Spontaneous Myocardial Infarction and Nitric Oxide Synthase

Masato Tsutsui*, Sei Nakata, Hiroaki Shimokawa, Yutaka Otsuji, and Nobuyuki Yanagihara

Myocardial infarction (MI) is caused by coronary atherosclerosis and/or arteriosclerosis. Because endothelial nitric oxide synthase (eNOS) exerts powerful antiatherosclerotic/antiarteriosclerotic effects, it is speculated that blockade of eNOS activity might result in MI. However, neither genetic disruption of eNOS nor pharmacologic inhibition of eNOS activity induces MI in animals. On the other hand, intriguingly, genetic disruption of all three nitric oxide synthase (NOS) isoforms (neuronal NOS, inducible NOS, and eNOS) spontaneously caused MI accompanied by multiple cardiovascular risk factors of metabolic origin in mice. This is the first in vivo demonstration showing that the defective NOS system is involved in the pathogenesis of spontaneous MI. Based on the evidence, this review summarizes our current knowledge of spontaneous MI and NOS. (Trends Cardiovasc Med 2008;18:275–279) © *2008, Elsevier Inc.*

• Introduction

Myocardial infarction (MI) is a disorder in which cardiac myocytes undergo necrosis as a consequence of interrupted coronary blood flow. This is commonly due to occlusion of an atherosclerotic/arteriosclerotic coronary artery. Myocardial infarction is the leading cause of death for both sexes all over the world. The molecular mechanisms for the pathogenesis of MI, however, remain to be fully elucidated (Antman and Braunwald 1997).

The nitric oxide (NO) synthase (NOS) system consists of three different isoforms: neuronal (nNOS), inducible (iNOS), and endothelial NOS (eNOS). The roles of the entire NOS system in vivo have been examined in pharmacologic studies with nonselective NOS inhibitors (Bredt and Snyder 1994, Furchgott 1984, Ignarro 1990, Moncada et al. 1991, Murad 1997, Shimokawa 1999). However, because the NOS inhibitors possess multiple nonspecific actions, the authentic roles of the NOS system in our body are still poorly understood. To address this issue, we have recently developed mice in which all three NOS genes are completely disrupted (triply *nNOS/iNOS/eNOS*$^{-/-}$ mice) (Morishita et al. 2005, Tsutsui et al. 2006). Notably, the triply *NOS*$^{-/-}$ mice manifested spontaneous MI with a shorter survival (Figure 1). These results provide the first direct evidence that complete deletion of all NOS isoforms causes spontaneous MI in mice in vivo, demonstrating the critical role of the defective NOS system in the pathogenesis of spontaneous MI (Nakata et al. 2008). Based on this finding, in the present article, we summarize our latest knowledge about spontaneous MI and NOS.

• History

Studies with eNOS-deficient Mice

It is well established that endothelium-derived NO, synthesized by eNOS, possesses a variety of antiatherosclerotic/antiarteriosclerotic actions. These include a stimulatory effect on vasodilation as well as inhibitory effects on vascular smooth muscle cell proliferation, platelet aggregation, monocyte adhesion, low-density lipoprotein (LDL) oxidation, and vascular inflammation (Harrison 1997, Luscher and Vanhoutte 1990, Sessa 1994). Thus, it is speculated that blockade of endothelial NO synthesis might result in the occurrence of MI. However, mice devoid of eNOS fail to exhibit spontaneous MI (Moroi et al. 1998, Nakata et al. 2008).

This inconsistency may be due to a compensatory mechanism by other NOS isoforms that are not genetically disrupted. Indeed, in the *eNOS*$^{-/-}$ mice, upregulation of vascular nNOS has been found (Huang et al. 2002, Lamping et al. 2000, Tsutsui 2004). In addition, we have also revealed that both NOS activity and NOx (nitrite plus nitrate) production are fairly well preserved in the genotype (Morishita et al. 2005).

Studies with NOS Inhibitors

L-Arginine analogues, including N^{ω}-nitro-L-arginine methyl ester and N^{G}-monomethyl-L-arginine, have been widely used as pharmacologic tools to inhibit all three NOS isoforms. Long-term oral treatment with the L-arginine analogues leads to the formation of coronary arteriosclerotic lesions in animals, the mechanism of which had been believed to mediate the inhibition of eNOS activity. However,

Masato Tsutsui and Nobuyuki Yanagihara are at the Department of Pharmacology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan. Sei Nakata and Yutaka Otsuji are at the Second Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan. Hiroaki Shimokawa is at the Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan.

* Address correspondence to: Masato Tsutsui, Department of Pharmacology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan. Tel.: (+81) 93 603 1611; fax: (+81) 93 601 6264; e-mail: mt2498@med.uoeh-u.ac.jp.

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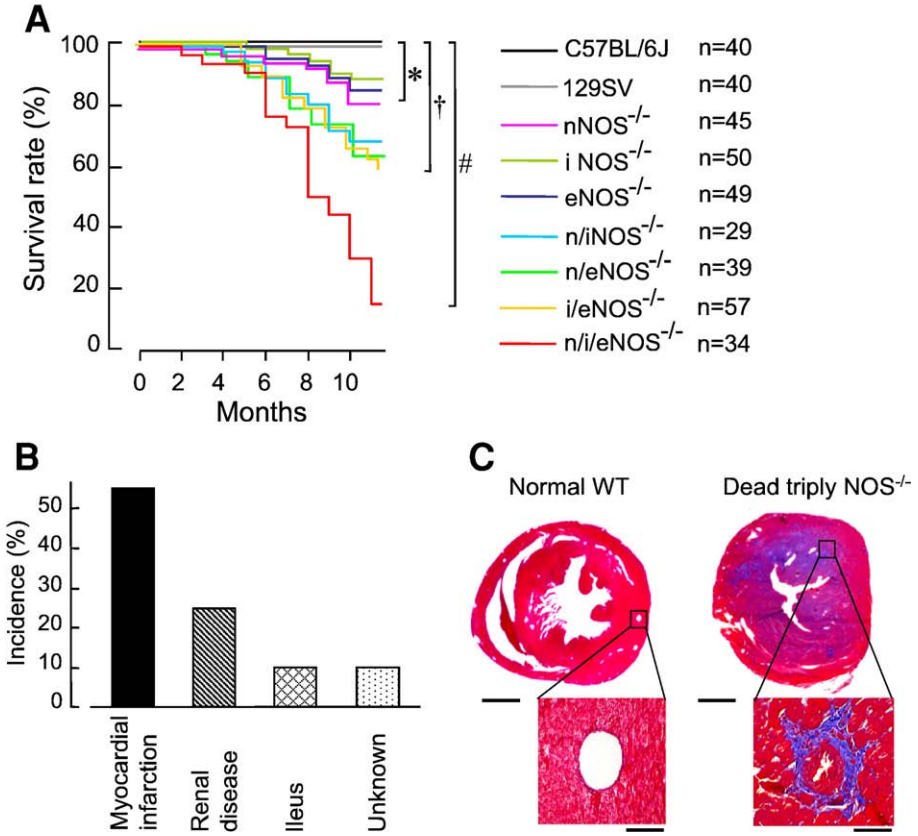

Figure 1. Decreased survival and spontaneous myocardial infarction (MI) in male triply *n/i/eNOS*$^{-/-}$ mice. (**A**) Survival rate (n = 29-57). *$P < .05$ between singly *NOS*$^{-/-}$ and C57BL/6J; †$P < .05$ between doubly *NOS*$^{-/-}$ and C57BL/6J; #$P < .05$ between triply *n/i/eNOS*$^{-/-}$ and C57BL/6J. (**B**) Causes of death (n = 20). (**C**) Acute MI and coronary arteriosclerotic lesion formation in male triply *n/i/eNOS*$^{-/-}$ mouse that died at 8 months of age (Masson-trichrome staining). Blue in the heart cross-section of the dead triply *n/i/eNOS*$^{-/-}$ mouse indicates anteroseptal acute MI (**right upper panel**). Adjacent coronary artery shows marked luminal narrowing, wall thickening, and perivascular fibrosis (blue) (**right lower panel**). WT indicates wild-type C57BL/6 mice. Scale bars in the heart, 1 mm. Scale bars in the coronary artery, 100 μm. In panel **C**, living WT mice were used. The "n" represents the number of mice used in each group. Data from *Circulation* 2008;117:2211-2223.

those vascular lesions are prominent at microvascular levels but not at macrovascular levels, and no MI is provoked (Baylis et al. 1992).

We have addressed the reason for this and revealed unexpected evidence that the long-term vascular effects of the L-arginine analogues are not mediated by the simple inhibition of NOS activity. Direct activation of the renin-angiotensin system and increased oxidative stress, independent of endogenous NO inhibition, appear to be involved in the long-term vascular effects of those analogues (Suda et al. 2002, 2004). Consistent with our results, various nonspecific actions of the L-arginine analogues, including antagonism of muscarinic acetylcholine receptors (Buxton et al. 1993), generation of superoxide anions (Heim et al. 1991), inhibition of cytochrome c reduction (Peterson et al. 1992), and inhibition of endothelium-independent relaxation induced by amiloride or 3′-5′-cyclic adenosine monophosphate (Thomas and Ramwell 1991) have been reported.

• Spontaneous Myocardial Infarction Caused by Defective NOS System

Male triply *n/i/eNOS*$^{-/-}$ mice showed markedly reduced survival as compared with male wild-type mice (Figure 1A). Notably, more than half of the triply *NOS*$^{-/-}$ mice died because of spontaneous MI accompanied by highly advanced coronary arteriosclerotic lesions (Figures 1B and C and 2A).

Marked coronary arteriosclerosis (intimal hyperplasia and medial thickening) without calcification were noted in the triply *NOS*$^{-/-}$ mice that died of MI (Figures 1C and 2A). Furthermore, in the dead triply *NOS*$^{-/-}$ mice, a marked infiltration of mast cells in the coronary artery adventitia was also observed (Figure 2B). Histamine released from adventitial mast cells is thought to cause coronary vasospasm with resultant MI in humans (Laine et al. 1999). Thus, it is possible that coronary arteriosclerosis and coronary vasospasm are involved in the cause of death in the triply *NOS*$^{-/-}$ mice (Figure 3).

• Mechanisms for Spontaneous Myocardial Infarction Caused by Defective NOS System

Cardiovascular Risk Factors

Because coronary arteriosclerotic lesions were noted in the triply *NOS*$^{-/-}$ mice, we then examined what cardiovascular risk factors were present. Intriguingly, the triply *NOS*$^{-/-}$ mice showed characteristics that resembled the metabolic syndrome in humans, including visceral obesity, hypertension, hypertriglyceridemia, and impaired glucose

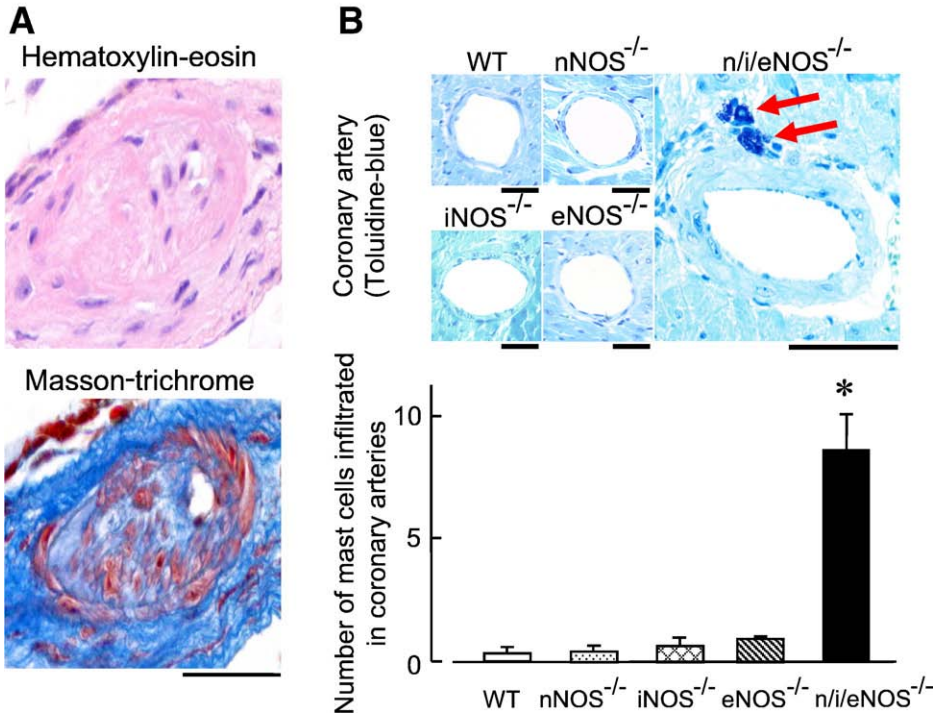

Figure 2. Coronary arteriosclerosis and adventitial mast cell infiltration in male triply *n/i/eNOS*$^{-/-}$ mice. (**A**) Arteriosclerotic lesion formation in serial sections of the infarct-related coronary artery (hematoxylin-eosin and Masson-trichrome staining). (**B**) Mast cell infiltration at the coronary artery adventitia (toluidine-blue staining) (n = 10-33). Red arrows indicate mast cells. WT, wild-type C57BL/6 mice. *$P < .05$ vs WT. Scale bars in the coronary artery, 100 μm. In panel **B**, living WT mice were used. The "n" represents the number of mice used in each group. Data from *Circulation* 2008;117:2211-2223.

tolerance (Ford 2005). Thus, a clustering of those cardiovascular risk factors could contribute to the development of arteriosclerotic lesion formation in the triply *NOS*$^{-/-}$ mice (Figure 3) (Antman and Braunwald 2005). Although in humans the metabolic syndrome is associated with atherosclerosis, such findings including atheroma formation

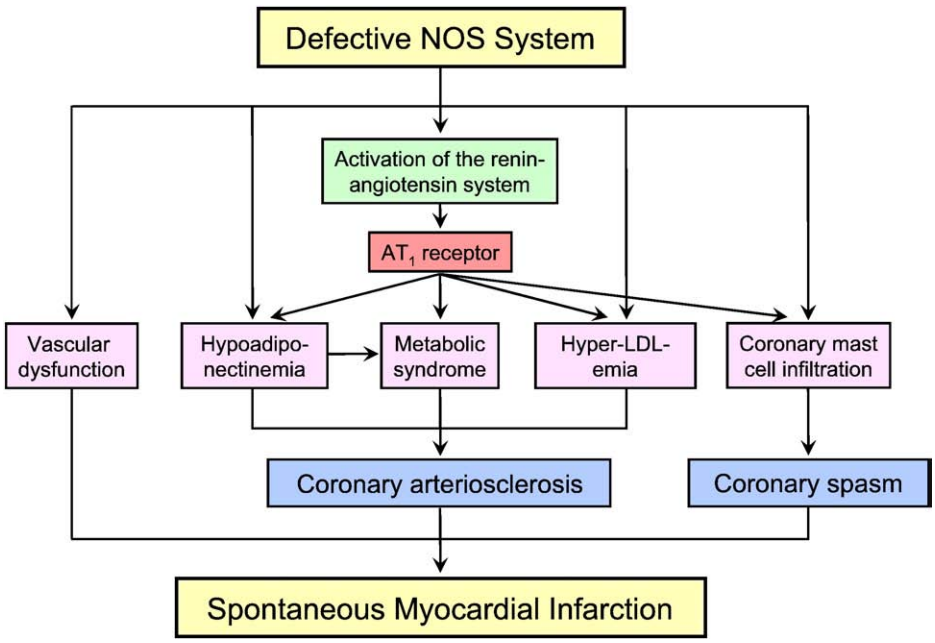

Figure 3. Schematic diagram showing mechanisms for spontaneous MI caused by the defective NOS system in mice in vivo. Complete deletion of all NOS isoforms caused the metabolic syndrome, hypoadiponectinemia, hyper-LDL-emia, coronary adventitial mast cell infiltration, and vascular dysfunction. Thus, those factors could contribute to the pathogenesis of spontaneous MI. Importantly, long-term pharmacologic blockade of the AT_1 receptor significantly reduced the incidence of MI, along with amelioration of the metabolic syndrome, hypoadiponectinemia, hyper-LDL-emia, and adventitial mast cell infiltration. It is therefore possible that the AT_1 receptor-mediated signal transduction pathway is involved, at least in part, in its molecular mechanism.

were rarely observed in the triply *NOS*$^{-/-}$ mice.

Plasma levels of low-density lipoprotein (LDL) cholesterol and small dense LDL particles, both of which are independent cardiovascular risk factors (Sniderman et al. 2003), were increased in the triply *NOS*$^{-/-}$ mice, suggesting that those alterations are also involved in the progression of arteriosclerotic lesion formation in the genotype (Figure 3).

Adiponectin

Adiponectin is an antiatherogenic adipocytokine, improving hypertriglyceridemia, glucose metabolism, and insulin resistance and inhibiting the progression of arteriosclerosis. Under the condition of obesity with adipocyte hypertrophy, synthesis of adiponectin is not increased, but rather decreased, and in patients with the metabolic syndrome, the circulating levels of adiponectin are reduced, in contrast to the increases in other adipocytokines levels. The deficiency of adiponectin is thought to play a pivotal role in the pathogenesis of the metabolic syndrome and its vascular complications (Matsuzawa et al. 2004). In the triply *NOS*$^{-/-}$ mice, plasma adiponectin levels were significantly reduced. Thus, the adiponectin deficiency may contribute to the development of the metabolic abnormalities and arteriosclerotic lesion formation in the triply *NOS*$^{-/-}$ mice (Figure 3).

Vascular Dysfunction

In the triply *NOS*$^{-/-}$ mice, endothelium-dependent relaxations to acetylcholine, which is a physiologic eNOS activator, were completely lacking, and contractions to phenylephrine, which is an α_1 adrenergic agonist, were markedly potentiated. Thus, these vascular dysfunctions could also be involved in the pathogenesis of MI in the triply *NOS*$^{-/-}$ mice (Figure 3).

Renin-angiotensin System

In the triply *NOS*$^{-/-}$ mice, the renin-angiotensin system was activated. Thus, we further examined our hypothesis that the AT_1 receptor-mediated mechanism is involved in the cardiovascular and metabolic abnormalities of the triply *NOS*$^{-/-}$ mice. Importantly, the long-term treatment with an AT_1 receptor blocker olmesartan potently inhibited coronary arteriosclerotic lesion formation, adventitial mast cell infiltration, and the occurrence of MI in the triply *NOS*$^{-/-}$ mice, with a resultant improvement of the prognosis. Furthermore, the treatment with olmesartan reversed all the abnormal metabolic phenotypes, along with amelioration of hypoadiponectinemia. These results suggest that the AT_1 receptor pathway is the main regulator of the defective NOS system (Figure 3).

How the defective NOS system leads to activation of the renin-angiotensin system remains to be elucidated. However, this may, at least in part, be caused by renal arteriosclerosis seen in the triply *NOS*$^{-/-}$ mice because reduced renal blood flow stimulates renin secretion. Further studies are needed to elucidate the mechanisms.

• Usefulness of Triply NOS$^{-/-}$ Mouse Model

The mouse lacking the high-density lipoprotein receptor SR-B1 and apolipoprotein E is known as the first and sole murine model of spontaneous MI (Braun et al. 2002). However, the longevity of this *SR-B1/apoE*$^{-/-}$ mouse is extremely short, and all mice die before 2 months of age (Braun et al. 2002). Thus, in this point, our triply *NOS*$^{-/-}$ mouse may be a more useful model of spontaneous MI.

Arteriosclerosis was seen in most of the vasculature in the triply *NOS*$^{-/-}$ mice, whereas atherosclerosis was observed in the aorta alone. Human MI results not only from coronary atherosclerosis but also other causes, including coronary arteriosclerosis and vasospasm (Antman and Braunwald 2005, Vanhoutte and Shimokawa 1989). The triply *NOS*$^{-/-}$ mouse would be a model of such non-atherosclerotic forms of MI. Although the triply *NOS*$^{-/-}$ mouse might also be a model of the metabolic syndrome in humans, this point remains to be studied in future studies.

• Clinical Implications

Several lines of evidence suggest an association of the defective NOS system with the metabolic syndrome, coronary arteriosclerosis, and MI in humans. First, it has been reported that plasma and/or urinary NOx levels, which are markers of NO production derived from all three NOSs in vivo, are reduced in patients with the metabolic syndrome and in those with coronary arteriosclerosis (Kurioka et al. 2000, Node et al. 1997, Piatti et al. 2003, Tanaka et al. 1997). Second, plasma concentrations of asymmetric dimethylarginine, which is an endogenous NOS inhibitor, have been shown to be elevated in patients with the metabolic syndrome, with arteriosclerosis, and with risk of MI (Cooke 2005). Finally, it has been revealed in humans that the gene polymorphisms of individual NOS are associated with the metabolic syndrome, arteriosclerosis, risk of MI, and low plasma NOx levels, although there has been no comparative analysis of all three NOSs (Cook 2006). These results may imply clinical significance of our findings with the triply *NOS*$^{-/-}$ mice.

• Conclusion and Future Perspectives

Complete deletion of all NOS isoforms caused spontaneous MI associated with the metabolic syndrome, hypoadiponectinemia, hyper-LDL-emia, adventitial mast cell infiltration, and vascular dysfunction in mice. These results provide the first direct evidence that the endogenous NOS system plays a critical role in preventing spontaneous MI in vivo. The present findings should contribute to a better understanding of the significance of the defective NOS system in the pathogenesis of spontaneous MI.

There are several questions that remain to be addressed. First, most human MI results from coronary atherosclerosis, whereas the evidence was seldom detected in the triply *NOS*$^{-/-}$ mice. Second, we have not evaluated histamine content or production of vasoconstrictor prostanoids in the vasculature of the triply *NOS*$^{-/-}$ mice. Third, we have not examined the cardiovascular phenotypes of the female triply *NOS*$^{-/-}$ mice. Fourth, there might be an involvement of signal transduction pathways other than the AT_1 receptor. Finally, it remains to be investigated whether or not pharmacologic interventions with NO donors are effective in the primary and secondary preventions of human MI. These issues need to be examined in future studies.

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