Editorial Comment

Is NO an Endogenous Antiatherogenic Molecule?

John P. Cooke, Philip S. Tsao

Endothelium-derived relaxing factor is now known to be nitric oxide (NO) or NO bound to a carrier molecule, possibly in the form of a nitrosothiol or nitrosoheme. NO is derived from the metabolism of l-arginine by NO synthase, which is expressed by a number of cell types, including endothelial cells, neurons, macrophages, platelets, and vascular smooth muscle cells.

NO is a potent vasodilator that activates soluble guanylate cyclase, thereby increasing intracellular levels of cyclic GMP. In addition, NO exerts an inhibitory influence on cell proliferation and adhesion. The proliferation of vascular smooth muscle cells or lymphocytes in culture is inhibited by exogenous NO donors as well as endogenous NO. Platelet adhesion and aggregation as well as monocyte adhesion and chemotaxis are negatively regulated by this molecule. These in vitro studies indicate that NO suppresses a number of key processes that are involved in atherogenesis. It seems logical, therefore, that a reduction in the activity of vascular NO would promote atherogenesis. Indeed, one of the earliest abnormalities to occur in hypercholesterolemic animals and humans is a reduction in the activity of vascular NO, as manifested by impaired endothelium-dependent vasodilation. This occurs well before any structural changes in the vessel wall.

This impairment in endothelium-dependent vasodilation is reversible in hypercholesterolemic animal models and humans by intravenous infusion of the NO precursor l-arginine. The effect of l-arginine to normalize endothelium-dependent vasodilation is likely due to its metabolism to NO, since the effect is not mimicked by d-arginine (which is not a substrate for NO synthase). Chronic administration of supplemental dietary arginine to hypercholesterolemic rabbits is also associated with an improvement in endothelium-dependent vasodilation. The sustained enhancement of vascular NO activity is paralleled by a striking inhibition of intimal lesion formation in these animals. Dietary arginine also exerts an inhibitory effect on neointimal lesion formation after balloon injury in this animal model. Therefore, the weight of the available evidence suggests that vascular NO exerts an inhibitory effect on key processes in atherogenesis.

In this issue of Arteriosclerosis and Thrombosis, Naruse and colleagues add support to this hypothesis by demonstrating that chronic inhibition of NO synthase accelerates atherogenesis. New Zealand White rabbits were fed standard chow or a 2% cholesterol diet; some animals in each group also received in their drinking water a low (80 mg/mL) or high (160 mg/mL) dose of nitro-l-arginine methyl ester (L-NAME), an inhibitor of NO synthase. After 8 or 12 weeks, the animals were killed to allow studies of vascular reactivity and histomorphometry. As expected, the hypercholesterolemic animals had impaired endothelium-dependent relaxation. Administration of L-NAME caused a further impairment of endothelium-dependent relaxation in hypercholesterolemic animals. Parallel changes were observed in vascular structure, with the thoracic aortas from the L-NAME-treated hypercholesterolemic animals manifesting greater lesion surface area. The data suggest that impairment of NO synthesis promotes atherogenesis. However, oral administration of L-NAME augmented serum cholesterol values in the hypercholesterolemic animals, and it is possible that this played some role in the acceleration of lesion development.

This confounding variable was obviated in the investigation by Cayatte and colleagues. These investigators administered a similar dose of l-NAME by osmotic minipump; subcutaneous delivery of L-NAME was not associated with any change in lipid profile or hemodynamics. It is possible that the effect of the NO synthase antagonist to alter the lipid profile is a unique effect of oral administration and may indicate an interesting interaction between the mesenteric NO synthase system and lipid absorption or metabolism. Despite this and other methodological differences between the two studies (composition of the cholesterol diet, length of treatment, and type of morphometric analysis), the findings are strikingly similar. In each case, chronic administration of NO synthase antagonists is associated with inhibition of endothelium-dependent vasodilation and a significant increase in the severity of intimal lesions.

The mechanisms of this intriguing effect remain unexplained. Moreover, it is not clear whether the effect of the NO synthase antagonist is due to inhibition of endothelium-derived NO and/or NO elaborated from...
other cell types (macrophages, platelets, or vascular smooth muscle cells).

Recent studies from our laboratory support a role for endothelium-derived NO. New Zealand White rabbits were fed normal chow or a 0.5% cholesterol diet; some animals received supplemental dietary arginine, whereas others received nitroarginine (the NO synthase antagonist). After 2 weeks the animals were killed and the thoracic aortas harvested for functional binding studies and measurement of nitrogen oxide by chemiluminescence. We found that the elaboration of vascular NO was increased in hypercholesterolemic animals that had received supplemental arginine; vascular NO release was inhibited in animals that had received nitroarginine. Binding of mononuclear cells to the vessel wall ex vivo was increased in hypercholesterolemic animals and was increased to a greater degree in animals that had received nitroarginine. By contrast, there was a reduction in cells bound to the thoracic aortas from hypercholesterolemic animals that had received dietary arginine. To conclude, changes in vascular NO elaboration were associated with reciprocal alterations in endothelial adhesiveness.

Is this alteration of endothelial adhesiveness due to an inhibitory effect of NO on the activation of adhesion molecules and/or the expression of chemotactic proteins? Bath and colleagues9 have found that exogenous NO inhibits adherence of mononuclear cells to porcine aortic endothelial cells in culture and inhibits in vitro chemotaxis stimulated by N-formyl-methionyl-leucyl phenylalanine. More recently, Zeiher and colleagues10 have reported that the NO donor SIN-1 inhibits mRNA expression and secretion of monocyte chemotactic protein-1 in primary cultures of human umbilical vein endothelial cells. Thus, endogenous NO may modulate endothelial-monocyte interactions. In addition, NO interferes with the elaboration of superoxide anion and may reduce the oxidative modification of low-density lipoprotein by macrophages.20

Although the available evidence suggests that vascular NO inhibits de novo formation of intimal lesions, the role of NO in the presence of preexisting lesions remains to be determined. It is possible that in the setting of established lesions, vascular NO could promote cell injury. Activated macrophages generate superoxide anion and NO, which combine to form peroxynitrite anion, a highly reactive free radical. The cytotoxic effects of peroxynitrite anion are in part mediated by membrane lipid peroxidation as well as nitrosation of tyrosine moieties regulating enzyme function and signal transduction. Furthermore, in large quantities, NO has cytotoxic effects mediated by nitrosylation, mono- and poly-ADP ribosylation, and interactions with metal-containing proteins that lead to the inhibition of enzymes required for cellular metabolism and DNA synthesis. However, even these effects of NO could serve to suppress cellular proliferation, inflammation, and lesion growth. To conclude, future investigations will be devised to elucidate the mechanisms and conditions under which endogenous NO inhibits atherogenesis. Knowledge of these mechanisms may lead to new therapeutic strategies for atherosclerosis and restenosis.

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