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REVIEW ARTICLE

Nitric Oxide: Implications for Vascular and Endovascular Surgery

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Nitric oxide has a key role in vascular homeostasis. It plays a protective role by suppressing abnormal proliferation of vascular smooth muscle following various pathological situations including atherosclerosis and restenosis after vascular interventions such as balloon angioplasty, stent deployment and bypass grafting. It also has strong antiplatelet and anti-thrombogenic properties. In this review, possible applications to daily vascular and endovascular surgery practice, including systemic use of NO donors, enhancing endogenous production of NO by L-arginine and gene therapy, local delivery strategies and coating stents and grafts with NO-delivering/enhancing chemicals are reviewed.

Key Words: Nitric oxide; Vascular surgery; Endovascular; Stents; Synthetic grafts; Homeostasis.

Introduction

Nitric oxide (NO) is the smallest known mammalian biological signalling molecule and plays an essential role in human physiology.[1] Here, the relationship between NO and intimal hyperplasia, and possible implications for vascular surgery, are reviewed.

Biochemistry, Synthesis and Functions of Nitric Oxide

Nitric oxide (NO) is a highly reactive free radical, which has the potential to participate in a wide variety of reactions to yield complex biological responses.[1–3] NO is an important regulatory determinant of vascular tissue homeostasis (Fig. 1) and acts through a variety of signalling elements, including the cGMP (cyclic guanosine monophophate) and related protein kinase G (PKG) systems.[4] PKG-independent pathways may also be important.[5]

NO is derived enzymatically from the oxidation of L-arginine[6] by nitric oxide synthase (NOS), which exits in three isoforms: nNOS, iNOS and eNOS. Neuronal NOS (nNOS) is found mainly in nerve terminals[7] in the central and peripheral nervous systems. nNOS regulates norepinephrine release, and its expression increases following physical and mechanical stress, nerve injury,[8] ischaemia, hypoxia,[9] changes in plasma osmolarity,[10] and during pregnancy.[11] Endothelial NOS (eNOS) is found in venous, arterial and capillary endothelial cells,[12] as well as myocytes[13] and platelets.[14] Haemodynamic shear stress, chronic exercise, thrombocyte growth factor-β (TGF-β) and chronic hypoxia increase eNOS transcription.[15–17] eNOS mediates endothelial cell proliferation and vasodilatation and inhibits vascular smooth muscle proliferation, platelet adhesion/aggregation, leukocyte and monocyte adhesion. Unlike these two isoforms that are often found at stable levels in their characteristic tissues, the inducible NOS (iNOS) is not found constitutively and its expression is induced after immunoactivation of macrophages, monocytes, and other immunocompetent cells.[18–20] Although exceptions exist, this induction generally reflects a pathophysiological cellular response to immunoactivation,[19–21] and elicits vasoplegia, myocardial depression, cytotoxic effects and apoptosis.[22]

NO is highly susceptible to oxidation by the superoxide anion, and the integrity and activity of NO depends on adequate scavenging of this radical.[23] Absence of nitric oxide synthesis also leads to superoxide accumulation. In many vascular diseases, superoxide anion levels increase, superoxide dismutase is overwhelmed, and the bioactivity of NO is thereby reduced.[24]

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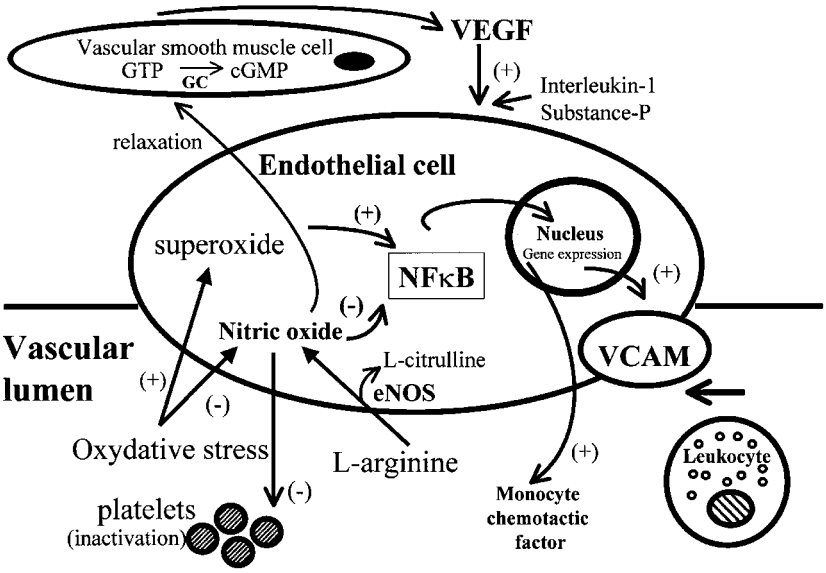

Fig. 1. Complex interactions between NO and surrounding milieu in vascular homeostasis. Nitric oxide (NO) is synthesised during the conversion of L-arginine to L-citrulline by Nitric Oxide Synthase (eNOS) in endothelial cells and counteracts with the effects of superoxide radical produced under oxydative stress. Oxydative stimulus causes Nuclear factor κB (NFκB) to travel to the nucleus and activate gene expression resulting in vascular cell adhesion molecule (VCAM-1) and monocyte chemotactic factor expressions. NO inhibits platelet adhesion and aggregation by inhibition of calcium influx, suppressing cellular signaling cascades, phosphorilation of thromboxane-A2 receptors, decreasing surface glycoprotein receptor expression and inhibition of eicosanoid mediator formation. NO also have an inhibitory effect on leukocyte and monocyte adhesion/migration and, therefore, may prevent the transformation of tissue macrophages into foam cells. NO causes relaxation of vascular smooth muscle cells via the activation of guanylyl cyclase (GC) and increased cyclic guanosine monophosphate (cGMP) formation. A number of mitogenic factors for endothelial cells including vascular endothelial growth factor (VEGF), Interleukin-1 and Substance-P requires NO production to exert their effects on endothelial cell growth during vascular healing.

Role of Nitric Oxide in Vascular Homeostasis

The primary role of NO as a physiological regulator derives from its constitutive production driven by the shear forces on the endothelium.[25] Following diffusion to smooth muscle cells, NO stimulates a variety of mechanisms, which causes relaxation by decreasing intracellular free calcium and also the sensitivity of contractile proteins to calcium. In addition to this paracrine control mechanism, NO participates in regulation of both sympathetic and parasympathetic control mechanisms. Many humoral substances, including acetylcholine and bradykinin, stimulate specific receptors on the endothelial surface to mediate an increase in endothelial cell calcium and NOS. Although NO is the primary vasodilator released from endothelial cells, it interplays with other vasoactive substances, including prostacycline, in regulating vascular tone.[26]

Vascular smooth muscle is the prototypical target tissue for NO, and principal effects are relaxation, growth inhibition and the promotion of apoptosis (programmed cell death).[27] Endothelial cells are the primary source of NO in the vascular system. NO release occurs in response to acetylcholine or shear stress. In contrast to its effects on vascular smooth muscle cell, NO has an anti-apoptotic effect in vascular endothelial cells, and stimulates endothelial cell growth, especially during angiogenesis and wound healing. Other major effects are to decrease endothelin release and to regulate vasodilatory prostaglandins (prostacyclin). NO also plays an important role in the maintenance of endothelial barrier function.[28] NO has an inhibitory effect on platelet activation[4] and may also affect bleeding times by interfering with the fibrinolytic system. It should be noted that as diminished NO production leads to an increase in the platelet aggregatory response, excessive NO bioactivity can lead to haemorrhagic states, as happens in sepsis and uraemia.[29]

Cytotoxicity, Apoptosis, and Nitric Oxide

High levels of NO assist in non-specific immune defence through its potent cytotoxicity against invading microorganisms, as well as tumour cells.[30,31] However, this is also destructive for the surrounding tissue. Both pro-[32] and anti-apoptotic[33] effects of NO have been demonstrated. Under physiologic conditions low levels of NO inhibits apoptosis of immune-competent cells and thereby supports immune defence, whereas chronic high levels may induce cell death. NO has an

anti-apoptotic effect on endothelial cells which leads to protection of the endothelium and, a pro-apoptotic effect on the smooth muscle cells that reduces neointimal proliferation, assists in remodelling but promotes atherosclerotic plaque destabilisation. Vascular endothelial growth factor (VEGF) is synthesised by smooth muscle cells and is a mitogen for endothelial cells. Arterial injury enhances VEGF gene expression and VEGF increases NO production in endothelial cells. Here, NO blocks apoptosis and enhances the mitogenic effects of VEGF, interleukin-1 (IL-1) and substance-P during endothelial wound healing and angiogenesis. NO released by regenerating endothelial cells has a negative feedback effect on VEGF expression.[34]

Cellular Proliferation, Vascular Injury Healing, Intimal Hyperplasia

The role of the adventitia in vascular remodelling

The adventitia plays an important role in normal vascular function and in diseases such as atherosclerosis, hypertension, and restenosis.[35] The major cell type within the adventitia is the fibroblast, which expresses iNOS upon inflammatory stimulation. There are many reports indicating that mast cells, also present in the adventitia, regulate leukocyte-endothelial cell relations via NO.[36] Mast cell membrane stabilising agents such as ketotifen and cromolyn may promote vascular homeostasis, by increasing iNOS expression and tissue cGMP levels, while reducing intercellular adhesion molecule-1 (ICAM-1) expression in the setting of lung ischaemia and reperfusion.[20] An *in vitro* study on human saphenous vein graft segments demonstrated that mast cell membrane stabilisation resulted in reduced vascular cell adhesion molecule (VCAM-1) and increased iNOS expression.[37]

The role of the endothelium and vascular smooth muscle in vascular remodeling

Bypass surgery, angioplasty, stent deployment, and endarterectomy are all complicated by aberrant healing characterised by excessive proliferation and migration of vascular smooth muscle cells. The main problem is the lack of function of the normal protective endothelial layer that lines arterial walls and secretes a variety of protective substances, including NO that inhibit platelet adhesion, aggregation, and thrombus formation.[38] Endothelial injury promotes its procoagulant, rather than anticoagulant, properties and leads to the expression of vasoactive molecules, cytokines and growth factors.[39]

Besides thrombogenity, disruption of the normal protective endothelial barrier results in a series of responses that may lead to restenosis. Exposure of subendothelial matrix elements results in platelet adhesion and activation with the release of chemotactic and mitogenic factors. Adhesion and migration of leukocytes releases additional growth factors, leading to migration and proliferation of vascular smooth muscle cells and myofibroblasts with formation of a neo-intima. NO can modulate this response to acute vascular injury by inhibiting several key components in the process including platelet adhesion and aggregation, leukocyte adhesion, and smooth muscle cell proliferation.

Endovascular stents

Endovascular stents were developed to overcome two major limitations of balloon angioplasty: Acute periprocedural vessel closure often related to dissection and intimal tears, which complicates 2–3% of attempts,[40] and chronic restenosis, which limits long-term patency in 20–50% of patients.[41] Because of their ability to seal intimal tears, stents may deal with threatened or abrupt vessel closure.[42] However, metallic stents are also very thrombogenic. In addition, by applying a continuous pressure on the arterial wall, further vascular injury may ensue and aggravate the restenotic process.

Synthetic vascular grafts

Synthetic material, being foreign and devoid of endothelial cells also represents a thrombogenic surface.[43] This attracts platelets and leukocytes, leading to thrombosis and intimal hyperplasia. This response is not a major problem with large-diameter grafts (8–20 mm in diameter) but is significant for small-diameter grafts between 2–6 mm in diameter. Over time, they develop an endothelial lining, which provides reasonable protection against thrombosis. However, vascular grafts are not effective at diameters less than 3 mm in diameter because they become occluded with intimal hyperplasia and thrombotic material.[44]

Systemic Use of NO Donors for Preventing Thrombosis and Restenosis after Angioplasty

NO donors

Systemic delivery of organic NO donor compounds such as molsidomine inhibits platelet adhesion and smooth muscle cell proliferation following balloon-induced carotid artery injury in pigs.[45] In this model, platelet thrombus formation was reduced by systemic administration of 3-morphplinosydnonimine (SIN-1), a spontaneous NO donor and molsidomine metabolite.[46] Administration of this agent also increased bleeding time and platelet cGMP. The ACCORD Study suggested that administration of NO donors linsidomine and molsidomine to patients undergoing coronary angioplasty lead to a modest improvement in the immediate angiographic outcome, with a greater minimal luminal diameter.[47] At the 6th-month angiographic follow-up, this modest improvement is sustained although there is no protection against late luminal loss.[47] Similarly, local delivery of a stable protein S-nitrosothiol reduces platelet deposition and inhibits neointimal proliferation after vascular injury.[48] Nitrosation of low-molecular-weight thiols or cysteinyl side chains of proteins occurs in the aqueous phase to yield RSNOs (s-nitrosothiol). RSNOs are formed in vivo by the reaction of bioavailable NO with sulfhydryl groups. RSNOs serve as a circulating reservoir of stable NO and allow NO to function in a paracrine fashion. These compounds further protect NO from reactive oxygen species. RSNOs contribute significantly to the pharmacological and physiological responses of the cardiovascular system, including maintenance of vascular tone, the response of the vessel wall to injury and inhibition of platelet aggregation. Attenuation of intimal and medial proliferation by local administration of RSNOs following balloon angioplasty suggests a therapeutic role for these agents in preventing restenosis. In a rabbit model of bilateral femoral artery injury, treatment with a polynitrosated S-nitroso-albumin demonstrated a significant decrease in platelet aggregation and intimal hyperplasia.[49] In summary, following arterial injury, augmenting NO production or providing exogenous NO reduces intimal hyperplasia in animal models. However, whether this is a benefit in human subjects remains unknown.

Enhancing endogenous NO production

L-arginine

In patients with severe vascular disease, intravenous L-arginine has been shown to improve limb blood flow.[50] A single intravenous dose of L-arginine (30 g over 60 min) results in 42% increase in femoral artery blood flow. Furthermore, daily intravenous administration of arginine for two weeks improves walking distance in patients with intermittent claudication.[51] Daily infusions of L-arginine (12.6 g) for 7 days increased calf blood flow and transcutaneous oxygen saturation, improved walking distance, shortened the time period to recovery from pain and reduced platelet aggregation and clot lysis time.[51,52] The enhancement in blood flow is extended to the microvascular circulation as well. Thirty grams of intravenous L-arginine was able to enhance calf muscle blood flow from 1.7 ± 0.1 to 2.2 ± 0.2 mL/min/100 g at 80 min following infusion.[53] Preliminary evidence suggests that administration of L-arginine-enriched food can increase walking distance and quality of life in patients with peripheral arterial disease. Consistent with observations in other patient populations, daily infusions of L-arginine (60 mmol) reduced ADP-induced platelet aggregation and shortened euglobin clot lysis time in patients with peripheral arterial disease who are known to have hyperaggregable platelets.[52,54,55] These antithrombotic effects of L-arginine may add to its vasodilatory effect to enhance overall walking ability and quality of life in this population. In addition, the improvement in endothelial function with L-arginine supplementation is associated with decreased neointima formation.[56]

Gene therapy approaches

NO production could be restored using gene transfer techniques to deliver the eNOS gene to the site of vascular injury. This might lead to inhibition of neointima formation and prevent platelet aggregation. Transfection with eNOS improves vascular NO production with restoration of endothelium-dependent relaxation and leads to a 70% reduction in neointima formation 14 days after balloon-induced injury in rat carotid arteries.[57] In a balloon injury model, it was demonstrated that retroviral-mediated overexpression of eNOS in smooth muscle cells can limit neointimal formation by inhibiting cell proliferation and increasing the production of NO.[58] In addition, iNOS gene transfer using an adhesive vector has been shown to inhibit neointimal hyperplasia following balloon injury in rats and pigs. This inhibition of neointimal proliferation by NOS gene transfer is, in part, the result of antiproliferative action on vascular smooth muscle cells.[59] eNOS expression inhibits DNA synthesis and reduces vascular smooth muscle cell proliferation following arterial injury, and subsequent enhancement in NO production is associated with an

inhibition of neointimal formation with a reduction in the intima-to-media ratio.[60]

Local Delivery of Chemical Agents for Augmentation of Tissue Nitric Oxide Levels

Local infusion strategies

The demonstrated efficacy of diazeniumdiolates as agents for inhibiting platelet adhesion in vivo lead to the hypothesis that their local application at the site of vascular graft placement would reduce the risk of thrombosis, even in the absence of systemic anticoagulation.[61] With the local administration of PROLI/NO (proline is the NO-carrying molecule here) via the boundary layer, dogs undergoing femoral artery endarterectomy immediately downstream displayed significantly less smooth muscle cell proliferation at the injury site 7 days after the surgery. PROLI/NO infused at an initial rate of 7 nmol/min reduced proliferation by 43% at the distal anastomosis and by 68% at the site of endarterectomy relative to the contralateral vessels infused with the carrier molecule proline instead.[62]

Local embedding strategies

A better approach might be to incorporate diazeniumdiolates into the graft itself, thereby eliminating the need for the infusion. The anionic complexes discussed above decompose spontaneously at physiological pH, some of them rapidly. Delivering NO to a targeted area before decomposition, and without interfering with other NO-dependent functions, remains a challenge. Periadventital application of diazeniumdiolates may be a possibility.[63] In a rabbit model of atheroma induction by placing a collar around the carotid artery; neointima formation after 14 days was reduced by 74% when the collars contained SPER/NO (spermine is the NO-carrying molecule) at an initial concentration of 1 mg/mL relative to contralateral control arteries whose collars contained carrier molecular spermine at similar concentrations.[63] In experiments with hypercholesterolaemic rabbits, jugular vein implants in the carotid artery were packed perivascularly with a biodegradable polymer containing SPER/NO. Intimal thickening was reduced by more than 40% in treated vein grafts relative to controls at 28 days after the implantation in arterial circulation.[64]

Coating blood-contact surfaces strategies

Insoluble NO-releasing polymers can be used to coat prosthetic vascular grafts. Diazeniumdiolates can be incorporated into polymeric matrices of different structural types. Prodrugs can be designed to protect the terminal oxygen; and binding of the prodrug facilitates its concentration in the target tissue or cell. This is achieved by cross-linking polyethylenimine onto commercial polytetrafluoroethylene grafts, then exposing them to NO to introduce the diazeniumdiolate functional group. Inserting of these grafts into the baboon arteriovenous shunt system led to dramatic reductions in platelet attachment over a 1-hour observation period. Grafts emitting NO at the rate of 1–2 nmol/min/mg remained patent, whereas control grafts blocked with thrombus within an hour.[65,66] An alternate approach is to embed microspheres of the same cross-linked polyethylenimine diazeniumdiolate into the pores of the polytetrafluoroethylene fabric.[67]

Coating endovascular stents

A stent offers a unique opportunity for local drug delivery, since it will be placed in very close contact with the acutely injured arterial wall and interfaces with the blood. Polymer coating of the Wall® stent (Schneider, Minneapolis, MN, U.S.A.) with cross-linked methane (Biogold) decreases early thrombosis of stents placed in pigs by 38% compared with uncoated stents. However, this polymer coating did not reduce neointimal hyperplasia significantly.[68] Another approach is to coat the stent with genetically engineered endothelial cells. Using retroviral-mediated gene transfer, the gene for human tissue-type plasminogen activator (t-PA) was inserted into cultured sheep endothelial cells.[69] These endothelial cells release PGI2, NO, and high concentrations of t-PA, but do not stick to bare metal stents. A substrate such as fibronectin is necessary before cells will seed.[69] However, fibronectin "glue" is itself thrombogenic[69] and if some seeded endothelial cells are released from the stent, the remained fibronectin-coated surface will be more thrombogenic than the bare stent itself. *In vivo* studies has been disappointing, possibly for this reason.[69] In addition, maintaining the viability of seeded endothelial cells is difficult. A different approach employs a fibrin film soaked in heparin that is then used as a circumferential coating on a stent.[70] These coated stents provide with less thrombotic occlusion, less neointimal hyperplasia and greater patency.[70] Preliminary studies with a heparin-coated

Benestent in patients also showed that the heparin-coated stent significantly decreased early thrombotic closure compared to an uncoated stent.[71] There was, however, no significant reduction in restenosis in this study.

Coating of synthetic grafts

Artificial surfaces in contact with blood quickly develop a coating of adsorbed plasma protein that governs subsequent interaction with blood cells. This initial protein adsorption occurs within seconds, before the blood platelets and other cellular components reach the arterial surface.[72] Much research has focused on the role of the most prevalent plasma proteins. Albumin is the most abundant protein in serum, and albumin coating has been used to make synthetic surfaces more thromboresistant.[73] By contrast, polymer surfaces coated with fibrinogen, another abundant protein in serum, promotes platelet activation and presents a very thrombogenic surface.[74] Thus, the initial protein layer adsorbed to the polymer surface is very important in determining the thrombogenicity of that surface.[72] Albumin has been used for coating vascular grafts to "passivate" surfaces in contact with blood and, thus, minimise surface-induced platelet activation.[75] Serum albumin can be modified to bear a covalently linked S-NO functional group that manifests nitrovasodilation and platelet inhibitory properties.[76] In addition, bovine serum albumin (BSA) has been chemically modified so that the molar ratio of S-NO to albumin is greater than 1:1.[59] This modification produces a polynitrosated albumin (pS-NO-BSA), which can be applied locally to foreign surfaces or to severely damaged arterial walls to make them less thrombogenic.[59,77,78] Among the most stable NO donating compounds are the S-nitroso-thiols, such as S-nitrosated albumin.[76] S-nitroso-thiols serve as carriers in the mechanism of action of NO by stabilising the labile NO.[76] In the dog, nitrosated albumin has been shown to inhibit *in vivo* platelet aggregation when given systemically[79] or locally.[78] It would seem reasonable that local delivery of an NO-like species (pS-NO-BSA) to restore or replace the relative deficiency of NO observed dysfunctional, acutely damaged endothelium could modulate the effect of vascular injury and potentially reduce platelet aggregation and reduce intimal proliferation after angioplasty or stent deployment. This has been examined in several recent studies, where platelets were exposed to collagen or other coated surfaces.

NO-releasing cross-linked polyetheleneimine microspheres can be incorporated into the pores of vascular grafts to deliver therapeutic amounts of NO (i.e. 10 nmol NO /mg with a half-life of 51 h) for the prevention of thrombosis and restenosis.[67] Incorporated [N(O)NO] group into polymeric matrices can be used for altering the time course of NO release.[65] The cross-linked poly-ethylenimine that has been exposed to NO inhibited the in vitro proliferation of rat aorta smooth muscle cells when added as a powder to a culture medium. It also showed potent antiplatelet activity when coated on a normally thrombogenic vascular graft situated in an intravenous shunt in a baboon's circulatory system.[65]

pS-NO-BSA might also be placed on stents and used for local delivery of NO. Palmaz-Schatz stents coated with pS-NO-BSA were compared to bare stents deployed in anesthetised pigs in six pairs of carotid arteries. pS-NO-BSA coating significantly retarded early platelet deposition.[80] The pS-NO-BSA-coated stents had a 40% reduction in neointimal area compared to bare stents.[80] There was also a statistically significant reduction in average neointimal thickness.[80] Thus, it appears that a pS-NO-BSA-coated stent offers promise for further studies to inhibit early thrombosis and late restenosis. One way to test the coating of damaged arterial walls is the direct application of pS-NO-BSA to the angioplastied femoral arteries of anaesthetised rabbits. After angioplasty of each femoral artery, one is exposed and coated with BSA, prepared for nitrosation (pS-BSA) but without NO incorporation. The other femoral artery is coated with chemically modified BSA (pS-NO-BSA) that does contain NO. The rabbits were then allowed to recover for 14 days. The artery receiving pS-NO-BSA had a significantly larger lumen $0.86 \times 10^5\ \mu M^2$ compared to the control $0.31 \times 10^5\ \mu M^2$ ($p = 0.01$) and the treated arteries had 81% less neointimal area ($p = 0.02$).[59] In these studies, the pS-NO-BSA also significantly inhibited radio labeled platelet binding to the damaged arterial surface compared to control surfaces. Finally, when the arteries treated with pS-NO-BSA were exposed to platelets for 1 min, they increased platelet cGMP levels more than fourfold compared to control, untreated arteries. Thus, the pS-NO-BSA reacted in a similar fashion when the NO donor was applied to implanted stents and when it was applied directly to damaged rabbit arteries.

Conclusion

Nitric oxide is an important regulatory determinant of vascular tissue homeostasis. It may play a protective role by inhibiting abnormal proliferation of vascular

smooth muscle following various pathological situations including atherosclerotic lesions, restenosis after balloon angioplasty, and vascular wall thickening in hypertension. It also has anti-platelet and anti-thrombogenic properties. Possible applications to daily vascular and endovascular surgery practice include systemic use of NO donors, enhancing endogenous production of NO by L-arginin and gene therapy, local delivery strategies and coating thrombogenic material such as endovascular stents and vascular grafts with NO-enhancing chemical agents.

Interest in NO biology has greatly built up over the past decade especially in cardiovascular medicine, as a consequent of our increasing understanding of its many roles in the vascular system. With the recent expansion of the field from the development of novel nitric oxide donors for the treatment of vascular disease to the development of gene therapy approaches for the restoration of endothelial function, the application of nitric oxide biology to the cardiovascular medicine and surgery is rapidly evolving. Accumulating our knowledge in nitric oxide biology may allow for designating futuristic therapeutic modalities in the treatment of vascular disease.

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References

1 Loscalzo J, Vita JA, eds. Nitric Oxide and the cardiovascular system. Humana Press Inc., New Jersey, 2000.
2 Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharm Rev* 1991; **43**: 109–142.
3 Wink DA, Darbyshire JF, Nims RW, Saavedra JE, Ford PC. Reactions of the bioregulatory agent nitric oxide in oxygenated aqueous media: determination of the kinetics for oxidation and nitrosation by intermediates generated in the N0/02 reaction. *Chem Res Toxicol* 1993; **6**: 23–27.
4 Lieberman EH, O'Neill S, Mendelsohn ME. S-nitrosocysteine inhibition of human platelet secretion is correlated with increases in platelet cGMP levels. *Circ Res* 1991; **68**: 1722–1728.
5 Gordge MP, Hothersall JS, Noronha-Dutra AA. Evidence for a cyclic GMP-independent mechanism in the anti-platelet action of S-nitrosoglutathione. *Br J Pharmacol* 1998; **124**: 141–148.
6 Palmer RM, Rees DD, Ashton DS, Moncada S. L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium dependent relaxation. *Biochem Biophys Res Commun* 1988; **153**: 1251–1256.
7 Chao DS, Hwang PM, Huang F, Bredt DS. Localization of neuronal nitric oxide synthase. *Methods Enzymol* 1996; **268**: 488–496.
8 Samdani AF, Dawson TM, Dawson VL. Nitric oxide synthase in models of focal ischemia. *Stroke* 1997; **28**: 1283–1288.
9 Shaul PW, North AJ, Brannon TS *et al.* Prolonged in vivo hypoxia enhances nitric oxide synthase type I and type III gene expression in adult rat lung. *Am J Respir Cell Mol Biol* 1995; **13**: 167–174.
10 O'Shea RD, Gundlach AL. Food or water deprivation modulates nitric oxide synthase (NOS) activity and gene expression in rat hypothalamic neurons: correlation with neurosecretory activity? *J Neuroendocrinol* 1996; **8**: 417–425.
11 Luckman SM, Huckett L, Bicknell RJ, Voisin DL, Herbison AE. Up-regulation of nitric oxide synthase messenger RNA in an integrated forebrain circuit involved in oxytocin secretion. *Neuroscience* 1997; **77**: 37–48.
12 Pollock JS, Nakane M, Buttery LK *et al.* Characterization and localization of endothelial nitric oxide synthase using specific monoclonal antibodies. *Am J Physiol* 1993; **265**: C1379–C1387.
13 Seki T, Hagiwara H, Naruse K *et al. In situ* identification of messenger RNA of endothelial type nitric oxide synthase in rat cardiac myocytes. *Biochem Biophys Res Commun* 1996; **218**: 601–605.
14 Sase K, Michel T. Expression and regulation of endothelial nitric oxide synthase. *Trends Cardiovasc Med* 1997; **7**: 25–34.
15 Nishida K, Harrison DG, Navas JP *et al.* Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J Clin Invest* 1992; **90**: 2092–2096.
16 Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res* 1994; **74**: 349–353.
17 Inoue N, Venema RC, Sayegh HS *et al.* Molecular regulation of the bovine endothelial cell nitric oxide synthase by transforming growth factor-beta$_1$. *Arterioscler Thromb Vasc Biol* 1995; **15**: 1255–1261.
18 Geller DA, Billiar TR. Molecular biology of nitric oxide synthases. *Cancer Metastasis Rev* 1998; **17**: 7–23.
19 Vural KM, Oz MC. Endothelial adhesivity, pulmonary hemodynamics and nitric oxide synthesis in ischemia-reperfusion. *Eur J Cardiothorac Surg* 2000; **18**: 348–352.
20 Vural KM, Liao H, Oz MC, Pinsky DJ. Effects of mast cell membrane stabilizing agents in a rat lung ischemia-reperfusion model. *Ann Thorac Surg* 2000; **69**: 228–232.
21 Morrissey JJ, McCracken R, Kaneto H *et al.* Location of an inducible nitric oxide synthase mRNA in the normal kidney. *Kidney Int* 1994; **45**: 998–1005.
22 Lopéz-Farré A, Sanchez de Miguel L, Caramelo C *et al.* Role of nitric oxide in autocrine control of growth and apoptosis of endothelial cells. *Am J Physiol* 1997; **272**: H760–H768.
23 Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 1986; **250**: H822–H827.
24 Cohen RA. The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. *Prog Cardiovasc Dis* 1995; **38**: 105–128.
25 Lamontagne D, Pohl U, Busse R. Mechanical deformation of vessel wall and shear stress determine the basal release of endothelium-derived relaxing factor in the intact rabbit coronary vascular bed. *Circ Res* 1992; **70**: 123–130.
26 Gryglewski RJ. Interactions between nitric oxide and prostacyclin. *Sem Thromb Hemost* 1993; **19**: 158–166.
27 Iwashina M, Shichiri M, Marumo F, Hirata Y. Transfection of inducible nitric oxide synthase gene causes apoptosis in vascular smooth muscle cells. *Circulation* 1998; **98**: 1212–1218.
28 Kanwar S, Wallace JL, Befus D, Kubes P. Nitric oxide synthesis inhibition increases epithelial permeability via mast cells. *Am J Physiol* 1994; **266**: G222–G229.
29 Remuzzi G, Perico N, Zoja C *et al.* Role of endothelium-derived nitric oxide in the bleeding tendency of uremia. *J Clin Invest* 1990; **86**: 1768–1771.
30 Granger DL, Hibbs JB Jr. High-output nitric oxide: weapon against infection? *Trends Microbiol* 1996; **4**: 46–47.
31 Dinapoli MR, Calderon CL, Lopez DM. The altered tumoricidal capacity of macrophages isolated from tumor-bearing mice

is related to reduce expression of the inducible nitric oxide synthase gene. *J Exp Med* 1996; **183**: 1323–1329.
32 Haendeler J, Weiland U, Zeiher AM, Dimmeler S. Effects of redox-related congeners on apoptosis and caspase-3 activity. *Nitric Oxide* 1997; **l**: 282–293.
33 Dimmeler S, Haendeler J, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of ICE-like and CPP32-like proteases. *J Exp Med* 1997; **185**: 601–608.
34 Tsurumi Y, Murohara T, Krasinski K *et al.* Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. *Nat Med* 1997; **3**: 879–886.
35 Shi Y, Pienick M, Fard A *et al.* Adventitial remodeling after coronary arterial injury. *Circulation* 1996; **93**: 340–348.
36 Hogaboam CM. Befus AD, Wallace JL. Modulation of rat mast cell reactivity by IL-1 beta. Divergent effects on nitric oxide and platelet-activating factor release. *J Immunol* 1993; **151**: 3767–3774.
37 Vural KM, Oz MC, Liao H, Batirel HF, Pinsky DJ. Membrane stabilization in harvested vein graft storage: effects on adhesion molecule expression and nitric oxide synthesis. *Eur J Thorac Cardiovasc Surg* 1999; **16**: 150–155.
38 Chester AH, O'Neil GS, Moncada S, Tadjkarimi S, Yacoub MH. Low basal and stimulated release of nitric oxide in atherosclerotic epicardial coronary arteries. *Lancet* 1990; **336**: 897–900.
39 Ross R. Atherosclerosis-an inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
40 DeFeyter PJ, DeJaegere PP, Murphy ES, Serruys PW. Abrupt coronary artery occlusion during percutaneous transluminal coronary angioplasty. *Am Heart J* 1992; **123**: 1633–1642.
41 Hirshfeld JW Jr, Schwartz JS. Jugo R *et al.* Restenosis after coronary angioplasty. a multivariate statistical model to relate lesion and procedure variables to restenosis. *J Am Coll Cardiol* 1991; **18**: 647–656.
42 Roubin GS, Cannon AD, Agrawal SK *et al.* Intracoronary stenting for acute and threatened closure complicating percutaneous transluminal coronary angioplasty. *Circulation* l992; **85**: 9l6–927.
43 Christenson JT, Al-Huneidi W, Owunwanne A. Early platelet deposition and distribution in various graft materials. *Surg Res Com* 1987; **l**: 151–158.
44 Leonard EF, Turitto VT, Vroman L, eds. Blood in Contact with Natural and Artificial Surfaces. New York Academy of Sciences, New York, 1987.
45 Groves PH, Banning AP, Penny WJ *et al.* The effects of exogenous nitric oxide on smooth muscle cell proliferation following porcine carotid angioplasty. *Cardiovasc Res* 1995; **30**: 87–96.
46 Groves PH, Lewis MJ, Cheadle HA, Penny WJ. SIN-1 reduces platelet adhesion and platelet thrombus formation in a porcine model of balloon angioplasty. *Circulation* l993; **87**: 590–597.
47 Lablanche JM, Grollier G, Lusson JR *et al.* Effect of the direct nitric oxide donors linsidomine and molsidomine on angiographic restenosis after coronary balloon angioplasty. The ACCORD Study (Angioplastic Coronaire Corvasal Diltiazem). *Circulation* 1997; **95**: 83–89.
48 Marks DS, Vita JA, Folts JS *et al.* Inhibition of neointimal proliferation in rabbits after vascular injury by a single treatment with a protein adduct of nitric oxide. *J Clin Invest* 1995; **96**: 2630–2638.
49 Pepke-Zaba J, Higgenbottam TW, Dinh Xuan AT, Stone D, Wallwork J. Inhaled nitric oxide as a cause of selective pulmonary vasodilation in pulmonary hypertension. *Lancet* 1991; **338**: 1173–1174.
50 Bode-Böger SM, Böger RH, Alfke H *et al.* L-arginine induces nitric oxide-dependent vasodilation in patients with critical limb ischemia. A randomized, controlled study. *Circulation* 1996; **93**: 85–90.
51 Böger R, Bode-Böger SM, Thiele W, Alexander K, Frolich JC. Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation* 1998; **95**: 2068–2074.
52 Gryglewski RJ, Grodzinska L, Kostka-Trabka E *et al.* Treatment with L-arginine is likely to stimulate generation of nitric oxide in patients with peripheral arterial obstructive disease. *Wien Klin Wochenschr* l996; **108**: 111–116.
53 Schellong SM, Böger RH, Burchert W *et al.* Dose-related effect of intravenous L-arginine on muscular blood flow of the calf in patients with peripheral vascular disease: a H215O positron emission tomography study. *Clin Sci (Colch)* 1997; **93**: 159–165.
54 Ichiki K, Ikeda H, Haramaki N, Ueno T, Imaizumi T. Long-term smoking impairs platelet-derived nitric oxide release. *Circulation* 1996; **94**: 3109–3114.
55 Cadwgan TM, Benjamin N. Evidence for altered platelet nitric oxide synthesis in essential hypertension. *J Hypertens* 1993; **11**: 4l7–4120.
56 Hamon M, Vallet B, Bauters C *et al.* Long-term oral administration of L-arginine reduces intimal thickening and enhances neoendothelium-dependent acetycholine-induced relaxation after arterial injury. *Circulation* 1994; **90**: 1357–1362.
57 von der Leyen HE, Gibbons GH, Morishita R *et al.* Gene therapy inhibiting neointimal vascular lesion: in vivo transfer of endothelial cell nitric oxide synthase gene. *Proc Natl Acad Sci USA* 1995; **92**: 1137–1141.
58 Chen L, Daum G, Forough R *et al.* Overexpression of human endothelial nitric oxide synthase in rat vascular smooth muscle cells and in balloon-injured carotid artery. *Circ Res* 1998; **82**: 862–870.
59 Miyagoshima M, Tzeng E, Shears LJ, Kawaharada N, Watkins Billiar TR. Perivascular iNOS gene transfer using a novel non-occlusive gene delivery device inhibits intimal hyperplasia. *Circulation* 1997; **96**: I–13.
60 Janssens S, Flaherty D, Nong Z *et al.* Human endothelial nitric oxide synthase gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation after balloon injury in rats. *Circulation* l998; **97**: l274–1281.
61 Saavedra JE, Southan GJ, Davies KM *et al.* Localizing antithrombotic and vasodilatory activity with a novel, ultrafast nitric oxide donor. *J Med Chem* 1996; **39**: 4361–4365.
62 Chen C, Hanson SR, Keefer LK *et al.* Boundary layer infusion of nitric oxide reduces early smooth muscle cell proliferation in the endarterectomized canine artery. *J Surg Res* l997; **67**: 26–32.
63 Yin ZL, Dusting GJ. A nitric oxide donor (spermine-NONOate) prevents the formation of neointima in rabbit carotid artery. *Clin Exp Pharmacol Physiol* 1997; **24**: 436–438.
64 Chaux A, Ruan XM, Fishbein MC *et al.* Perivascular delivery of a nitric oxide donor inhibits neointimal hyperplasia in vein grafts implanted in the arterial circulation. *J Thorac Cardiovasc Surg* 1998; **115**: 604–614.
65 Smith DJ, Chakravarthy D, Pulfer S *et al.* Nitric oxide-releasing polymers containing the [N(O)NO]- group. *J Med Chem* 1996; **39**: 1148–1156.
66 Hanson SR, Hutsell TC, Keefer LK, Mooradian DL, Smith DJ. Nitric oxide donors: a continuing opportunity in drug design. *Adv Pharmacol* l995; **34**: 383–398.
67 Pulfer SK, Ott D, Smith DJ. Incorporation of nitric oxide-releasing crosslinked polyethyleneimine microspheres into vascular grafts. *J Biomed Mater Res* 1997; **37**: 182–189.
68 Van Der Giessen WJ, Van Beusekom HM, Van Houten CD *et al.* Coronary stenting with polymer-coated and uncoated self-expanding endoprotheses in pigs. *Coron Artery Dis* 1992; **3**: 631–640.
69 Dichek DA, Neville RF, Zwiebel JA *et al.* Seeding of intravascular stents with genetically engineered endothelial cells. *Circulation* 1989; **80**: 1347–1353.
70 Holmes DR Jr, Camrud AR, Jorgenson MA, Edwards WD, Schwartz RS. Polymeric stenting in the porcine coronary artery model: differential outcome of exogenous fibrin sleeves versus polyurethane-coated stents. *J Am Coll Cardiol* 1994; **24**: 525–531.
71 Hardhammar PA, van Beusekom HM, Emanuelsson HU *et al.* Reduction in thrombotic events with heparin-coated Palmaz-Schatz stents in normal porcine coronary arteries. *Circulation* l996; **93**: 423–430.

72 Brash JL. Protein interactions with artificial surfaces. In: Salzman EW, ed. *Interaction of the Blood with Natural and Artificial Surfaces.* Marcel Dekker, New York, 1981, p. 37.
73 Ishikawa Y, Sasakawa S, Takase M, Osada Y. Effect of albumin immobilization by plasmapolymerization on platelet reactivity. *Thromb Res* 1984; **35**: 193–202.
74 Park K, Mosher DF, Cooper SL. Acute surface-induced thrombosis in the canine *ex vivo* model: importance of protein composition of the initial monolayer and platelet activation. *J Biomed Mater Res* 1986; **20**: 589–612.
75 Amiji M, Park H, Park K. Study on the prevention of surface induced platelet activation by albumin coating. *J Biomater Sci Polymer* 1992; **3**: 375–388.
76 Stamler JS, Simon DI, Osborne JA *et al.* S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc Natl Acad Sci USA* 1992; **89**: 444–448.
77 Folts JD. Coating Palmaz–Schatz stents with a unique NO donor reduces the need for post-procedure anticoagulation when placed in pig carotid arteries. *J Invest Med* 1995; **43**: 476A.
78 Folts JD, Keaney JF Jr, Loscalzo J. Local delivery of nitrosated albumin to stenosed and damaged coronary arteries inhibits platelet deposition and thrombosis. *J Am Coll Cardiol* 1995; **Special Edition**: 377A.
79 Keaney JF Jr, Stamler JS, Scharfstein J, Folts JD, Loscalzo J. NO forms a stable adduct with serum albumin that has potent antiplatelet properties *in vivo. Clin Res* 1992; **40**: 194A.
80 Maalej N, Albrecht R, Loscalzo J, Folts JD. The potent platelet inhibitory effect of S-nitrosated albumin coating of artificial surfaces. *J Am Coll Cardiol* 1999; **33**: 1408–1014.

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