

Endothelial nitric oxide synthase overexpression attenuates myocardial reperfusion injury

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Jones, Steven P., James J. M. Greer, Aman K. Kakkar, P. Derek Ware, Richard H. Turnage, Michael Hicks, Rien van Haperen, Rini de Crom, Seinosuke Kawashima, Mitsuhiro Yokoyama, and David J. Lefer. Endothelial nitric oxide synthase overexpression attenuates myocardial reperfusion injury. *Am J Physiol Heart Circ Physiol* 286: H276–H282, 2004. First published September 11, 2003; 10.1152/ajpheart.00129.2003.—Previous studies indicate that deficiency of endothelial nitric oxide (NO) synthase (eNOS)-derived NO exacerbates myocardial reperfusion injury. We hypothesized that overexpression of eNOS would reduce the extent of myocardial ischemia-reperfusion (MI/R) injury. We investigated two distinct strains of transgenic (TG) mice overexpressing the eNOS gene (eNOS TG). Bovine eNOS was overexpressed in one strain (eNOS TG-Kobe), whereas the human eNOS gene was overexpressed in the other strain (eNOS TG-RT). Non-TG (NTG) and eNOS TG mice were subjected to 30 min of coronary artery occlusion followed by 24 h of reperfusion, and the extent of myocardial infarction was determined. Myocardial infarct size was reduced by 33% in the eNOS TG-Kobe strain ($P < 0.05$ vs. NTG) and by 32% in the eNOS TG-RT strain ($P < 0.05$ vs. NTG). However, postischemic cardiac function (cardiac output, fractional shortening) was not improved in the eNOS TG-Kobe mouse at 24 h of reperfusion [$P =$ not significant (NS) vs. NTG]. In additional studies, eNOS TG-Kobe mice were subjected to 30 min of myocardial infarction and 7 days of reperfusion. Fractional shortening and the first derivative of left ventricular pressure were measured in eNOS TG-Kobe and NTG mice, and no significant differences in contractility were observed ($P =$ NS) between the eNOS TG mice and NTG controls. Left ventricular end-diastolic pressure was significantly ($P < 0.05$ vs. NTG) reduced in the eNOS TG-Kobe strain at 7 days of reperfusion. The cardioprotective effects of eNOS overexpression on myocardial infarct size were ablated by *N*^ω-nitro-L-arginine methyl ester (300 mg/kg) pretreatment. Thus genetic overexpression of eNOS in mice attenuates myocardial infarction after MI/R but fails to significantly protect against postischemic myocardial contractile dysfunction in mice.

myocardial infarction; cardiac dysfunction; murine model

NITRIC OXIDE (NO) is constitutively produced by the vascular endothelium by endothelial NO synthase (eNOS) and serves to protect against cardiovascular disease. NO promotes vasodilation (4, 8), regulates leukocyte-endothelial cell interactions (17), inhibits platelet adhesion and aggregation (9, 22), attenuates smooth muscle cell proliferation (7), and may modulate cardiac myocyte

function (16). Since the discovery of endothelium-derived relaxing factor (EDRF) in 1980, NO has been implicated in numerous disease states as both a beneficial and a deleterious moiety. Attenuated eNOS function and reduced NO generation is a critical early event in many cardiovascular diseases (9). NO therapy utilizing physiological levels of NO is beneficial in a number of pathophysiological states including hypercholesterolemia (9), diabetes mellitus (9), and ischemia-reperfusion (I/R) injury (11, 17, 18).

A number of previous studies (3, 20, 28–30) have demonstrated that treatment with various NO-donating compounds is highly effective in the setting of myocardial I/R (MI/R) injury. NO has also recently emerged as a crucial modulator of myocardial preconditioning, and both eNOS-derived and inducible NOS (iNOS)-derived NO are thought to mediate cardioprotection during the preconditioning process (33, 36). Furthermore, MI/R injury is significantly enhanced in eNOS-deficient animals compared with wild-type controls (12). Pharmacological inhibition of NO synthesis exacerbates MI/R injury (29), whereas administration of the NO precursor L-arginine can ameliorate MI/R injury (23, 28, 32).

Recently, transgenic (TG) mice that overexpress the eNOS gene have been developed (25, 31). Previous studies of eNOS overexpression have focused primarily on shock states (35) and atherosclerosis (27, 31). While genetic overexpression of eNOS has been shown to protect against endotoxin shock (35), the effects of eNOS overexpression in the setting of hypercholesterolemia and atherogenesis are somewhat controversial at present with conflicting reports in the literature (27, 31).

In the present study, we endeavored to determine whether overexpression of eNOS could affect the severity of MI/R injury in an in vivo murine model of acute myocardial ischemia and reperfusion injury. Specifically, we sought to examine two distinct strains of eNOS TG mice and to evaluate the effects of eNOS overexpression on myocardial infarct size and postischemic myocardial contractile function.

METHODS

eNOS Transgenic Mice

Two distinct strains of eNOS TG mice were utilized in the present study. One eNOS TG mouse was developed in Kobe, Japan (eNOS

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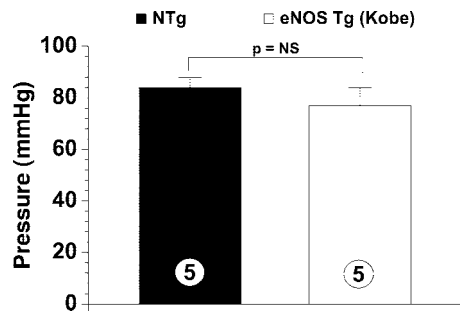


Fig. 1. Mean arterial blood pressure (in mmHg) in nontransgenic (NTG) and endothelial nitric oxide synthase (eNOS) transgenic (TG) mice developed in Kobe, Japan (eNOS TG-Kobe mice). Blood pressures were measured in anesthetized mice. No differences were observed in blood pressure between the NTG and eNOS TG mice. Numbers inside the bars are numbers of mice per group. NS, not significant.

TG-Kobe), and featured the murine preendothelin-1 promoter (GenBank accession no. U07982) and bovine eNOS cDNA (GenBank accession no. M99057). This mouse was developed on a C57BL/6 background and has been extensively characterized previously (15, 25–27, 34, 35).

An additional eNOS TG mouse was recently developed in Rotterdam (31), The Netherlands (eNOS TG-RT), and these mice were also used in the present study of MI/R injury. A DNA fragment containing the human eNOS gene was isolated from a homemade human genomic cosmid library (10) using eNOS cDNA (kindly donated by Dr. S. Janssens, Leuven, Belgium) as a probe (31). In addition, the DNA fragment contained ~6 kb of the 5' natural flanking sequence, including the native eNOS promoter, and ~3 kb of the 3' sequence to the gene. Thus promoter DNA elements were included that have been shown to be essential in transcriptional regulation as well as elements responsible for the tissue distribution of eNOS expression (19). Also, the endothelium enhancer element that is located 4.9 kb upstream from the transcription start site of the eNOS gene is included in this construct. Vector sequences were removed by restriction endonucleases. A solution of 1–2 $\mu\text{g}/\text{ml}$ DNA was used for microinjection of fertilized oocytes from FVB donor mice and transplanted into the oviducts of pseudopregnant B10xCBA mice. Founder mice and offspring were genotyped by PCR on DNA isolated from tail biopsies. The primers used were as follows: 5'-GTCCTGCAGACCGTG-CAGC-3' (sense) and 5'-GGCTGTTGGTGTCTGAGCCG-3' (anti-sense). Mice were backcrossed to C57BL/6 mice for at least seven generations (~99% C57BL/6). The individual performing all experiments was blinded to the mouse genotype until all data were fully analyzed. All experiments performed at Louisiana State University Health Sciences Center conformed to state and federal regulations regarding animal experimentation and with American Physiological Society guidelines.

Myocardial Infarction Protocol

Ligation of the left main coronary artery was performed similar to methods described previously (12, 14). Briefly, mice were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) and pentobarbital sodium (50 mg/kg). The animals were then attached to

a surgical board with their ventral side up. The mice were orally intubated with polyethylene (PE)-90 tubing connected to PE-240 tubing and then connected to a model 683 rodent ventilator (Harvard Apparatus; Natick, MA). The tidal volume was set at 2.2 ml, and the respiratory rate was set at 122 breaths/min. The mice were supplemented with 100% oxygen via the ventilator side port. A median sternotomy was performed using an electric cautery, and the proximal left main coronary artery was visualized and completely ligated with 7-0 silk suture mounted on a tapered needle (BV-1, Ethicon).

In additional experiments, both non-TG (NTG) and eNOS TG mice (eNOS TG-Kobe) were subjected to coronary artery ligation for 30 min and 24 h of reperfusion. All of the mice were treated with 5-hydroxydecanoic acid (5-HD) at a dose of 10 mg/kg 30 min before the left main coronary artery ligation (24). An additional group of eNOS TG-Kobe mice ($n = 7$) were treated with 300 mg/kg *N*^ω-nitro-L-arginine methyl ester (L-NAME; intraperitoneal injection) 30 min before the left main coronary artery ligation. The mice receiving L-NAME were subjected to 30 min of left main coronary artery ligation and 24 h of reperfusion. Additional experiments were also performed in C57BL/6 mice ($n = 6$ mice/group) treated with the NO donor dipropyleneetriamine (DPTA) NONOate (Alexis Pharmaceuticals; San Diego, CA). In these experiments, mice were subjected to 30 min of myocardial ischemia and 24 h of reperfusion. DPTA NONOate (100 $\mu\text{g}/\text{kg}$) was administered 5 min before coronary artery reperfusion via direct injection into the left ventricular (LV) cavity.

Myocardial Infarct Size Determination

At 24 h of reperfusion, the mice were anesthetized as described previously, intubated, and connected to a rodent ventilator. A catheter (PE-10 tubing) was placed in the common carotid artery to allow for Evans blue dye injection. A median sternotomy was performed, and the left main coronary artery was religated in the same location as before. Evans blue dye (1.2 ml of a 2% solution) was injected into the carotid artery catheter into the heart to delineate the ischemic zone from the nonischemic zone. The heart was rapidly excised and serially sectioned along the long axis in 1-mm-thick sections, which were then incubated in 1.0% 2,3,5-triphenyltetrazolium chloride for 5 min at 37°C to demarcate the viable and nonviable myocardium within the risk zone. Each of the five 1-mm-thick myocardial slices was weighed, and the areas of infarction, risk, and nonischemic LV were assessed by a blinded observer using computer-assisted planimetry (NIH Image 1.57). All of the procedures for area at risk (AAR) and infarct size determination have been previously described (12, 14).

Evaluation of Arterial and LV Hemodynamics

At 7 days after myocardial ischemia and reperfusion, a 1.4-Fr Millar (SPR-671; Houston, TX) pressure transduction catheter was inserted similar to methods described previously (21). Data for each animal were calculated from at least 10 s of chart recording (arithmetic mean of at least 50 cardiac cycles).

Echocardiographic Assessment of LV Function

Transthoracic echocardiography of the LV using a 15-MHz linear array transducer (15L8) interfaced with a Sequoia C256 (Acuson) was performed in groups of mice after either 24 h or 7 days of reperfusion after 30 min of left main coronary artery ligation. Two-dimensional

Table 1. Baseline cardiac function in the Kobe NTG and Kobe eNOS TG mice

Group	Heart Rate, beats/min	Stroke Volume, μl	Cardiac Output, $\mu\text{l}/\text{min}$	LVEDD, mm	LVESD, mm	FS, %
NTG	340 ± 18	37.9 ± 1.6	605 ± 20	3.54 ± 0.13	2.64 ± 0.11	26.7 ± 0.3
eNOS TG	333 ± 10	−34.6 ± 2.6	540 ± 33	3.56 ± 0.10	2.50 ± 0.10	29.9 ± 1.8

Values are means ± SE; $n = 10$ –11 Kobe nontransgenic (NTG) and endothelial nitric oxide synthase (eNOS) transgenic (TG) mice. LVEDD and LVESD, left ventricular end-diastolic and end-systolic diameter, respectively; FS, fractional shortening.

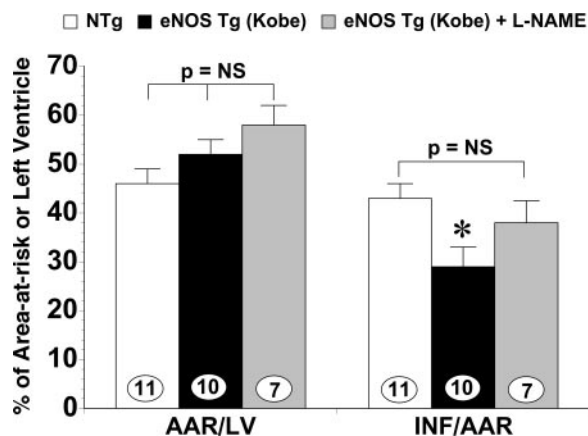


Fig. 2. Area at risk (AAR) per left ventricle (LV) and infarction (INF) per AAR in NTG and eNOS TG-Kobe mice after 30 min of coronary artery ligation and 24 h of reperfusion. The AAR was determined using Evans blue dye injection, and the infarction area was determined using 2,3,5-triphenyltetrazolium chloride staining. Myocardial infarction (MI) was significantly ($*P < 0.05$ vs. NTG) reduced in the eNOS TG-Kobe mouse. Numbers inside the bars are numbers of mice per group. L-NAME, *N*^ω-nitro-L-arginine methyl ester.

echocardiography was performed as described previously (13, 14). All data were calculated from 10 cardiac cycles/experiment.

Statistical Analyses

Data were analyzed by two-way ANOVA with post hoc analysis using StatView software (version 5.0, SAS Institute; Cary, NC). The Bonferroni test was used for post hoc analysis. Data are reported as means \pm SE with differences accepted as significant when $P < 0.05$.

RESULTS

eNOS TG-Kobe Mice

Hemodynamics. Mean arterial blood pressure (Fig. 1) was measured in the eNOS TG-Kobe mice and NTG control animals. Blood pressure was 84 ± 4 mmHg in the NTG group and 77 ± 7 mmHg in the eNOS TG group [$P =$ not significant (NS)]. Similarly, baseline heart rates (Table 1) were similar in the NTG (340 ± 18 beats/min) and eNOS TG (333 ± 10 beats/min) groups.

Myocardial infarct size. Myocardial AAR per LV and infarct size per AAR data after 30 min of left main coronary artery occlusion and 24 h of reperfusion are presented for NTG controls ($n = 11$) and eNOS TG mice ($n = 10$) in Fig. 2. The AARs per LV were similar ($P =$ NS) in the NTG and eNOS TG-Kobe mice ($46 \pm 3\%$ and $52 \pm 3\%$, respectively). Myocardial infarct size per AAR was $43 \pm 3\%$ in the NTG group and $29 \pm 4\%$ in the eNOS TG group ($P > 0.05$ between the groups). In eNOS TG mice treated with L-NAME ($n = 7$) before myocardial ischemia, the AAR per LV was $58 \pm 4\%$ ($P =$ NS compared with NTG and eNOS TG alone), and the myocardial infarct size per AAR was $37 \pm 4\%$ ($P =$ NS

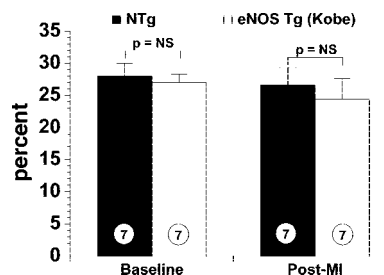


Fig. 3. Fractional shortening (in %) as measured using two-dimensional echocardiography in NTG and eNOS TG-Kobe mice after 30 min of coronary artery occlusion and 7 days of reperfusion. No significant differences were observed between the NTG and eNOS TG-Kobe groups. Numbers inside the bars are numbers of mice per group.

compared with the NTG group and $P < 0.05$ compared with the eNOS TG group). In additional experiments, we investigated the effects of L-NAME treatment of eNOS TG mice at dosages of 50 and 100 mg/kg and failed to observe any changes in myocardial infarct size compared with vehicle (data not shown).

Baseline and postischemic cardiac function after 30-min myocardial infarction and 24 h of reperfusion. Baseline and postischemic cardiac function data are presented in Tables 1 and 2. No group differences in heart rate, stroke volume, cardiac output, LV end-diastolic diameter, LV end-systolic diameter, or fractional shortening were observed at baseline. After myocardial ischemia and reperfusion, the heart rate was significantly ($P < 0.05$) reduced in both the NTG and eNOS TG groups compared with baseline values. Furthermore, stroke volume and fractional shortening were also significantly ($P < 0.05$ vs. baseline) depressed in the NTG and eNOS TG groups after 30 min of myocardial ischemia and 24 h of reperfusion.

Baseline and postischemic cardiac function after 30-min myocardial infarction and 7 days of reperfusion. An additional group of eNOS TG-Kobe mice was subjected to myocardial ischemia for 30 min followed by 7 days of reperfusion, and cardiac function was assessed. LV fractional shortening data (two-dimensional echocardiography) are presented in Fig. 3. Baseline fractional shortening was $28 \pm 2\%$ in NTG mice and $27 \pm 1\%$ in eNOS TG mice ($P =$ NS). After MI/R, fractional shortening was $27 \pm 3\%$ in the NTG group and $24 \pm 3\%$ in the eNOS TG group ($P =$ NS). Additional data for LV pressures (LV Millar catheter) are presented in Figs. 4 and 5 after myocardial infarction and 7 days of reperfusion. LV end-systolic pressure was 90 ± 2 mmHg in the NTG group and 85 ± 9 mmHg in the eNOS TG group ($P =$ NS). LV end-diastolic pressures were 16 ± 6 mmHg in the NTG group and 3 ± 2 in the eNOS TG group ($P < 0.05$). LV developed pressure was 74 ± 5 mmHg in the NTG mice and 82 ± 9 in the eNOS TG mice ($P =$ NS).

Data for the first derivative of LV pressure (LV dp/dt) are presented in Fig. 5. Positive dp/dt was similar ($P =$ NS) in the

Table 2. Cardiac function in Kobe NTG and Kobe eNOS TG mice after myocardial ischemia and 24 h of reperfusion

Group	Heart Rate, beats/min	Stroke Volume, μ l	Cardiac Output, μ l/min	LVEDD, mm	LVESD, mm	FS, %
NTG	$463 \pm 13^*$	$23.1 \pm 2.0^*$	567 ± 60	3.33 ± 0.05	2.63 ± 0.14	$21.4 \pm 1.6^*$
eNOS TG	$470 \pm 16^*$	$23.7 \pm 2.9^*$	538 ± 38	3.47 ± 0.09	2.79 ± 0.08	$19.7 \pm 2.4^*$

Values are means \pm SE; $n = 10$ – 11 mice/group. $*P < 0.05$ vs. baseline.

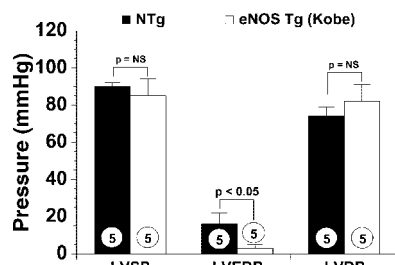


Fig. 4. LV end-systolic pressure (LVSP), LV end-diastolic (LVEDP), and LV developed pressure (LVDP) as measured using a 1.4-Fr Millar catheter placed in the LV cavity. Data were collected in NTG and eNOS TG-Kobe mice after 30 min of coronary artery occlusion and 7 days of reperfusion. No significant differences in LVSP or LVDP were observed between the groups. LVEDP was significantly ($P < 0.05$) lower in the eNOS TG-Kobe mice. Numbers inside the bars are numbers of mice per group.

NTG and eNOS TG groups ($4,675 \pm 1,195$ and $6,300 \pm 1,980$ mmHg/s, respectively). Negative dP/dt was $-3,750 \pm 992$ mmHg/s in the NTG animals and $-6,700 \pm 2,215$ mmHg/s in the eNOS TG-Kobe animals ($P = NS$).

eNOS TG-RT Mice

Myocardial ischemia-reperfusion. Myocardial ischemia (30 min) and reperfusion (24 h) experiments were also performed in the eNOS TG-RT mouse strain. The results of these experiments are presented in Figs. 6 and 7. Mean arterial blood pressure data are shown in Fig. 6. Baseline blood pressure was 86 ± 5 mmHg in the NTG mice and 80 ± 3 mmHg in the eNOS TG mice ($P = NS$). The blood pressure during ischemia was reduced to 59 ± 5 mmHg in the NTG group and to 55 ± 3 mmHg in the eNOS TG group ($P < 0.05$ vs. baseline for both groups; $P = NS$ between groups). After reperfusion, mean arterial blood pressure remained significantly depressed in both groups ($P < 0.05$ vs. baseline).

Myocardial infarct data for the eNOS TG-RT mice are presented in Fig. 7. The AAR per LV was $51 \pm 4\%$ in the NTG controls and $48 \pm 7\%$ in the eNOS TG group ($P = NS$). Myocardial infarct size per AAR was significantly ($P < 0.05$) reduced in the eNOS TG group ($34 \pm 3\%$) compared with the NTG group ($50 \pm 7\%$).

eNOS TG-Kobe Mice with 5-HD Treatment

Myocardial infarct size. The effects of the mitochondrial ATP-sensitive K^+ (K_{ATP}) channel blocker 5-HD on myocar-

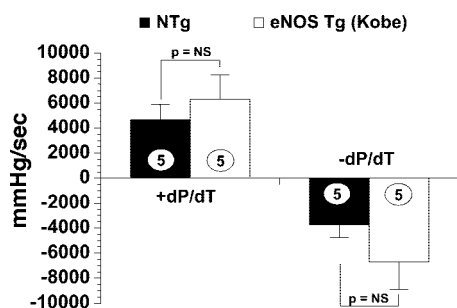


Fig. 5. Positive and negative first derivatives of LV pressure (dP/dt ; in mmHg/s) measured in NTG and eNOS TG-Kobe mice after 30 min of coronary artery occlusion and 7 days of reperfusion. No significant group differences were observed. Numbers inside the bars are numbers of mice per group.

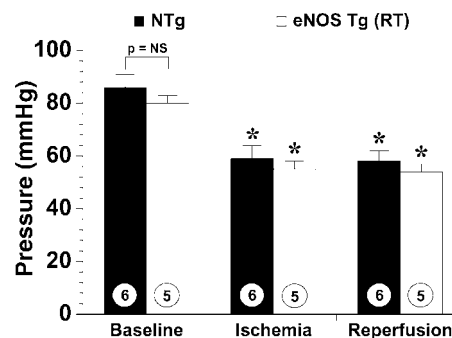


Fig. 6. Mean arterial blood pressure (in mmHg) measured in NTG and eNOS TG mice from Rotterdam, The Netherlands (eNOS TG-RT mice), at baseline and during ischemia and reperfusion. Blood pressure was measured in anesthetized mice. Blood pressure was measured at 25 min of ischemia and also at 2 h after reperfusion. Numbers inside the bars are numbers of mice per group. * $P < 0.05$ vs. baseline.

dial infarct size in NTG mice and the eNOS TG-Kobe strain of mice are presented in Fig. 8. Treatment with 5-HD failed to attenuate myocardial infarct size in both the eNOS TG group ($P = NS$ vs. no treatment) and the NTG group ($P = NS$ vs. no treatment).

Wild-Type Mice with NO Donor Therapy

Myocardial infarct size. The effects of NO donor therapy (DPTA NONOate) were evaluated in NTG wild-type mice ($n = 6$ mice/group), and these data are presented in Fig. 9. The AAR per LV was $61 \pm 4\%$ in untreated wild-type mice and $63 \pm 4\%$ in wild-type mice treated with DPTA ($P = NS$ between groups). Infarct size per AAR was $45 \pm 3\%$ in wild-type controls and $21 \pm 2\%$ in mice treated with DPTA ($P < 0.01$ between groups).

DISCUSSION

Data from the present study provide evidence for a beneficial role of genetic overexpression of eNOS in the setting of MI/R injury. Myocardial infarct size was reduced by $\sim 33\%$ in two distinct strains of eNOS TG mice. The bovine eNOS gene was overexpressed in one mouse strain, whereas the human eNOS gene was overexpressed in the other mouse strain. eNOS genetic expression varied from ~ 6 - to 12-fold in these TG mice, and eNOS protein expression was confined primarily to

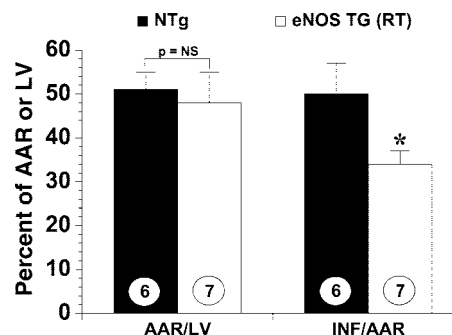


Fig. 7. Myocardial AAR per LV and infarct size per AAR in NTG and eNOS TG-RT mice after 30 min of coronary artery ischemia and 24 h of reperfusion. No significant group differences in AAR per LV were observed. Myocardial infarct size was significantly (* $P < 0.05$) reduced in eNOS TG-RT mice. Numbers within the bars are numbers of mice per group.

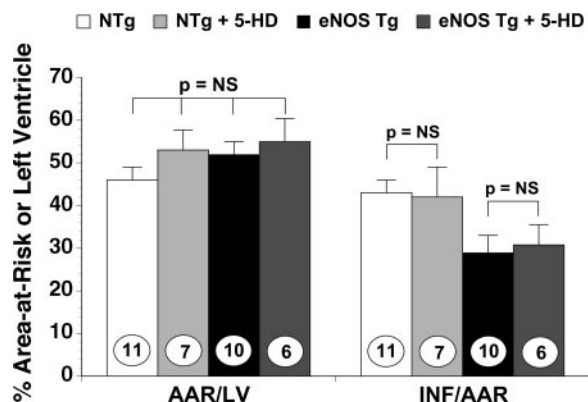


Fig. 8. Myocardial infarct size in NTG and eNOS TG-Kobe mice treated with the mitochondrial ATP-sensitive K^+ channel inhibitor 5-hydroxydecanoic acid (5-HD) at a dose of 10 mg/kg before myocardial ischemia and reperfusion. Treatment with 5-HD did not alter myocardial infarct size in the NTG or eNOS TG mice. Numbers within the bars are numbers of mice per group.

the vascular endothelium (13, 25, 27, 31). All of these improvements occurred in the eNOS TG mice without changes in systemic hemodynamics or differences in baseline ventricular morphology and function. Despite the significant reduction in myocardial infarct size, we failed to observe any significant preservation of postischemic myocardial contractile function as assessed by echocardiography or by LV direct catheterization experiments when mice were evaluated at 24 h or 7 days after myocardial infarction. It is likely that the reduction in myocardial infarct size, although significant, was not of a great enough magnitude to induce protection against postischemic contractile dysfunction. Treatment of eNOS TG mice with the NOS inhibitor L-NAME attenuated the cardioprotection observed with eNOS overexpression. These data support a cardioprotective role for eNOS in MI/R injury and further support previous studies of eNOS-deficient mice and studies of NO donors in acute MI/R injury.

Previous experimental studies of eNOS TG mice have revealed a very interesting phenotype that results from genetic overexpression of eNOS. eNOS TG mice have been shown to be protected against endotoxin shock (35) and to display systemic hypotension (25, 31). The eNOS TG mice display reduced NO-mediated vasodilator responses and appear to exhibit a nitrate tolerance (25, 34). Additionally, eNOS overexpression has been shown to limit neointimal lesion formation and medial thickening in a model of vascular remodeling (15). Overexpression of eNOS has also been shown to protect against skeletal muscle I/R injury (26). Reductions in skeletal muscle I/R injury were accompanied by a reduction in leukocyte-endothelial cell adhesion molecules and leukocyte trafficking into the ischemic tissue (26). Our laboratory has recently demonstrated (13) that genetic overexpression of eNOS significantly attenuates the severity of congestive heart failure after severe myocardial infarction in mice. Our previous study (13) clearly demonstrated significant improvements in survival and cardiac function at 30 days after myocardial infarction in the eNOS TG-RT mouse compared with NTG controls. The role of eNOS-derived NO in atherogenesis has been extensively investigated. Two strains of eNOS TG mice have been bred with apolipoprotein E-deficient mice as a means to study the potential role of NO in atherosclerosis. Interestingly, con-

flicting reports have emerged, with one group reporting that eNOS overexpression accelerates atherosclerotic lesion formation (27) and the other group reporting attenuated atherosclerotic lesion formation (31). Finally, both eNOS and iNOS have been selectively overexpressed within cardiac myocytes (1, 6). Cardiac myocyte-specific eNOS overexpression has been shown to blunt cardiac myofilament Ca^{2+} sensitivity without any alterations in systemic hemodynamics (1), whereas cardiac-specific iNOS overexpression did not alter cardiac function (6).

Our laboratory and others (3, 20, 23, 28–30) have previously reported that NO-donating agents significantly reduce the extent of myocardial reperfusion injury in various animal model systems. In addition, our laboratory and others have previously demonstrated that exogenous NO therapy does protect against postischemic myocardial contractile dysfunction (3, 28, 29) and that inhibition of NO promotes postischemic myocardial contractile dysfunction (5, 28). Furthermore, genetic deficiency of the eNOS enzyme significantly exacerbates MI/R injury (12). The present study provides additional support for the cardioprotective actions of NO in the setting of myocardial reperfusion injury. However, the extent of myocardial infarct size reduction observed in the present study (i.e., 32–33%) was significantly less than that reported in previous studies of exogenous NO donors (i.e., >50–65% reductions in infarct size). Furthermore, we administered the NO donor DPTA NONOate to wild-type mice in the present study and observed a highly significant 53% reduction in myocardial infarct size. One possible explanation for this apparent discrepancy is that the eNOS TG mice are exposed to elevated NO levels from the time of birth (i.e., chronic exposure), whereas NO donors have been administered to wild-type animals only for a short period of time. Thus chronic exposure to elevated eNOS and NO levels may induce myocardial tolerance, resulting in a reduction in myocardial protection compared with treatment of naïve mice with NO-donating agents. This observation may have important implications for the use of gene therapy. A previous study (34) has revealed that eNOS TG mice are resistant to NO/cGMP signaling. A recent study (2) has also demonstrated that eNOS overexpression protects isolated perfused hearts against myocardial reperfusion injury. Brunner et al. (2) demonstrated significant cardioprotection in gene-targeted mice with cardiac myocyte-specific overexpression of eNOS. Exper-

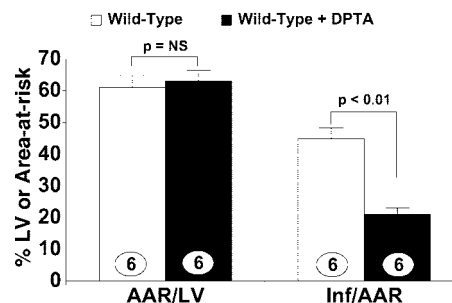


Fig. 9. Myocardial infarct size in wild-type mice treated with the NO donor dipropylentriamine NONOate (DPTA), at a dose of 100 μ g/kg 5 min before reperfusion. DPTA was injected directly into the LV. Mice were subjected to 30 min of myocardial ischemia and 24 h of reperfusion. The AAR per LV was not significantly different between groups; however, the infarct size per AAR was significantly ($P < 0.01$) attenuated in mice receiving DPTA. Numbers within the bars are numbers of mice per group.

iments utilizing a conditional transgenesis approach may provide important new insights regarding eNOS overexpression and MI/R injury.

The primary mechanism(s) responsible for the observed cardioprotective effects in the two strains of eNOS TG mice remains unknown. Future studies in our laboratory will focus on the elucidation of the precise cellular mechanisms responsible for cytoprotection in the eNOS TG mice. NO derived from eNOS has a highly diverse biological profile that includes antiplatelet (21) and antileukocyte actions (16), vasodilator effects (7, 26), and direct cytoprotective actions (11). It is likely that the cardioprotective actions observed in eNOS TG mice are the result of a number of these actions of NO. Recently, experimental evidence indicated that NO can modulate mitochondrial K_{ATP} channels in the setting of myocardial preconditioning and thereby induce myocardial protection (23). We treated both NTG and eNOS TG mice with a selective inhibitor of mitochondrial K_{ATP} channels before the onset of myocardial ischemia and observed no attenuation of myocardial infarct size. These data suggest that the reduction in myocardial infarct size observed in the eNOS TG mouse is not dependent on activation of mitochondrial K_{ATP} channels in the myocardium.

In summary, overexpression of the eNOS enzyme significantly attenuates the extent of myocardial infarct size after coronary artery ischemia and reperfusion. These data provide clear evidence of the beneficial role of enhanced eNOS-derived NO production during myocardial reperfusion injury. Future studies should be directed toward identifying the mechanism of this protective effect. In the future, therapies might be developed to improve vascular eNOS function as a means to improve outcomes in patients suffering from acute myocardial infarction.

GRANTS

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