REVIEW ARTICLE

Nitric oxide and arterial disease

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Nitric oxide (NO) is a molecule that has gained recognition as a crucial modulator of vascular disease. NO has a number of intracellular effects that lead to vasorelaxation, endothelial regeneration, inhibition of leukocyte chemotaxis, and platelet adhesion. Its role in vascular disease has been intensively investigated and further elucidated over the past two decades. It is important in the pathogenesis of many cardiovascular diseases, including atherosclerosis, intimal hyperplasia, and aneurysmal disease. In addition, NO has been used as a therapeutic tool to treat diseases that range from recurrent stenosis to inhibiting thrombotic events. Many commonly used medications have their therapeutic actions through the production of NO. This review highlights the vascular biologic characteristics of NO, its role in the pathogenesis of cardiovascular disease processes, and its potential therapeutic applications. (J Vasc Surg 2004;40:187-93.)

Nitric oxide (NO) is a molecule that has gained increasing attention in the vascular community since being named *Science* magazine's "Molecule of the Year" in 1992, followed by the awarding of the Nobel Prize in Physiology and Medicine in 1998 to Drs Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad "for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system." Over the past two decades the vascular actions of NO have been investigated intensively. Here we review the biologic characteristics of NO as it relates to the cardiovascular system, disorders of NO metabolism as they relate to specific clinical disorders, and the potential vascular clinical applications of this elusive molecule.

BIOLOGIC CHARACTERISTICS OF NO

NO is a gas with a half-life of several seconds. It is synthesized by a family of NO synthase (NOS) enzymes that produce NO and citrulline through a five-electron oxidation of the guanidine-nitrogen terminal of L-arginine. Three distinct isoforms of NOS have been identified in human beings and other organisms. Two of these are constitutively expressed: neuronal NOS (nNOS; also known as NOS-1, because it was the first isoform discovered) and endothelial NOS (eNOS; NOS-3). Both of these are regulated by calcium and calmodulin and by post-translational modifications of the enzymes. The third isoform is inducible NOS (iNOS; NOS-2). It is regulated by cytokine stimulation, and produces quantities of NO far exceeding those produced by the other two isoforms. These enzymes all require several cofactors for proper function, including tetrahydrobiopterin (BH_4), nicotinamide-adenine-dinucleotide phosphate (NADPH), flavin adenine dinucleotide, and flavin mononucleotide. (For a full bibliography, see Appendix, online only.)

NOS enzymes are expressed by a variety of cell types. eNOS has been identified in endothelial and smooth muscle cells (SMCs), cardiac myocytes, bone cells, and neurons. nNOS has been found in neurons, skeletal muscle, the pancreas, and the kidneys. iNOS can be expressed in almost any cell type under cytokine stimulation, but is also constitutively expressed in some tissues such as the bowel wall. The list of tissues and cells that can express any or all of these NOS isoforms continues to grow, which supports the importance of NO in a variety of physiologic and pathophysiologic processes.

The first realization that NO was pivotal in biologic processes occurred when it was identified as endothelium-derived relaxing factor (EDRF). In 1980 EDRF was described as a factor that mediates vasodilation and is released by the vascular endothelium in response to acetylcholine. Independent work conclusively revealed that NO had identical properties and characteristics as those of EDRF. Since these early works, NO has been further characterized and found to possess a number of physiologic functions essential to vascular homeostasis (Fig).

The molecular signaling pathways through which NO transmits its message are many, and are still being elucidated. However, one prevalent pathway in the vasculature involves the activation of soluble guanylyl cyclase, which then produces cyclic GMP (cGMP). cGMP appears to be the second messenger responsible for mediating vasorelaxation and antiplatelet functions. NO inhibits SMC proliferation and migration through cGMP, although cGMP-independent pathways for these actions also have been found. NO can also act directly on calcium-dependent potassium channels, leading to the relaxation of smooth muscle. The vasoprotective effects of NO extend beyond

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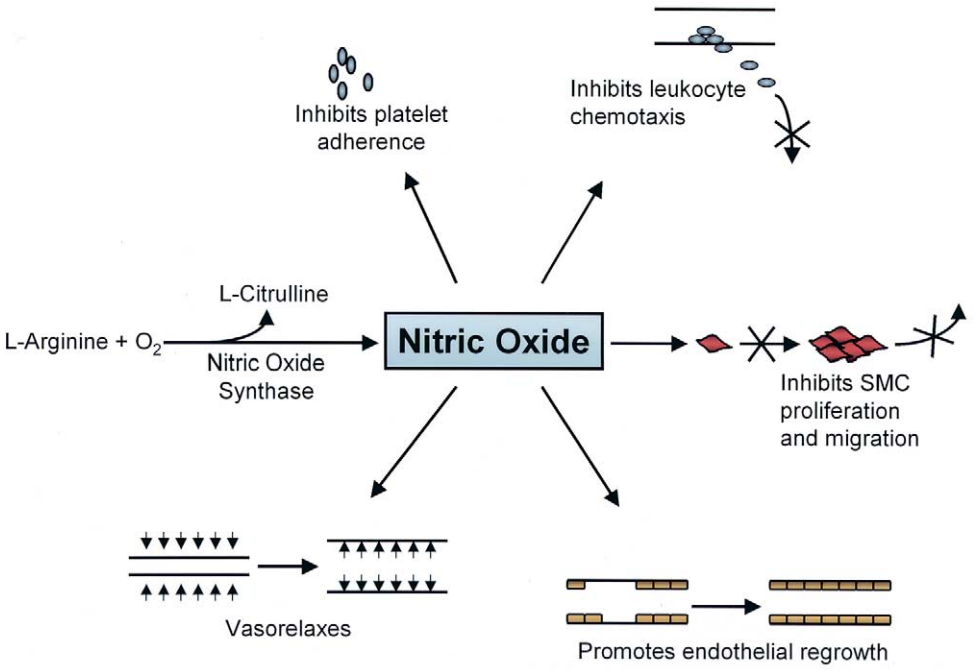

Summary of vascular effects of nitric oxide.

the SMCs, and include promotion of endothelial cell proliferation, protection of endothelial cells from apoptosis, and inhibition of adherence of inflammatory cells.

DISEASE PROCESSES

Atherosclerosis. Atherosclerosis is the number 1 cause of morbidity and mortality in the United States. It involves a cascade of pathologic processes that culminate in vascular lesion development and the consequences of reduced tissue perfusion, such as myocardial infarction and limb loss. Once atherosclerotic lesions have developed, plaque composition determines its predilection for rupture and subsequent ischemic events. In 1986, it was reported that acetylcholine induces vasodilation in the coronary vessels of healthy patients without atherosclerosis, but not in patients with angiographic evidence of atherosclerosis. Since then it has clearly been shown that not only is there impaired endothelial production of and vascular response to NO in atherosclerosis, but NO also plays a crucial part in the development of these lesions.

One of the earliest processes identifiable in the development of atherosclerosis is endothelial dysfunction. The elaboration of NO is crucial to the normal homeostatic function of the endothelium. Patients with developing atherosclerosis have reduced NO bioavailability in both the coronary and peripheral vasculature. The importance of NO in atherogenesis was suggested in mice deficient in apolipoprotein E (apoE), in which atherosclerotic lesions developed spontaneously when eNOS was also deleted. Compared with ApoE knockout mice, ApoE-eNOS double-knockout mice showed accelerated atherosclerotic plaque formation. Similarly, NOS inhibition in ApoE knockout mice increased atherogenesis. The overexpression of eNOS in transgenic mice leads to a decrease in blood pressure and plasma cholesterol levels, and a 40% reduction in atherosclerotic lesion formation. However, evidence also suggests that NO may be involved in atherogenesis. ApoE-iNOS double-knockout mice demonstrate reduced atherosclerotic plaque formation. This suggests that NO derived from eNOS may protect the vasculature from atherosclerosis, whereas NO from iNOS may promote lesion formation.

The understanding of the pathogenesis of atherosclerosis has evolved substantially over the last several decades. It is now clear that inflammatory mediators, in addition to endothelial dysfunction, are intimately involved in this process. After the initial endothelial dysfunction and reduced NO levels, a pro-inflammatory phenotype is seen in animals predisposed to atheromatous plaque formation, with increased neutrophil adherence. Processes involved in the generation of oxidative stress, such as the generation of superoxide anion, are activated, leading to cellular injury and peroxidation of lipid components. This produces oxidized low-density lipoproteins (LDLs), which are key mediators of atherosclerosis.

A number of specific factors can alter the production of NO and therefore influence the progression of atherosclerosis. BH_4 is a necessary cofactor for the proper functioning of all NOS enzymes. When it is in short supply within the cell, NOS uncouples and behaves as an NADPH oxidase, leading to production of superoxide rather than NO. Vitamin C exerts beneficial effects on the cardiovascular system

by increasing eNOS activity by increasing intracellular concentrations of BH_4. Supplementation with BH_4 in apoE-deficient mice increases eNOS activity, but does not affect iNOS activity. Recent studies have also demonstrated the cardiovascular protection afforded by folate supplementation, with a trend toward increased basal NO production and improvement in endothelial function. Folate can augment BH_4 biosynthesis and increase NOS activity, providing endothelial protection against development of atherosclerosis.

Hypercholesterolemia and hyperlipidemia contribute to atherosclerotic plaque formation. These molecules mediate endothelial dysfunction, in part by consuming NO and forming reactive oxygen species that further damage the vasculature. Pharmacologic reduction of plasma cholesterol and lipid concentrations can reduce lesion formation by preventing consumption of NO. These medications work to reduce lipid levels through inhibition of 5-hydroxy-3-methylglutaryl coenzyme A reductase activity. They also have a direct effect on eNOS activity, increasing expression of the enzyme and subsequent NO synthesis. Alternatively, statin agents can help decrease nitrotyrosine-bound proteins (a toxic breakdown product of NO) and decrease the progression of atherosclerosis.

A number of other factors also contribute to the progression of plaque formation. Oxidized lipids (eg, oxidized LDL) can displace eNOS from its position in the intracellular caveolae and therefore disrupt eNOS activity. This interference with eNOS can be prevented by elevating high-density lipoprotein levels, which can fully restore eNOS activity. Oxidized lipids also consume NO within the vessel wall, resulting in nuclear factor κB activation and setting into motion a number of inflammatory mediators. Of interest, human beings have endogenous NOS inhibitors, including N^{ω}-monomethyl-L-arginine and asymmetric dimethylarginine (ADMA), the latter being more prevalent in plasma. ADMA levels are elevated in both animals and human beings with hypercholesterolemia, and in the setting of peripheral occlusive disease. Increasing the L-arginine–ADMA ratio in patients with intermittent claudication by administration of L-arginine increased pain-free walking intervals by more than 200% and absolute walking distance by 150% over control. Therefore finding ways to decrease ADMA levels may prove efficacious in attempts to limit atherosclerotic lesion progression.

Diabetic vascular disease. Diabetes mellitus is not itself a vascular disease; however, the pathophysiologic mechanisms behind development of vascular disease are accentuated in diabetes. Diabetes affects more than 150 million persons worldwide, greater than 90% of whom have type II, or non-insulin-dependent diabetes. These patients with type II diabetes constitute a large percentage of patients with advanced cardiovascular disease. They have a more aggressive form of arterial disease than do their counterparts without diabetes, and also respond less favorably to a variety of vascular interventions, with higher morbidity and poorer outcomes after angioplasty and bypass surgery. In patients with diabetes, neointimal areas develop more than twofold that in patients without diabetes after angioplasty and stent placement. Recent trials have demonstrated that reversing the metabolic derangements of diabetes, including hyperglycemia, hyperlipidemia, and hypertension, can reduce cardiovascular complications by as much as 50%.

One of the hallmarks of diabetes mellitus is dysfunction of the endothelium and SMCs, the major components of the blood vessel wall. A number of studies have correlated this dysfunction with impaired production or reduced bioavailability of NO. Impaired vasodilation after brachial artery infusions of sodium nitroprusside, an exogenous NO donor, and methacholine chloride, a stimulator of endothelium-derived NO, in patients with diabetes suggests that there is both impaired release of NO in response to agonists and impaired response to exogenously delivered NO in these patients. Similarly, impaired relaxation to NO donors has also been documented in the coronary circulation in patients with type II diabetes at intravascular ultrasound scanning.

Emerging data show that not only is the production of NO crucial in the maintenance of vascular integrity, but the availability of NO and the local redox state of the vessel also have important roles. Vessels in patients with diabetes have an increased concentration of advanced glycation end products, their corresponding receptor, and oxidized LDLs, all of which can consume locally produced NO and limit its bioavailability. Diabetes and chronic hyperglycemia also lead to augmented superoxide production. Superoxide can react with NO to produce peroxynitrite, which is cytotoxic, proinflammatory, and can contribute to further vascular damage and dysfunction.

Of interest, a number of medications used in the management of diabetes have beneficial effects on the vasculature through NO. The thiazolidinediones, including rosiglitazone, are a class of potent insulin-sensitizing agents used in the treatment of type II diabetes mellitus. These medications act through peroxisome proliferators–activated receptor-γ, a transcription factor that regulates lipid and glucose metabolism. In mice, these drugs increase NO availability by decreasing NADPH oxidase activity and thereby decreasing superoxide production and reducing the quenching effect on NO. Despite its pro-proliferative effects on SMCs, insulin can also exert anti-inflammatory effects, in part through NO-mediated down-regulation of intracellular adhesion molecule-1 expression in endothelial cells. Furthermore, insulin activates the phosphatidylinositol-3 kinase pathway to stimulate eNOS synthesis of NO. Angiotensin II can increase generation of reactive oxygen species and oppose the local action of NO. Angiotensin converting enzyme inhibitors may be vasoprotective by decreasing this superoxide production, increasing NO availability and decreasing ADMA concentrations.

Aneurysms. Aneurysmal degeneration of the blood vessel wall presents a formidable diagnostic and therapeutic dilemma for those who care for patients with vascular disease. The development of aneurysms has been an intense area of research, because surveillance programs have proven

unsatisfactory. Therefore, identifying and treating patients at risk has taken on an even more important role in this disease process. Increasingly it has become clear that NO has an important role in the pathogenesis of aneurysm formation.

Experimental models of aortic aneurysm formation lend insight into the potential causes of aneurysmal degeneration of the vascular wall. One of the more commonly used methods in animal models for creating aneurysmal dilatation of the aorta is elastase infusion. In a rat model, elastase infusion results in aneurysm formation and increased iNOS and matrix metalloproteinase-9 expression in the aortic wall at days 1 and 7, respectively. Along with iNOS, a variety of other genes involved with oxidative stress are up-regulated. NOS inhibition with N(G)-nitro-L-arginine methyl ester (L-NAME) or aminoguanidine significantly reduced aortic dilatation in response to elastase. In a similar murine model, eNOS and nNOS were down-regulated in the aorta after elastase infusion, with a concomitant increase in iNOS located predominantly within the inflammatory cells. iNOS knockout mice, however, had a similar extent of aneurysm formation after elastase treatment compared with wild-type controls, suggesting that iNOS is not essential for aneurysm formation in this experimental model.

Understanding the role of NO in the formation of aneurysms is much more difficult in human beings, because the pathogenesis is poorly understood and experimental models with elastase perfusion are clearly different from the human condition. Some information can be gleaned from the available data in human beings, however. Patients with abdominal aortic aneurysms have impaired endothelium-dependent and endothelium-independent vasodilation despite elevated serum nitrate levels. Of interest, these patients also have moderately decreased ADMA levels. Aortic specimens from patients undergoing elective aortic aneurysm repair confirmed the presence of iNOS in the medial and adventitial layers of the wall. iNOS was confined largely to B and T lymphocytes, macrophages, and SMCs. Nitrotyrosine, the reaction product of peroxynitrite and proteins, was also found within the macrophages and smooth muscle of the aneurysm tissue, which suggests the presence of cytotoxic reactive nitrogen species. No iNOS expression or staining for nitrotyrosine was found in normal aortic tissue. These findings support that iNOS is a component of the inflammatory process involved in aneurysm formation. However, it is still unclear whether iNOS-derived NO is directly responsible for vascular degeneration or whether it is merely a bystander within the inflammatory cells localized within the aneurysm wall.

Intimal hyperplasia. Intimal hyperplasia is an exuberant healing process that occurs in the vessel wall after injury limiting the patency rates of vascular interventions, including bypass grafts, stents, and balloon angioplasty. Five-year patency rates for femoropopliteal disease treated with percutaneous transluminal angioplasty range from 38% to 70%, and with bypass range from 65% to 80%. Intimal hyperplasia is a complex process characterized by loss or dysfunction of the vascular endothelium, proliferation and migration of SMCs, and a local inflammatory reaction with recruitment of inflammatory cells and circulating myofibroblast precursors. These events all contribute to the progressive obliteration of the vessel lumen. Despite intensive investigation, no pharmacologic agent has been developed that can reliably inhibit intimal hyperplasia and improve the outcomes of vascular interventions.

The pathogenesis of intimal hyperplasia is intimately linked to loss of local NO synthesis. The initiating event is endothelial denudation. Loss of the endothelium results in loss of endothelium-derived NO production and the attendant loss of its homeostatic function. This opens the door for platelet aggregation, inflammatory cell infiltration, and SMC proliferation and migration. Intimal hyperplasia occurs after a variety of interventions, including balloon angioplasty, at the sites of vascular anastomoses, and within and at the edges of vascular stents. One of the major limitations of endovascular stents has been development of in-stent recurrent stenosis. Patients with impaired endothelium-derived NO production have higher rates of in-stent stenosis. Patients with genetic mutation of the eNOS enzyme have higher rates of cardiac death after coronary stent placement, and higher rates of in-stent stenosis.

THERAPEUTIC APPLICATIONS OF NO

Inhibition of intimal hyperplasia. Because the loss of local endothelial NO production has an important role in the development of neointimal lesions, it logically follows that restoration of NO or even providing supraphysiologic levels of NO to sites of vascular injury may inhibit intimal hyperplasia. Some of the methods used to augment NO production in attempting to prevent intimal hyperplasia formation are summarized in the Table. Early studies showed that supplementation of the NOS substrate L-arginine in rodent models of vascular injury significantly inhibited intimal hyperplasia. Subsequent studies were performed with systemic administration of NO donor compounds, which blocked intimal hyperplasia by 70% to 80% in rodent models of vascular injury. Even NO delivered via inhalation reduced neointima formation in rodents, but required NO administration for the duration of the experiment.

Few studies have looked directly at the effect of NO or NO donors on the prevention of intimal hyperplasia in human beings. One such study, however, was ACCORD (*A*ction to *C*ontrol *C*ardiovascular *R*isk in *D*iabetes), in which 700 patients undergoing elective percutaneous transluminal coronary angioplasty were randomized to receive either NO donors (infusion of linsidomine followed by oral molsidomine) or calcium channel blockers for 6 months. While no improvement was documented in clinical outcomes such as death, nonfatal myocardial infarction, or need for repeat procedures, there was a reduction in recurrent stenosis rate (defined as $\geq$50% stenosis), from 47% to 38%, in patients who received the NO donors.

The systemic effects of NO are not fully understood at this time, and the potential for systemic hypotension with

Various methods used to examine the therapeutic effect of NO on the process of neointimal hyperplasia

Form of NO	*Percent inhibition of IH*	*Species*	*Model*	*Time period*	*Method of delivery*	*Reference/year*
L-Arginine	39	Rabbit	Balloon angioplasty	2 wk	Oral	McNamara et al[1] 1993
NO-albumin	77	Rabbit	Balloon angioplasty	2 wk	Local	Marks et al[2] 1995
NO	43	Rat	Balloon angioplasty	2 wk	Inhaled	Lee et al[3] 1996
NO donor	18	Human	Coronary angioplasty	6 mo	Oral	Lablanche et al[4] 1997
L-Arginine	37	Rabbit	Iliac injury	4 wk	Local	Schwarzacher et al[5] 1997
Adenoviral eNOS	72	Rat	Balloon angioplasty	2 wk	Local gene transfer	Janssens et al[6] 1998
Adenoviral iNOS	>95 (rat) 52(pig)	Rat/pig	Balloon angioplasty	6 wk (rat) 21 d (pig)	Local gene transfer	Shears et al[7] 1998
Adenoviral eNOS	28	Pig	Coronary angioplasty	28 d	Local gene transfer	Varenne et al[8] 1998
NO-releasing aspirin	23	Mouse	Balloon angioplasty	21 d	Oral	Napoli et al[9] 2001
NO-eluting stent	0	Pig	Stent deployment	28 d	Eluting stent	Yoon et al[10] 2002
Adenoviral iNOS	37	Pig	Coronary angioplasty with stent	28 d	Local gene transfer	Wang et al[11] 2003
NO donor	0	Human	Coronary angioplasty	6 mo	Oral	Wohrle et al[12] 2003
NO donor	46	Pig	Coronary angioplasty	8 wk	Local	Harnek et al[13] 2003
NO-eluting stent	32	Rabbit	Stent deployment	28 d	Eluting stent	Do et al[14] 2004

NO, Nitric oxide; *IH*, intimal hyperplasia.

use of systemic NO donors at a dose that might be required for local inhibition of intimal hyperplasia is of real concern. With the advent of gene transfer technologies, the potential for localized NO delivery became a reality. The first published study exploring NOS gene therapy used modified liposomes to express eNOS within injured rat carotid arteries and demonstrated a 70% reduction in intimal hyperplasia and restoration of vasoresponsiveness to endothelium-dependent vasodilators.

Since that landmark study, many other studies have also demonstrated successful inhibition of neointima formation with NOS gene transfer with eNOS, nNOS, and iNOS. Most studies have been performed with iNOS. The greater specific activity of iNOS gives it a theoretical advantage over eNOS and nNOS, and may allow lower titers of vectors to be used, thereby averting toxicity. This is an important consideration, because one of the greatest limitations of gene transfer technologies is the ability to express sufficient levels of therapeutic product without using toxic quantities of vector. Adenovirus-mediated iNOS gene transfer to balloon-injured rat carotid arteries inhibited intimal hyperplasia by 98% with an extremely low dose of viral vector, which supports this potential advantage of iNOS. Subsequent studies in pigs showed that iNOS gene transfer inhibited intimal hyperplasia by 55% in injured iliac arteries and 30% within vein bypass grafts. It was also effective in a pig model of coronary angioplasty and stenting, in which in-stent stenosis was reduced by almost 40%.

With the surge in endovascular techniques for treating vaso-occlusive disease, methods to reduce in-stent recurrent stenosis have been intensively investigated by industry. Rapamycin and paclitaxel-coated stents have recently been proven to limit in-stent stenosis, and there are several encouraging studies that have used NO in a similar fashion. NO eluting stents exert a local biologic effect on the stented vessel, including increasing cGMP levels, but initially failed to prevent in-stent stenosis in a porcine model. More recently, however, in-stent stenosis was reduced by 32% in a rabbit model with a stent coated with a biodegradable polymer with NO-releasing capacity. Overexpression of the human RAD50 gene, a DNA repair protein, via gene transfer in a porcine stent model also prevents in-stent stenosis through a NO-dependent mechanism. These results certainly provide promise for the use of NO-eluting stents and NOS gene transfer to inhibit intimal hyperplasia.

Therapeutic angiogenesis. While prevention of intimal hyperplasia is a major focus of biomedical investigations, therapies aimed at promoting angiogenesis have received equal or greater attention over the past decade. A significant number of patients with peripheral limb ischemia and myocardial ischemia represent excessive operative risk. Others have diffuse disease not amenable to angioplasty or bypass techniques. These patients require techniques that generate new blood supply from the existing vasculature for end-organ salvage.

Angiogenesis involves the generation of new capillaries from preexisting vessels. It is intimately tied to the proliferation and differentiation of endothelial cells and the interaction with their extracellular matrix. A number of growth factors, the most prominent being vascular endothelial growth factor (VEGF), regulate this process at the biochemical level, and can stimulate generation of new

vessels, which can then supply ischemic regions with critically needed blood flow.

NO is intimately involved in angiogenesis. eNOS gene transfer induced angiogenesis in animal models of hind limb ischemia in which the arterial flow to the lower limb had been ligated. This increase in limb blood flow induced by NOS gene transfer was abrogated with administration of the NOS inhibitor L-NAME, which indicates that the beneficial effect was mediated by NO synthesis. Furthermore, these animals were found to have up-regulated VEGF expression in the ischemic limbs. Similar findings have been reported in a stroke model in which NO donors were administered in rats after embolic stroke. These animals exhibited greater angiogenesis compared with control animals, mediated through the NO-cGMP pathway.

Many growth factors are involved in angiogenesis, the most important of which may be VEGF. VEGF stimulates angiogenesis through an increase in eNOS-mediated NO production within the endothelium both in vivo and in culture. Another key growth factor is fibroblast growth factor (FGF). FGF also functions through a NO-dependent pathway. NO appears to be crucial for FGF-induced endothelial tube formation, an early process in angiogenesis.

There is currently no Food and Drug Administration–approved protocol for use of NOS gene therapy to induce angiogenesis. There are, however, a number of protocols involving other growth factors to stimulate angiogenesis, either in peripheral ischemia or inoperable myocardial ischemia. The first use of gene therapy for this indication was reported in 1996 when VEGF plasmid DNA was administered intramuscularly in a single patient with lower extremity ischemia. Since then, more than a dozen clinical trials of VEGF gene transfer have been performed, with successful generation of new collateral circulation within ischemic extremities and within the ischemic myocardium. The most common adverse event has been development of transient edema. Similar angiogenesis trials have been performed with FGF, with comparable results. Because the angiogenic effect of VEGF appears to be mediated by NO, there are likely to be further attempts to use the NOS enzyme as a targeted means to induce vessel growth in ischemic tissues.

Anti-thrombotic agents. Limb ischemia and bypass graft occlusion often result from acute thrombotic events. NO has a number of effects on thrombosis and the coagulation system. NO is generated not only in the vessel wall, but also within circulating platelets, which possess NOS. NO can have direct effects on platelets, causing a decease in platelet aggregation and adhesion through an increase in platelet-derived cGMP. Therefore patients with injured or dysfunctional endothelium, such as those with atherosclerosis, are affected disproportionately with thrombotic events. The conventional treatments of these events include anticoagulation therapy, administration of thrombolytic agents, and surgical intervention.

A number of investigators have used NO to modulate thrombosis under experimental conditions. Gene transfer of C-type natriuretic peptide, a secreted polypeptide, reduced neointima formation and thrombosis in a rabbit carotid injury model. The beneficial effect of C-type natriuretic peptide was associated with induction of iNOS. Others have used eNOS and iNOS gene transfer to inhibit thrombosis in similar models. Alternatively, blocking NOS function with inhibitors such as L-NAME can induce microvessel and macrovessel thrombosis, in part through angiotensin II–induced tissue factor expression in the endothelium and generation of superoxide. Infusion of the NO precursor L-arginine inhibits activation of the coagulation cascade in human beings, increasing bleeding time, prothrombin time, and activated partial thromboplastin time.

Two common pharmacologic agents used to inhibit thrombosis, aspirin and cilostazol, act in part through NO. The beneficial effect of aspirin on cardiac mortality and stroke has largely been attributed to its anti-platelet activity mediated by the irreversible inhibition of platelet cyclooxygenase and thromboxane formation. Aspirin, however, also has a number of protective effects on the endothelium. These effects appear to be mediated through eNOS. Cilostazol, used in the treatment of claudication, is a phosphodiesterase III inhibitor. It has an endothelium-dependent vasorelaxant effect in rat thoracic aorta preconstricted with phenylephrine. This effect was abrogated in the presence of NOS inhibition. Cilostazol possesses vasorelaxant properties in the coronary circulation, a phenomenon attributable to the actions of NO. It has been used clinically to inhibit recurrent stenosis after percutaneous interventions.

NO donor drugs are a class of therapeutic agents that bind a nitrate compound to another existing drug. Medications such as glyceryl trinitrate, isosorbide mononitrate, and isosorbide dinitrate are NO donors used for antianginal effects stemming from NO-mediated vasodilation. This concept has been extended to other drugs in which a nitrate group is attached to a pharmacologically active compound. One of the more heavily investigated such drugs is NO-releasing aspirin. The NO donor aspirin NCX-4016 was used in a model of age-related recurrent stenosis, and showed a marked reduction in intimal hyperplasia after balloon injury compared with that in animals given aspirin alone. These animals also had fewer gastrointestinal side effects than did those given aspirin, although future compounds using NO-donating nonsteroidal anti-inflammatory compounds should limit this occurrence. NO-donating aspirin also has antiproliferative effects, and increases cGMP formation in saphenous vein grafts ex vivo, holding promise as a potential tool for prevention of thrombosis and intimal hyperplasia in vein grafts.

CONCLUSION

Without question, NO has a critical role in a variety of cardiovascular diseases. Its production is crucial for maintenance of normal vascular endothelial integrity. A deficiency of NO is a cardinal feature of atherosclerosis, intimal hyperplasia, thrombosis, and aneurysm formation. As our understanding of the complex biochemical features of NO has evolved, its vasoprotective properties have been further

elucidated and their import more fully appreciated. Moreover, we have come to appreciate the therapeutic possibilities of NO, and that a number of common clinical therapies produce their vasoprotective effects through generation of NO. New technologies, such as gene therapy and drug modification, will no doubt offer an even better understanding of the biologic properties of NO and enable application of NO to even more cardiovascular therapies.

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