INVITED BASIC SCIENCE REVIEW

Nitric oxide and the vascular surgeon

Raymond G. Makhoul, MD, Charles E. Fields, MD, and **Anthony D. Cassano, MD,** *Richmond, Va*

In 1980, the simple but seminal observation was made that, when vascular rings of rabbit aorta were suspended in an organ bath, acetylcholine dilated the vessels only if the endothelium was intact. This effect was attributed to a short-lived humoral substance, which was called endothelium-derived relaxing factor. By 1986, it was confirmed that endothelium-derived relaxing factor was identical to nitric oxide (NO), a free radical gas. What followed was an explosion of investigative work that culminated in the awarding of the Nobel Prize in Medicine in 1998 for the discovery of NO as a signaling molecule in the cardiovascular system. In addition to its key role in cardiovascular physiology, NO is also important in the nervous system, the immune system, the gastrointestinal system, and the pulmonary system. In view of the almost daily discoveries concerning the critical role of NO in vascular biology, it is imperative that the vascular surgeon have a basic understanding of this important molecule.

BIOCHEMISTRY OF NITRIC OXIDE

Unlike most messenger molecules in humans, which are quite complex, NO is a simple, lipophilic, free-radical gas with a half life of 3 to 5 seconds. Because of these unique characteristics, NO is able to easily diffuse through cell membranes and thus does not act via membrane receptors. In humans and other mammals, NO is synthesized from the amino acid L-arginine by a family of enzymes called NO synthases (NOS). The reaction is a two-step process that yields the intermediate product, N-hydroxy-L-arginine, and the final products, NO and L-citrulline. The short half life of NO is the result of its rapid oxidation to nitrite (NO_2) and nitrate (NO_3) by reactions with O_2 and superoxide anion O_2^-.

Within the vessel wall, the ultimate target of NO is the enzyme-soluble guanylate cyclase. NO binds to the heme moiety of this enzyme, which results in an elevation of the second messenger cyclic guanosine monophosphate (cGMP). It is this rise in cGMP that is responsible for the physiologic actions of NO, such as vasorelaxation and antiplatelet activity (Fig 1). NO may also modify the activity of several other enzymes that contain heme or non-heme iron and thus exhibit different effects. Other indirect actions of NO result from its interaction with O_2 or O_2^-. The reaction of NO and oxygen may form N_2O_3, which is able to modify thiol groups through S-nitrosylation to form stable NO carriers that act as reservoirs of endogenous NO. When NO reacts with O_2^-, the powerful oxidant peroxynitrite is formed. This extremely reactive product is cytotoxic and, in excessive amounts, may cause tissue injury. Given the numerous interactions NO may have, its ultimate effect may be beneficial or deleterious.

The NOS enzymes in blood vessels can be broadly categorized into two types: constitutive and inducible. The constitutive enzyme is always present, localized to the endothelium and platelets, and produces the relatively small amounts of NO that are needed for vascular homeostasis. The stimulation of the endothelial cell by acetylcholine, bradykinin, substance P, calcium ionophores, thrombin, serotonin, and cyclic stress and shear stress results in NO production. In contrast, the inducible isoform (iNOS) is expressed in macrophages, vascular smooth muscle cells, hepatocytes, and neutrophils only after stimulation with endotoxins or cytokines, such as interferon-gamma, interleukin-1, and tumor necrosis factor. Once induced, iNOS produces high concentrations of NO and is therefore more important in pathophysiologic states.

PHYSIOLOGY OF NITRIC OXIDE IN THE VESSEL WALL

The first described and best known action of NO in the blood vessel is that of vasorelaxation. The NO

From the Division of Vascular Surgery, Department of Surgery, Medical College of Virginia Campus at Virginia Commonwealth University.
Reprint requests: Dr Raymond G. Makhoul, Medical College of Virginia, Department of Surgery, Division of Vascular Surgery, PO Box 980108, Richmond, VA 23298.
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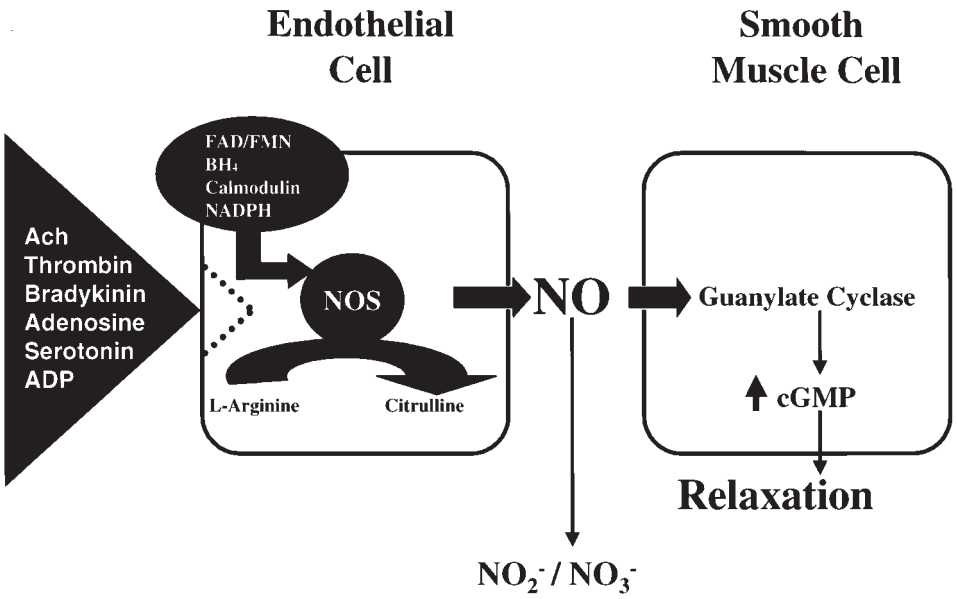

Fig 1. Metabolic pathway of nitric oxide *(NO)* in blood vessel wall. Endothelial cells secrete NO in response to variety of external stimuli. NO is released during conversion of L-arginine to citrulline with NO synthase *(NOS)*. This enzyme is dependent on cofactors flavin adenine dinucleotide/flavin mononucleotide *(FAD/FMN)*, tetrahydrobiopterin *(BH_4)* calmodulin, and reduced nicotinamide adenine dinucleotide phosphate *(NADPH)*. After release from endothelial cell, NO diffuses into extracellular milieu where it can react with smooth muscle cells or undergo variety of other reactions. *cGMP,* Cyclic guanosine monophosphate; *Ach,* acetycholine; *ADP,* adenosine diphosphate.

produced by endothelial cells is the major physiologic regulator of basal blood vessel tone with its continual release into the circulation. Subsequently, other important influences of NO on blood elements and the vessel wall have been described. NO is a potent platelet inhibitor, also via a cGMP dependent mechanism. In addition, NO inhibits platelet adhesion to collagen fibrils, endothelial cell matrix, and endothelial cell monolayers in vitro. Leukocyte adhesion to the endothelium is also attenuated by NO. Because platelets and leukocytes accumulated at the site of vessel injury may contribute many of the mediators of vascular smooth muscle cell proliferation, the inhibitory actions of NO likely play a pivotal role in the modulation of the responses to these mediators (Fig 2).

There is also ample evidence that NO directly inhibits vascular smooth muscle cell proliferation and migration and the collagen that comprises the extracellular matrix in the vessel wall. Taken together, it is apparent that NO not only plays a key role in the maintenance of vasomotor function but that it is also vitally important in the opposition of thrombosis and vascular lesion formation.

NITRIC OXIDE AND PATHOPHYSIOLOGIC CONDITIONS

There are a number of pathophysiologic conditions that impact on the capacity of the vessel to produce NO and result in what has been termed *endothelial dysfunction.* This endothelial dysfunction may shift the balance in the vessel wall, which would make it more vulnerable to vasospasm, thrombosis, and lesion formation and the resultant clinical consequences (Fig 3). The mechanisms that are potentially responsible for this decreased production include direct endothelial cell injury and loss, limitations in substrate availability (L-arginine), alterations of the NO synthase enzymes, and increased destruction of NO by superoxide.

Hyperlipidemia and atherosclerosis. It is well known that many cellular and subcellular events, such as cell growth and vasomotor function, are abnormal in atherosclerotic arteries. Given that NO can interact with a number of the key components in the formation of atherosclerosis, it is not surprising that a relationship between NO and atherosclerosis has been well documented. In humans, hypercholesterolemia causes an impairment of endothelial-

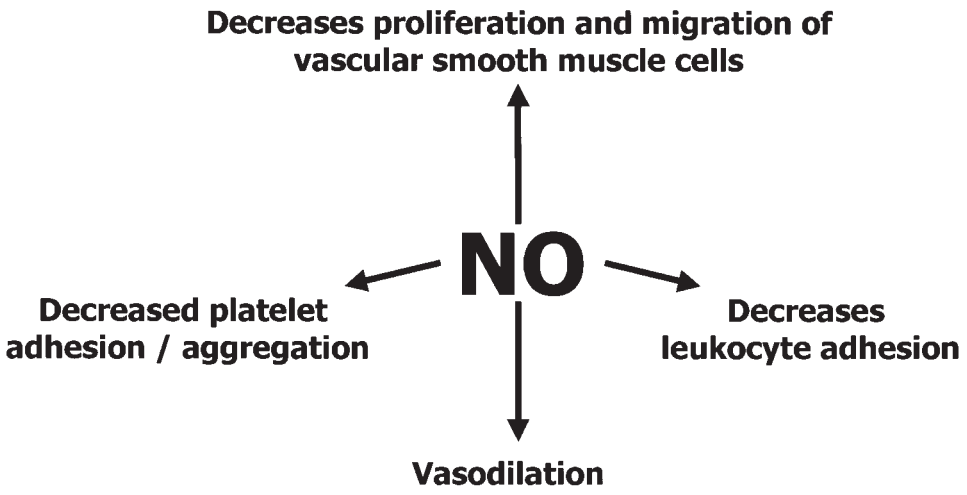

Fig 2. Beneficial effects of nitric oxide *(NO)* on blood vessel wall.

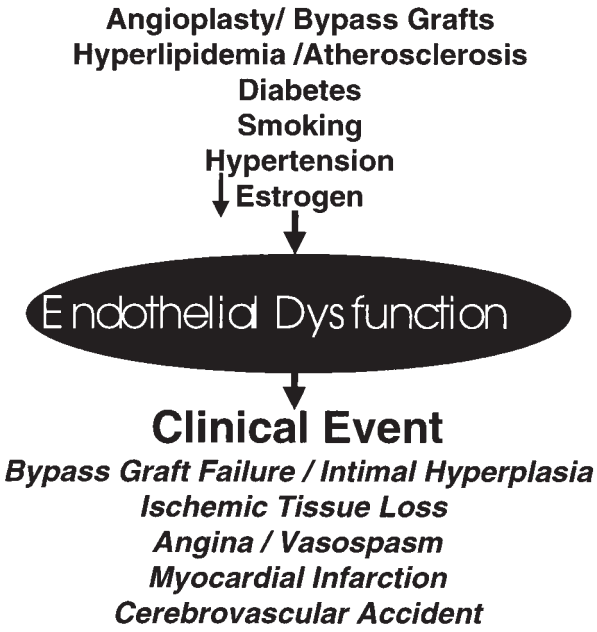

Fig 3. Causes of endothelial dysfunction and their relationship to clinical events.

dependent vasorelaxation that occurs well before any structural changes are seen in the vessel wall. Interestingly, this endothelial dysfunction is reversed by the exogenous administration of the NO precursor L-arginine and by lowering cholesterol levels. Additional support for the protective role of NO in atherosclerosis is provided by experimental animal studies that show accelerated plaque formation by inhibition of NO production.

Hypertension. Hypertension is a well-recognized risk factor in the development of vascular disease. In patients with hypertension, NO production is impaired, although the exact mechanism remains unclear. The diminished vasorelaxation is likely caused by endothelial rather than smooth muscle cell dysfunction because the response to nitroprusside (a NO-producing drug) was identical in patients who were hypertensive and normotensive.

Diabetes. In diabetes mellitus, there is a generalized vasculopathy that is associated with impaired NO-dependent vasorelaxation. The mechanism for this seems to differ between patients with type I and

type II diabetes. In the patient with type I diabetes, the vasomotor defects are related to vascular smooth muscle cell resistance to endothelial cell–generated NO. In the patient with type II diabetes, there appears to be both endothelial and smooth muscle cell dysfunction. The endothelial dysfunction is probably related to the hyperglycemia and the creation of an osmotic gradient across the endothelial cell wall, which results in cell swelling and dysfunction. In addition, glucose metabolism generates an abundance of superoxide anions, which react with NO and cause its depletion and the production of deleterious free radicals. Other conditions that have been associated with diminished NO production and endothelial dysfunction include chronic cigarette smoking and diminished estrogen levels in women.

NITRIC OXIDE AND VASCULAR INTERVENTIONS

After endarterectomy, vein bypass grafting, and balloon angioplasty, there is a denudation of the endothelium and a stretching of the vessel wall with damage to the media. This is followed by platelet adherence and degranulation, with the release of the potent mitogens platelet–derived growth factor and fibroblast growth factor. Smooth muscle cell proliferation ensues, with extracellular matrix formation and collagen deposition. Endothelial repair begins with the ingrowth of regenerated endothelial cells, which have an altered morphologic appearance. The endothelium regenerated after these manipulations is dysfunctional with respect to NO activity and its inhibition of vascular smooth muscle cell growth and vessel thrombosis.

An almost immediate response to endothelial injury is an increased gene expression of iNOS from the smooth muscle cells. This is associated with an increase in NO production, which could represent an inherent protective mechanism whereby the injured vessel resists thrombosis and restenosis.

Veins and arteries differ with respect to structure, function, and NO production. The release of NO from human saphenous vein is markedly diminished as compared with the internal mammary artery, possibly accounting for the inferior patency of coronary vein grafts as compared with internal mammary grafts. NO activity in human veins is significantly impaired during routine intraoperative handling of the grafts before implantation. Many of the endothelial cells are initially lost after implantation, which exposes the subendothelium to leukocytes and platelets and makes vein grafts prone to platelet aggregation and the release of products that favor vasospasm and thrombosis in the short term and the development of intimal hyperplasia in the long term. Furthermore, the NO synthases are present in human vein grafts and their expression seems to be a function of the unique environmental influences that include flow rates and shear stress.

CONCLUSION

The discovery of NO and its role in physiologic and pathophysiologic conditions stands as one of the greatest medical advances of this century. There is great potential for the treatment of atherosclerosis and intimal hyperplasia with NO augmentation. Already, studies in animal models aimed at increasing NO production in vessels with L-arginine administration, NO donor drugs, and gene transfer of the NO synthases have been promising. With almost daily advances in our understanding of this simple molecule, effective therapeutic strategies in humans are likely to be just on the horizon.

SUGGESTED READINGS

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