Review

Inducible nitric oxide synthase and vascular injury

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Abstract

The role nitric oxide (NO) plays in the cardiovascular system is complex and diverse. Even more controversial is the role that the inducible NO synthase enzyme (iNOS) serves in mediating different aspects of cardiovascular pathophysiology. Following arterial injury, NO has been shown to serve many vasoprotective roles, including inhibition of platelet aggregation and adherence to the site of injury, inhibition of leukocyte adherence, inhibition of vascular smooth muscle cell (VSMC) proliferation and migration, and stimulation of endothelial cell (EC) growth. These properties function together to preserve a normal vascular environment following injury. In this review, we discuss what is known about the involvement of iNOS in the vascular injury response. Additionally, we discuss the beneficial role of iNOS gene transfer to the vasculature in preventing the development of neointimal thickening. Lastly, the pathophysiology of transplant vasculopathy is discussed as well as the role of iNOS in this setting. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Nitric oxide (NO), while outwardly a simple molecule, has been shown to play very diverse roles in the physiology and pathophysiology of the cardiovascular system. Since the discovery of the NO pathway in 1987 and the elucidation that NO is intimately involved in the regulation of vasomotor tone, NO has earned the conflicting reputation of being both protective and deleterious to vascular homeostasis. The loss of endothelial ability to constitutively synthesize and release NO has been suggested to be the inciting event in atherogenesis [1,2]. In contrast, however, inducible NO synthase (iNOS) has been detected in atherosclerotic plaques and the local release of large amounts of NO has been linked to the production of harmful oxidative products such as peroxynitrite [3–5]. The focus of this review will be limited to iNOS and its involvement in the vascular injury response, an area that more clearly demonstrates the potential benefits of NO production. Additionally, the pathophysiology of transplant vasculopathy, a process that greatly resembles the arterial injury response, as well as the role of iNOS in this disease process will be reviewed.

2. Nitric oxide synthase isoforms and nitric oxide

NO is synthesized by a family of enzymes called NO synthases (NOS). The three different isoforms include endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). These isoforms, while clearly distinct, share a number of similarities. The enzymatic reaction involves the 5-electron oxidation of one of the guanidino nitrogens of L-arginine in the presence of molecular oxygen to generate NO and its byproduct, L-citrulline [6,7]. They all require the cofactors NADPH, FAD, FMN, heme, and tetrahydrobiopterin to catalyze this reaction [6,7]. In general, eNOS and nNOS are constitutively expressed enzymes and NO production is regulated predominantly by intracellular Ca²⁺ fluxes that permit calmodulin binding which activates the enzymes [7]. In contrast, iNOS is transcriptionally regulated and is not

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normally produced by most cells [7,8]. Typically, iNOS is expressed in response to cellular stress and generates 100–1000-fold more NO than its constitutive counterparts whose roles are involved in physiologic regulation [8,9].

NO is a highly diffusible molecule and can reach distant cells quickly [10]. The biologic half-life of NO is on the order of seconds which restricts its actions to the local environment [10]. NO is avidly bound by hemoglobin which then converts it to the inactive end products, nitrate and nitrate, another mechanism by which the body prevents systemic toxicity [10]. NO can also interact with superoxide to produce peroxynitrite and other potentially toxic oxidants that may be involved in tissue injury and organ dysfunction [11].

3. The arterial injury response

To fully appreciate the beneficial role iNOS plays in the arterial injury response, a brief review of the events that occur following arterial injury is required (for reviews, see Refs. [12–18]). Injury to the arterial wall, either through balloon angioplasty or multiple passes of a balloon catheter, leads to endothelial denudation and possible rupture of the internal elastic lamina (IEL) with damage to the underlying smooth muscle cell (SMC) layer. Following endothelial denudation and exposure of the underlying IEL and SMC layer, platelets immediately aggregate and adhere to the site of injury. Leukocyte chemotaxis then follows and an array of cytokines and growth factors are secreted which have multiple effects. Basic fibroblast growth factor (bFGF) is responsible for the first wave of smooth muscle cell proliferation following injury [19]. Platelet derived growth factor (PDGF) is mainly responsible for the migration of SMCs from the media to the intima [20], and transforming growth factor-β (TGF-β) potently stimulates interstitial collagen gene expression by human SMC, leading to extracellular matrix deposition [21].

Activation of SMCs by these growth factors converts them from a contractile to a synthetic phenotype (for reviews, see Refs. [12–18]). Smooth muscle proliferation then follows as early as 24 h following injury and continues for at least 2 weeks. In order for the SMCs to migrate from the media to the intima, the extracellular matrix and IEL must undergo some degree of degradation as these act as barriers to migration. Plasminogen activators, proteases that lyse clots and activate matrix-degrading enzymes, and matrix metalloproteinases, which degrade collagen and elastin, are upregulated following injury. This degradation of the extracellular matrix allows SMCs to migrate to the intima between 1–3 days following injury, where they continue to proliferate for several weeks. Concurrently, endothelial regeneration occurs through the stimulation of bFGF within 24 h after injury, and can continue for 6–10 weeks [22]. Lastly, following injury, there is a marked upregulation of the genes that encode for extracellular matrix proteins, such as collagen and elastin. Deposition of the extracellular matrix is a significant component of the vascular remodeling that occurs after injury. As an end result of this complex process intimal thickening occurs at the site of injury, and this encroachment on the lumen may be countered by vascular remodeling which leads to a compensatory increase in vessel diameter.

4. Mechanisms of nitric oxide mediated vasoprotection

4.1. Role of platelet adherence

NO has been shown to be vasoprotective through a variety of different mechanisms (Fig. 1). One of the initial observations was the capacity of NO to prevent platelet adherence and aggregation [23]. The inhibition of platelet adhesion was found to be mediated by cGMP, not cAMP, by using selective inhibitors of cGMP and cAMP phosphodiesterase [24]. More recently, human platelets have been shown to express iNOS and eNOS isoforms [25]. Porcine platelets upon activation and aggregation express iNOS, potentially leading to a negative feedback loop to further suppress excessive platelet aggregation [26].

Arterial injury results in the exposure of collagen to circulating blood elements causing platelets to aggregate at the site of injury. Injury also results in the upregulation of iNOS in vascular smooth muscle cells (VSMC) in the arterial wall [27,28]. In 1996, Yan [28] showed that this upregulation of iNOS in vivo not only prevented the adherence of platelets to the injured site but also preserved blood flow. These investigators injured carotid arteries with a balloon catheter and exposed vessels to the NOS inhibitor L-NAME. L-NAME resulted in a 3-fold increase in 111In-labeled platelets to the site of injury and a 24% reduction of blood flow. Because the injury resulted in the endothelial denudation with loss of eNOS, it was concluded that the upregulation of iNOS represents a protective mechanism against platelet adherence that compensates for the loss of the endothelium.

4.2. Role of nitric oxide inhibition of leukocyte chemotaxis

Leukocyte accumulation at the site of arterial injury leads to the production of a several growth factors and cytokines that stimulate VSMC proliferation and migration. NO has been shown to inhibit this leukocyte chemotactic response and hence negatively effect the downstream events. In 1991, Kubes et al. [29] infused cat mesenteric preparations with inhibitors of NO production and observed single venules by intravital video microscopy. Both L-NAME and L-NMMA increased leukocyte adherence.
Fig. 1. The vasoprotective effects of nitric oxide. Nitric oxide (NO) production at the site of injury can inhibit platelet aggregation and adherence, leukocyte adherence, vascular smooth muscle cell (VSMC) proliferation, and VSMC migration. Additionally, NO can stimulate endothelial cell proliferation and protect the endothelial cells from apoptosis. These properties of NO favor reestablishment of a normal vascular environment following arterial injury.

more than 15-fold. Additionally, L-NAME induced adhesion was reversed by L-arginine but not D-arginine, suggesting that this is a NO-specific event. To specifically evaluate the role of iNOS in the leukocyte–endothelium interaction, iNOS knock out mice were studied [30]. Using a model of endotoxemia the authors demonstrated that the number of rolling and adherent leukocytes in the cremasteric postcapillary venules and liver postsinusoidal venules of iNOS-deficient mice were significantly greater compared to the wild-type mice. These results suggest that NO produced following iNOS upregulation may function as a homeostatic regulator of leukocyte recruitment and may play an important role in vascular inflammation.

Inducible NOS expression has also been shown to limit monocyte adherence to the vessel wall. Peng et al. [31] demonstrated that iNOS prevented monocyte adhesion to endothelial cells through the downregulation of VCAM-1 gene transcription by inhibiting nuclear factor-kappa B (NFκB). Using endothelial and RAW cell co-cultures, they stimulated iNOS production with bacterial LPS and IFN-γ and measured VCAM-1 expression and monocyte adherence to the endothelial surface. Expression of iNOS was associated with decreased VCAM-1 mRNA and reduced monocyte–endothelial cell interaction. Both effects were prevented in the presence of a nonspecific NOS inhibitor. Using immunofluorescent studies with antibodies to the RelA (p65) subunit of NFκB, they then demonstrated that NO produced from iNOS inhibited the activation of NFκB. Thus, iNOS upregulation in the vessel wall can impair both PMN and monocyte accumulation. Demonstration that this occurs following vascular injury has not been reported. Although, since the upregulation of iNOS following vascular injury is a relatively delayed event (24 h) it is possible that iNOS expression serves to resolve inflammation through this mechanism.

4.3. Nitric oxide inhibition of VSMC proliferation

In 1989, Garg et al. [32] demonstrated that NO-generating vasodilators could inhibit rat aortic smooth muscle cell (RASMC) proliferation. They administered sodium nitroprusside (SNP), S-nitroso-N-acetylpenicillamine (SNAP), and isosorbide dinitrate exogenously to RASMC and demonstrated a dose-dependent inhibition of RASMC proliferation assessed by serum-induced [3H]thymidine incorporation. Several studies following this publication demonstrated the beneficial effect of delivering excess NO to the site of vascular injury to prevent the development of intimal hyperplasia in vivo. In 1993, McNamara et al. [33] administered the nitric oxide precursor L-arginine to rabbits from 2 days prior to 2 weeks following catheter-induced injury to the rabbit thoracic aorta. Animals receiving L-arginine displayed 39% less intimal hyperplasia compared to control untreated animals. This reduction in intimal
hyperplasia was reversed by co-administration of l-NAME. In 1995, Marks et al. [34] showed that a single treatment with a protein adduct of NO that possesses a prolonged biologic half-life prevented intimal hyperplasia in response to vascular injury. Rabbit femoral arteries were injured with balloon-catheter denudation and exposed to the NO protein adduct for 15 min. The single treatment with the NO protein adduct inhibited the development of intimal hyperplasia by 77% compared to the controls at 14 days. To demonstrate efficacy of NO in preventing the injury response in a model more closely resembling human conditions, Groves et al. [35] showed that 3 weeks of treatment with an oral NO donor molsidomine resulted in a 32% reduction in intimal thickness following angioplasty in porcine carotid arteries. Lee et al. [36] showed that chronic inhalation of NO following balloon-induced arterial injury in the rat carotid artery resulted in a 43% decrease in the intima/media area ratio at 2 weeks. The specific role of endogenous iNOS in this injury response was first determined by Hansson et al. in 1994 [27]. They found that iNOS mRNA was upregulated in the medial smooth muscle cells following balloon-induced endothelial denudation from 1–14 days following injury. In 1996, the same group demonstrated that this upregulation of iNOS in vivo following arterial injury also prevented adherent platelets to the injured vasculature and preserved blood flow by administering inhibitors of iNOS [28]. More recently, Yan et al. [37] demonstrated that not only did medial SMCs show upregulation of iNOS but that neointimal cells displayed a greater expression of iNOS by both Western blot and Northern blot analysis. Interestingly, this upregulation by the neointimal cells was due to more efficient activation of the iNOS promoter as demonstrated by transfection studies using a reporter gene under the control of the iNOS promoter. However, the neointimal cells were less responsive to the antiproliferative effects of NO than the medial smooth muscle cells as measured by the inhibition of cellular proliferation. As with the other protective actions of iNOS following vascular injury, the expression of iNOS possibly functions to resolve the injury response and limit excessive SMC proliferation. Much work has gone into determining how NO inhibits VSMC proliferation. One mechanism is through the induction of a G0/G1 cell cycle arrest, preventing cells from entering the synthesis phase of the cell cycle and undergoing proliferation. Sarkar et al. [38] treated cultured RASMCs with SNAP and S-nitrosoglutathione and observed a 50% decrease in the cells in the G2/M and S phases of the cell cycle and a corresponding increase in the cells in G0/G1. This cell cycle arrest was reversible upon withdrawal of the NO donors. Ishida et al. [39] in 1997 demonstrated that as SNAP induced a G0/G1 cell cycle arrest that was associated with inhibition of VSMC proliferation. NO also induced a significant upregulation of the cell cycle inhibitor p21. Additionally, they demonstrated that NO inhibited the activity of cdk2 and the subsequent phosphorylation of the retinoblastoma protein, both of which are necessary for cell cycle progression. Most recently, this inhibition of proliferation and upregulation was determined to be independent of the tumor suppressor gene p53 [40]. p53 has long been shown to upregulate p21 transcription and it has been thought that one way p53 is able to induce cell cycle arrest is thought its ability to upregulate p21. Since NO has been shown to increase the expression of p53 [41], it was thought that this was one mechanism by which NO could lead to inhibition of VSMC proliferation. We isolated SMCs from p53 knock out mice, and found that NO was able to inhibit VSMC proliferation in these p53−/− cells and that it was still associated with the upregulation of p21 by Western blot analysis [40]. Hence NO upregulates p21 and inhibits VSMC proliferation independent of p53.

4.4. Effects of nitric oxide on VSMC migration

Just as VSMC proliferation is imperative to the formation of the neointima, so is migration of the SMCs from the media to the neointima. NO has also been shown to retard this process. In 1995, Dubey et al. [42] showed that SNP and SNAP could inhibit angiotensin II induced SMC migration by ~60%. RASMC migration was assessed using a modified Boydens chamber. To determine if NO produced from iNOS would inhibit this migratory process, they stimulated iNOS expression using IL-1β and showed that NO could also inhibit migration of the RASMCs by ~60%. Sarkar et al. [43] demonstrated that three other donors of NO, namely diethylamine NONO-ate, spermine NONOate, and S-nitrosoglutathione, all exhibited concentration-dependent inhibition of both the number of SMCs migrating and the maximal distance migrated following wounding of a confluent culture of RASMCs. This inhibition was reversible upon removal of the NO donors. Measurements of VSMC protein synthesis and mitochondrial respiration indicated that inhibition of migration by these NO donors was not due to metabolic cytostasis.

4.5. Protective effects of nitric oxide on endothelial cells

In addition to the action of NO on VSMC responses following injury, NO has also been shown to effect endothelial cell pathophysiology during the injury response. Some evidence indicates that NO stimulates the growth of endothelial cells. One example comes from studies examining angiogenesis, a process that requires the stimulation of proliferation and migration of endothelial cells. In 1994, Ziche et al. [44] evaluated the effects of NO donors and endogenous NO on the angiogenesis process. They monitored the angiogenic response in rabbit corneas and found that sodium nitroprusside (SNP) potentiated angiogenesis and this was inhibited by l-NAME. By examining coronary endothelial cells in culture, they found that NO generating compounds such as SNP, isosorbide
dinitrate, and glyceryl trinitrate, produced a dose-dependent increase in endothelial cell proliferation as measured by \(^{3}H\)thymidine incorporation. Additionally, migration assessed using a modified Boyden's chamber was also potentiated by NO generating agents. Endogenous production of NO following stimulation of the coronary endothelial cells with substance P also resulted in an increased proliferative and migratory response in these cells, both of which were prevented with pre-treatment with NOS inhibitors. Hence, these data demonstrate the potential for NO to function as an autocrine regulator of vascular events necessary for re-endothelialization and perhaps angiogenesis. These properties favor healing of the injured vasculature in the sense that more rapid coverage of the injured site with endothelium will diminish the stimulus for platelet adherence and leukocyte chemotaxis, and hence the stimulation of growth factors and cytokines that continually drive vascular VSMC proliferation and migration. In support of this conclusion, Guo et al. [45] showed that administration of a NO donor in vivo accelerated the regrowth of the endothelium following carotid injury in rats. This same group found that the NO donor dose-dependently inhibited PDGF-BB stimulated RAMSC proliferation while it stimulated RAEC proliferation. Again, demonstrating that the effects of NO in the vessel wall are cell specific and aimed at promoting vessel repair.

Most recently, in 1997, Tzeng et al. [46] demonstrated yet another beneficial effect of NO on endothelial cells. Overexpression of iNOS using a adenoviral vector increased endothelial proliferation and suppressed apoptosis. This work continues the study of Dimmeler et al. [47] that showed that NO is a potent inhibitor of apoptosis in endothelial cells and that the inhibition of apoptosis occurs through the suppression of caspase 3-like protease activity.

5. Inducible nitric oxide synthase gene transfer

5.1. Arterial injury

Given all of the vasoprotective properties of NO, it seemed reasonable that overexpression of iNOS locally at the site of vascular injury would be beneficial in inhibiting the development of intimal hyperplasia. Systemic administration of NO donors can be associated with deleterious side-effects such as hypotension and may require continuous administration. Gene therapy using iNOS is an attractive approach to locally increase NO production in a sustained manner and all three NOS isoforms have been used with some success [48–53]. Although many vectors may prove useful, the adenoviral vector has a relatively high transfer efficiency to the vasculature compared to other vectors, and the duration of expression of the transgene can be approximately 2 weeks. Theoretically, this may be the optimal period of NO production. iNOS is capable of producing large quantities of NO in a sustained, calcium-independent fashion. Therefore, iNOS may have advantages over eNOS or nNOS which both require post-translational modifications for activity. With these greater NO producing characteristics one can hypothesize that less adenovirus could be used to effectuate the same results. This is beneficial given the host immune response evoked with current first and second generation adenoviral vectors. Using less virus might lead to less of an inflammatory response and possibly persistence of the transgene for extended periods.

Prior to proceeding with iNOS gene therapy, Tzeng et al. [54] studied the requirements for assembly of the iNOS enzyme subunits into an active dimer. They used a retrovirus to transfect NIH 3T3 cells with the human iNOS cDNA, NIH 3T3 cells were utilized because of their deficiency of de novo tetrahydrobiopterin (H\(_{2}\)B) biosynthesis. Cells infected with iNOS that were not exposed to exogenous H\(_{2}\)B synthesized low levels of NO compared to cells exposed to exogenous supplemental H\(_{2}\)B in the form of either tetrahydrobiopterin or its precursor sepiapterin. The increased amount of NO produced in response to supplemental H\(_{2}\)B was not due to an increase in iNOS protein. Instead, it was due an increased percentage of dimeric forms of iNOS, raising from 20 to 66% in the groups receiving H\(_{2}\)B. Thus, this demonstrates that H\(_{2}\)B plays a critical role in the dimerization and activation of iNOS, revealing a post-translational mechanism by which intracellular H\(_{2}\)B can regulate iNOS expression. Further studies revealed that the requirement for H\(_{2}\)B could be met by the co-transfer of the cDNA for GTP-cyclohydrolase, the rate-limiting enzyme for H\(_{2}\)B biosynthesis. Whether H\(_{2}\)B will be limiting in vivo remains to be determined.

In 1996, Tzeng et al. [55] delivered retroviral-mediated human iNOS to isolated porcine arterial segments ex vivo. Vessels infected with iNOS produced more nitrite and cGMP compared to vector infected vessels. Additionally, there was complete inhibition of the development of myointimal thickening following balloon catheter injury in the ex vivo system despite the 1% transfer efficiency observed. This very low iNOS gene transfer had significant effects on vessel wall responses.

To demonstrate the efficacy of iNOS overexpression in the vasculature following injury, Shears et al. [52] demonstrated that adenoviral delivery of the human iNOS gene to both rat carotid and pig iliac arteries following injury significantly inhibited the development of intimal hyperplasia. First, they demonstrated that adenoviral iNOS (AdiNOS) infection of RASMC produced 100-fold more nitrite than control and AdlacZ-infected cells. AdiNOS or AdlacZ was then transferred intralumenally to the rat carotid artery following balloon-induced injury using a very low titer of 2×10\(^{6}\) pfu per artery. Evaluation of the carotid arteries 2 weeks following injury and infection resulted in a 96.7% inhibition of neointimal thickening. The investigators then evaluated the efficiency of iNOS gene transfer in a model more similar to humans, namely
the pig iliac artery injury model. Delivery of $5 \times 10^3$ pfu per artery of AdiNOS or AdLacZ resulted in a 51.8% reduction in the intima/media area ratio in the vessels infected with AdiNOS. This study demonstrated that very low titers of adenovirus encoding the iNOS gene had profound effects in two different animal models of arterial injury. These are among the lowest titers reported to be effective for any gene therapy approach using adenoviral vectors in the cardiovascular system [56]. These titers are well below the threshold associated with inflammation and the increased intimal hyperplasia associated with adenovirus used in some studies [57,58]. Thus, the efficacy as well as feasibility of iNOS gene transfer to the vasculature to prevent the arterial injury response has been successfully demonstrated. The applicability of this approach in a clinical setting however remains to be determined. The above studies were performed in normal arteries, therefore it will be imperative to determine the efficacy of iNOS gene transfer in atherosclerotic animal models, as this cellular environment may respond differently to adenoviral gene transfer and NO overproduction.

5.2. Transplant vasculopathy

One of the long-term limitations of cardiac transplantation is accelerated graft coronary arteriosclerosis. This disease can be detected by coronary angiography in 6–18% of cardiac recipients at 1 year and as many as 50% at 5 years [59,60]. The hallmark manifestation of this process is diffuse, progressive narrowing of the coronary arteries due to severe intimal thickening comprised of phenotypically modulated vascular smooth muscle cells [61]. Similar to restenosis following balloon angioplasty, allograft arteriosclerosis involves the proliferation and migration of SMCs from the media to the intima under the influence of mitogenic signals. One theory for the etiology of this complex process is that cellular and humoral immune responses initiate a cascade of cytokines and growth factors that induce immune-mediated endothelial injury [62]. VSMCs exposed to these mitogenic stimuli convert from a contractile to synthetic phenotype, begin to proliferate, migrate from the media to the intima, and lead to the development of diffuse coronary artery graft neointimal thickening.

The role of NO and iNOS in transplant vasculopathy has been quite complex. Several groups have demonstrated an increase in plasma and urine nitrite levels following allogeneic cardiac transplant rejection but not in syngeneic transplants [63,64]. The increase in urinary nitrite production was completely inhibited with L-NMMA, demonstrating that this was due to increased production of NO. iNOS expression was later found to correlate with the production of NO within the cardiac allografts during acute rejection [65] and it was demonstrated that coronary arteries in transplanted cardiac grafts suffering from transplant coronary artery disease (TCAD) also displayed an increase in iNOS mRNA and protein [66]. Therefore, it was suggested that iNOS may be playing a detrimental role in the pathophysiology of transplant vasculopathy.

More definitive studies on the role of iNOS in transplant vasculopathy were performed in the past 2 years. Shears et al. [67] delivered an inhibitor of iNOS, L-NIL, to rats receiving an aortic allograft systemically via an alzet osmotic pump. They found that rats receiving L-NIL had 57% more intimal thickening at 4 weeks, indicating that NO suppresses the development of allograft arteriosclerosis. To extend these observation they performed gene transfer with an adenoviral vector carrying the human iNOS gene to allogeneic rat aortic transplants and evaluated the transplanted aortae for transplant vasculopathy. Following delivery of just $2 \times 10^3$ pfu of adenoviral iNOS to explanted aortae prior to transplantation they found a near complete inhibition of intimal thickening at 28 days post-transplantation. This study would predict that effective supplementation of NO would limit coronary transplant vasculopathy.

The negative role of iNOS was confirmed by Koglin et al. [68]. They transplanted allogeneic hearts into iNOS $-/-$ and iNOS $+/+$ mice and evaluated the coronary arteries for signs of transplant vasculopathy. Interestingly, the iNOS $-/-$ recipients displayed significantly more neointimal thickening compared to the iNOS $+/+$ recipients. From these two studies it appears that iNOS may play a protective role in the short term development of transplant arteriosclerosis, suppressing neointimal smooth muscle cell accumulation.

6. Summary

Following injury to the vasculature, the expression of iNOS is upregulated with subsequent synthesis of large quantities of NO. This NO production has been found to serve as a powerful vasoprotective agent by inhibiting platelet aggregation, leukocyte chemotaxis, VSMC proliferation, VSMC migration, and by promoting endothelial cell survival and growth. Many groups have demonstrated the therapeutic potential of NO in preventing the arterial injury response in animal models of arterial injury. Much remains to be learned about how NO regulates these processes at the molecular level, and to determine how NO is able to execute such diverse actions in different cell types. It seems likely that effective therapies based on NO and specifically iNOS will positively impact on cardiovascular pathology.

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