Therapeutic Application of Nitric Oxide in Human Diseases

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Abstract – Nitric oxide (NO), synthesized from L-arginine by three isoforms of NO synthase (NOS), is a gaseous signaling molecule with an astonishingly wide range of biological and pathophysiological activities, including vasorelaxation, angiogenesis, anti-inflammation, and anti-apoptosis in mammalian cells. Recent studies have shown that NO donors and inhaled NO convert to biologically active NO under biological conditions and act as a signaling molecule in pathophysiological conditions. This review will discuss the roles of NO and its potential therapeutic implication in various human diseases, such as tumor, vascular regeneration, hypertension, wound healing, and ischemia-reperfusion injury.

Keywords: Nitric oxide, Therapy, Nitric oxide donors, Nitric oxide synthase, Tumor, Ischemia-reperfusion injury

INTRODUCTION

NO, first characterized as a major endothelial-derived relaxing factor (Furchgott and Zawadzki, 1980), is a gaseous molecule with a wide range of physiological and pathological activities (Ignarro et al., 1987), including smooth muscle relaxation, inhibition of platelet aggregation, and neurotransmission. NO, synthesized from L-arginine by the reaction of three NOS isozymes, such as eNOS expressed in endothelial cells, iNOS induced in immune cells including macrophages, and nNOS expressed in neuronal cells, has received intense media coverage due to its role as a biological messenger and was named ‘molecule of the year’ in 1992 by the journal Science. Moreover, this molecule has been investigated for its critical role in vascular physiology, immune response to bacterial infections, and neurotransmission. In 1998, Drs. Furchgott, Ingnarro and Murad shared the Noble Prize in Physiology and Medicine for ‘the first discovery that a gas can act as a signal molecule’, emphasizing that NO produced by mammalian cells is an important signaling mediator in various biological systems such as immunology, physiology, and neuroscience.

Various biological activities of NO are highly associated with chemical reactivity for intracellular target molecules, such as transition metal, free thiol (sulfhydryl) group, tyrosine residues, superoxide anion, and molecular oxygen. The earliest described intracellular receptors for NO are heme and non-heme iron-containing proteins, including hemoglobin, soluble guanylyl cyclase (sGC), and aconitase. In particular, binding of NO to the heme group of sGC promotes its catalytic activity, increasing the conversion of GTP to cGMP, which in turn activates protein kinase G (Murad, 1986). The NO-cGMP pathway plays an important role in NO-mediated physiological events, such as vasodilation and penile erection. Despite identification of sGC as the first receptor against NO for eliciting cellular function, it has become clearly demonstrated that NO can exert most of its cellular influence in a cGMP-independent manner. NO interacts with sulfhydryl groups of proteins and non-protein biomolecules to generate S-nitrosothiol, designated S-nitrosylation. S-nitrosylation exhibits a wide rage of cellular effects of NO on pathophysiological processes in the cardiovascular system and vascular disorders (Lima et al., 2010). In addition, S-nitrosylation on catalytic thiols of caspase family proteases prevents cells from apoptotic cell death (Kim et al., 1997b; Li et al., 1997).
Reaction of NO with superoxide anion generates the strong oxidant peroxynitrite, leading to cytotoxicity or apoptotic cell death. However, NO reaction with molecular oxygen decreases its biological effect in pathophysiological conditions as a result of reduced bioavailability via the production of the stable and inert oxidation products, nitrite and nitrate. Taken together, NO plays versatile functions in various pathophysiological conditions via reactions with different intracellular receptors and target molecules. Here, we review the biological roles and pathophysiological mechanisms of action of NO and discuss the therapeutic potential of NO in various human diseases.

**NO AND NITRIC OXIDE SYNTHESSES**

NO is a small, odorless, colorless, endogenous, and free radical molecule, which highly diffuses in water and through cellular membranes. However, its high reactivity limits its short half-life in biological systems. Interestingly, NO elicits different reactions in cells in a concentration-dependent manner. At low concentrations, it is a potent biological messenger in a variety of tissues, with a wide range of physiological functions such as vasodilation, inhibition of platelet aggregation, regulation of neurotransmission and natural defense of the immune system. Conversely, high concentrations of NO are involved in the immune system during cytotoxicity of tumor cells, infection of microorganisms, and inflammation (Nathan, 1997).

NO is endogenously produced by a group of homodimeric NOS enzymes through enzymatic oxidation of the guanidine group of L-arginine in the presence of oxygen (Ignarro et al., 1987; Nathan, 1992). It is hydroxylated to generate L-hydroxyarginine, which is further oxidized, yielding L-citrulline and NO (Marletta et al., 1998) (Fig. 1). All three NOS isoforms possess reductase and highly conserved oxygenase polypeptide domains within each monomer; thus, this enzyme catalyzes two sequential NADPH- and O2-dependent mono-oxygenase reactions to produce NO from L-arginine. These isoforms are denoted by descriptive terms, based on the requirement of intracellular calcium oscillation for full activity as well as gene expression (Table I). Two NOS isoforms, eNOS and nNOS, are constitutively expressed and operate their catalytic activities in a calcium-dependent manner, consequently producing NO at low concentrations (nM - pM) (Forstermann et al., 1995; Gath et al., 1996). These constitutive NOS isoforms require a transient increase in intracellular calcium levels and several cofactors for their enzymatic activity, which promotes the release of NO over the course of several minutes. Specifically, these NOS isoforms increase their enzymatic activity at a specific level of intracellular calcium, but inactivate at a low level of calcium. These constitutive isoforms are key modulators in physiological processes such as memory, long-term potentiation, and depression in the nervous system and regulate blood pressure (vasorelaxation) in the vascular system.

The comparatively small quantities of NO produced by

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**Table I. Isoforms of nitric oxide synthases**

<table>
<thead>
<tr>
<th>Isoform Type</th>
<th>Expression Type</th>
<th>M.W (kDa)</th>
<th>Regulated by</th>
<th>Location</th>
<th>Main Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal NOS (nNOS, NOS1)</td>
<td>Constitute</td>
<td>160</td>
<td>Ca2+/calmodulin</td>
<td>Brain, other neuronal tissues/cells</td>
<td>Neurotransmission</td>
</tr>
<tr>
<td>Inducible NOS (iNOS, NOS2)</td>
<td>Inducible</td>
<td>125</td>
<td>Cytokine, endotoxin</td>
<td>Macrophages, neutrophiles, hepatocytes, chondrocytes</td>
<td>Cytotoxicity against tumor cells and bacteria</td>
</tr>
<tr>
<td>Endothelial NOS (eNOS, NOS3)</td>
<td>Constitute</td>
<td>135</td>
<td>Ca2+/calmodulin</td>
<td>Endothelial cells, cardiac myocytes</td>
<td>Vasodilation</td>
</tr>
</tbody>
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**Fig. 1.** Reaction pathway of NO production from L-arginine by NOS.