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Vascular Protection

Molecular Mechanisms, Novel Therapeutic Principles and Clinical Application

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Our knowledge of vascular biology has expanded dramatically in the last decade. Recognizing the increasing complexity and multidisciplinary nature of this field has prompted us to create this book, which integrates several new aspects of research—from basic sciences to clinical application so as to provide a comprehensive overview of the most recent progress in this field. This book is based, in part, on the material presented during “The International Symposium on Vascular Protection: From Basic Sciences to the Clinic” which was held in Los Angeles, California in December 1998.

The chapters, written by leading experts of their respective field, collectively emphasize the mechanisms and pathologic consequences of vascular disorders, highlighting the clinical ramifications of these insights and the potential for new therapeutic strategies.

Vascular protection is an ideal theme for exploring advances in vascular biology and how they translate into innovations in drug therapy for vascular disease. The broad spectrum of scientific subjects covered includes recent progress in basic knowledge about endothelial vasoactive factors (eg, nitric oxide) and other biologically active molecules (eg, growth factors, cytokines, coagulation and fibrinolytic factors, and adhesion and chemoattractant molecules), endothelial cell activation/dysfunction, atherosclerosis, thrombosis and fibrinolysis, free radicals, interventional technology, and gene therapy. Based on breakthroughs and worldwide awareness in recent years the book also covers novel themes such as the role of apoptosis and sex steroids in vascular biology.

The editors believe that this book, which is the first of its kind, provides critical new information of interest to researchers and clinicians, as well as to industrial scientists in pursuit of novel therapies of cardiovascular disorders.

The Editors would like to express their gratitude to the authors for their excellent contribution to this book and to the staff of Harwood Academic Publishers for their professional and efficient publication of this book.
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1. “Nitric Oxide Deficiency” in Cardiovascular Diseases: Cardiovascular Protection by Restoration of Endothelial Nitric Oxide Production

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INTRODUCTION

Cardiovascular diseases are often associated with endothelial dysfunction represented by diminished endothelium-dependent vasodilation. Endothelium derived nitric oxide (NO), a vasodilator molecule produced by the intact endothelial layer of the vascular wall, plays a key role in the maintenance of vascular integrity by acting via multiple mechanisms of action. These include inhibition of platelet aggregation, prevention of leukocyte adhesion, attenuation of smooth muscle proliferation and inhibition of vasospasm. NO also reacts with oxygen free radicals and interferes with redox-sensitive transcription of pro-inflammatory molecules.

Impaired NO activity is an early symptom in cardiovascular diseases including atherosclerosis, systemic and pulmonary hypertension, heart failure, peripheral arterial occlusive disease as well as cardiovascular complications of diabetes. The apparent “NO-deficiency” is the net result of several different pathological processes interfering with NO availability and bioactivity in the vascular wall. These processes can decrease the amount of endothelial NO at different levels of its production.

Availability of endothelial NO can be regulated by the expression of its generating enzyme, nitric oxide synthase-III (NOS-III), as well as by the activity of the NOS-III enzyme, which is tightly controlled by cofactor and substrate availability, posttranslational modifications (myristoylation, palmitoylation and phosphorylation), protein-protein interactions (caveolin, Hsp90) and cellular localization. In addition, accumulation of endogenous NOS inhibitors and increased oxidative degradation of NO could also lead to diminished availability of endothelial NO.

The pathogenic link between decreased NO production and atherogenesis is demonstrated by experiments with hypercholesterolemic rabbits and apoE-deficient mice. These studies reported accelerated development and progression of atherosclerosis as a result of chronic pharmacological inhibition of NO synthesis.

Therapies with demonstrated efficacy in atherosclerotic diseases, such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)-inhibitors, angiotensin converting enzyme (ACE)-inhibitors, antioxidant vitamins, estrogens and L-arginine all improve NO-mediated vasorelaxation. The effect of the HMG CoA-
inhibitor, simvastatin, in stroke and the ACE-inhibitor, ramiprilate, in myocardial infarction was abolished in NOS-III deficient mice indicating the obligatory role of endothelial NO in mediating cardiovascular benefit by these treatments. Experiments with cultured primary human aortic endothelial cells and isolated vessels from atherosclerotic animal models show that these therapies target different molecular mechanisms contributing to the regulation of endothelial NO production.

NOS-III ENZYME

Endothelial NO is produced by the endothelium as a result of the oxidation of L-arginine to L-citrulline by the endothelial isoform of nitric oxide synthase, NOS-III. NOS-III belongs to the family of NOS isoenzymes, which form homodimers, and contain a heme oxygenase domain and a cytochrome P-450 reductase domain. All isoforms require the same cofactors: NADPH, FAD, FMN, tetrahydrobiopterin (BH4), calmodulin and haem (List et al. 1997). NADPH provides the electrons for the oxidation of L-arginine. The electron transfer to the haem is catalyzed by the FAD and FMN containing cytochrome P-450 reductase domain, which requires bound calmodulin for its activity. NOS-I (neuronal isoform) and NOS-III are constitutively expressed, calcium-calmodulin dependent enzymes. NOS-II (inducible isoform) is induced upon cytokine stimulation and does not require additional calcium for activity. Calmodulin is tightly bound to NOS-II in contrast to the constitutive isoforms, probably due to the lack of an autoinhibitory loop on NOS-II (Salerno et al. 1997).

PHYSIOLOGICAL ROLE OF ENDOTHELIAL NO

Under normal physiological conditions NOS-III derived NO, released by receptor activation or shear stress, freely diffuses from the endothelium towards the lumen and the vessel wall. NO plays a key role in the maintenance of vascular homeostasis (Rubanyi 1993) (Figure 1).

NO has been shown to inhibit platelet adhesion and aggregation (Stamler et al. 1989, Cooke et al. 1990) and prevent thrombosis (Shultz and Raij 1992). Platelet aggregation is enhanced by incubation with inhibitors of NOS and antagonized by the NOS substrate L-arginine (Chen and Mehta 1996).

NO is also a potent inhibitor of leukocyte adhesion (Kubes et al. 1991, Gaboury et al. 1993) and transmigration by preventing the redox-sensitive transcription of proinflammatory molecules (VCAM-1, ICAM-1, MCP-1, MCSF, etc.) via the inhibition of NF-kB activation (Peng et al. 1995, Zeiher et al. 1995).

Oxidatively modified LDL (oxLDL) is a major contributor to vascular wall activation during the pathogenesis of atherosclerosis. NO has also been shown to inhibit oxidative modification of LDL (Wang et al. 1994).

NO also attenuates smooth muscle proliferation and inhibits neointima formation (Tarry and Makhoul 1994). On the other hand NO protects endothelial cells from apoptotic stimuli (Dimmeler et al. 1998) and mediates the angiogenic effect of vascular endothelial growth factor (VEGF) (Murohara et al. 1998).

Finally NO is a potent vasodilator, which led to its discovery as EDRF in 1980 (Furchgott and Zawadzki 1980) and later to its identification as NO (Moncada et al. 1991) using bioassay systems allowing the
assessment of its biological half-life (Griffith et al. 1984, Rubanyi et al. 1985). Continuous synthesis of endothelial NO plays an important role in the regulation of normal blood pressure. Administration of NOS inhibitors increases blood pressure in experimental animals, as well as in humans (Moncada et al. 1991).

**NOS-III DEFICIENT MOUSE**

Proof for the numerous physiological, mostly vasculoprotective role of endothelial NO was provided by the development of the NOS-III deficient (NOS-III-KO) mouse, in which NOS-III expression was genetically disrupted (Huang et al. 1995).

Homozygous NOS-III-KO mice have 30% elevated mean arterial blood pressure, consistent with the role of endothelial NO in the regulation of blood pressure and vascular tone (Huang et al. 1995, Shesely et al. 1996). Isolated aortic rings with intact endothelium from NOS-III-KO mice do not relax to acetylcholine, which provides genetic evidence that the NOS-III gene is required for the EDRF activity. These mice showed markedly decreased bleeding times (Freedman et al. 1999), exhibited enhanced leukocyte adhesion associated with elevated surface expression of P-selectin in the microcirculation (Lefer et al. 1999) and impaired angiogenic response (Lee et al. 1999).

In addition, myocardial ischemia and reperfusion injury was significantly exacerbated in the absence of endothelial cell nitric oxide synthase using NOS-III-KO mice (Jones et al. 1999). NOS-III deficiency also resulted in enlarged cerebral infarcts following permanent middle cerebral artery occlusion (MCAO) (Huang et al. 1996). These results confirmed the protective role of NOS-III in cardiovascular injury.

**REGULATION OF ENDOTHELIAL NO AVAILABILITY**

NO production by NOS-III is under complex intracellular and extracellular control mechanisms (Figure 2). The different cofactors involved in NO formation provide potential points for regulation of enzyme activity,
besides other transcriptional and posttranscriptional mechanisms. These regulatory pathways may as well involve modulation of substrate availability or the metabolism of enzyme cofactors. As for other important signaling molecules, subcellular localization of NOS-III is under dynamic control by different posttranslational modifications. The fate of NO, once its made, may also be controlled by intracellular and extracellular pathways that importantly influence its biological activity.

**Regulation of NOS-III Expression**

**Regulation at the transcriptional level**

The promoter of the NOS-III gene, like that of other constitutively expressed housekeeping genes, does not contain a TATA-like element (Marsden et al. 1993). However, NOS-III expression and endothelial NO production appear to be under tight physiological control. One of its most important physiological regulators is shear stress (Rubanyi et al. 1986, Miller et al. 1986). The presence of AP-1, AP-2, SP-1, NF-1, p53, sterol regulatory elements and half palindromic sequences of estrogen response elements (ERE) in the NOS-III promoter suggests potential regulation of NOS-III expression by several different factors (Venema et al. 1994). Lysophosphatidylcholine (Cieslik et al. 1998), shear stress (Uematsu et al. 1995), transforming growth factor-β (Inoue et al. 1995), protein kinase C (Ohara et al. 1995, Li et al. 1998), phenolic antioxidants (Ramasamy et al. 1999) and estrogens (Kleinert et al. 1998) represent the examples of exogenous stimuli known to modify NOS-III gene transcription.

**NOS-III mRNA stability**

Post-transcriptional regulation is also an important modulator of the steady-state NOS-III mRNA level under pathophysiological conditions. Tumor necrosis factor-α (TNFα) (Yoshizumi et al. 1993, Marsden et al. 1993).