Increased Inducible Nitric Oxide Synthase Expression Contributes to Myocardial Dysfunction and Higher Mortality After Myocardial Infarction in Mice

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***Background*—**Inducible nitric oxide synthase (iNOS) is expressed in the myocardium after myocardial infarction (MI) and in heart failure. Its pathophysiological role in these conditions, however, is not clear. We hypothesized that increased NO production from iNOS expression causes myocardial dysfunction and results in higher mortality after MI.

***Methods and Results*—**MI was induced by left coronary artery ligation in iNOS$^{-/-}$ mutant and wild-type mice. Mortality was followed up for 30 days. MI resulted in a significant increase in mortality in both iNOS$^{-/-}$ and wild-type mice compared with sham operation ($P<0.01$). Mortality was significantly decreased and LV myocardial contractility was increased, however, in iNOS$^{-/-}$ mice compared with the wild-type mice ($P<0.05$). Five days after MI, myocardial iNOS mRNA expression, plasma nitrate and nitrite concentrations, and myocardial and plasma nitrotyrosine levels were significantly increased in wild-type compared with iNOS$^{-/-}$ mutant mice ($P<0.05$). Both basal LV +dP/dt and its response to dobutamine were significantly increased in iNOS$^{-/-}$ compared with the wild-type mice ($P<0.05$).

***Conclusions*—**Increased NO production from iNOS expression contributes to myocardial dysfunction and mortality after MI in mice. **(*Circulation*. 2001;104:700-704.)**

Key Words: heart failure ■ nitric oxide ■ nitric oxide synthase ■ myocardial infarction

Nitric oxide (NO) is produced from L-arginine by a family of NO synthases. Three distinct isoforms of nitric oxide synthase (NOS), derived from separate genes, are neural NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS).[1] Whereas eNOS and nNOS are calcium-dependent enzymes and produce small amounts of NO on stimulation, iNOS is a calcium-independent enzyme often induced by cytokines and produces high levels of NO. Basal generation of NO by eNOS plays an important role in the regulation of basal vascular tone, blood pressure, and tissue perfusion.[2,3] High levels of NO produced by activated macrophages not only may be toxic to undesired microbes, parasites, or tumor cells but also may harm healthy cells.[1]

Cardiac myocytes have been demonstrated to produce iNOS protein and activity within several hours of treatment with cytokines.[4] Recent studies have shown that iNOS expression and activity are increased in the myocardium of failing hearts and result in increased NO levels in the circulation.[5–9] Although increased NO production from iNOS may decrease vascular resistance, which is beneficial, high levels of NO may also depress myocardial contractility and, through formation of peroxynitrite, may cause myocardial damage.[10] In the present study, we hypothesized that increased NO production from iNOS expression causes myocardial dysfunction and results in high mortality after myocardial infarction (MI). To test this hypothesis, we occluded the left coronary artery in iNOS$^{-/-}$ mutant and wild-type mice and investigated the role of iNOS in myocardial dysfunction and disease progression after MI.

Methods

Animals

Animals used in this study were handled in accordance with the guidelines of the Animal Care Committee at the University of Western Ontario, Canada. Breeding pairs of iNOS$^{-/-}$ mutant (stock 2609) and C57BL6 wild-type mice were purchased from Jackson Laboratory. A breeding program was carried out to produce adult mice (age 3 to 6 months) for the experiments. Mice were genotyped by a polymerase chain reaction (PCR) method using genomic DNA extracted from the tail.

Induction of MI

Mice were randomly selected to undergo coronary artery ligation or sham surgery by a technique similar to that described in rats.[5,11] Mice were anesthetized with sodium pentobarbital (50 mg/kg IP). Atropine (0.05 mg SC) was administered to reduce airway excretion. Animals were intubated and artificially ventilated with a respirator (SAR-830, CWE, Inc). A left intercostal thoracotomy was performed. After the pericardium had been opened, the left coronary artery was ligated by a suture. The lungs were then hyperinflated,

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and the thorax was closed. Sham-operated mice underwent the same surgery minus the coronary artery ligation. The infarct size was measured at the end of the experiment and was expressed as a fraction of the total cross-sectional endocardial circumference of the left ventricle (LV).[5,11]

Hemodynamic Measurements

Mice were anesthetized with sodium pentobarbital (50 mg/kg IP) for catheter placements. The right carotid artery was cannulated with a Millar tip transducer catheter (model SPR-261, 1.4F). After arterial blood pressure and heart rate measurements were obtained, the catheter was advanced to the LV for measurement of LV systolic and end-diastolic pressures as well as the maximal rate of pressure development (+dP/dt) and rate of relaxation (−dP/dt) of LV.

Isolated Heart Preparation

Mice were killed by cervical dislocation. Hearts were rapidly removed and placed on a Langendorff apparatus perfused with Krebs solution at 37°C. Contractility was measured by use of ultrasound crystals.[12] The advantage of this technique over the classic Langendorff preparation in studying infarcted hearts is that a balloon is not required in the LV chamber. LV pressures were monitored by a fluid-filled catheter connected to a pressure transducer. Both atria were cut open to drain perfusate. The crystals (0.7 and 1.0 mm) were fixed on the heart surface to allow long- and short-axis measurement. The ultrasound and pressure signals were measured by a Digital Sonomicrometer (Sonometrics). Maximum and minimum distances as well as percent shortening were calculated.[12]

Nitrate/Nitrite Assay

Plasma nitrate/nitrite (NO_x) levels were measured as we previously described.[5] Briefly, nitrate was converted to nitrite with *Aspergillus* nitrate reductase, and the total nitrite was measured with the Griess reagent. The absorbance was determined at 540 nm with a spectrophotometer.

Nitrotyrosine Measurements

Nitrotyrosine, the fingerprint of peroxynitrite in the myocardium, was determined by ELISA according to the manufacturer's instructions (Cayman Chemical). Briefly, the noninfarcted LV myocardium was homogenized, and the supernatant was obtained. Plasma and tissue supernatant were concentrated to 2 to 4 times before they were incubated overnight with anti-nitrotyrosine rabbit IgG (Chemicon International) and nitrotyrosine acetylcholinesterase tracer in precoated (mouse anti-rabbit IgG) microplates followed by color development with Ellman's reagent. The absorbance was measured at 405 nm. Intra-assay and interassay variabilities were 7% and 9%, respectively. To determine cellular localization of nitrotyrosine in the myocardium, immunohistochemical staining was performed in paraffin-embedded sections of the heart by use of the same antibodies as above. Sections were counterstained with hematoxylin.

Reverse Transcription–PCR

Total RNA was isolated from the noninfarcted LV myocardium with Trizol reagent and reverse transcribed into first-strand cDNA by use of the Moloney murine leukemia virus reverse transcriptase (RT) system. The cDNAs of iNOS and GAPDH were amplified by PCR with the same primers and conditions as we described previously.[13] Equal aliquots of cDNA were amplified for 38 and 20 cycles for iNOS and GAPDH, respectively. PCR products of iNOS (189 bp) and GAPDH (297 bp) were electrophoresed in 1.5% agarose gels.

Statistical Analysis

Data were expressed as the mean±SEM. ANOVAs were performed with the Student-Newman-Keuls test to detect significance in multiple groups or Student's *t* test between 2 groups. Survival was analyzed by the method of Kaplan and Meier. Differences were considered significant at the level of $P<0.05$.

TABLE 1. General Characteristics of $iNOS^{-/-}$ and Wild-Type Mice Subjected to MI or Sham Operation

	MI		Sham	
	Wild	$iNOS^{-/-}$	Wild	$iNOS^{-/-}$
n	58	58	10	14
Sex, M/F	53/5	52/6	9/1	12/2
Age, d	100±5	97±5	121±26	110±13
Body weight, g	27.0±0.4	28.1±0.5	26.6±0.8	28.9±1.0
Infarct size, %	33.8±0.8	35.1±0.9	0	0

Note, there was no statistical difference between $iNOS^{-/-}$ and wild-type mice in any of the above parameters within the MI or sham group.

Results

Mortality After MI

A total of 99 wild-type and 97 $iNOS^{-/-}$ mice were subjected to MI or sham operation. Animals were excluded from analysis for 2 reasons: (1) perioperative death, within the first 24 hours after surgery (28 wild-type and 23 $iNOS^{-/-}$) or (2) infarct size <20% of the LV (3 wild-type and 2 $iNOS^{-/-}$). The remaining 68 wild-type and 72 $iNOS^{-/-}$ mice were included in the study, and their mortality was followed up for 30 days after surgery. General characteristics of these animals are shown in Table 1. There were no differences in age, sex, body weight, or infarct size between $iNOS^{-/-}$ mutant and wild-type mice subjected to MI (P=NS). MI resulted in a significant increase in mortality in both $iNOS^{-/-}$ and wild-type mice compared with sham operation ($P<0.001$, Figure 1). The 30-day survival in $iNOS^{-/-}$ mice (58.6%, or 34/58), however, was significantly increased compared with the wild-type mice (37.9%, or 22/58, $P=0.034$, Figure 1).

Thirty days after MI, plasma NO_x levels were significantly increased in the wild-type mice (Table 2). There were no significant differences in infarct size, heart rate, mean arterial pressure, or LV systolic pressure between $iNOS^{-/-}$ and wild-type mice. LV dP/dt, however, was increased in $iNOS^{-/-}$ compared with the wild-type mice ($P<0.01$, Table 2). Myocardial contractile function after MI was also studied in a modified Langendorff preparation. Basal LV end-diastolic pressure was 0.2±0.4 and 0.5±0.5 mm Hg in wild-type and $iNOS^{-/-}$ mice (n=3 per group), respectively. In response to dobutamine 3 μg/mL, LV end-diastolic pressure was −0.2±0.6 and 0.5±0.5 mm Hg in wild-type and $iNOS^{-/-}$ mice (n=3 per

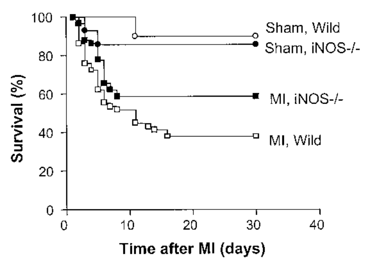

Figure 1. Survival after MI in $iNOS^{-/-}$ mutant and wild-type mice. Animals were followed up for 30 days after surgery. Post-MI survival was significantly increased in $iNOS^{-/-}$ mutants (n=58) vs wild-type mice (n=58, $P<0.05$). There was no significant difference in survival after sham operation between $iNOS^{-/-}$ (n=14) and wild-type (n=10) mice.

TABLE 2. Changes in Plasma NO_x and Hemodynamic Parameters in Anesthetized $iNOS^{-/-}$ Mutant and Wild-Type Mice 30 Days After MI

Parameters	Wild-Type	$iNOS^{-/-}$	*P*
n	9	8	
Plasma NO_x, μmol/L	88.4±10.5	24.3±11.1	<0.01
Weight			
Body, g	30.6±1.2	31.2±1.5	NS
LV, mg	137.9±9.3	137.2±4.3	NS
RV, mg	38.2±5.5	30.7±2.5	NS
Heart, mg	171.3±15.3	170.8±3.6	NS
LV/body weight ratio, mg/g	4.6±0.4	4.4±0.2	NS
Heart/body weight ratio, mg/g	5.4±0.6	5.4±0.2	NS
HR, bpm	422±16	425±18	NS
MAP, mm Hg	67±3	69±4	NS
LVSP, mm Hg	85±2	93±3	NS
LVEDP, mm Hg	9.1±1.2	6.3±1.0	0.09
LV +dP/dt, mm Hg/s	4036±228	5188±205	<0.01
LV −dP/dt, mm Hg/s	4214±291	5250±236	<0.05
Infarct size, %	34.3±2.5	34.4±2.2	NS

RV indicates right ventricle; HR, heart rate; MAP, mean arterial pressure; LVSP, LV systolic pressure; and LVEDP, LV end-diastolic pressure.

group), respectively. There were no significant changes in perfusion pressure during the experiment (data not shown). Dimensions of the heart at baseline were similar between wild-type and $iNOS^{-/-}$ mice (long axis 9.98±0.39 versus 10.06±0.21 mm; short axis 8.71±0.26 versus 8.49±0.20 mm, n=6 per group, *P*=NS). Shortening of the long axis, however, was significantly increased in $iNOS^{-/-}$ compared with wild-type mice ($P<0.05$). Mice deficient in iNOS also had a better response to dobutamine 3 μg/mL than did wild-type mice ($P<0.05$, Table 3).

Because most of the animals died within 5 days after MI, myocardial function, iNOS mRNA expression, and plasma NO_x levels were determined in a separate experiment 5 days after MI.

iNOS Expression, NO Production, and Nitrotyrosine Generation

Five days after MI, iNOS mRNA expression in the noninfarcted myocardium was determined by RT-PCR (Figure 2A). There was no iNOS mRNA expression after MI in $iNOS^{-/-}$ mutant mice or after sham operations. Significant iNOS mRNA expression was present, however, in the noninfarcted myocardium of wild-type mice after MI. Consistent

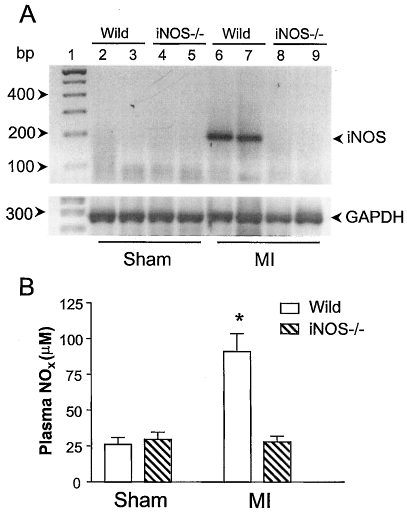

Figure 2. A, Expression of iNOS mRNA in LV myocardium 5 days after MI. Both iNOS and GAPDH mRNAs were determined by RT-PCR. Representative gels (1.5% agarose) of 3 independent experiments are shown. Each lane represents an individual animal. B, Plasma NO_x concentrations in $iNOS^{-/-}$ mutant and wild-type mice 5 days after MI. n=5 per group, *$P<0.05$ vs all 3 groups.

with myocardial iNOS expression, plasma NO_x concentrations were significantly increased after MI in wild-type mice compared with $iNOS^{-/-}$ mutant ($P<0.01$) as well as sham-operated mice ($P<0.01$, Figure 2B). Immunohistochemical staining demonstrated that nitrotyrosine was present in cardiomyocytes of the noninfarcted LV myocardium in both wild-type (n=3) and $iNOS^{-/-}$ mice (n=4). The intensity of nitrotyrosine staining was much stronger in wild-type than $iNOS^{-/-}$ mice (Figure 3D and 3E). The staining was inhibited by nitrotyrosine preincubation with the anti-nitrotyrosine antibody (Figure 3B) but not by tyrosine (Figure 3C), indicating specificity of nitrotyrosine staining. Nitrotyrosine levels determined by ELISA were increased in the plasma (31.0±3.2 versus 21.2±1.7 ng/mL) and LV myocardium (29.0±1.8 versus 21.5±1.9 ng/mg protein) in wild-type compared with $iNOS^{-/-}$ mice (n=5 per group, $P<0.05$).

Hemodynamic Changes

Hemodynamic measurements were made 5 days after MI in anesthetized $iNOS^{-/-}$ (n=8) and wild-type (n=7) mice. There were no significant differences in infarct size, heart rate, mean arterial pressure, or LV systolic pressure between $iNOS^{-/-}$ and wild-type mice (data not shown). LV end-dia-

TABLE 3. Percent Shortening in Isolated Hearts From $iNOS^{-/-}$ and Wild-Type Mice 30 Days After MI

		Baseline			Dobutamine 1 μg/mL			Dobutamine 3 μg/mL		
	Infarct Size, %	% Shortening			% Shortening			% Shortening		
		Long Axis	Short Axis	HR, bpm	Long Axis	Short Axis	HR, bpm	Long Axis	Short Axis	HR, bpm
Wild, n=6	34±4	0.90±0.10	1.18±0.15	229±11	1.06±0.11	1.18±0.09	239±29	1.01±0.18	1.22±0.05	235±27
$iNOS^{-/-}$, n=6	33±3	1.38±0.18	1.93±0.33	223±27	1.74±0.52	1.91±0.39	251±35	2.11±0.60	2.44±0.40	295±46
P	NS	<0.05	0.08	NS	NS	NS	NS	0.09	<0.05	NS

HR indicates spontaneous heart rate. Data were compared by unpaired Student's *t* test between wild-type and $iNOS^{-/-}$ mice.

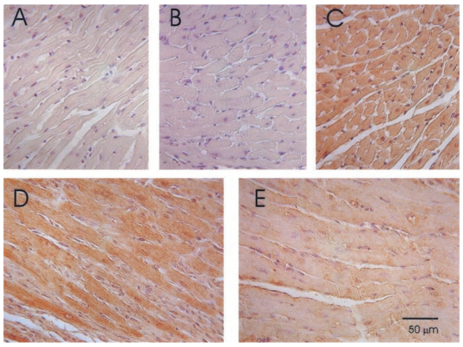

Figure 3. Nitrotyrosine levels in wild-type and iNOS$^{-/-}$ mutant mice 5 days after MI. A through E, Immunohistochemical staining of nitrotyrosine. Negative controls (A) were performed without anti-nitrotyrosine antibody. Anti-nitrotyrosine antibody (1:50) was preincubated with nitrotyrosine (200 μmol/L, B) or tyrosine (200 μmol/L, C) for 1 hour at room temperature before antibody was incubated with tissue section. Tissues in A through D were all from wild-type mice. Representative nitrotyrosine staining in noninfarcted LV myocardium from wild-type (D) and iNOS$^{-/-}$ mutant (E) mice.

stolic pressure was decreased (8.1±1.2 versus 12.4±1.4 mm Hg, $P<0.05$), however, and LV +dP/dt was increased in iNOS$^{-/-}$ mutants compared with the wild-type mice ($P<0.05$, Figure 4A). In response to dobutamine 4 μg/kg IV, the increase of LV +dP/dt was significantly enhanced in iNOS$^{-/-}$ compared with the wild-type mice ($P<0.05$, Figure 4B). Basal and dobutamine-stimulated LV −dP/dt were not statistically different between the 2 groups (P=NS).

Discussion

The main finding of the present study was the significant increase of survival after MI in iNOS$^{-/-}$ mutants compared with the wild-type mice. After MI, iNOS expression was induced in the LV myocardium and resulted in elevations of NO and nitrotyrosine levels in the wild-type compared with the iNOS$^{-/-}$ mutant mice. Furthermore, increases in NO production and nitrotyrosine levels in the wild-type mice were associated with decreased myocardial function. The results suggest that increased NO production and peroxynitrite formation from iNOS expression contribute to myocardial dysfunction and heart failure progression in mice after MI.

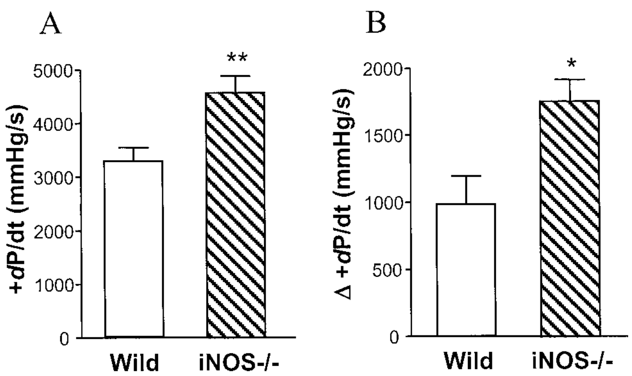

Figure 4. LV +dP/dt in iNOS$^{-/-}$ and wild-type mice 5 days after MI. A, Basal levels of LV +dP/dt. B, Increases of LV +dP/dt after dobutamine (4 μg/kg IV). n=7 to 8 per group. *$P<0.05$, **$P<0.01$ vs wild-type mice.

A number of cellular constituents of cardiac muscle, including the endothelium and smooth muscle of the cardiac microvasculature, the endocardial endothelium, and cardiac myocytes, are now known to be capable of expressing iNOS in response to lipopolysaccharide and specific cytokines.[14,15] Myocardial iNOS expression has been demonstrated in humans and animals with induced heart failure regardless of pathogenesis.[6–9,16–19] Consistent with this notion, the present study showed a marked iNOS expression in the noninfarcted area of the LV myocardium after MI in the wild-type mice. Mechanisms of the increased iNOS expression and NO production after MI are still not fully understood. Cytokines such as tumor necrosis factor-α are increased in rats with MI[20] and in patients with heart failure.[17,21] Many factors, such as activation of angiotensin II and α-adrenergic receptors, may also promote iNOS expression in cardiac myocytes after MI.[22]

Myocardial iNOS induction has been demonstrated to cause contractile dysfunction in various preparations, including isolated myocytes, isolated perfused working hearts, and in vivo animal preparations.[4,14,15,23] NO produced by iNOS within cardiac myocytes is reported to be responsible for diminished inotropic responsiveness to isoproterenol in an autocrine and/or paracrine fashion.[24] In patients with heart failure due to idiopathic dilated cardiomyopathy, inhibition of NO synthesis potentiates the positive inotropic response to β-adrenergic stimulation.[25] The physiological sequelae of iNOS induction may not be limited to a reversible decline in myocyte contractile function. Expression of iNOS has been shown to induce apoptosis in macrophages[26,27] and vascular smooth muscle cells.[28] Our recent studies have demonstrated that iNOS expression induces apoptosis in cardiomyocytes.[13] The contribution of NO-induced apoptosis in cardiac dysfunction after MI, however, requires further investigation.

To investigate the specific role of iNOS in the development of heart failure, we used iNOS$^{-/-}$ mutant mice. As expected, there was no iNOS expression in the myocardium, and plasma NO_x levels were not elevated in the iNOS$^{-/-}$ mutant mice after MI. Basal myocardial contractility was better preserved in iNOS$^{-/-}$ mutant mice than wild-type mice 5 days after MI. In response to the β-adrenergic agonist dobutamine, the increase of LV +dP/dt was enhanced in iNOS$^{-/-}$ mutant mice compared with the wild-type mice. Better basal contractility and enhanced response to dobutamine were also observed in the isolated hearts of iNOS$^{-/-}$ mice. Our results agree with a recent study that showed that selective inhibition of iNOS activity improves cardiac performance in rabbits with acute MI.[29] To further examine the role of iNOS in development of heart failure, mice were followed up for 30 days after MI. Although the infarct size was similar, survival was significantly increased in iNOS$^{-/-}$ mice. Furthermore, the iNOS$^{-/-}$ survivors had better LV contractility than wild-type survivors. Therefore, the present study demonstrated both a significant increase in survival and improved myocardial function after MI in iNOS$^{-/-}$ compared with wild-type mice.

Many of the toxic actions of NO are mediated by peroxynitrite, the reaction product of NO and superoxide (O_2^-).[30] The detrimental effects of peroxynitrite include oxidation of lipids, nitration of protein tyrosine residues to form nitrotyrosine products, oxidation of free protein-associated thiols, and stimulation of apoptosis.[30] A recent study demonstrated that peroxynitrite is a major contributor to cytokine-induced myocardial dysfunction.[10] In the present study, nitrotyrosine levels, the fingerprints of peroxynitrite, were significantly increased in the LV myocardium and plasma of wild-type mice after MI compared with iNOS$^{-/-}$ mice. Our results support the notion the peroxynitrite is involved in the myocardial dysfunction in mice with MI.

Formation of peroxynitrite depends on the balance between local concentrations of NO, O_2^-, and superoxide dismutase (SOD).[30] In the isolated perfused hearts, a 5-fold increase in NO production was associated with <1-fold increase in nitrotyrosine formation,[10] clearly indicating that other factors, not just NO, contribute significantly to the formation of peroxynitrite. In the present study, marked NO production was associated with only a moderate increase (35% to 46%) in nitrotyrosine in wild-type mice after MI. The reason for this is not clear. SOD is increased in rats after MI.[31] The increased SOD activity enhances the clearance of O_2^-. Furthermore, formation of nitrate and nitrite is a major decomposition pathway of NO in vivo because oxyhemoglobin in red blood cells rapidly combines with NO to yield methemoglobin and nitrate.[32] These mechanisms may explain a moderate increase in peroxynitrite production and nitrotyrosine formation in the present study. Factors that contributed to basal levels of nitrotyrosine in the myocardium and plasma of iNOS$^{-/-}$ mice are not known. Reactive species, such as nitrogen dioxide and acidified nitrite, can produce nitrotyrosine.[30] Moreover, myeloperoxidase and horseradish peroxidase also oxidize nitrite in the presence of H_2O_2 into species able to nitrate tyrosine.[33] The contribution of these factors to the production of nitrotyrosine after MI requires further investigation.

In summary, MI results in myocardial iNOS expression and NO production and higher nitrotyrosine levels, leading to myocardial dysfunction and increased mortality. Further studies are necessary to investigate the therapeutic potential of inhibiting iNOS activity versus reducing peroxynitrite formation in heart failure.

Acknowledgments

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References

1. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. 1991;43:109–142.
2. Huang PL, Huang Z, Mashimo H, et al. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature*. 1995;377:239–242.
3. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*. 1989;2:997–1000.
4. Schulz R, Nava E, Moncada S. Induction and potential biological relevance of a Ca^{2+}-independent nitric oxide synthase in the myocardium. *Br J Pharmacol*. 1992;105:575–580.
5. Feng Q, Fortin AJ, Lu X, et al. Effects of L-arginine on endothelial and cardiac function in rats with heart failure. *Eur J Pharmacol*. 1999;376:37–44.
6. Haywood GA, Tsao PS, von der Leyen HE, et al. Expression of inducible nitric oxide synthase in human heart failure. *Circulation*. 1996;93:1087–1094.
7. Heymes C, Vanderheyden M, Bronzwaer JG, et al. Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. *Circulation*. 1999;99:3009–3016.
8. Drexler H, Kastner S, Strobel A, et al. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. *J Am Coll Cardiol*. 1998;32:955–963.
9. Vejlstrup NG, Bouloumie A, Boesgaard S, et al. Inducible nitric oxide synthase (iNOS) in the human heart: expression and localization in congestive heart failure. *J Mol Cell Cardiol*. 1998;30:1215–1223.
10. Ferdinandy P, Danial H, Ambrus I, et al. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. *Circ Res*. 2000;87:241–247.
11. Feng Q, Lu X, Fortin AJ, et al. Elevation of an endogenous inhibitor of nitric oxide synthesis in experimental congestive heart failure. *Cardiovasc Res*. 1998;37:667–675.
12. Jones DL, Narayanan N. Defibrillation depresses heart sarcoplasmic reticulum calcium pump: a mechanism of postshock dysfunction. *Am J Physiol*. 1998;274:H98–H105.
13. Song W, Lu X, Feng Q. Tumor necrosis factor-alpha induces apoptosis via inducible nitric oxide synthase in neonatal mouse cardiomyocytes. *Cardiovasc Res*. 2000;45:595–602.
14. Balligand JL, Ungureanu D, Kelly RA, et al. Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. *J Clin Invest*. 1993;91:2314–2319.
15. Brady AJ, Poole-Wilson PA, Harding SE, et al. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am J Physiol*. 1992;263:H1963–H1966.
16. Winlaw DS, Smythe GA, Keogh AM, et al. Increased nitric oxide production in heart failure. *Lancet*. 1994;344:373–374.
17. Habib FM, Springall DR, Davies GJ, et al. Tumor necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy. *Lancet*. 1996;347:1151–1155.
18. Fukuchi M, Hussain SNA, Giaid A. Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure. *Circulation*. 1998;98:132–139.
19. Stein B, Eschenhagen T, Rudiger J, et al. Increased expression of constitutive nitric oxide synthase III, but not inducible nitric oxide synthase II, in human heart failure. *J Am Coll Cardiol*. 1998;32:1179–1186.
20. Irwin MW, Mak S, Mann DL, et al. Tissue expression and immunolocalization of tumor necrosis factor-α in postinfarction dysfunctional myocardium. *Circulation*. 1999;99:1492–1498.
21. Levine B, Kalman J, Mayer L, et al. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med*. 1990;323:236–241.
22. Ikeda U, Murakami Y, Kanbe T, et al. Alpha-adrenergic stimulation enhances inducible nitric oxide synthase expression in rat cardiac myocytes. *J Mol Cell Cardiol*. 1996;28:1539–1545.
23. Gardiner SM, Kemp PA, March JE, et al. Cardiac and regional haemodynamics, inducible nitric oxide synthase (NOS) activity, and the effects of NOS inhibitors in conscious, endotoxaemic rats. *Br J Pharmacol*. 1995;116:2005–2016.
24. Balligand JL, Ungureanu Longrois D, Simmons WW, et al. Cytokine-inducible nitric oxide synthase (iNOS) expression in cardiac myocytes: characterization and regulation of iNOS expression and detection of iNOS activity in single cardiac myocytes in vitro. *J Biol Chem*. 1994;269:27580–27588.
25. Hare JM, Givertz MM, Creager MA, et al. Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: potentiation of β-adrenergic inotropic responsiveness. *Circulation*. 1998;97:161–166.
26. Albina JE, Cui S, Mateo RB, et al. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. *J Immunol*. 1993;150:5080–5085.
27. Shimaoka M, Iida T, Ohara A, et al. NOC, a nitric-oxide-releasing compound, induces dose dependent apoptosis in macrophages. *Biochem Biophys Res Commun*. 1995;209:519–526.
28. Geng YJ, Wu Q, Muszynski M, et al. Apoptosis of vascular smooth muscle cells induced by in vitro stimulation with interferon-γ, tumor necrosis factor-α, and interleukin-1β. *Arterioscler Thromb Vasc Biol*. 1996;16:19–27.
29. Wildhirt SM, Suzuki H, Horstman D, et al. Selective modulation of inducible nitric oxide synthase isozyme in myocardial infarction. *Circulation*. 1997;96:1616–1623.
30. Beckman JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. *Am J Physiol*. 1996;271:C1424–C1437.
31. Hill MF, Singal PK. Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. *Am J Pathol*. 1996;148:291–300.
32. Doyle MP, Hoekstra JW. Oxidation of nitrogen oxides by bound dioxygen in hemoproteins. *J Inorg Biochem*. 1981;14:351–358.
33. van der Vliet A, Eiserich JP, Halliwell B, et al. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite: a potential additional mechanism of nitric oxide-dependent toxicity. *J Biol Chem*. 1997;272:7617–7625.