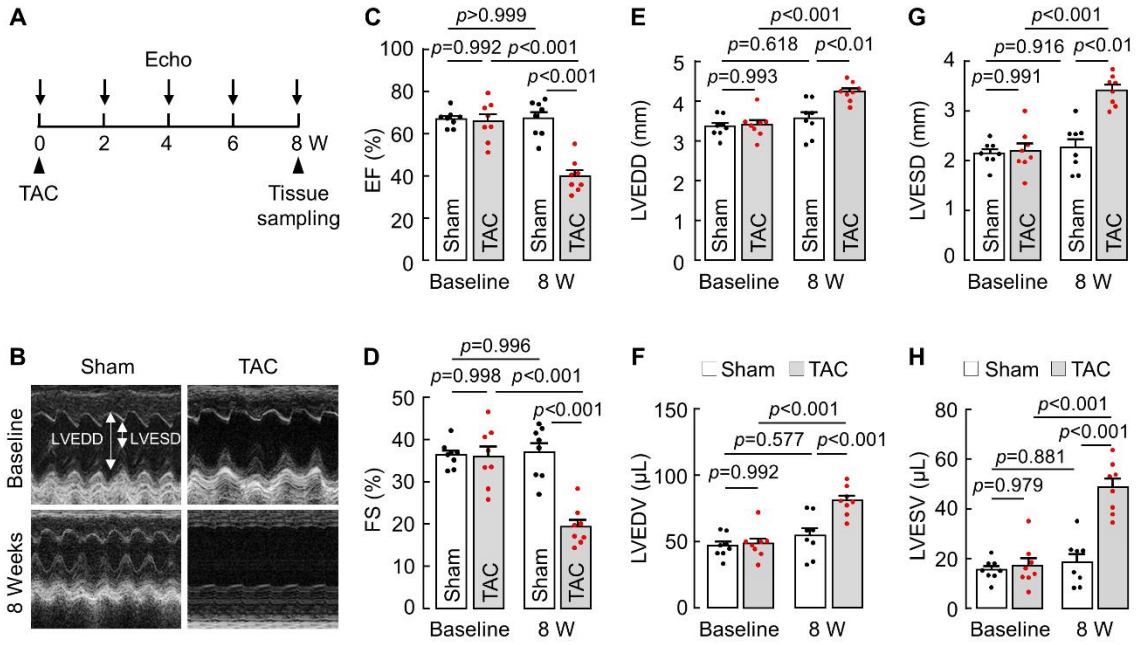
Tissues were collected at one week post-surgery. **A**, Representative heart sections stained by Masson's trichrome. Scar tissues are in blue. Scale bars: 100 μm. **B**, Serial heart sections from the left coronary artery ligation site forward the apex were stained by triphenyl tetrazolium chloride (TTC). Scale bars: 200 μm. **C**, Representative lung sections stained by Prussian blue to indicate iron-containing macrophages (arrows). **D**, Quantitative data of Prussian blue-stained lung sections. Data are mean ± SEM. *P* values were analyzed by Student's *t* test.

Figure S4. sCorin treatment in MI-induced mouse HF model.



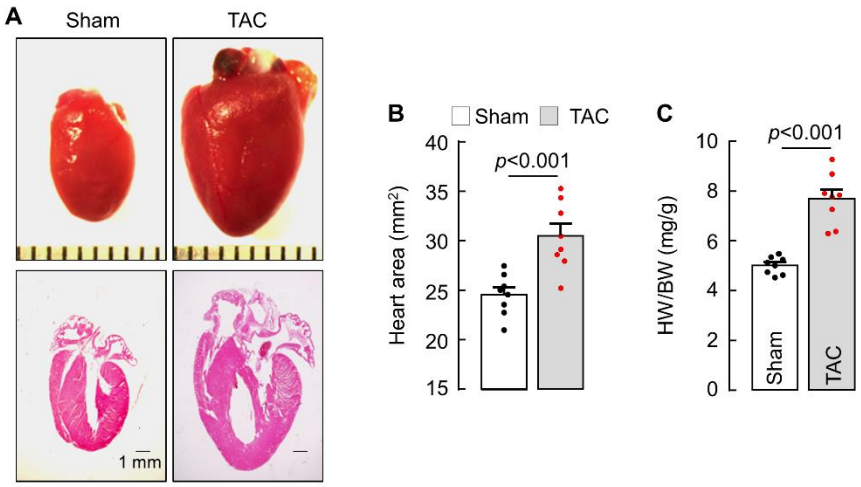
A, Illustration of echocardiography and sCorin or vehicle (Veh) treatment in mice. **B**, Representative echocardiographic images in sham-operated mice and mice with MI that were treated with vehicle (Veh) or sCorin.

Figure S5. Transverse aortic constriction (TAC)-induced HF model in mice.



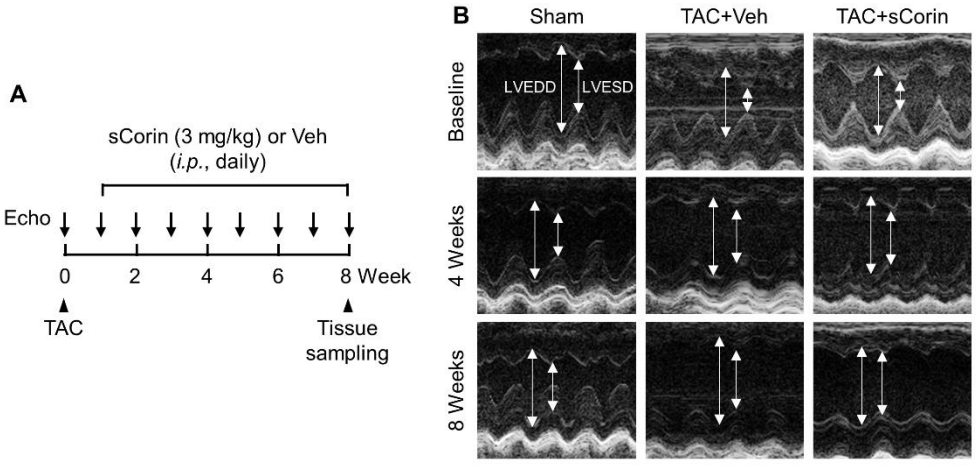
TAC or sham operation was performed in male C57BL/6 mice (10-12-week old) (n = 8 per group). Echocardiography was used to assess cardiac function before (baseline at 0 w) and after (2-8 w) the surgery, as illustrated in (**A**). Representative echocardiographic images are shown in (**B**). Values of ejection fraction (EF) (**C**), fractional shortening (FS) (**D**), LV end diastolic dimension (LVEDD) (**E**) and volume (LVEDV) (**F**), and LV end systolic dimension (LVESD) (**G**) and volume (LVESV) (**H**) at 8 weeks post-surgery are shown. Data are mean ± SEM. *P* values were analyzed by one-way ANOVA and Tukey's post hoc analysis.

Figure S6. Analysis of heart tissues in sham-operated mice and mice with TAC.



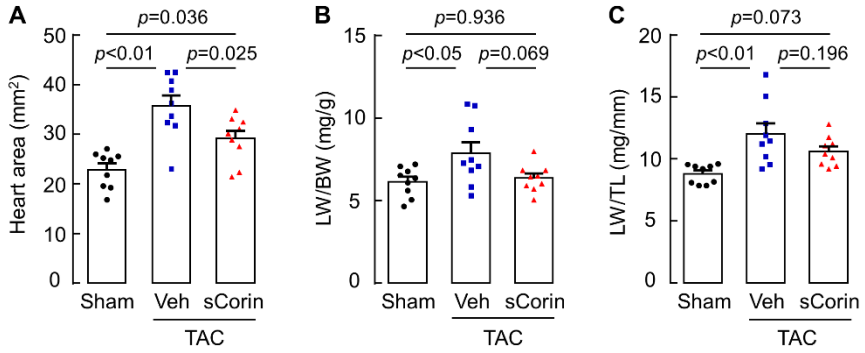
Tissues were collected at 8 weeks post-surgery. **A**, Representative heart images (top panels) and sections stained by H&E (lower panels). Scale bars: 1 mm. **B**, Relative heart sizes indicated by areas of heart images analyzed by Image-Pro-Plus software. **C**, Ratios of heart weight (HW) to body weight (BW). Data are mean ± SEM. *P* values were analyzed by Student's *t* test.

Figure S7. sCorin treatment in TAC-induced mouse HF model.



A, Illustration of echocardiography and sCorin or vehicle (Veh) treatment in mice. **B**, Representative echocardiographic images in sham-operated mice and mice with TAC that were treated with vehicle (Veh) or sCorin.

Figure S8. Analysis of hearts and lungs in sham-operated mice and mice with TAC.



A, Hearts from sham-operated mice and mice with TAC with vehicle (Veh) or sCorin treatment were collected at 8 weeks post-surgery. Relative heart sizes indicated by areas of heart images analyzed by Image-Pro-Plus software. **B** and **C**, Values of lung weight (LW) were normalized with body weight (BW) (**B**) or tibia length (TL) (**C**). Data are mean ± SEM. *P* values were analyzed by one-way ANOVA and Tukey's post hoc analysis.