Beneficial Effects of Neuronal Nitric Oxide Synthase in Atherosclerosis

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Endothelial nitric oxide synthase (eNOS) is the only NOS isoenzyme expressed in normal coronary arteries. However, all 3 NOS isoforms—eNOS, inducible NOS (iNOS), and neuronal NOS (nNOS)—are found in human atherosclerotic plaques. Precise studies of knockout mice have uncovered the role of eNOS and iNOS in atherogenesis (Figure). The eNOS isoform protects against atherosclerosis, because mice lacking eNOS have increased atherosclerotic plaques. In contrast, iNOS is proatherogenic: iNOS knockout mice have decreased atherosclerotic plaque area. However, the role of nNOS in atherogenesis has remained a mystery until now.

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In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Kuhlencordt and colleagues explore the effects of deleting nNOS alleles on atherogenesis, by comparing the atherosclerotic burden in apoE−/− mice and in nNOS−/− apoE−/− mice fed a Western diet. The authors observed that nNOS decreases lesion area by 66% in male mice and by 31% in female mice. Unexpectedly, nNOS also improves survival: apoE−/− mice also lacking nNOS had a 30% increase in mortality compared with apoE−/− mice. Thus nNOS is doubly beneficial to mice predisposed to atherosclerosis: nNOS protects against atherosclerosis and decreases mortality. How?

Neuronal NOS might normally protect mice from atherosclerosis through indirect mechanisms. Expression of nNOS has been detected outside of the cardiovascular system in neurons, skeletal muscle, lung epithelia, and the kidney. It has been detected outside of the cardiovascular system in neurons, skeletal muscle, lung epithelia, and the kidney. However, mice lacking nNOS have increased atherosclerotic plaques.

Perhaps nNOS limits atherosclerosis through more direct mechanisms. The current study did not detect nNOS in normal coronary arteries, but found nNOS expressed in atherosclerotic plaques, in cells that appear to be smooth muscle cells or macrophages. Others have also found nNOS in atherosclerotic plaques. If nNOS is expressed within the plaque, how could NO derived from nNOS directly suppress atherogenesis? NO can simultaneously affect several atherogenic pathways: NO inhibits endothelial inflammation, NO decreases leukocyte trafficking, NO restrains smooth muscle cell proliferation, and NO limits platelet adherence and aggregation. Kuhlencordt et al found no difference in macrophage infiltrates between mice with and without nNOS. However, mice lacking nNOS had particularly prominent smooth muscle cell staining; although this difference was not significant, it suggests that nNOS might affect the cellular composition of the atherosclerotic plaque.

Additionally, nNOS might modulate atherosclerosis by directing the flow of NO toward particular targets. The amino terminus of nNOS contains a PSD/Discs-large/ZO-1 homologous (PDZ) domain which anchors nNOS to other PDZ domain proteins in discrete subcellular regions within neurons and skeletal muscle. Localization of nNOS within smooth muscle cells might direct NO toward intracellular components of pathways regulating migration and proliferation, such as growth factor receptors, cell cycle phosphatases, or G proteins. Although the current study did not define the subcellular localization of nNOS, it is tempting to speculate that nNOS localized toward the vessel lumen might even decrease leukocyte and platelet adhesion to the coronary artery, whereas nNOS closer to the adventitia might limit smooth muscle cell proliferation.

Another striking aspect of the current study is that nNOS improved survival in apoE-deficient mice fed a Western diet. Mice lacking both nNOS and apoE alleles had ~70% survival of mice merely lacking apoE alleles after 30 weeks. Why? The simplest explanation is that the double-knockout mice die from myocardial infarctions attributable to excessive coronary artery disease. Another possible cause of death is arrhythmias, because nNOS knockout mice have decreased heart rate variability compared with wild-type mice.

In addition to providing insight into the role of nNOS in atherogenesis, this landmark study by Kuhlencordt and colleagues raises intriguing questions. What mechanisms activate the expression of nNOS in smooth muscle cells and macrophages? Is nNOS localized within vascular cells, and if so, does it interact with proteins containing PDZ-domains? Furthermore, the nNOS knockout mice used in this and other studies are not truly deficient in nNOS; these mice lack the second exon of nNOS, blocking expression of the predominant nNOS splice variant nNOS-alpha, but permitting expression of a minor splice variant nNOS-gamma. What would be the effect on atherosclerosis of a complete absence of all nNOS splicing variants? Does NO derived from nNOS also protect brain vessels from atherosclerosis? Are vessels...
innervated by nNOS expressing neurons protected from atherosclerosis, whereas other vascular beds lacking nNOS neurons are more susceptible? Why does NO derived from nNOS (or eNOS) limit atherosclerosis but NO from iNOS neurons are more susceptible? Why does NO derived from atherosclerosis increase atherosclerosis? Last and most intriguing of all, how does nNOS promote survival in mice prone to atherosclerosis? This pioneering study has raised important questions that deserve further study.

Disclosure(s)

None.

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