Improvement in Left Ventricular Remodeling by the Endothelial Nitric Oxide Synthase Enhancer AVE9488 After Experimental Myocardial Infarction

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Background—Reduced endothelial nitric oxide (NO) bioavailability contributes to the progression of heart failure. In this study, we investigated whether the transcription enhancer of endothelial NO synthase (eNOS) AVE9488 improves cardiac remodeling and heart failure after experimental myocardial infarction (MI).

Methods and Results—Starting 7 days after coronary artery ligation, rats with MI were treated with placebo or AVE9488 (25 ppm) as a dietary supplement for 9 weeks. AVE9488 therapy versus placebo substantially improved left ventricular (LV) function, reduced LV filling pressure, and prevented the rightward shift of the pressure-volume curve. AVE9488 also attenuated the extent of pulmonary edema, reduced LV fibrosis and myocyte cross-sectional area, and prevented the increases in LV gene expression of atrial natriuretic factor, brain natriuretic peptide, and endothelin-1. eNOS protein levels and calcium-dependent NOS activity were decreased in the surviving LV myocardium from placebo MI rats and normalized by AVE9488. The beneficial effects of AVE9488 on LV dysfunction and remodeling after MI were abrogated in eNOS-deficient mice. Aortic eNOS protein expression and endothelium-dependent NO-mediated vasorelaxation were significantly enhanced by AVE9488 treatment after infarction, whereas increased vascular superoxide anion formation was reduced. Moreover, AVE9488 prevented the marked depression of circulating endothelial progenitor cell levels in rats with heart failure after MI.

Conclusions—Long-term treatment with the eNOS enhancer AVE9488 improved LV remodeling and contractile dysfunction after MI. Molecular alterations, circulating endothelial progenitor cell levels, and endothelial vasomotor dysfunction were improved by AVE9488. Pharmacological interventions designed to increase eNOS-derived NO constitute a promising therapeutic approach for the amelioration of postinfarction ventricular remodeling and heart failure. (Circulation. 2008;118:818-827.)

Key Words: endothelium □ heart failure □ myocardial infarction □ nitric oxide □ nitric oxide synthase □ remodeling

Cardiac remodeling after myocardial infarction (MI) involves myocyte hypertrophy, chamber dilation, and interstitial fibrosis. Alterations in cardiomyocytes and collagen matrix lead to contractile dysfunction and contribute to the progression of ventricular enlargement and heart failure. Nitric oxide (NO) generated by endothelial NO synthase (eNOS) plays an important role in the pathophysiology of postinfarction ventricular remodeling and heart failure. NO regulates vascular tone and cardiomyocyte contractility and protects against myocyte apoptosis and hypertrophy. Moreover, NO acts as an antithrombogenic and antiatherogenic agent by inhibiting vascular smooth muscle proliferation, platelet aggregation, and leukocyte adhesion.

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Genetic ablation of eNOS impaired contractile function and promoted left ventricular (LV) hypertrophy and dilatation after infarction. The beneficial effects of angiotensin-converting enzyme inhibition and AT1 antagonists on LV remodeling were almost abolished in mice lacking eNOS, emphasizing the importance of eNOS-derived NO in the cardioprotective effects of various established pharmacological interventions. On the other hand, infarcted mice with targeted overexpression of the eNOS gene within the vascular endothelium had improved survival and reduced heart failure progression. In addition, cardiomyocyte-specific eNOS over-
expression\textsuperscript{10} improved cardiac performance and attenuated myocyte hypertrophy after MI.

Therefore, compounds that specifically upregulate eNOS appear to be a useful tool for improving LV remodeling and function after infarction. In the present study, we investigated whether long-term treatment with AVE9488, a low-molecular-weight compound shown to enhance eNOS expression and NO production,\textsuperscript{11} improves cardiac remodeling and heart failure after MI in rats.

**Methods**

All procedures conformed to the guiding principles of the American Physiological Society and were approved by the institutional Animal Research Committee.

**Pharmacokinetics of AVE9488 in Rats**

The pharmacokinetics of AVE9488 was studied in male Wistar rats. After a single oral dose of AVE9488 (2 mg/kg), the serum area under the curve was 4,844 hours, maximum plasma concentration (Cmax) was 0.598 ng/mL, time to reach Cmax (tmax) was 0.5 hours, elimination half-life (t1/2) was 4.8 hours, and bioavailability was 83.8%.

**MI and Study Protocols**

Left coronary artery ligations were performed in adult male Wistar rats.\textsuperscript{11} Seven days after coronary ligation or sham operation, rats were randomized to receive either placebo (untreated) or AVE9488 (25 or 250 ppm) for 9 weeks. In preliminary experiments, long-term treatment with AVE9488 at a low (25 ppm) and high (250 ppm) dose led to similar effects on hemodynamics, LV remodeling, and endothelial function. Therefore, we used the treatment with 25-ppm AVE9488 for further analyses. Treatments were given as food additives. Twice weekly, rats were weighed and their food intake was measured. The average drug dose of AVE9488 received by the rats was 1.25 mg per 1 kg body weight per day.

**Hemodynamic and Volume Measurements**

Hemodynamic and volumetric measurements were performed after 9 weeks of treatment. Mean arterial pressure, LV systolic and end-diastolic pressures, dP/dt, and heart rate were measured under intraperitoneal pentobarbital anesthesia (30 mg/kg body weight) as described.\textsuperscript{13} The time constant of LV pressure isovolumic decay [r, regression of log(pressure) versus time] was calculated by the Weiss method. Correlation coefficients for all studies were \( r > 0.99 \). The in vivo LV pressure-volume relationship was analyzed with a conductance catheter (SPR-774, Millar Instruments, Houston, Tex.). Pressure-volume signals were acquired by BioBench software (National Instruments, Austin, Tex.). Pyan software (Millar) was used to analyze all pressure-volume loop data recorded at steady state and during injection of hypertonic saline for the calibration of parallel conductance volume (\( V_p \)). Dilution maneuvers with a regression coefficient and/or percentage amplitude <85% and 15%, respectively, were rejected. LV volume showed intravariability in the range of 2% to 5%. LV volume was calculated for each rat from the conductance volume corrected by the relative \( V_p \) as described.\textsuperscript{12}

**Infarct Size and Pulmonary Edema**

The right ventricle and LV, including the septum, were separated in the cold on ice and weighed. Infarct size was quantified histologically by planimetry. The LV was cut into 3 transverse sections: apex, middle ring (~3 mm), and base. From the middle ring, 5- \( \mu \)m sections were cut at 100- \( \mu \)m intervals and stained with picrosirius red.\textsuperscript{12} Pulmonary edema was assessed as net fluid weight (difference between the wet and dry weights).

**Plasma Norepinephrine**

After hemodynamic measurement, a blood sample was collected from the right carotid artery. Plasma norepinephrine was measured with high-performance liquid chromatography.\textsuperscript{12}

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**Table 1. Sequences of PCR Primers and Detection Probes (TaqMan)**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Sequence (5’→3’): Accession No. in GenBank</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>(+)AAGCTGTTGATCATGATGGAAAC M17701</td>
</tr>
<tr>
<td></td>
<td>(−)GAAGACGCGTACAGTCCACAG</td>
</tr>
<tr>
<td></td>
<td>FAM-ATCCTTGAGCGCGCCATCGG-TAMRA</td>
</tr>
<tr>
<td>ANF</td>
<td>(+)AAATCCCGTGATACAGTGCG M27428</td>
</tr>
<tr>
<td></td>
<td>(−)GAGGGCATGTACCTACCTTC</td>
</tr>
<tr>
<td></td>
<td>FAM-TCAAGAGCTTCAGTTAGACCCACTGAGGAG-TAMRA</td>
</tr>
<tr>
<td>BNP</td>
<td>(+)CCAGAACATCCACAGATCCG RATABP</td>
</tr>
<tr>
<td></td>
<td>(−)TGAGAAGCTTCCTCCTGAGG-TAMRA</td>
</tr>
<tr>
<td>ET-1</td>
<td>(+)CTCCCTTGTATGACGAAAG RATET1</td>
</tr>
<tr>
<td></td>
<td>(−)CTTGTACGTTGCTGTCATGG</td>
</tr>
<tr>
<td></td>
<td>FAM-AGCAGTGGTGGAGGAAAGA-A-TAMRA</td>
</tr>
</tbody>
</table>

(+) Indicates forward primers; (−), reverse primers; FAM-TAMRA, probe; and ET-1, endothelin-1.

**Biopterin Levels**

Biopterin content was analyzed with high-performance liquid chromatography and a differential oxidation method as performed previously.\textsuperscript{14}

**Myocyte Size and Interstitial Fibrosis**

Tissue sections (5 \( \mu \)m) from the central portion of the LV were stained with hematoxylin and eosin. For mean myocyte size, the cross-sectional area of at least 100 cells in which the nucleus and a clear staining of the cell borders could be visualized was averaged from at least 4 LV sections. The myocyte outlines were traced, and the cell areas were measured with the lasso tool in Adobe Photoshop (Adobe, San Jose, Calif). Quantitative myocardial collagen assessment was performed as previously described\textsuperscript{14} with minor modifications. Briefly, 7- \( \mu \)m picrosirius red sections of the interventricular septum were examined under either blue-filtered bright-field or polarized light. Images were analyzed with Scion Image Beta 4.02 program (Scion Corp, Frederick, Md).

**LV Gene Expression**

Total RNA was isolated from LV samples (noninfarcted LV myocardium) with TRIzol reagent (Invitrogen, Carlsbad, Calif) according to the manufacturer’s instructions. Total RNA samples (1 \( \mu \)g) were reverse transcribed with oligo(dT) primers by use of Superscript II (Invitrogen). Quantification of cardiac gene expression was determined by real-time polymerase chain reaction with the Bio-Rad iCycler iQ system (Bio-Rad Laboratories, Hercules, Calif). Published primer and probe sequences (Table 1) were used to amplify and detect atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), endothelin-1, and GAPDH. For each gene, a standard was constructed by cloning the specific cDNA-amplified fragment into pCR2.1-TOPO vector (Invitrogen). Serial 10-fold dilutions of the generated plasmid were used as the standard curve. A given mRNA level was expressed as a ratio with respect to the level of mRNA for GAPDH.

**Western Blot Analysis and Immunohistochemistry**

LV samples (noninfarcted LV myocardium) and aortic samples were homogenized in ice-cold Tris buffer. The homogenates were centrifuged at 8000 \( g \) for 20 minutes at 4\( ^\circ \)C, and the supernatants were used as total tissue lysates. To obtain a membrane-enriched fraction for eNOS determination, the supernatants were then centrifuged at 120 000 \( g \) for 60 minutes at 4\( ^\circ \)C. The resulting pellets were resuspended in Tris buffer and solubilized with 1% Triton X-100 and 1%...
NOS Activity Assay

NOS activity was determined by assessing the conversion of L-[guanidino-15N2]arginine. Calcium-independent activity was determined by assessing the conversion of L-[guanidino-15N2]arginine in the presence of 5 mmol/L CaCl2, 500 nmol/L calmodulin, and 5 mmol/L -dioctadecyl-3,3,3',1,1'-tetramethylindocarbocyanine perchlorate-labeled acetylated low-density lipoprotein (dil-acLDL; Molecular Probes, Carlsbad, Calif) and FITC-conjugated lectin from Ulex europeus (UEA-1; Sigma, St Louis, Mo) in serum-free EB2 media, cells were washed twice and incubated for 90 minutes at 37°C in 10 mmol/L BH4, 1 mmol/L NADPH, 5 μmol/L CHAPS, 10 mmol/L CaCl2, 500 nmol/L calmodulin, and 5 mmol/L L-arginine. Calcium-independent activity was determined in the presence of 5 mmol/L EGTA and in the absence of calcium/calmodulin.

Vascular Reactivity Studies and Superoxide Anion Formation

Vascular reactivity studies were performed after 9 weeks of treatment. The descending thoracic aorta was dissected after removal of the heart and cleaned of connective tissue. One section (10 mm) was used for measurement of superoxide anion production; the remainder was cut into rings (3 mm in length) mounted in an organ bath (Föhr Medical Instruments, Seeheim, Calif), nitrotyrosine (Abcam, Cambridge, Mass), eNOS, inducible NOS (BD Biosciences PharMingen, San Jose, Calif), nitrotyrosine (Abcam, Cambridge, Mass), β-actin, phospho-

Endothelial Progenitor Cell Levels

Peripheral blood mononuclear cells were isolated by Ficoll density centrifugation as described.17 Peripheral blood mononuclear cells (2×10⁷) were cultured on fibronectin-precoated 24-well plates in EB2 culture medium supplemented with EGM SingleQuots (Cambrex, Belgium) and 20% FCS for 3 days. To exclude contamination with mature circulating endothelial cells, we carefully removed culture supernatant 8 hours after initial seeding and placed it on new fibronectin-precoated chamber slides. After dilution of 1:1 of diocadecyl-3,3,3’,3'-tetramethylindocarbocyanine perchlorate–labeled acetylated low-density lipoprotein (dil-acLDL; Molecular Probes, Carlsbad, Calif) and FITC-conjugated lectin from Ulex europeus (UEA-1; Sigma, St Louis, Mo) in serum-free EB2 media, cells were washed twice and incubated for 90 minutes at 37°C in EB2 medium containing 10 μg/mL dil-acLDL and 10 μg/mL UEA-1. After washing, cells were detached by trypsin/EDTA treatment, and the amount of double-positive (dil-acLDL and UEA-1) cells was investigated by flow cytometry. Unlabelled cells were used as negative controls.

### Results

In rats without coronary ligation, AVE9488 treatment (25 or 250 ppm in chow) for 9 weeks did not significantly affect mean arterial pressure (placebo, 120±8 mm Hg; AVE9488 [25 ppm], 107±3 mm Hg; AVE9488 [250 ppm], 113±5 mm Hg; P=NS), LV systolic pressure (placebo, 146±3 mm Hg; AVE9488 [25 ppm], 140±4 mm Hg; AVE9488 [250 ppm], 138±5 mm Hg; P=NS), LV function, or endothelial-dependent relaxation compared with placebo treatment. Therefore, for further experiments, a dosage of 25 ppm was chosen.
ppm was used, resulting in plasma concentrations of 10 to 13 ng/mL.

Long-term AVE9488 treatment (25 ppm) increased eNOS mRNA and protein levels in the LV myocardium (see the Figure in the online-only Data Supplement).

Global Parameters, Hemodynamics, and LV Dilation

Starting on the seventh postoperative day, sham-operated animals received placebo treatment (sham; n = 10), and surviving MI rats (n = 33) were randomly allocated to placebo (placebo MI; n = 19) and AVE9488 (AVE9488 MI; n = 14) treatment. Five MI rats died during the 9 weeks of treatment: 4 placebo and 1 AVE9488. Only rats with extensive infarcts (>40%) were included in the study (placebo MI, n = 12; AVE9488 MI, n = 12).

Infarct size and body weight were similar among the experimental groups (Table 2). LV weight was unchanged by infarction or treatment. Right ventricular weight and pulmonary fluid accumulation were elevated in placebo-treated MI rats compared with sham-operated animals and were significantly reduced by AVE9488 therapy (Table 2).

LV systolic pressure was reduced in MI rats regardless of treatment (Table 2). MI rats on placebo developed elevated LV end-diastolic pressure, lower LV dP/dtmax (Figure 1B), dP/dtmax divided by instantaneous pressure (dP/dtmax · IP⁻¹), and dP/dtmin, and prolonged τ (Table 2). AVE9488 decreased LV end-diastolic pressure; improved dP/dtmax, dP/dtmax · IP⁻¹, and dP/dtmin; and shortened τ. Chronic MI resulted in a rightward shift of the LV pressure-volume loops to high volumes (Figure 1C). AVE9488 prevented the rightward shift of LV volume and significantly decreased LV end-systolic and end-diastolic volumes compared with placebo. LV ejection fraction was markedly lower in placebo MI rats and significantly improved by AVE9488 treatment (Table 2).

Plasma Norepinephrine

Rats with chronic MI displayed an increase in circulating norepinephrine levels (sham, 224 ± 35 pg/mL; placebo MI, 518 ± 120 pg/mL; P = 0.07), which were nearly normalized by AVE9488 treatment (323 ± 62 pg/mL).

Interstitial Fibrosis and Myocyte Size

Interstitial collagen density in the failing LV myocardium was increased in placebo MI rats and nearly normalized by AVE9488 therapy (Figure 2A). Similarly, myocyte cross-sectional area was increased in placebo rats and significantly reduced by AVE9488 treatment (Figure 2B).

LV Gene Expression

Myocardial endothelin-1 gene expression was increased in placebo MI rats and suppressed by AVE9488 (Figure 2C).
RNA expression of ANF and BNP in the noninfarcted LV myocardium was examined as molecular markers of the hypertrophic response to infarction. ANF and BNP mRNAs were increased in placebo MI rats and reduced by AVE9488 treatment (Figure 2C).

**LV eNOS Expression and Activity, Tetrahydrobiopterin, and Nitrotyrosine**

eNOS protein expression in the failing LV myocardium was decreased in placebo MI rats and significantly enhanced by AVE9488 therapy (Figure 3A). Consistently, AVE9488 also increased eNOS activity, which was lower in placebo MI rats (Figure 3B). Myocardial tetrahydrobiopterin (BH4) levels were similar in sham-operated rats and MI rats treated with placebo or AVE9488 (Figure 3C). LV inducible NOS protein levels did not significantly change after MI (ratio of inducible NOS to β-actin: sham, 0.45±0.09; placebo MI 0.62±0.29; AVE9488 MI 0.54±0.15; P=NS). Calcium-independent NOS activity tended to be enhanced in placebo MI rats compared with sham and to be reduced by AVE9488 (sham, 14±1 nmol/µg; placebo MI, 27±4 nmol/µg; AVE9488 MI, 19±3 nmol/µg protein×1000 [15N]nitrate formation from [guanidino 15N2]L-arginine). Myocardial nitrotyrosine levels as determined by Western blot analysis were increased in placebo MI rats compared with sham-operated animals and reduced by AVE9488 (Figure 3E).

**Vascular Function, Superoxide Anion Formation, Aortic eNOS, and Nitrotyrosine Levels**

Chronic MI increased the aortic contractile response to phenylephrine, which was prevented by long-term treatment with AVE9488 (Figure 4A). Aortas from placebo MI rats showed a profound decrease in acetylcholine-induced endothelium-dependent relaxation. Long-term treatment with AVE9488 significantly improved the response to acetylcholine (Figure 4B). Endothelium-independent relaxation induced by sodium nitroprusside did not differ among the groups (Figure 4C). In parallel, to the beneficial effects on vascular reactivity, AVE9488 treatment significantly diminished the increased superoxide anion production in rats with MI (Figure 4D). Aortic eNOS protein expression was reduced in placebo MI rats and markedly increased by AVE9488 treatment (Figure 4E).

Immunohistochemical staining showed increased nitrotyrosine, localized mainly in the endothelium, in placebo MI rats compared with sham-operated and AVE9488 MI rats (Figure 4F). In addition, long-term treatment with AVE9488 significantly increased vascular VASP phosphorylation at serine 239, an index of the integrity of the NO/cGMP pathway, in MI rats (ratio of phospho-VASP to VASP: placebo, 1.43±0.08; AVE9488, 1.72±0.07; P<0.05).
Circulating Endothelial Progenitor Cells
Chronic MI was characterized by a depression of circulating endothelial progenitor cell (EPC) levels, which were normalized by long-term AVE9488 treatment (Figure 5).

Hemodynamic and Volume Measurements in eNOS-Knockout Mice
To define the specific role of eNOS in mediating the beneficial effects of AVE9488 in heart failure, we investigated the effects of long-term AVE9488 treatment on LV dilation and dysfunction in eNOS-deficient mice after MI. In these mice, AVE9488 treatment did not affect LV filling pressure and end-diastolic and end-systolic volumes. Moreover, infarcted eNOS−/− mice treated with placebo or AVE9488 showed similar LV dP/dtmax, dP/dtmin, IP−1, dP/dtmin, τ, and LV ejection fraction (Table 3). Thus, the beneficial effects of AVE9488 on LV remodeling were completely abolished in mice lacking eNOS, emphasizing the importance of eNOS in the cardioprotective effects of AVE9488 pharmacological intervention.

Discussion
Long-term treatment with the pharmacological eNOS enhancer AVE9488 ameliorated LV remodeling and contractile dysfunction in rats with heart failure after infarction. AVE9488 prevented LV dilation, hypertrophy, fibrosis, and molecular alterations; increased circulating EPC levels; and improved endothelial vasomotor dysfunction.

Endothelial NO bioavailability and cardiac NO production are markedly diminished in congestive heart failure.3,4,21–24 Genetic ablation of eNOS impaired contractile function and promoted LV hypertrophy and dilation after infarction.7 On the other hand, targeted overexpression of eNOS within the vascular endothelium ameliorated survival, cardiac function, and pulmonary congestion in mice with heart failure after MI.9 Cardiomyocyte-specific eNOS overexpression improved LV performance and remodeling after infarction,10 suggesting that strategies to increase eNOS-derived NO production may be promising treatments to improve LV remodeling and function in the failing heart. In the present study, treatment with the eNOS enhancer AVE9488 increased eNOS expression and restored eNOS activity in the failing LV myocardium, leading to an improvement in LV remodeling and contractile dysfunction. No indication was found that, in rats with chronic MI treated with either placebo or AVE9488, eNOS was uncoupled, producing superoxide rather than NO as observed, for example, in diabetes mellitus.25 Accordingly,

Figure 3. eNOS protein levels (A), eNOS activity (B), BH4 levels (C), eNOS dimer-to-monomer ratio (D), and immunoblotting for nitrotyrosine (E) in the LV myocardium of sham-operated rats and rats with MI. Rats were treated with placebo or AVE9488 for 9 weeks. Values are mean±SEM (n=5 to 12). *P<0.05 vs sham; ‡P<0.05 vs placebo MI.
we did not detect any differences in BH4 levels or changes in
the dimer-to-monomer ratio among the groups. In addition to
the improvement in total NO bioavailability, one might also
speculate that AVE9488 treatment interferes with the intra-
cellular compartmentalization of NO; this needs to be ad-
dressed in further studies.26

The cardioprotective effects of AVE9488 were abrogated
in infarcted mice lacking eNOS, emphasizing the importance
of eNOS in the beneficial effects of AVE9488 pharmacolog-
ical intervention. Several mechanisms may underlie the
beneficial effects of this novel treatment. We suppose that the
positive modulation of LV function and remodeling by
long-term AVE9488 treatment was mediated in part by the
reduction in reactive fibrosis in the remote noninfarcted LV
myocardium, a major determinant of ventricular remodeling
in ischemic cardiomyopathy.2 Several alterations in the collagen
matrix raise cardiac muscle stiffness and impair LV perfor-
mance, leading to progressive dysfunction and heart failure.2
NO displays a negative trophic role and attenuates cardiac
fibrosis; NO-generating compounds inhibit collagen synthe-
sis in vascular smooth muscle cells.28 NO mediates
bradykinin-induced downregulation of extracellular matrix
production in cardiac fibroblasts29 and decreases angiotensin
II–induced cardiac fibrosis.30 Therefore, increased NO pro-
duction by AVE9488 may beneficially affect postinfarction
LV fibrosis by dual mechanisms: enhanced inhibitory action
of bradykinin and reduced promoting effect of angiotensin II.
Although cardiomyocyte-specific eNOS overexpression10 did
not prevent LV fibrosis after MI, our data suggest that the
nonrestricted effect of the eNOS enhancer in the present study
confers more pronounced cardiac protection postinfarction.

AVE9488 therapy also prevented pathological hypertro-
phy, as evidenced by the reduction in myocyte cross-sectional
area and expression of fetal genes such as ANF and BNP.
NO, via cGMP-dependent protein kinase type I, inhibits
cardiomyocyte hypertrophy31 and plays an important role in
the antihypertrophic effect of bradykinin.32 Enhanced eNOS-
derived NO production may thereby protect against detrimen-
tal hypertrophy after MI, as also shown by attenuated myo-
cyte hypertrophy in cardiomyocyte-specific eNOS transgenic
mice,10 and increased LV hypertrophy in eNOS−/− mice.7
Furthermore, eNOS deficiency abrogated the beneficial effects
of statins on LV remodeling after MI, thus proving the essential
role of eNOS-derived NO for the attenuation of cardiac fibrosis
and hypertrophy in response to statin treatment.33

In addition, the marked reduction in myocardial
endothelin-1 expression likely contributed to the benefit of
AVE9488 therapy by preventing the adverse effects of
endothelin on postinfarction LV fibrosis and dilation. Inter-
actions between the endothelin and NO pathways have been

Figure 4. Phenylephrine (PE)-induced contraction (A), endothelium-dependent relaxation induced by acetylcholine (B; ACh), endothelium-
independent relaxation by sodium nitroprusside (C; SNP), superoxide anion formation (D), eNOS protein expression (E), and immunohis-
tochemical staining for nitrotyrosine (F; brown; magnification ×400) in aortic rings of sham-operated rats and rats with MI treated
with placebo (PLA) or AVE9488 for 9 weeks. Values are mean±SEM (n=10 to 12). *P<0.05 vs sham; ‡P<0.05, †P<0.01 vs placebo MI.

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described. Stimulation of endothelium-derived NO production inhibits the expression and production of endothelin. Furthermore, NO inhibits the expression of endothelin-1 induced by hypoxia. Prevention of right ventricular hypertrophy and pulmonary edema by AVE9488 treatment may be mediated through an improvement in LV failure. The hypertrophic growth of the right ventricle after MI is related to LV dilation and pump failure, resulting in increased pressure load on the ventricle and accumulation of pulmonary fluid. However, a direct attenuation of endothelin-1 synthesis by eNOS-derived NO may contribute to the pronounced improvement in right ventricular remodeling and pulmonary edema, which was also the most eminent observation in mice with endothelium-targeted eNOS overexpression after MI. The hemodynamic improvement likely accounted for reflex inhibition of sympathetic activation. The reduction in plasma norepinephrine may have further contributed to the benefit of AVE9488 therapy by preventing the adverse cardiovascular effects of excessive sympathetic stimulation.

Table 3. Hemodynamic and Volume Measurements in eNOS-Knockout Mice Treated With Placebo or AVE9488 for 8 Weeks After MI

<table>
<thead>
<tr>
<th></th>
<th>Placebo MI (n=6)</th>
<th>AVE9488 MI (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI size, %</td>
<td>29±2</td>
<td>28±4</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>102±8</td>
<td>92±4</td>
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<td>LVEDP, mm Hg</td>
<td>15±2</td>
<td>15±3</td>
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<td>dP/dtmax, mm Hg/s</td>
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<td>4976±420</td>
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<td>103±10</td>
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<td>dP/dtmin, mm Hg/s</td>
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<td>5305±512</td>
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<td>τ, ms</td>
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<tr>
<td>LVESV, μL</td>
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<td>LVEDV, μL</td>
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<td>EF, %</td>
<td>26±3</td>
<td>24±3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>423±23</td>
<td>455±21</td>
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</table>

LVEDP indicates LV end-diastolic pressures. Other abbreviations as in Table 2. Values are mean±SEM.
tion such as $\tau$ and $\frac{dP/dt_{\text{max}} \cdot IP}{IP^3}$ were significantly improved by the eNOS enhancer, direct effects on the myocardium seem to mediate at least part of the beneficial effects.

Circulating EPC levels are reduced in patients with severe congestive heart failure and endothelial dysfunction.\textsuperscript{17,42} Long-term AVE9488 treatment increased circulating EPCs, which were markedly reduced in placebo MI rats. Because EPCs may provide a circulating pool of cells that could replace dysfunctional endothelium,\textsuperscript{32} amelioration of postinfarction endothelial dysfunction by treatment with the eNOS enhancer may relate to increased circulating EPC. Because eNOS-derived NO plays an essential role in EPC mobilization,\textsuperscript{43} enhanced NO production induced by AVE9488 likely contributed to the increase in EPC levels. Treatment of bone marrow mononuclear cells from patients with ischemic cardiomyopathy with AVE9488 enhanced NO formation and improved their impaired functional activity.\textsuperscript{11}

Conclusions

The eNOS enhancer AVE9488 substantially improved LV remodeling and function, LV molecular alterations, and endothelial dysfunction after experimental MI. Cardioprotection by AVE9488 was absent in infarcted eNOS-deficient mice. Pharmacological interventions aiming to increase eNOS-derived NO production constitute a promising therapeutic approach to the prevention of postinfarction ventricular remodeling and heart failure.

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Disclosures

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References

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Nitric oxide (NO) generated by the endothelial NO synthase (eNOS) plays a key role in vascular tone and cardiomyocyte contractility and protects against hypertrophy and atherosclerosis. Cardiac and vascular NO bioavailability is reduced in heart failure, contributing to contractile dysfunction, ventricular hypertrophy, and remodeling, as well as endothelial dysfunction. Overexpression of the eNOS gene in endothelial cells or cardiomyocytes improved cardiac performance in mice after myocardial infarction. Various established pharmacological interventions in heart failure enhance NO bioavailability. Nitrates are powerful NO donors widely used in coronary artery diseases and heart failure. However, nitrate tolerance and the induction of reactive oxygen species formation limit their benefits. In the present study, we tested the concept of directly augmenting eNOS by pharmacological intervention. The novel compound AVE9488, shown to elevate eNOS expression and NO production, improved left ventricular remodeling and contractile dysfunction in rats after coronary artery ligation. Myocardial molecular alterations were prevented by AVE9488; endothelial vasomotor dysfunction and superoxide formation were attenuated; and levels of circulating endothelial progenitor cell were elevated. Importantly, the phenomenon of eNOS uncoupling, which may lead to production of superoxide anions instead of NO, was not observed after treatment with AVE9488. Thus, a pharmacological intervention to increase eNOS expression and subsequent NO formation constitutes a promising therapeutic approach for the amelioration of postinfarction ventricular remodeling and heart failure.
Improvement in Left Ventricular Remodeling by the Endothelial Nitric Oxide Synthase Enhancer AVE9488 After Experimental Myocardial Infarction
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Legend to online figure:

(A) eNOS mRNA expression and (B) eNOS protein levels as determined by Western blot analysis in myocardial extracts. (C) Representative immunohistochemical staining of myocardial tissue. Rats were treated with placebo or with AVE9488 for 9 weeks. Values are mean±SEM (n=7-10). *p<0.01.
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