Inhibition of Endostatin/Collagen XVIII Deteriorates Left Ventricular Remodeling and Heart Failure in Rat Myocardial Infarction Model

Kazuya Isobe, MD*; Keiji Kuba, MD**;†; Yasuhiro Maejima, MD*; Jun-ichi Suzuki, MD*;††; Shunichiro Kubota, MD‡; Mitsuaki Isobe, MD*

Background: Although therapeutic angiogenesis is a most promising strategy for the treatment of myocardial infarction (MI), it remains unknown if and how endogenous angiogenesis inhibitors, such as endostatin, regulate angiogenesis in MI. In the present study the role of endostatin in left ventricular (LV) remodeling and heart failure was tested in a rat MI model.

Methods and Results: When exposed to hypoxia, rat cardiomyocytes showed increased expression of endostatin. After MI induction in the rat MI model, endostatin expression was upregulated in cardiomyocytes, and serum endostatin levels were significantly elevated. Anti-endostatin antibody treatment resulted in significantly higher mortality of MI rats than controls. The MI rats with endostatin neutralization displayed adverse LV remodeling and severe heart failure compared with control MI rats. Although angiogenesis was increased, tissue remodeling and interstitial fibrosis were further exaggerated in post-MI hearts by endostatin neutralization. Furthermore, the expression and protease activity of matrix metalloproteinases -2 and -9, and of angiotensin-converting enzyme were markedly elevated by endostatin neutralization.

Conclusions: Neutralization of endostatin worsens the symptoms and outcomes of MI in a rat model. The results imply that endogenous endostatin/collagen XVIII may suppress aberrant LV remodeling and heart failure after MI. (Circ J 2010; 74: 109–119)

Key Words: Anti-angiogenesis; Endostatin; Heart failure; Left ventricular remodeling; Myocardial infarction

Therapeutic angiogenesis aims to increase coronary collateralization, which improves the prognosis of patients after myocardial infarction (MI) and other obstructive coronary artery diseases (CAD). Collateral limit infarct size and improve ventricular function and overall perfusion in the ischemic myocardium. The development of coronary collaterals appears to be initiated by an occlusive event, resulting in opening of preexisting anastomotic channels through increased shear forces and pressure or by formation of novel capillary sprouts (angiogenesis) from the ischemic area. Subsequent maintenance and maturation of these vessels (arteriogenesis) is believed to be mediated by a balance of pro- and anti-angiogenic factors that favor neovascularization. The concept of using the genes or proteins of pro-angiogenic growth factors (eg, vascular endothelial growth factor) for therapeutic angiogenesis is well established and currently being tested in clinics. On the other hand, the role of endogenous angiogenesis inhibitors in angiogenesis in ischemic heart disease and subsequent disease pathology and prognosis has been poorly investigated.

Editorial p45

Endostatin is an endogenous inhibitor of angiogenesis that was originally isolated from the culture supernatant of hemangoendothelioma cells. It is an endogenous 20-kD protein that is the non-collagenous carboxy-terminal fragment of collagen XVIII, produced through proteolytic cleavage by elastase, cathepsin L, and various other enzymes. Endostatin exhibits potent anti-angiogenic activity by inhibiting the proliferation and migration of endothelial cells and has been shown to inhibit the growth of many types of murine cancers.
by inhibiting tumor angiogenesis and inducing dormancy. A variety of malignancies are associated with elevated circulat ing serum endostatin levels. Recently, studies of human patients with CAD demonstrated that endostatin protein levels are significantly upregulated in the ischemic heart, which correlates with reduced angiogenesis and poorly developed collaterals. This implicates endostatin as an angiogenesis inhibitor in the angiogenesis of ischemic myocardium.

The physiological process of tissue remodeling and repair, which includes angiogenesis, is essential for compensating for the impaired function of hearts damaged by MI. However, this process frequently causes overt left ventricular (LV) remodeling of non-infarcted myocardium, leading to LV dilatation and associated interstitial fibrosis. This adverse LV remodeling is an independent determinant of morbidity and mortality in MI patients. Destruction and reconstitution of extracellular matrix (ECM) is a key feature of LV remodeling. Zinc-dependent matrix metalloproteinases (MMP), such as MMP2 and MMP9, play significant roles in ECM remodeling, and their levels are increased after MI in humans and animals. Cardiac dysfunction and mortality are significantly suppressed by MMP inhibitors, as well as in MMP2-null or MMP9-null mice, after MI. On the other hand, various vasoactive peptides, including angiotensin II and norepinephrine, have been recently identified as pro-inflammatory and directly promoting LV remodeling in MI. Angiotensin-converting enzyme (ACE) is a critical protease for the generation of angiotensin II in the renin–angiotensin system. Clinical studies demonstrate that ACE inhibitors (ACEI) significantly reduce the onset and mortality of MI. Inhibition of myocyte hypertrophy, collagen deposition and inflammation may contribute to these clinical beneficial effects of ACEI in addition to a reduction in cardiac afterload.

For this study, we hypothesized that inhibition of an endogenous angiogenesis inhibitor, endostatin, might be beneficial in improving heart function and thus mortality by facilitating neoangiogenesis in CAD. We first observed increased expression of endostatin mediated by hypoxia in infarcted myocardium of rats. We next examined the effects of endostatin inhibition on angiogenesis in the ischemic heart by using neutralizing anti-endostatin IgG.

**Methods**

**Reagents**

Endostatin polyclonal antibody was generated against the peptide sequence, IFSDGKDVLHPTWPQKV, from human endostatin/collagen XVIII in rabbits, and it specifically detects human and rat endostatin/collagen XVIII in Western blotting and immunohistochemistry. We found that endostatin induced apoptosis of calf pulmonary artery endothelial cells. The viability of cells induced by 1 μg/ml endostatin was 29.7±15.4% (P<0.001) at 48 h compared with that at 0 h (96.0±1.0%). The anti-endostatin antibody (10 μg/ml) significantly blocked the apoptosis induced by endostatin (unpublished data). Non-specific cytotoxic effects of the antibody on other cell types were not observed, and endotoxin levels were below detection limits determined by the limulus amoebocyte lysate assay (Cambrex).

**Rat MI Model**

Myocardial ischemia was surgically introduced in rats as described previously. Briefly, 7-week-old male Sprague–Dawley rats (250–300g) were anesthetized with inhaled pentobarbital sodium (40–60mg/kg) and intubated orally with a polyethylene tube for artificial respiration. After a thoracotomy was performed at the 4th intercostal space, the pericardium was gently removed to expose the heart. Myocardial ischemia was produced by ligation of the left anterior descending coronary artery (LAD). The chest was then closed, and the animals were allowed to recover in a warm, clean cage. The study was approved by the institutional Animal Research Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996).

**Treatment Protocol**

Animals were assigned randomly to 1 of 4 treatment groups: (1) anti-endostatin antibody injection (3mg/kg in sterile saline) twice weekly for 28 days (MI+anti-endostatin antibody group, n=20); (2) control IgG injection (intraperitoneally) twice weekly for 28 days (MI control group, n=20); (3) sham-operation with anti-endostatin antibody or (4) control IgG (thoracotomy with LAD isolation but without ligation, n=10 each).

**Echocardiography**

Transthoracic echocardiography was performed on animals anesthetized with pentobarbital sodium as described. An echocardiographic machine with a 7.5-MHz transducer (Nemio, Toshiba) was used for M-mode LV echocardiographic recording. A 2D-targeted M-mode echocardiogram was obtained along the short-axis view of the LV at the level of the papillary muscles. LV fractional shortening (LVFS) was calculated from the M-mode echocardiograms over 3 consecutive cardiac cycles according to the American Society for Echocardiography leading edge method. Measurements were made offline by 2 independent investigators.

**Measurement of Infarct Size and Histological Analysis**

Tissue sections of the LV were stained with Masson-trichrome stain, and the area stained blue was defined as the infarcted area. Infarct size was then calculated as the circumference of the infarct area divided by the total LV circumference (n=8, each group) as described. The area of myocardial fibrosis in the non-infarcted myocardium (middle portion of LV septal wall) (n=6; each group) was measured and corrected for the area of the microscopic field in a blinded manner at a magnification of ×200 with Scion Image 4.0.2 software (Meyer Instruments, Houston, TX, USA). Three fields of each section were averaged.

**Cell Culture and Treatment**

Neonatal cardiomyocytes from 1- or 2-day-old Wistar rats were isolated, subjected to Percoll gradient centrifugation and cultured in vitro as described previously. The cardiomyocytes were incubated in Eagle’s minimum essential medium (MEM) (Sigma) supplemented with 5% calf serum (JRH Biosciences, Lenexa, KS, USA) at 37°C. After the growth medium was changed, cells were exposed to hypoxia/reoxygenation conditions. To create a hypoxic stimuli, cells were exposed to severe hypoxic conditions in a Plexiglass chamber containing serum-free medium purged with a constant stream of water-saturated 95% N2/5% CO2. The cells were subsequently incubated under normoxic conditions with 5% calf serum medium for reperfusion. Zero time point was assigned at the beginning of the reperfusion step.

**Confocal Immunofluorescent Analysis**

Frozen sections and cells were stained with specific first anti-
bodies (endostatin, tropomyosin, or von Willebrand Factor) followed by incubation with Alexa Fluor 488 (FITC), or 568 (Rhodamine)-conjugated secondary antibodies (Molecular Probes). Nuclei were stained with DAPI. Sections were mounted using a Perma Fluor (Thermo Shandon) and observed under LSM510 laser scanning confocal microscope (Carl Zeiss).

Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)
Real-time RT-PCR was performed with the following primers: GAPDH (5'-AATGTATCCGTGTGGATCTGA-3' and 5'-GCTTACACCACCTCTGTGATG-3'), type XVIII collagen (5'-CTTTTCGATGCGAGACAGC-3' and 5'-AGCCACAGCATACGCTT-3'), MMP2 (5'-GCACACGCAGAGTATGAC-3' and 5'-ACCCACAGTGCACTGAC-3'), MMP9 (5'-CCTCTGCAAGAGACGAGACATAA-3' and 5'-GGTCAGGGTTAGACGCCAG-3'), collagen I (5'-CACCCGCAGGAGACAGA-3' and 5'-GACCCAGGGAGACAGA-3'), collagen III (5'-CTGCCCGCGAGAGAGG-3' and 5'-ATGTCCTGCGAGAGAGG-3') and collagen XVIII (5'-CGACGTCG-3' and 5'-GACGTCG-3'). Briefly, 1 μg of DNase-treated total RNA extracted from either the rat heart or cardiomyocytes was reverse transcribed using First-Strand Beads (GE Healthcare) with random primers. SYBR green real-time PCR reactions were performed with an iQ-Cycler (Bio-Rad).

Western Blot Analysis and ELISA
Rat serum was collected intravenously (n=8 each) and the concentration of endostatin was determined with an ELISA kit (BioSource International, Camarillo, CA, USA) according to the manufacturer’s instructions. Western blotting was performed for cell and tissue lysates using anti-endostatin antibody and anti-β-actin antibody (MAB3128; Millipore).
Bands were visualized with an enhanced chemiluminescence system (GE Healthcare).

**Gelatin Zymography**

Tissues were snap-frozen in liquid nitrogen and homogenized in ice-cold lysis buffer (containing 50 mmol/L Tris-HCl buffer (pH 7.6), 0.15 mol/L NaCl, 10 mmol/L CaCl2, 0.05% Brij 35, 0.02% NaN3). Samples were then centrifuged, the supernatant collected, and the protein concentration was determined with a BCA protein assay (Pierce Biotechnology). The protein samples (each 25 μg) were separated with 6% gelatin gel, which was renatured by zymogram renaturing buffer (Invitrogen). The gel was stained with Coomassie Blue, and MMP activity was measured as described previously.28

**ACE Activity Assay**

Tissue ACE activity was measured with a colorimetric assay (ALPCO Diagnostics, Windham, NH, USA), using the

---

**Figure 2.** Hypoxia-induced expression of endostatin in neonatal rat cardiomyocytes in vitro. (A) Immunofluorescent staining of collagen XVIII/endostatin in neonatal rat cardiomyocytes after 24-h culture under hypoxic conditions. Red: troponin I; Green: endostatin; Blue: DAPI. (B) Time-dependent increase in collagen XVIII mRNA expression in hypoxic cardiomyocytes. Total RNA was isolated from neonatal rat cardiomyocytes and subjected to real-time PCR analyses. Collagen XVIII levels were normalized to control GAPDH mRNA (n=12 per group). (C, D) Endostatin protein in culture supernatants and cell lysates of neonatal rat cardiomyocytes under hypoxia. Endostatin (20 kDa) in supernatant (C) and cell lysate (D) of cardiomyocytes measured by Western blotting. β-actin is shown as loading control. All values B–D are mean±SEM; *P<0.01.
synthetic substrate, N-hippuryl-L-histidyl-L-leucine. Tissue extracts was incubated with substrate, and the released hippuric acid was colorimetrically measured at the absorbance of 382 nm. Results are expressed as ACE units, defined as the amount of enzyme required to release 1 μmol/L of hippuric acid per min per L tissue extract. Samples were measured in duplicate.

**Results**

**Expression of Endostatin in MI**

We first measured the serum endostatin levels in rats with MI, in which LV infarction had been induced by surgical ligation of the LAD. Consistent with the results from a recent study of MI patients, the serum endostatin level was significantly increased at 24 h after MI as compared with sham-operated control rats (Figure 1A). Because endostatin is generated from the C-terminus of collagen XVIII by proteolytic cleavage, we next determined the mRNA expression of collagen XVIII in the heart by real-time RT-PCR. Collagen XVIII expression was markedly elevated in the ischemic heart (Figure 1B). Consistently, immunofluorescent staining of endostatin in the ischemic area (Figure 1C). To further examine the hypoxic induction of endostatin expression in cardiomyocytes, we exploited an in vitro cardiomyocyte primary culture system. In vitro rat neonatal cardiomyocytes showed higher immunoreactivity for anti-endostatin antibody under hypoxic condition (0% oxygen) than those in control normoxia (Figure 2A). Both the mRNA and protein of collagen XVIII accumulated in cardiomyocytes in a time-dependent manner in response to hypoxia (Figure 2B). Notably, endostatin protein levels in both culture supernatant and cell lysates (20kDa in molecular weight) were significantly upregulated by hypoxic stimuli (Figures 2C, D). Thus, both transcriptional induction and proteolytic cleavage of collagen XVIII are likely to increase the expression of collagens XVIII.

**Table. Infarct Size, Organ Weights, Plasma BNP, and Echocardiographic Assessment**

<table>
<thead>
<tr>
<th></th>
<th>Sham + control IgG</th>
<th>Sham + anti-endostatin IgG</th>
<th>MI + control IgG</th>
<th>MI + anti-endostatin IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>0</td>
<td>0</td>
<td>50.1±1</td>
<td>50.7±2.2</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>350±12</td>
<td>350±18</td>
<td>330±22</td>
<td>336±26</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>90±5</td>
<td>92±4</td>
<td>83±4</td>
<td>80±5</td>
</tr>
<tr>
<td>BW (g)</td>
<td>378±22</td>
<td>386±12</td>
<td>388±24</td>
<td>392±23</td>
</tr>
<tr>
<td>Lung/BW (mg/g)</td>
<td>3.6±0.4</td>
<td>3.5±0.3</td>
<td>4.1±0.1</td>
<td>6.0±0.6*</td>
</tr>
<tr>
<td>LV/BW (mg/g)</td>
<td>2.6±0.2</td>
<td>2.6±0.3</td>
<td>2.6±4</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.4±0.1</td>
<td>0.4±0.2</td>
<td>0.5±0</td>
<td>0.6±0.0*</td>
</tr>
<tr>
<td>Plasma BNP (pg/ml)</td>
<td>ND</td>
<td>ND</td>
<td>110.7±6.3</td>
<td>166.6±12.9*</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>5.2±0.1</td>
<td>5.0±0.2</td>
<td>8.6±0.1</td>
<td>9.7±0.3*</td>
</tr>
<tr>
<td>LVEDA (mm²)</td>
<td>23.1±2.5</td>
<td>24.0±3.5</td>
<td>55.2±3.4</td>
<td>73.2±3.2*</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>80.0±6.1</td>
<td>79.0±7.0</td>
<td>41.0±2.7</td>
<td>37.7±2.9*</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>49.1±1.7</td>
<td>48.5±1.9</td>
<td>19.6±1.2</td>
<td>17.3±1.6*</td>
</tr>
</tbody>
</table>

Values are mean±SE. *P<0.05 vs MI+control IgG.

BNP, brain natriuretic peptide; MI, myocardial infarction; HR, heart rate; BP, blood pressure; BW, body weight; LV, left ventricular weight; RV, right ventricular weight; ND, not done; LVEDD, left ventricular end-diastolic dimension; LVEDA, left ventricular end-diastolic area.
endostatin in hypoxic cardiomyocytes. Accordingly, endostatin expression is upregulated in both the cardiomyocytes of ischemic hearts in vivo and the cultured cardiomyocytes under hypoxia in vitro.

Anti-Endostatin Antibody Treatment in Rat MI
To dissect the functional role of endostatin in MI, we evaluated the effects of the neutralizing antibody for endostatin on the mortality of rats with MI. The survival rate within 24 h of ligation of the LAD was ~40%. Surviving rats were assigned to the MI+control IgG-treatment group (n=20) or the MI+anti-endostatin antibody-treatment group (n=20). The survival rate up to 28 days was significantly lower in the MI+anti-endostatin antibody-treatment group than in the MI+control IgG-treatment group (P<0.05). The number of rats that survived up to 28 days was 13 in the MI+control IgG-treatment group and 8 in the anti-endostatin antibody-treatment group (Figure 3). However, there were no differences in infarct size at day 28 after MI; the MI+control IgG-treatment group had an average infarct size of 50.1±1.6%, and the MI+anti-endostatin antibody-treatment group had an average infarct size of 50.7±2.2% (P=0.99) (Table). This result suggests that the higher mortality after anti-endostatin antibody-treatment is because of post-MI pathological events, such as heart failure (HF) and/or LV remodeling, but not because of a difference in the area of initial ischemia induced by coronary artery ligation.

Severe HF and Enhanced LV Remodeling After Neutralization of Endostatin
At 28 days after the induction of MI, the right ventricle weight-to-body weight ratio and lung weight-to-body weight ratio were significantly higher in the MI+anti-endostatin antibody-treatment group than in the MI+control IgG-treatment group (Figure 4). Impaired cardiac function of post-myocardial infarction hearts by neutralization of endostatin. (A) M-mode and short-axis echocardiograms of the MI+anti-endostatin IgG group and MI+control IgG group 28 days after MI induction by coronary artery ligation. (B, C) MI+anti-endostatin IgG group show dilated left ventricles compared with the MI+control IgG group. MI+anti-endostatin IgG group show increased left ventricular diameter in the diastolic phase (LVEDD) (B) and decreased fractional shortening (C) compared with the MI+control IgG group (n=8 per group). Values are mean±SEM. *P<0.05 vs MI+control IgG group.
ratio were significantly greater in the MI group than in the sham-operated group (Table). Treatment with anti-endostatin antibody increased both ratios and also upregulated plasma B-type natriuretic peptide (BNP) levels remarkably in comparison with the vehicle-treated MI group (Table), indicating that development of HF was augmented by anti-endostatin antibody. We next examined heart function by 2-dimensional and M-mode echocardiography. In the MI rats, the LV end-diastolic diameter increased and percent fractional shortening (FS) decreased significantly compared with...
sham-operated animals at day 28 after ligation of the LAD (Table). Consistent with the higher mortality, anti-endostatin antibody-treatment further increased the LV diameter and significantly impaired contractile function at 28 days after MI compared with vehicle-treated rats (Figure 4, Table).

The impaired heart function caused by neutralization of endostatin was already seen at 3 days after MI induction and persisted over the observation period of days 3 to 28 (data not shown). In addition, anti-endostatin antibody decreased the wall thickness in the infarcted region while it increased...
Role of Endostatin in Rat MI

that of the non-infarcted areas (Figure 5A). Moreover, histological quantification of myocyte cross-sectional area demonstrated that anti-endostatin antibody further increased the size of cardiomyocytes in MI, suggesting that cardiac hypertrophy in the post-MI heart is promoted by endostatin blockade (Figure 5B). Therefore, endostatin neutralization enhances LV remodeling and augments impaired heart contractility in MI.

**Enhanced Angiogenesis and Tissue Remodeling Induced by Endostatin Inhibition**

To address the mechanisms of severe HF and enhanced LV remodeling by endostatin neutralization, we evaluated angiogenesis by quantifying von Willebrand factor-positive capillary blood vessels in the infarcted heart (Figures 5C, D). As expected from the anti-angiogenic property of endostatin, its neutralization significantly increased newly forming blood vessels in the post-MI heart. However, when tissue fibrosis was measured by Masson-trichrome staining, endostatin-neutralized hearts showed increased interstitial fibrosis in the non-infarcted areas compared with control IgG-treated hearts (Figures 5E, F). Thus, endostatin inhibition certainly enhances angiogenesis in the post-MI heart, but not enough to exert beneficial effects. Endostatin neutralization rather

![Figure 7.](image-url)
strongly stimulates tissue remodeling and fibrosis and thereby augments HF in MI.

**Protease Activities of MMP2, MMP9 and ACE After Endostatin Neutralization**

In the post-MI heart, MMPs play a crucial role in LV remodeling. In the present study, gel gelatin zymography showed that the gelatinase activity of MMP9 (95/88 kDa) and MMP2 (72/66 kDa) was significantly increased in the myocardium of the MI group at 7 days after induction of MI compared with the sham-operated group. These increased activities of MMP2 and MMP9 were further enhanced in the MI + anti-endostatin antibody-treatment group compared with the control IgG-treatment MI group (Figure 6A). The mRNA expression levels of MMP2, MMP9, collagen I and collagen III were consistently upregulated in post-MI heart tissue, and anti-endostatin antibody further increased the MMPs expression (Figures 6A–C). Thus, endostatin neutralization enhances matrix proteinase activity in MI.

Furthermore, we investigated the activity of ACE in the myocardium, because it is a key molecule of the renin–angiotensin system, which promotes post-MI HF. The mRNA expression and activity of ACE were significantly upregulated by treatment with anti-endostatin antibody, as shown by immunohistochemistry, ACE activity assay and real-time quantitative RT-PCR (Figures 7A–C). ACE activity was remarkably increased by anti-endostatin antibody (Figure 7C). Therefore, multiple proteinase activities, such as MMP2, MMP9 and ACE, are elevated by endostatin inhibition in MI, leading to enhanced LV remodeling, impaired heart function, and higher mortality.

**Discussion**

The present study provides the first evidence of the physiological role of endogenous endostatin/collagen XVIII in LV remodeling and HF after MI. Rat cardiomyocytes in both in vitro hypoxic conditions and in-vivo infarcted hearts showed upregulated expression of endostatin/collagen XVIII through transcriptional induction of collagen XVIII expression. Of remarkable interest was our finding that inhibition of endostatin by its neutralizing antibody enhanced LV remodeling and severe HF and thus increased mortality. Although endostatin neutralization promoted neangiogenesis in the myocardium, it dramatically induced tissue fibrosis and remodeling, leading to worse outcomes. Importantly, the expression and activities of ACE, MMP2 and MMP9 are significantly increased by endostatin inhibition. These results suggest that endogenous endostatin/collagen XVIII may play a pivotal role in protecting the myocardium from pathological LV remodeling and contractile dysfunction in MI.

Angiogenesis is a critical physiological phenomenon for coronary collateralization and functional compensation in the ischemic heart of CAD patients. Endostatin exhibits potent anti-angiogenic activity by inhibiting the proliferation and migration of endothelial cells and our results clearly demonstrated the pro-angiogenic effects of endostatin inhibition in MI. However, endostatin inhibition-mediated angiogenesis did not improve but rather worsened LV remodeling and function. Because treatment of sham-operated rats with anti-endostatin IgG showed no alteration in their hearts’ function or histological appearance, endostatin/collagen XVIII does not seem important for cardiac homeostasis under normal conditions. In mouse gene-targeting studies, collagen XVIII/endostatin-null mice are apparently normal except for iris abnormalities, which reflects human pigment dispersion syndrome. In contrast, in atherosclerosis models, endostatin/collagen XVIII expression is elevated in the aorta, and loss of collagen XVIII enlarged plaque lesions through enhanced neovascularization and vascular permeability. Thus, in the cardiovascular system, anti-angiogenesis function of endostatin is likely to be exerted in pathological settings leading to cardiovascular protection.

One important question we addressed in this study is how endostatin inhibition deteriorates LV remodeling and HF. Based on a previous study of genetic abrogation of endostatin in atherosclerosis, it can be speculated that endostatin inhibition may also generate prematurely leaky blood vessels and increase vascular permeability in MI. However, our results clearly indicated the obvious interstitial fibrosis of the non-infarcted myocardium, increased LVEDD and increased levels of the proteases MMP2, MMP9 and ACE in the myocardium after endostatin inhibition. Similar to these results, endostatin was previously shown to block the activation of MMP2, MMP9 and MMP13. Tissue levels of MMPs have been shown to increase remarkably after MI in human patients and animal models. Pharmacological and genetic inhibition of MMP2 and MMP9 improves LV remodeling and dysfunction after MI. However, little is known about any effects of endostatin on ACE activity, although ACE inhibitors or angiotensin II receptor blockers are currently used for CAD patients in clinical practise. Interestingly, a recent study showed that the internal peptide sequence of endostatin resembles an ACE-inhibitory peptide, called bradykinin-potentiating peptide, and that the synthetic peptide of the sequence indeed inhibited ACE catalytic activity. Thus, endostatin or its degraded peptide fragments are implicated as a putative ACEI. Taking all the evidence together, endostatin is a negative regulator of protease-mediated adverse tissue remodeling in the infarcted heart. Nevertheless, further studies are required to understand the molecular mechanisms of endostatin-mediated protease regulation.

The clinical relevance of our findings is strongly supported by recent studies of endostatin in human CAD patients. Pericardial fluid levels of endostatin harvested from CAD patients were significantly lower in hearts with well-developed collaterals than in those with poor collateralization. In addition, endostatin was detected in the coronary circulation of CAD patients, and increased coronary endostatin levels correlated with severe coronary stenosis and poor collateral development. Thus, it can be speculated that physiological induction of endostatin expression might compensate for cardiac stress in MI, and is downregulated after sufficient compensation of the infarcted heart’s functions.

In summary, this study provides the first evidence of physiological cardioprotective functions of endostatin/collagen XVIII in suppressing adverse LV remodeling and HF in rat MI. Although the detailed molecular mechanisms of these endostatin functions remain to be elucidated in future studies, our results suggest a potential new therapeutic strategy of using endostatin/collagen XVIII in CAD patients.

**Acknowledgment**

The authors acknowledge the assistance of Ms Keiko Yasuda with the endostatin ELISA assay.

**Funding**

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
Role of Endostatin in Rat MI

Disclosures

All authors report no conflicts.

References