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Roles of Nitric Oxide Synthases in Arteriosclerotic Vascular Disease: Insights from Murine Genetic Models

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Abstract

Nitric Oxide (NO) exerts a variety of biological actions under both physiological and pathological conditions. NO is synthesized by three distinct NO synthase (NOS) isoforms, encoded by three distinct NOS genes, including neuronal (nNOS), inducible (iNOS), and endothelial NOS (eNOS), all of which are expressed in the human vascular system. Although the roles of the NOSs in arteriosclerotic vascular diseases have been described in pharmacological studies with selective and non-selective NOS inhibitors, the selectivity and specificity of the NOS inhibitors continue to be an issue of debate. To solve this issue, genetically altered animals have been established. All types of NOS gene-deficient animals have been developed, including singly, doubly, and triply NOS-deficient mice and various types of NOS Gene-Transgenic (TG) animals have also been generated, including conditional and non-conditional TG mice bearing site-specific overexpression of each NOS gene. The roles of individual NOS isoforms as well as the entire NOSs system in arteriosclerotic vascular diseases have been extensively investigated in those mice, providing pivotal insights into an understanding of the pathophysiological significance of the NOSs in human arteriosclerotic vascular diseases. The present review, which is based on studies with the murine NOS genetic models, summarizes the latest knowledge about the NOSs and arteriosclerotic vascular diseases.

Keywords: Arteriosclerotic vascular disease; Nitric oxide synthase; Knockout mice; Transgenic mice; Myocardial infarction

Introduction

Nitric oxide (NO) exerts a variety of biological actions, and plays an important role in maintaining vascular homeostasis [1-7]. NO is synthesized by three distinct NO synthase (NOS) isoforms, encoded by three distinct NOS genes: neuronal (nNOS; also known as NOS-1), inducible (iNOS; also known as NOS-2) and endothelial NOS (eNOS; also known as NOS-3).

It was initially indicated that nNOS and eNOS are constitutively expressed mainly in the nervous system and the vascular endothelium, respectively, synthesizing a small amount of NO in a calcium-dependent manner under both basal conditions and upon stimulation, and that iNOS is induced only when stimulated by microbial endotoxins or certain proinflammatory cytokines, producing a greater amount of NO in a calcium-independent manner [6,7]. However, recent studies have revealed that both nNOS and eNOS are subject to expressional regulation and that iNOS is constitutively expressed even under physiological conditions [8-14]. All three NOS isoforms have been reported to be expressed in the vascular system under both physiological and pathological conditions [13,15].

Genetically engineered animals are a powerful experimental tool to study the function of target genes *in vivo*. All types of NOS gene-knockout (KO) animals have been generated, including singly, doubly, and triply NOS-KO mice [16-28] (Table 1). Various types of NOS Gene-Transgenic (TG) animals have also been established, including conditional and non-conditional TG mice with site-specific overexpression of each NOS isoform [29-40] (Table 2). By using those genetically engineered mice, the roles of the NOSs in the pathogenesis of arteriosclerotic vascular diseases have been extensively studied, and the findings provide pivotal insights into the significance of the NOSs in human arteriosclerotic vascular diseases. In the present review, we summarize the current knowledge of the NOSs and arteriosclerotic vascular diseases, based on research outcomes obtained from the murine NOS genetic models.

Role of eNOS in Arteriosclerotic Vascular Disease

Endothelium-specific eNOS-TG mice with an 8-fold increase in vascular NOS activity showed decreased neointimal formation after carotid artery ligation and another strain of endothelium-specific eNOS-TG mice with a 10-fold increase in vascular NOS activity similarly exhibited a reduction in atherosclerotic vascular lesion formation induced by breeding with apoE-KO mice [38,41]. Consistent with these findings, eNOS-KO mice displayed increased neointimal formation, accelerated medial thickening, and abnormal vascular remodeling in response to carotid artery ligation and cuff placement around the femoral artery [42-44] (Figure 1). Furthermore, eNOS-KO/apoE-KO mice exhibited exacerbated formation of atherosclerotic vascular lesion as compared with apoE-KO mice [45,46]. These lines of evidence indicate a vasculoprotective role of eNOS in vascular lesion formation. On the other hand, there are also reports of inconsistent opposite results that diet-induced atherosclerotic vascular lesion formation by crossbreeding with apoE-KO mice is accelerated in endothelium-specific eNOS-TG mice with an 8-fold increase in

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NOS$^{-/-}$ Mice	Sites of gene deletion	References
nNOS$^{-/-}$	Exon 2 (#1) Exon 6 Exon 6	*Cell* 1993;75:1273-1286 *Endocrinology* 2002;143:2767-2774 *PNAS* 2003;100:9566-9571
Renal collecting duct-specific nNOS$^{-/-}$	Exon 6	*Hypertension* 2013;62:91-98
iNOS$^{-/-}$	Proximal 585 bases of promoter plus exons 1-4 (#2) Near exons 1-5 Exons 12 and 13 and a part of exon 11 (#3)	*Cell* 1995;81:641-650 *Nature* 1995;375:408-411 *PNAS* 1995;92:10688-10692
eNOS$^{-/-}$	Exons 24-26 (#4) Exon 12 (#5) Exons 24 and 25	*Nature* 1995;377:239-242 *PNAS* 1996;93:13176-13181 *Circ Res* 1998;82:186-194
n/iNOS$^{-/-}$	#1 and #3 #1 and #2	*Mol Reprod Dev* 2003;65:175-179 *PNAS* 2005;102:10616-10621
n/eNOS$^{-/-}$	#1 and #4 #1 and #5 #1 and #4	*Cell* 1996;87:1015-1023 *Mol Reprod Dev* 2003;65:175-179 *PNAS* 2005;102:10616-10621
i/eNOS$^{-/-}$	#3 and #5 #2 and #4	*Mol Reprod Dev* 2003;65:175-179 *PNAS* 2005;102:10616-10621
n/i/eNOS$^{-/-}$	#1, #2 and #4	*PNAS* 2005;102:10616-10621

NOS, Nitric Oxide Synthase; Nnos, Neuronal NOS; Inos, Inducible NOS; Enos, Endothelial NOS; NOS-/-, NOS-Deficient

Table 1: Mice Lacking the NOS Genes That Have Thus Far Been Established.

TG Mice	Overexpression site	Promoter used	References
nNOS-TG	Myocardium (Conditional)	α-MHC	Circ Res 2007;100:e32-e44
	Myocardium (Conditional)	α-MHC	Circulation 2008;117:3187-3198
	Brain	CaMKIIα	Cell Mol Biol 2005;51:269-277
iNOS-TG	Myocardium (Conditional)	α-MHC	J Clin Invest 2002;109:735-743
	Myocardium	α-MHC	Circ Res 2002;90:93-99
	Pancreatic β Cell Retina	insulin rod opsin	J Biol Chem 1998;273:2493-2496 PLoS One 2012;7:e43089
	Liver	albumin	J Biol Chem 011;286:34959-34957
eNOS-TG	Endothelium	preproendothelin-1	J Clin Invest 1998;109:735-743
	Endothelium	eNOS	J Biol Chem 002;277:48803-48807
	Myocardium	α-MHC	Circulation 2001;104:3097-3102
	Myocardium	α-MHC	Circ Res 2004;94:1256-1262

CaMKII: Calcium-Calmodulin Multifunctional Kinase II; MHC: Myosin Heavy Chain; TG: Transgenic

Table 2: Mice Overexpressing the NOS Gene That Have Thus Far Been Established.

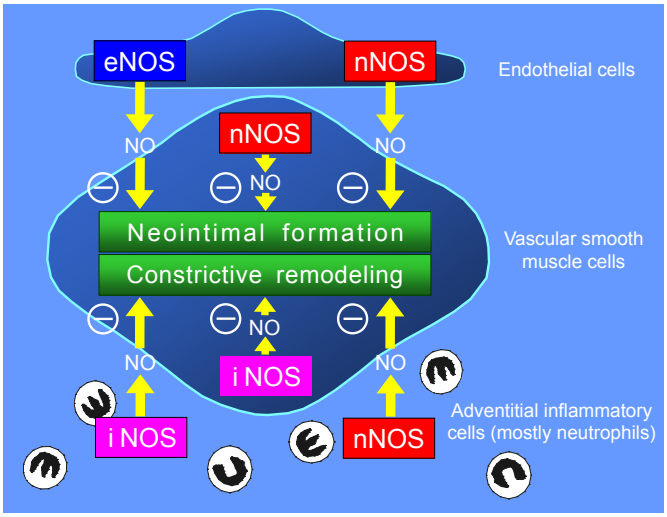

Figure 1: Different vasculoprotective roles of the three NOS isoforms in a mouse carotid artery ligation model.
Studies with each NOS isoform-/- mice demonstrated that eNOS inhibits neointimal formation, that iNOS attenuates constrictive vascular remodeling, and that nNOS suppresses both neointimal formation and constrictive vascular remodeling. Thus, individual NOS isoforms have different vasculoprotective actions against vascular lesion formation in mice *in vivo* inhibition [7].

vascular NOS activity and that fatty streak formation is paradoxically reduced in eNOS-KO mice [47,48]. eNOS-derived NO has multiple vasculoprotective effects, including the dilation of blood vessels and the inhibition of vascular smooth muscle cell proliferation, platelet aggregation, leukocyte-endothelial cell adhesion, and Low-Density Lipoprotein (LDL) oxidation, whereas, under certain conditions such as deficiency of a substrate (e.g., L-arginine) or a cofactor (e.g., tetrahydrobiopterin), NOSs produce superoxide anions rather than NO, with resultant production of a potent oxidant peroxynitrite (which phenomenon is referred to as 'NOS uncoupling') [49,50]. Thus, eNOS uncoupling may be partly involved in these discrepant results.

When 12 eNOS-KO/apoE-KO mice were fed on a Western-type diet for 16 weeks, 3 mice developed abdominal aortic aneurysms, and 2 developed aortic dissections (Stanford type B) spontaneously [46]. These results indicate that eNOS deficiency introduces abdominal aortic aneurysms and aortic dissections in the presence of severe hyperlipidemia, suggesting a protective role of eNOS in aortic diseases.

Role of iNOS in Arteriosclerotic Vascular Disease

The role of iNOS in vascular lesion formation seems to be complicated. Deletion of the iNOS gene in mice exacerbated pathological vascular remodeling in a carotid artery ligation model and in a cardiac transplant model; however, it conversely ameliorated neointimal formation in a carotid cuff placement model and lipid-rich

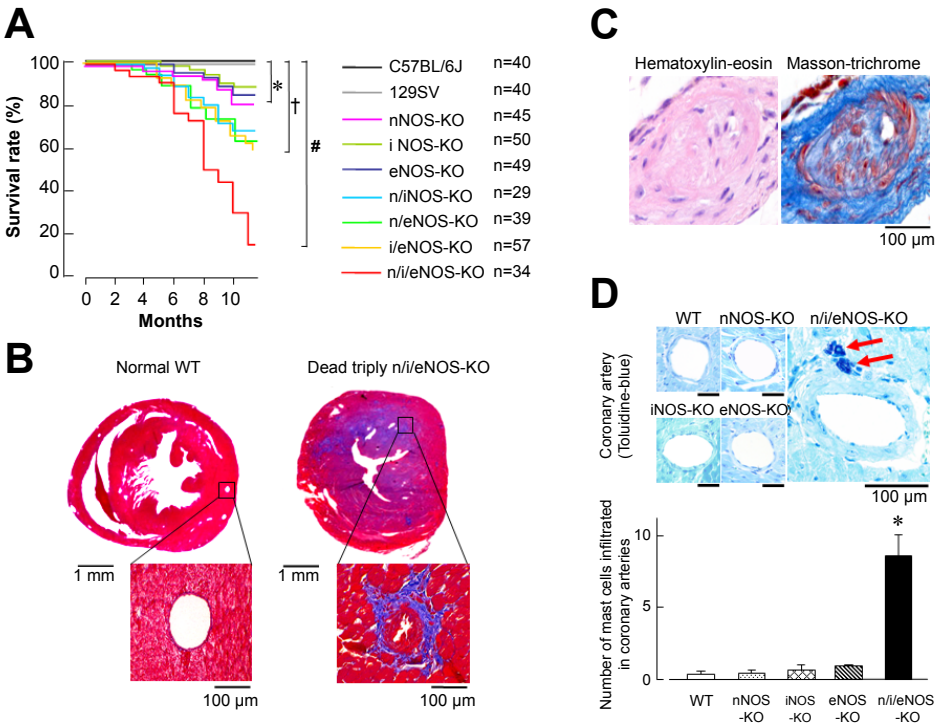

Figure 2: Decreased survival, spontaneous Myocardial Infarction (MI), coronary arteriosclerosis and mast cell infiltration in male triply n/i/eNOSs$^{-/-}$ mice. (A) Survival rate (n=29-57). The red line represents markedly reduced survival in the triply n/i/eNOSs$^{-/-}$ mice. *, †, and #: P<0.05 between wild-type (WT) C57BL/6J vs. singly, doubly, and triply NOS-KO, respectively. (B) Acute MI and coronary arteriosclerotic lesion formation in a triply n/i/eNOSs$^{-/-}$ mouse that died at 8 months of age (Masson-trichrome staining). Blue in the heart cross-section of the dead triply n/i/eNOSs$^{-/-}$ mouse indicates antero-septal acute MI. Adjacent coronary artery shows marked luminal narrowing, wall thickening, and perivascular fibrosis (blue). (C) Arteriosclerotic lesion formation in serial sections of the infarct-related coronary artery. (D) Mast cell infiltration in the coronary artery adventitia (toluidine-blue staining) (n=10-33). Red arrows indicate mast cells. *P<0.05 vs. WT [62].

atherosclerotic vascular lesion formation in apoE-KO mice [43,51-53] (Figure 1). Thus, iNOS appears to have two faces. This discrepancy may be explainable in part by the oxidant and antioxidant properties of iNOS in the presence and absence, respectively, of iNOS uncoupling [54].

The extent of elastase-induced abdominal aneurismal dilatation was comparable between male iNOS-KO and wild-type mice, whereas it was greater in female iNOS-KO than in female wild-type mice, which effect was reversed by previous ovariectomy [55]. It is thus likely that iNOS deficiency also leads to the occurrence of abdominal aortic aneurysms induced by elastase solely in the female.

Role of nNOS in Arteriosclerotic Vascular Disease

Expression of nNOS is up-regulated in the neointima, endothelial cells and macrophages in both early and advanced human atherosclerotic lesions [15]. Although the regulatory roles of eNOS and iNOS in vascular lesion formation have been widely studied, little was known about the role of nNOS. We addressed this point in nNOS-KO mice and demonstrated that nNOS gene deficiency caused a worsening of neointimal formation and constrictive vascular remodeling (a reduction in the vascular cross-sectional area) following carotid artery ligation [56] (Figure 1). In agreement with our evidence, nNOS-KO/apoE-KO mice showed accelerated atherosclerotic vascular lesion formation as compared with apoE-KO mice [57]. These results suggest that nNOS also plays a role in suppressing arteriosclerotic/atherosclerotic vascular lesion formation [12]. Up-regulation of nNOS may play a compensatory role in the presence of reduced eNOS activity (e.g. inflammation and arteriosclerosis) to maintain vascular homeostasis [12]. We revealed that inflammatory and proliferative stimuli (angiotensin II, interleukin-1β, and platelet-derived growth factor) and a statin increase vascular nNOS expression [10,11]. Hypoxic and hypertensive situations have also been shown to up-regulate vascular nNOS expression [58-60].

Role of the Whole NOSs System in Arteriosclerotic Vascular Disease

Because all NOSs play a role in the vascular system, we conceived a project to investigate the roles of the whole NOSs system *in vivo*. The roles of the NOSs system in the human body have been investigated in pharmacological studies with non-selective NOS inhibitors and in studies with NOS isoform-KO mice. However, because of the non-specificity of the agents and of compensation among NOS isoforms, the authentic roles of the NOSs system were still poorly understood. To address this important issue, we developed mice in which the entire NOSs system is completely disrupted (triply nNOS/iNOS/eNOS-KO mice) [22,61]. The triply n/i/eNOSs-KO mice, but not any singly NOS-KO mice, spontaneously developed arteriosclerotic vascular lesions (neointimal formation, medial thickening, and perivascular fibrosis) in the coronary and renal arteries, and lipid-rich atherosclerotic vascular lesions in the aorta, even on a normal chow diet, suggesting a vasculoprotective role of the entire NOSs system in vascular lesion formation [62,63] (Figure 2).

Myocardial Infarction (MI) is the leading cause of death for both genders all over the world [64,65]. The molecular mechanisms for the pathogenesis of MI, however, remain to be fully elucidated. It is well

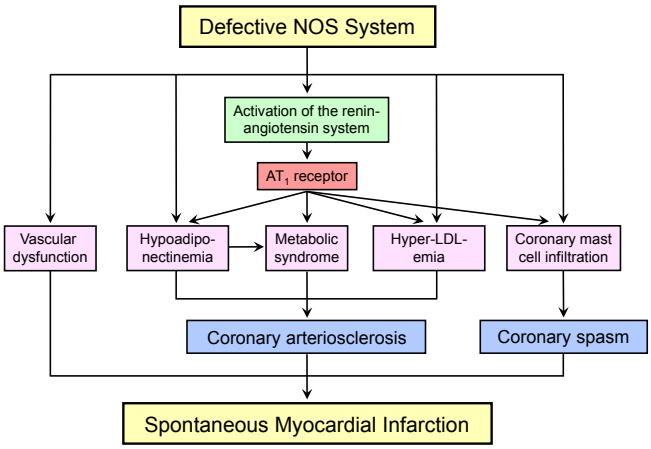

Figure 3: Mechanisms for spontaneous MI caused by the defective NOS system in mice *in vivo*.
Genetic disruption of all NOSs caused metabolic syndrome, hypoadiponectinemia, hyper-low-density-lipoprotein (LDL)-emia, coronary adventitial mast cell infiltration, and vascular dysfunction. Those factors could contribute to the pathogenesis of spontaneous MI. Importantly, long-term pharmacological blockade of the angiotensin II type 1 (AT1) receptor significantly reduced the incidence of MI, along with amelioration of those risk factors. It is therefore possible that the AT1 receptor pathway is involved in its molecular mechanism [62].

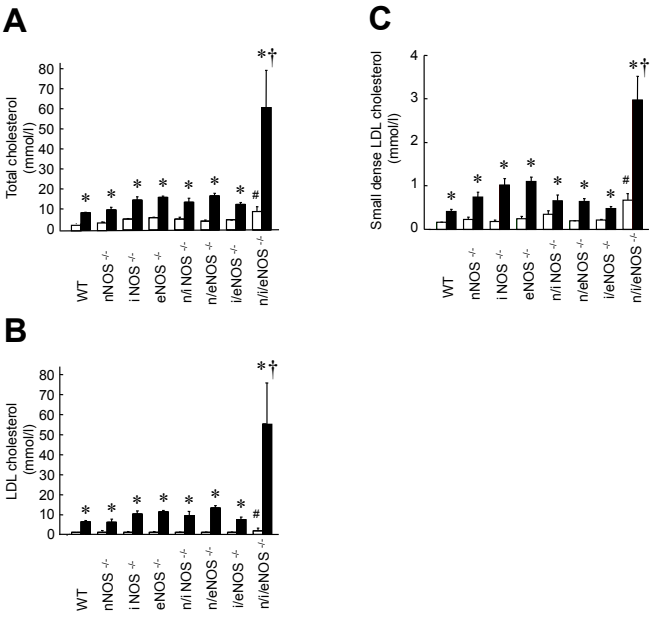

Figure 4: Serum lipid profile in WT and NOS$^{-/-}$ mice fed a regular or high-cholesterol diet for 3 months (n=6-11). White and black bars indicate the regular and high-cholesterol diets, respectively. WT, C57BL/6. *P<0.05 vs. the regular diet; †P<0.05 vs. WT mice fed the high-cholesterol diet; #P<0.05 vs. WT mice fed the regular diet [68].

established that eNOS has powerful anti-arteriosclerotic and anti-atherosclerotic effects [1-7]. However, neither deletion of the eNOS gene nor pharmacological inhibition of eNOS activity induce MI in animals. On the other hand, intriguingly, our triply n/i/eNOSs-KO mice experienced spontaneous MI and sudden cardiac death [63] (Figure 2). This was the first *in vivo* demonstration showing that the defective NOS system is involved in the pathogenesis of spontaneous MI.

Arteriosclerosis was seen in most of the vasculature in the triply NOS-KO mice, whereas atherosclerosis was observed in the aorta alone. MI in humans results not only from coronary atherosclerosis, but also from other causes, including coronary intimal hyperplasia, medial thickening, and coronary vasospasm [64,66]. Marked coronary intimal hyperplasia and medial thickening were noted in our triply n/i/eNOS-KO mice that died of MI and, furthermore, marked infiltration of mast cells at the coronary artery adventitia was also observed in those mice [63] (Figure 2). Histamine released from adventitial mast cells is thought to cause coronary vasospasm with resultant MI in humans [67]. It is thus possible that coronary arteriosclerosis and coronary vasospasm are involved in the cause of death in the triply NOS-KO mice (Figure 3).

In our triply n/i/eNOS$^{-/-}$ mice, endothelium-dependent relaxations to acetylcholine, which is a physiological eNOS activator, were completely lacking, and contractions to phenylephrine, which is an α_1 adrenergic agonist, were markedly potentiated [63]. These vascular dysfunctions could also be involved in the pathogenesis of MI in the triply NOSs-KO mice (Figure 3). In our triply n/i/eNOS$^{-/-}$ mice, metabolic syndrome-like phenotypes, including visceral obesity, hypertension, dyslipidemia, impaired glucose tolerance, and insulin resistance were noted in association with reduced plasma levels of adiponectin, which is an anti-atherogenic adipocytokine, improving metabolic syndrome [63]. Thus, metabolic syndrome and hypoadiponectinemia could also be involved in the pathogenesis of MI in the triply NOSs-KO mice (Figure 3).

When wild-type, singly, doubly, and triply NOSs-KO mice were fed a high-cholesterol diet for 3-5 months, the serum levels of total cholesterol, LDL cholesterol, and small-dense LDL cholesterol were significantly increased in all the genotypes as compared with the regular diet. Importantly, when compared with the wild-type genotype, those levels in the high-cholesterol diet were markedly elevated only in the triply NOSs$^{-/-}$ genotype, but not in any singly or doubly NOS$^{-/-}$

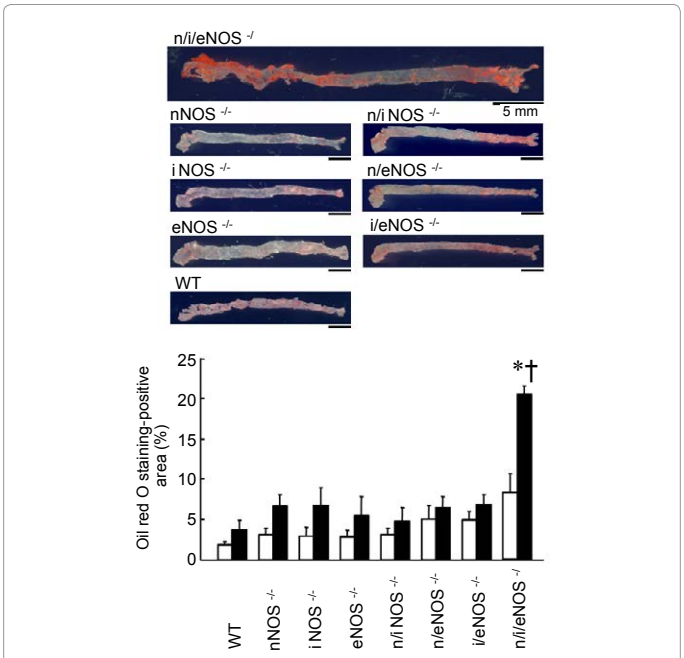

Figure 5: Lipid accumulation in longitudinally opened aortas of WT and NOS$^{-/-}$ mice fed a high-cholesterol diet (oil red O staining) (n=6-11).
Red color indicates positive staining. White and black bars represent the regular and high-cholesterol diets, respectively. WT, C57BL/6. *P<0.05 vs. the regular diet; †P<0.05 vs. WT mice fed the high-cholesterol diet [68].

genotypes and this was associated with remarkable atherosclerosis and sudden cardiac death, which occurred mainly in 4-5 months after the high-cholesterol diet [68] (Figures 4 and 5). Out of 15 dead triply NOSs$^{-/-}$ mice fed the high-cholesterol diet, myocardial infarction was detected in 1 mouse, giant organized thrombi in the left and right ventricles were seen in 2 mice, and marked neointimal formation and perivascular fibrosis of the coronary artery and pulmonary congestion were noted in all the dead mice. These results suggest the protective role of the whole endogenous NOSs system in the pathogenesis of dyslipidemia and atherosclerotic vascular disease. Hepatic LDL receptor expression was markedly reduced only in the triply NOS$^{-/-}$ genotype, accounting for the diet-induced dyslipidemia in the genotype.

Bone marrow-derived vascular progenitor cells in the blood accumulate in injured arteries, differentiate into vascular wall cells, and contribute to arteriosclerotic vascular lesion formation. All NOSs have been reported to be expressed in bone marrow cells. However, whether NOSs in bone marrow cells play a role in vascular lesion formation remained to be clarified. We addressed this point in the triply NOS$^{-/-}$ mice and in bone marrow transplantation experiments. We previously reported that, in Wild-Type (WT) mice that underwent bone marrow transplantation from Green Fluorescent Protein (GFP)-TG mice, GFP-positive fluorescence was detected in the ligated carotid arteries, confirming the involvement of bone marrow-derived vascular progenitor cells in vascular lesion formation after carotid artery ligation [69]. In a comparison of the NOSs$^{-/-}$ genotype that received NOSs$^{-/-}$ bone marrow transplantation and the NOSs$^{-/-}$ genotype that received WT bone marrow transplantation, the extent of neointimal formation and the extent of constrictive remodeling were both significantly less in those that received the WT bone marrow transplantation, along with significantly higher NOS activities in the ligated carotid arteries [70]. Furthermore, in a comparison of the WT genotype with WT bone marrow transplantation and the WT genotype with NOSs$^{-/-}$ bone marrow transplantation, the extent of neointimal formation and the extent of constrictive remodeling were both significantly greater in the WT genotype with NOSs$^{-/-}$ bone marrow transplantation, and this was associated with significantly lower NOS activities in the ligated carotid arteries [70]. These results indicate that NOSs in bone marrow cells exert an inhibitory effect on vascular lesion formation caused by blood flow disruption in mice *in vivo*, demonstrating a novel vasculoprotective role of NOSs in bone marrow-derived vascular progenitor cells.

Clinical Implications

Several lines of evidence suggest an association of the defective NOSs system with arteriosclerotic vascular disease in humans. First, it has been reported that plasma and/or urinary NOx levels, which are markers of NO production, are reduced in patients with the arteriosclerotic risk factors and in those with coronary arteriosclerosis [71-74]. Second, plasma concentrations of asymmetric dimethylarginine, which is an endogenous NOS inhibitor, have been shown to be elevated in patients with arteriosclerotic risk factors, with arteriosclerosis, and with risk of MI [75]. Finally, it has been revealed in humans that the gene polymorphisms of individual NOSs are associated with arteriosclerotic risk factors, arteriosclerosis, risk of MI, and low plasma NOx levels [76]. These results may imply a clinical significance of the findings with the NOSs$^{-/-}$ mice.

Judging from the results of the murine genetic models, it is conceivable that eNOS is involved in the pathogenesis of endothelial dysfunction, arteriosclerosis, aortic dissection, and abdominal aortic aneurysm, that iNOS contributes to the pathogenesis of arteriosclerosis, aortic dissection, and abdominal aortic aneurysm, that nNOS serves functions in the pathogenesis of arteriosclerosis, and that entire NOSs play roles in the pathogenesis of endothelial dysfunction, coronary vasospasm, arteriosclerosis, and myocardial infarction. The roles of NOSs in human arteriosclerotic vascular diseases remain to be examined in future clinical studies.

Therapeutic Potential of NOS Activators

A number of NOS activators, such as eNOS transcriptional enhancers (AVE9488 and AVE3085), tetrahydrobiopterin, statins, trans-resveratrol, vanadate, protein kinase C inhibitor midostaurin, and pentacyclic triteroenoids ursolic acid and betulinic acid, have been reported to increase NOS expression and activity or ameliorate NOS uncoupling [77-79]. These NOS activators may have therapeutic potential in the treatment of arteriosclerotic vascular diseases.

Concluding Remarks

The mouse is the most ideal genetically modifiable mammalian presently available [80]. Studies with mice that are deficient in or overexpressing NOSs provide pivotal insights into the roles of the NOSs in the pathogenesis of arteriosclerotic vascular diseases. In general, eNOS, nNOS, and the whole NOSs system exert vasculoprotective roles, while iNOS seems to exert dual effects in the vascular system. The observations with the genetically modified animals have greatly advanced our understanding of the roles of the NOSs system in the pathogenesis of arteriosclerotic vascular diseases. Further studies are certainly needed to clarify whether these observations can be translated to human patients with arteriosclerotic vascular diseases.

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References

1. Bredt DS, Snyder SH (1994) Nitric oxide: a physiologic messenger molecule. Annu Rev Biochem 63: 175-195.
2. Ignarro LJ (1990) Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol 30: 535-560.
3. Moncada S, Palmer RM, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109-142.
4. Murad F (1997) What are the molecular mechanisms for the antiproliferative effects of nitric oxide and cGMP in vascular smooth muscle? Circulation 95: 1101-1103.
5. Shimokawa H (1999) Primary endothelial dysfunction: atherosclerosis. J Mol Cell Cardiol 31: 23-37.
6. Tsutsui M, Shimokawa H, Otsuji Y, Ueta Y, Sasaguri Y, et al. (2009) Nitric oxide synthases and cardiovascular diseases: insights from genetically modified mice. Circ J 73: 986-993.
7. Tsutsui M, Shimokawa H, Otsuji Y, Yanagihara N (2010) Pathophysiological relevance of NO signaling in the cardiovascular system: novel insight from mice lacking all NO synthases. Pharmacol Ther 128: 499-508.
8. Dudzinski DM, Igarashi J, Greif D, Michel T (2006) The regulation and pharmacology of endothelial nitric oxide synthase. Annu Rev Pharmacol Toxicol 46: 235-276.
9. Förstermann U, Boissel JP, Kleinert H (1998) Expressional control of the ‚constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). FASEB J 12: 773-790.
10. Nakata S, Tsutsui M, Shimokawa H, Tamura M, Tasaki H, et al. (2005) Vascular neuronal NO synthase is selectively upregulated by platelet-derived growth factor: involvement of the MEK/ERK pathway. Arterioscler Thromb Vasc Biol 25: 2502-2508.

11. Nakata S, Tsutsui M, Shimokawa H, Yamashita T, Tanimoto A, et al. (2007) Statin treatment upregulates vascular neuronal nitric oxide synthase through Akt/NF-kappaB pathway. Arterioscler Thromb Vasc Biol 27: 92-98.

12. Tsutsui M (2004) Neuronal nitric oxide synthase as a novel anti-atherogenic factor. J Atheroscler Thromb 11: 41-48.

13. Buchwalow IB, Podzuweit T, Bocker W, Samoilova VE, Thomas S, et al. (2002) Vascular smooth muscle and nitric oxide synthase. FASEB J 16: 500-508.

14. Park CS, Park R, Krishna G (1996) Constitutive expression and structural diversity of inducible isoform of nitric oxide synthase in human tissues. Life Sci 59: 219-225.

15. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, et al. (1997) Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. Arterioscler Thromb Vasc Biol 17: 2479-2488.

16. Gödecke A, Decking UK, Ding Z, Hirchenhain J, Bidmon HJ, et al. (1998) Coronary hemodynamics in endothelial NO synthase knockout mice. Circ Res 82: 186-194.

17. Gyurko R, Leupen S, Huang PL (2002) Deletion of exon 6 of the neuronal nitric oxide synthase gene in mice results in hypogonadism and infertility. Endocrinology 143: 2767-2774.

18. Huang PL, Dawson TM, Bredt DS, Snyder SH, Fishman MC (1993) Targeted disruption of the neuronal nitric oxide synthase gene. Cell 75: 1273-1286.

19. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, et al. (1995) Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377: 239-242.

20. Laubach VE, Shesely EG, Smithies O, Sherman PA (1995) Mice lacking inducible nitric oxide synthase are not resistant to lipopolysaccharide-induced death. Proc Natl Acad Sci U S A 92: 10688-10692.

21. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, et al. (1995) Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. Cell 81: 641-650.

22. Morishita T, Tsutsui M, Shimokawa H, Sabanai K, Tasaki H, et al. (2005) Nephrogenic diabetes insipidus in mice lacking all nitric oxide synthase isoforms. Proc Natl Acad Sci U S A 102: 10616-10621.

23. Packer MA, Stasiv Y, Benraiss A, Chmielnicki E, Grinberg A, et al. (2003) Nitric oxide negatively regulates mammalian adult neurogenesis. Proc Natl Acad Sci U S A 100: 9566-9571.

24. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, et al. (1996) Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 93: 13176-13181.

25. Son H, Hawkins RD, Martin K, Kiebler M, Huang PL, et al. (1996) Long-term potentiation is reduced in mice that are doubly mutant in endothelial and neuronal nitric oxide synthase. Cell 87: 1015-1023.

26. Tranguch S, Huet-Hudson Y (2003) Decreased viability of nitric oxide synthase double knockout mice. Mol Reprod Dev 65: 175-179.

27. Wei XQ, Charles IG, Smith A, Ure J, Feng GJ, et al. (1995) Altered immune responses in mice lacking inducible nitric oxide synthase. Nature 375: 408-411.

28. Hyndman KA, Boesen EI, Elmarakby AA, Brands MW, Huang P, et al. (2013) Renal collecting duct NOS1 maintains fluid-electrolyte homeostasis and blood pressure. Hypertension 62: 91-98.

29. Brunner F, Andrew P, Wölkart G, Zechner R, Mayer B (2001) Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. Circulation 104: 3097-3102.

30. Burkard N, Rokita AG, Kaufmann SG, Hallhuber M, Wu R, et al. (2007) Conditional neuronal nitric oxide synthase overexpression impairs myocardial contractility. Circ Res 100: e32-44.

31. Heger J, Gödecke A, Flögel U, Merx MW, Molojavyi A, et al. (2002) Cardiac-specific overexpression of inducible nitric oxide synthase does not result in severe cardiac dysfunction. Circ Res 90: 93-99.

32. Janssens S, Pokreisz P, Schoonjans L, Pellens M, Vermeersch P, et al. (2004) Cardiomyocyte-specific overexpression of nitric oxide synthase 3 improves left ventricular performance and reduces compensatory hypertrophy after myocardial infarction. Circ Res 94: 1256-1262.

33. Loyer X, Gomez AM, Milliez P, Fernandez-Velasco M, Vangheluwe P, et al. (2008) Cardiomyocyte overexpression of neuronal nitric oxide synthase delays transition toward heart failure in response to pressure overload by preserving calcium cycling. Circulation 117: 3187-3198.

34. Mungrue IN, Gros R, You X, Pirani A, Azad A, et al. (2002) Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. J Clin Invest 109: 735-743.

35. Ohashi Y, Kawashima S, Hirata Ki, Yamashita T, Ishida T, et al. (1998) Hypotension and reduced nitric oxide-elicited vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. J Clin Invest 102: 2061-2071.

36. Packer MA, Hemish J, Mignone JL, John S, Pugach I, et al. (2005) Transgenic mice overexpressing nNOS in the adult nervous system. Cell Mol Biol (Noisy-le-grand) 51: 269-277.

37. Takamura T, Kato I, Kimura N, Nakazawa T, Yonekura H, et al. (1998) Transgenic mice overexpressing type 2 nitric-oxide synthase in pancreatic beta cells develop insulin-dependent diabetes without insulitis. J Biol Chem 273: 2493-2496.

38. van Haperen R, de Waard M, van Deel E, Mees B, Kutryk M, et al. (2002) Reduction of blood pressure, plasma cholesterol, and atherosclerosis by elevated endothelial nitric oxide. J Biol Chem 277: 48803-48807.

39. Wu GS, Jiang M, Liu YH, Nagaoka Y, Rao NA (2012) Phenotype of transgenic mice overexpressed with inducible nitric oxide synthase in the retina. PLoS One 7: e43089.

40. Shinozaki S, Choi CS, Shimizu N, Yamada M, Kim M, et al. (2011) Liver-specific inducible nitric-oxide synthase expression is sufficient to cause hepatic insulin resistance and mild hyperglycemia in mice. J Biol Chem 286: 34959-34975.

41. Kawashima S, Yamashita T, Ozaki M, Ohashi Y, Azumi H, et al. (2001) Endothelial NO synthase overexpression inhibits lesion formation in mouse model of vascular remodeling. Arterioscler Thromb Vasc Biol 21: 201-207.

42. Moroi M, Zhang L, Yasuda T, Virmani R, Gold HK, et al. (1998) Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice. J Clin Invest 101: 1225-1232.

43. Yogo K, Shimokawa H, Funakoshi H, Kandabashi T, Miyata K, et al. (2000) Different vasculoprotective roles of NO synthase isoforms in vascular lesion formation in mice. Arterioscler Thromb Vasc Biol 20: E96-96E100.

44. Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, et al. (1998) Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. J Clin Invest 101: 731-736.

45. Knowles JW, Reddick RL, Jennette JC, Shesely EG, Smithies O, et al. (2000) Enhanced atherosclerosis and kidney dysfunction in eNOS(-/-)Apoe(-/-) mice are ameliorated by enalapril treatment. J Clin Invest 105: 451-458.

46. Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, et al. (2001) Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation 104: 448-454.

47. Ozaki M, Kawashima S, Yamashita T, Hirase T, Namiki M, et al. (2002) Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. J Clin Invest 110: 331-340.

48. Shi W, Wang X, Shih DM, Laubach VE, Navab M, et al. (2002) Paradoxical reduction of fatty streak formation in mice lacking endothelial nitric oxide synthase. Circulation 105: 2078-2082.

49. Vásquez-Vivar J, Kalyanaraman B, Martásek P, Hogg N, Masters BS, et al. (1998) Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci U S A 95: 9220-9225.

50. Wang W, Wang S, Yan L, Madara P, Del Pilar Cintron A, et al. (2000) Superoxide production and reactive oxygen species signaling by endothelial nitric-oxide synthase. J Biol Chem 275: 16899-16903.

51. Koglin J, Glysing-Jensen T, Mudgett JS, Russell ME (1998) Exacerbated transplant arteriosclerosis in inducible nitric oxide-deficient mice. Circulation 97: 2059-2065.

52. Chyu KY, Dimayuga P, Zhu J, Nilsson J, Kaul S, et al. (1999) Decreased neointimal thickening after arterial wall injury in inducible nitric oxide synthase knockout mice. Circ Res 85: 1192-1198.

53. Kuhlencordt PJ, Chen J, Han F, Astern J, Huang PL (2001) Genetic deficiency of inducible nitric oxide synthase reduces atherosclerosis and lowers plasma lipid peroxides in apolipoprotein E-knockout mice. Circulation 103: 3099-3104.

54. Goss SP, Hogg N, Kalyanaraman B (1997) The effect of nitric oxide release rates on the oxidation of human low density lipoprotein. J Biol Chem 272: 21647-21653.

55. Lee JK, Borhani M, Ennis TL, Upchurch GR Jr, Thompson RW (2001) Experimental abdominal aortic aneurysms in mice lacking expression of inducible nitric oxide synthase. Arterioscler Thromb Vasc Biol 21: 1393-1401.

56. Morishita T, Tsutsui M, Shimokawa H, Horiuchi M, Tanimoto A, et al. (2002) Vasculoprotective roles of neuronal nitric oxide synthase. FASEB J 16: 1994-1996.

57. Kuhlencordt PJ, Hötten S, Schödel J, Rützel S, Hu K, et al. (2006) Atheroprotective effects of neuronal nitric oxide synthase in apolipoprotein e knockout mice. Arterioscler Thromb Vasc Biol 26: 1539-1544.

58. Ward ME, Toporsian M, Scott JA, Teoh H, Govindaraju V, et al. (2005) Hypoxia induces a functionally significant and translationally efficient neuronal NO synthase mRNA variant. J Clin Invest 115: 3128-3139.

59. Boulanger CM, Heymes C, Benessiano J, Geske RS, Lévy BI, et al. (1998) Neuronal nitric oxide synthase is expressed in rat vascular smooth muscle cells: activation by angiotensin II in hypertension. Circ Res 83: 1271-1278.

60. Ebrahimian T, Mathieu E, Silvestre JS, Boulanger CM (2003) Intraluminal pressure increases vascular neuronal nitric oxide synthase expression. J Hypertens 21: 937-942.

61. Tsutsui M, Shimokawa H, Morishita T, Nakashima Y, Yanagihara N (2006) Development of genetically engineered mice lacking all three nitric oxide synthases. J Pharmacol Sci 102: 147-154.

62. Tsutsui M, Nakata S, Shimokawa H, Otsuji Y, Yanagihara N (2008) Spontaneous myocardial infarction and nitric oxide synthase. Trends Cardiovasc Med 18: 275-279.

63. Nakata S, Tsutsui M, Shimokawa H, Suda O, Morishita T, et al. (2008) Spontaneous myocardial infarction in mice lacking all nitric oxide synthase isoforms. Circulation 117: 2211-2223.

64. Antman EM, Braunwald E (2005) ST-elevation myocardial infarction: pathology, pathophysiology and clinical features. In: Zipes DP, Libby P, Bonow RO ,Braunwald Braunwald's Heart Disease. (7th edn.) Elsevier Saunders, Philadelphia, USA: 1141-1166.

65. Kohro T, Hayashi D, Okada Y, Yamazaki T, Nagai R (2008) Demographics and changes in medical/interventional treatment of coronary artery disease patients over a 3.5-year period in Japan: the Japanese Coronary Artery Disease Study: trend examination. Circ J 72: 1397-1402.

66. Vanhoutte PM, Shimokawa H (1989) Endothelium-derived relaxing factor and coronary vasospasm. Circulation 80: 1-9.

67. Laine P, Kaartinen M, Penttilä A, Panula P, Paavonen T, et al. (1999) Association between myocardial infarction and the mast cells in the adventitia of the infarct-related coronary artery. Circulation 99: 361-369.

68. Yatera Y, Shibata K, Furuno Y, Sabanai K, Morisada N, et al. (2010) Severe dyslipidaemia, atherosclerosis, and sudden cardiac death in mice lacking all NO synthases fed a high-fat diet. Cardiovasc Res 87: 675-682.

69. Sasaguri Y, Wang KY, Tanimoto A, Tsutsui M, Ueno H, et al. (2005) Role of histamine produced by bone marrow-derived vascular cells in pathogenesis of atherosclerosis. Circ Res 96: 974-981.

70. Furuno Y, Morishita T, Toyohira Y, Yamada S, Ueno S, et al. (2011) Crucial vasculoprotective role of the whole nitric oxide synthase system in vascular lesion formation in mice: Involvement of bone marrow-derived cells. Nitric Oxide 25: 350-359.

71. Kurioka S, Koshimura K, Murakami Y, Nishiki M, Kato Y (2000) Reverse correlation between urine nitric oxide metabolites and insulin resistance in patients with type 2 diabetes mellitus. Endocr J 47: 77-81.

72. Node K, Kitakaze M, Yoshikawa H, Kosaka H, Hori M (1997) Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. Hypertension 30: 405-408.

73. Piatti P, Di Mario C, Monti LD, Fragasso G, Sgura F, et al. (2003) Association of insulin resistance, hyperleptinemia, and impaired nitric oxide release with in-stent restenosis in patients undergoing coronary stenting. Circulation 108: 2074-2081.

74. Tanaka S, Yashiro A, Nakashima Y, Nanri H, Ikeda M, et al. (1997) Plasma nitrite/nitrate level is inversely correlated with plasma low-density lipoprotein cholesterol level. Clin Cardiol 20: 361-365.

75. Cooke JP (2005) ADMA: its role in vascular disease. Vasc Med 10 Suppl 1: S11-17.

76. Cook S (2006) Coronary artery disease, nitric oxide and oxidative stress: the „Yin-Yang" effect--a Chinese concept for a worldwide pandemic. Swiss Med Wkly 136: 103-113.

77. Förstermann U, Li H (2011) Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing eNOS uncoupling. Br J Pharmacol 164: 213-223.

78. Papapetropoulos A, Fulton D, Lin MI, Fontana J, McCabe TJ, et al. (2004) Vanadate is a potent activator of endothelial nitric-oxide synthase: evidence for the role of the serine/threonine kinase Akt and the 90-kDa heat shock protein. Mol Pharmacol 65: 407-415.

79. Rochette L, Lorin J, Zeller M, Guilland JC, Lorgis L, et al. (2013) Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: possible therapeutic targets? Pharmacol Ther 140: 239-257.

80. Mungrue IN, Husain M, Stewart DJ (2002) The role of NOS in heart failure: lessons from murine genetic models. Heart Fail Rev 7: 407-422.

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