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Review

Nitric oxide and nitrite-based therapeutic opportunities in intimal hyperplasia

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ABSTRACT

Vascular intimal hyperplasia (IH) limits the long term efficacy of current surgical and percutaneous therapies for atherosclerotic disease. There are extensive changes in gene expression and cell signaling in response to vascular therapies, including changes in nitric oxide (NO) signaling. NO is well recognized for its vasoregulatory properties and has been investigated as a therapeutic treatment for its vasoprotective abilities. The circulating molecules nitrite (NO_2^-) and nitrate (NO_3^-), once thought to be stable products of NO metabolism, are now recognized as important circulating reservoirs of NO and represent a complementary source of NO in contrast to the classic L-arginine–NO–synthase pathway. Here we review the background of IH, its relationship with the NO and nitrite/nitrate pathways, and current and future therapeutic opportunities for these molecules.

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Vascular intimal hyperplasia

Atherosclerosis and vascular disease are responsible for approximately 50% of all deaths in the developed world [1]. The idea that vascular disease develops in response to vessel injury and involves inflammation and vessel remodeling is well accepted [2]. Current interventions for treating vascular disease include surgical bypass and percutaneous interventions. Early failure is related to problems in surgical technique and thrombosis [3–5]. Alternatively, late failure occurs when the mechanical vascular injury incites a distinct pathobiological response that leads to intimal hyperplasia (IH) [6–8].

Intimal hyperplasia is the exaggerated healing process initiated by injury that occurs as a result of the therapeutic intervention. The initial injury is often characterized by endothelial denudation or apoptosis, platelet adhesion and aggregation, and leukocyte chemotaxis. This results in extracellular matrix changes, and vascular smooth muscle cell proliferation and migration [9]. Additionally, it has been demonstrated in rabbits that hypercholesterolemia accelerates IH due to expansion of cellular proliferation and accumulation of foamy macrophages within the vessel wall [10]. There are many factors involved in the vascular response to injury and it is accepted that inflammatory mediators play an essential role in both the initiation and progression of the injury resulting in constrictive vascular remodeling [11].

Numerous techniques and strategies have been investigated and employed to limit the formation of IH. Minimally invasive treatment alternatives include distal atherectomy [12], cryotherapy [13], brachytherapy [14], photodynamic therapy [15], and angioplasty with or without stenting [16]. All are in different stages of investigation with different levels of implementation in patients. Sirolimus, a natural macrocyclic lactone, and paclitaxel,

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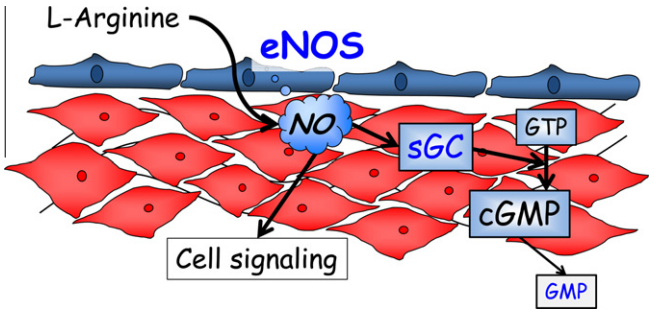

Fig. 1. The 'classical' arginine/nitric oxide synthase/nitric oxide signaling pathway and vascular signaling. L-Arginine, with cofactors NADH and oxygen, is catabolized by nitric oxide synthase (NOS) to nitric oxide (NO) and L-citrulline. NO acts as a second messenger activating cyclic guanosine monophosphate (cGMP) and non-cGMP dependent signaling. NO activates soluble guanylate cyclase to convert GTP to cGMP.

a cytotoxic agent, are immunosuppressive and anti-proliferative drugs that can be sprayed or impregnanted within stents or angioplasty balloons. Multi-center randomized clinical trials have demonstrated that drug-eluting stents and balloons are safe and efficient in preventing restenosis after percutaneous transluminal coronary angioplasty [17–22]. However, evidence for drug-eluting devices in the peripheral vascular system is lacking and whether these devices are clinically and cost effective is still emerging [23].

Nitric oxide

The discovery of a biological role of NO and identification as an 'endothelium-derived relaxing factor' in the late 1980s led to a multitude of studies in the 1990s defining the vasoregulatory properties of this gaseous molecule [24]. The oxidation of one of the amidine nitrogens of L-arginine by a group of enzymes known as NO synthases (NOS) was found to be responsible for the production of endogenous NO and L-citrulline [25]. These enzymes produce NO through the metabolism of L-arginine, NADPH, and molecular oxygen [26]. The vasodilating effects of NO are produced via action on soluble guanylate cyclase to produce cyclic guanosine monophosphate (cGMP) [27–29] (Fig. 1). There are two constitutively expressed NOS isoforms (endothelial NOS; eNOS; NOS3, and neuronal NOS; nNOS; NOS1) and one inducible isoform within many cells and tissues (inducible NOS; iNOS; NOS2). Cellular stress activates iNOS which then generates 100- to 1000-fold more NO than its constitutive counterparts [30,31]. The short half-life and readily diffusible ability of NO make it an effective autocrine or paracrine signaling molecule.

Nitric oxide and the vascular system

In the vascular system, eNOS, as its name implies, is the isoform that is expressed constitutively by the endothelium. Thus, eNOS plays a crucial role in NO production within the vessel wall and in regulating vascular tone. The generation of nearly 70% of systemic NO formation in the body is by eNOS in the vascular endothelium and is predominantly regulated by intracellular calcium fluxes that allow calmodulin binding, which activates the enzyme [32].

A lack of NO is often associated with IH. Impairment of endothelial function is often marked by decreased expression of eNOS. At the very least, established vascular disease is often defined by a dysfunctional endothelium and eNOS protein [33]. Following injury, impaired vasorelaxation and accelerated cellular proliferation results from decreased NO production by the endothelium and decreased responsiveness to NO [34,35]. Thus, decreased NO has been attributed to systemic hypertension, pulmonary arterial hypertension, atherosclerosis, and vasospasm. In disease states, not only is there a decreased production of NO by NOS enzymes, but the enzymes may be 'uncoupled' secondary to decreased substrate or cofactor availability [36–39]. This may lead to the production of reactive oxygen species that can exacerbate vascular injury.

Although over-production of NO has also been associated with deleterious cardiovascular consequences, these are usually in the setting of acute processes such as sepsis. The systemic hypotension of sepsis has been attributed in part to expression of iNOS and high NO production. Similarly, microcirculatory dysfunction may occur from excess NO produced with resultant inhibition of mitochondrial oxidative phosphorylation and oxygen utilization.

Nitric-oxide-related therapies for intimal hyperplasia

Nitric oxide has been shown to inhibit IH through multiple mechanisms of action. Initially, NO prevents platelet aggregation and adhesion [40,41]. Several studies have illustrated that NO can also limit vascular inflammation by diminishing leukocyte recruitment and leukocyte chemotaxis [42,43]. Smooth muscle cell proliferation and migration occur once a pro-inflammatory and pro-proliferative state is established in the vessel wall. Others and we have shown the ability of NO to limit smooth muscle proliferation and migration via both cGMP-dependent and independent mechanisms [44–50]. A number of studies have illustrated that NO inhibits smooth muscle cell proliferation and cell cycle progression via effects on mitogen activated protein kinase signaling and increased expression of cyclin dependent kinase inhibitor proteins, such as $p21^{Waf1/Cip1}$. Furthermore, NO inhibits extracellular matrix proliferative changes [51–57] and stimulates endothelial cell proliferation and regeneration of a healthy endothelium [58–64].

Based upon such salutary actions of NO in the vasculature, many studies have investigated approaches to harness the therapeutic benefits of the arginine/NOS/NO signaling pathway (Fig. 1). Inhalational NO has demonstrated the ability to reduce IH in a rat model after 14 days of continuous therapy [65], however systemic side effects and feasibility have limited the application of continuous gas treatment. Systemic administration of NO donors has also been investigated. In several preclinical mouse and pig models, encouraging results were found demonstrating reduction of IH using the oral NO donor molsidomine, oral NO-releasing aspirin, and intravenous linsidomine [66–69]. However, these treatments have not demonstrated efficacy in human trials [70,71]. Short-comings of systemic NO delivery by pharmacological donors includes the fact that delivery is not targeted and can have dramatic systemic effects, including vasodilation, hypotension, headaches, and increased bleeding complications.

To overcome this, several studies have attempted targeted delivery of pharmacological NO donors directly to the site of vascular injury. A variety of donors have been investigated in animal models and among them are a polythiolated form of bovine serum albumin (ps-BSA) altered to carry *S*-nitrosothiol groups (ps-NO-BSA), SPER/NO, SNAP gel on rabbit vein grafts, molsidomine on a hydrogel-coated catheter, and 3-morpholino-sydnonimine [53,72–76]. All have shown the ability to inhibit IH formation in animal models.

An additional approach has been to increase arginine availability as a substrate for NOS enzymes. Oral L-arginine administered to rats and rabbits did successfully limited IH development [77–79]. These encouraging results led to human trials with oral L-arginine supplementation that unfortunately met with mixed results. Where short to medium-term treatment led to improved symptoms in cardiovascular disease and long-term studies have shown