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Review

Specificity of inducible nitric-oxide synthase inhibitors: prospects for their clinical therapy

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ABSTRACT

Nitric oxide (NO) is a ubiquitous, naturally occurring molecule found in a variety of cell types and organ systems. It is a double-edged sword, beneficial as a messenger or modulator and for immunology self-defense, but potentially toxic. The formation and signal function of nitric oxide are mainly modulated by nitric-oxide synthase (NOS). Up to the present, a number of diseases, including circulatory shock, atherosclerosis, cardiac allograft rejection, chronic inflammation, cardiac infarction, cancer and so on, have been demonstrated that their pathogenesis may be involved in the sustained production of large quantities of nitric oxide. Animal studies and human studies have shown that specific inhibitors of inducible nitric-oxide synthase may be useful in the therapy of a variety of diseases associated with induction of nitric-oxide synthase. In this review, we compare and contrast these inhibitors along with examples of their use in the studies of medicine.

INTRODUCTION

NO is a short-lived, gaseous radical that is the smallest biosynthetically derived secretory product of mammalian cell. The key role of NO as an important bioregulatory signaling molecule is now recognized[1,2]. Its activity, mediated in most cases by elevation of intracellular 3′, 5′ cyclic guanosine monophosphate (cGMP), is fundamental in a variety of physiological process, including the endothelial regulation of vascular smooth muscle tone and proliferation, inhibition of platelet adhesion and aggregation, peripheral and central neurotransmission and macrophages toxicity. NO can potentially influence cardiac function, by altering peripheral and coronary vascular tone and thus cardiac loading and coronary perfusion respectively. In recent years, it has been established that NO may also exert direct effects on myocardial contractile function. The effects of NO in unstimulated myocardium appear to modulate relaxation and diastolic tone[3-5], whereas in circumstances where there is a β-adrenergic stimulation, NO may modulate inotropic and chronotropic response[6]. The inducible isoforms of nitric-oxide synthase (iNOS) are induced by cytokines or bacterial endotoxin in macrophage, endothelial cells, vascular cells, and cardiac myocytes. The induction of iNOS was accompanied by increase in cGMP in heart tissue, impaired ventricular function and evidence of myocardial infiltration with macrophages and lymphocytes, interstitial edema and necrosis of cardiac muscle cells. Recent studies have shown that the selective inhibitors of iNOS can play an important role in circulatory shock[7], chronic inflammation[8], transplant rejection[9], and cancer[10].

BIOSYNTHESIS OF NITRIC OXIDE

The biosynthesis of NO requires the oxidation of

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the terminal guanidino nitrogen of *L*-arginine to NO via an intermediate compound identical as N^G-hydroxy-*L*-arginine, with *L*-citrulline as equimolar coproduct. This reaction is enantiomerically specific and is inhibited by some arginine derivatives, such as N^G-monomethyl-*L*-arginine. NO is generated from *L*-arginine by the family of enzymes known as NO synthase, which were originally described in the vascular endothelium, the brain[11], and the macrophage[12]. The endothelial and neuronal enzymes require calcium and calmodulin. They are involved in the physiological control of vascular and neuronal function. The iNOS is an cytokine-inducible and Ca^{2+}-independent enzyme, which was originally described in the murine macrophage[12]. In these cells, the enzyme is not normally expressed; however, in the presence of the appropriate cytokine signals, gene transcription is induced and, nitric oxide is generated. In human, the expression of the iNOS has been documented in hepatocytes[13], chondrocytes[14], monocytes[15], macrophages, and cardiac myocytes[16]. At present, some selective inhibitors of iNOS are being under investigation, these inhibitors may be of significant therapeutic benefit in human disease.

NOS ISOFORMS (NOSs)

NOSs are homodimers whose monomers are themselves with two enzymes fused, a cytochrome reductase and a cytochrome, which require three cosubstrates (*L*-arginine, NADPH, and O_2) and five cofactors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobiopterin, and heme). To date, three distinct isoforms of NOS have been identified. Each represents the product of a separate gene, and each has been cloned and sequenced and identified in a diverse number of cell types[17]. The constitutive isoform first identified in brain (ncNOS or NOS_1) has recently been found to be expressed in skeletal muscle cells[18]. The inducible isoform (iNOS or NOS_2) was first characterized in a macrophage cell line and is now known to be expressed in a wide variety of cells after stimulation with inflammatory mediators, including adult cardiac myocytes. The third isoform to be identified originally was found to be constitutively expressed in large vessel endothelial cells (ecNOS or NOS_3). The activities of the constitutively active NOS isoenzymes (NOS_1 and NOS_3) identified to date are believed to be regulated by Ca^{2+} and calmodulin within the physiologically relevant range of intracellular Ca^{2+} level. These isoenzymes can also be phosphorylated by cAMP-dependent protein kinase C, and the Ca^{2+}/calmodulin-dependent protein kinase, with consequent regulation of activity. The cytokine-inducible NOS isoenzyme (iNOS or NOS_2) does not appear to be regulated by Ca^{2+} within the physiological range. iNOS transcription and synthesis in macrophages, cardiac myocytes, and many additional cell types are induced by lipopolysaccharide (LPS) and inflammatory cytokines, including interleukin-1β (IL-1β) and interferon-γ[19-21]. In 1992, iNOS were first cloned and expressed in activated murine macrophages.

iNOS in infiltrating macrophages Wildhirt *et al*[22] have demonstrated that iNOS was localized in infiltrating inflammatory cells, which could be identified as mononuclear cells/macrophage. Induction of NOS in macrophage has also been shown by cardiac allograft rejection in rats and myocardial infarction in rabbits[23]. It is known that NO from macrophage-derived iNOS is cytotoxic for neighbouring cells, the persist, local release of NO derived from macrophage iNOS may affect the myocardium and its preserved vasculature. This is partially mediated by the binding of nitric oxide to iron-containing enzymes, ie enzymes in the mitochondrial electron transfer chain, aconitase or ribonucleotide reductase[24]. Furthermore, superoxide may bind to NO to form peroxynitrite thereby leading to the production of hydroxyl radicals, thus resulting in enhanced regional tissue destruction[25]. Macrophage-derived iNOS also inhibits the contractile response of cultured rat cardiac myocytes to sympathetic stimuli, indicating that local iNOS induction may account for the cardiac depression often seen in myocardial infarction. These findings suggest that under pathological conditions, macrophage-derived iNOS may have cardiodepressant and cytotoxic effects.

iNOS expression in cardiac myocytes In cardiac myocytes, 1L-1β, TNFα, and interferon-γ each individually induces iNOS transcription, and the combination of 1L-1β, TNFα with interferon-γ results in a synergistic induction of iNOS gene expression[26]. Ikeda and colleagues[27] have shown that both angiotension Ⅱ and the α-adrenergic agonist phenylephrine

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augment cytokine-induced NOS_2 expression in cardiac myocytes. In addition, agents that increase intracellular cAMP or membrane permeated cAMP analogues augment NOS_2 messenger RNA level in cardiac myocytes in response to IL-1β[28]. It has been shown that human cardiomyocytes have the capacity to express iNOS in pathological states, such as a dilated cardiomyopathy and transplant rejection[29]. These findings indicate that an overproduction of nitric oxide from cardiomyocyte-derived iNOS may contribute to myocardial contractile dysfunction and the development of heart failure.

ROLE OF SPECIFIC INHIBITORS OF iNOS

L-Arginine analogues A number of *L*-arginine analogues, including N^G-cyclopropyl-*L*-arginine (*L*-NCPA), N^G-*L*-aminoarginine (*L*-NAA), N^G-*L*-aminohomoarginine (*L*-NAHA), N^G-nitro-*L*-arginine (*L*-NNA) or its methyl ester (*L*-NAME), N^G-iminoethyl-*L*-ornithine (NIO), N^G-nitro-*L*-*P*-anilide (NAPNA), and N^G-mono-methyl-*L*-arginine (*L*-NMMA), are inhibitors of nitric oxide synthesis[29-31]. All of the above *L*-arginine analogues can be regarded as competitive inhibitors of NOS, for their inhibitory effects are reversed to varied degrees by excess of *L*-arginine. The order of potency of these agents as inhibitors of the constitutive NOS *in vitro* is *L*-NAME > *L*-NAA = *L*-NCPA > *L*-NMMA. In contrast, the rank order of potency of various *L*-arginine analogues as of the iNOS is NIO = *L*-NAA > *L*-NAHA > *L*-NMMA > *L*-NAME > *L*-NCPA. At present, it has been shown that *L*-NAME and *L*-NAA may be regarded as a selective inhibitor of iNOS, the enhanced depression of contractile function by IL-1β plus TNFα is prevented by the NOS inhibitor *L*-NAME and *L*-NAA. The finding that NAPNA is a selective inhibitor of neuronal NOS relative to endothelial NOS may well be due to preferential hydrolysis of NAPNA to *L*-NAA by homogenates. Thus, it is conceivable that drugs can be designed that target NOS inhibitors to specific tissues based on different distribution of metabolizing enzymes. The development of selective inhibitors of iNOS may be a prerequisite to achieve the full therapeutic benefit of inhibition nitric oxide synthesis in cardiovascular disease.

Glucocorticoids In 1990, Radomski and colleagues[32] first demonstrated that dexamethasone and hydrocortison inhibited the expression of a calcium-independent NOS in endothelial cells activated with endotoxin and interferon-γ. It is now well established that glucocorticoids inhibit the endotoxin or cytokine-induced NOS activity in various cells, including endothelial cells, vascular smooth muscle cells, macrophages, neutrophils, and hepatocytes[32-34]. Dexamethasone significantly inhibits the expression of iNOS in infarcted rabbit myocardium, as a result, the cGMP level is no longer elevated after administration of glucocorticoids. Singh (1996) reported that the synthetic glucocorticoid dexamethasone markedly increased osteopontin expression in ventricular myocytes and microvascular endothelial cells *in vitro* and in cardiac muscle *in vivo*. Increased synthesis of osteopontin may limit the extent of iNOS induction in the heart and may be one mechanism through which glucocorticoids restrict iNOS expression.

Aminoguanidine Aminoguanidine (AMG) is a more potent inhibitor of iNOS than N^{ω}-substituted arginine analogues. In addition, AMG is less potent as an inhibitor of the constitutive NOS activity in cultured cells, isolated blood vessels and enzyme preparation *in vitro*[35,36]. AMG, a relatively selective inhibitor of iNOS, attenuates the delayed circulatory failure associated with endotoxic shock in the rat and improves the survival in a murine model of endotoxeamia[37]. In particular, AMG can attenuate the delayed fall in blood pressure and the vascular hyporeactivity to noradrenaline elicited by prolonged period of endotoxeamia in the rat. AMG combined with low doses of cycloporine can reduce the allogeneic response across major histoincompatibilities in rodent lung transplantation[38]. These findings suggest that AMG as a selective inhibitor of iNOS may be an effective adjuvant to conventional immunosuppressive and useful in the therapy of patients with circulatory shock and allograft rejection.

Isothioureas analogues A number of *s*-substituted isothioureas analogues, including *s*-methyl-isothiourea (SMT), *s*-aminoethyl-isothiourea (SAET), *s*-ethyl-isothiourea (SET), and *s*-isopropyl-isothiourea (SIPT), are inhibitors of iNOS[39,40]. Inhibition of iNOS activity by these *s*-substituted isothioureas is dose-dependently prevented by excess *L*-arginine suggesting that these isothioureas

competitive inhibitors of iNOS at the *L*-arginine binding site. SAET, a selective inhibitor of iNOS activity, improves liver circulation and oxygen metabolism in a porcine model of endotoxemia, restores hepatic arterial blood flow and increases hepatic oxygen consumption[41]. SMT, a non-amino acid analogue of *L*-arginine, is a potent and preferential inhibitor of human and rat iNOS, and it significantly improves left ventricular performance after acute myocardial infarction and increases myocardial blood flow in the surviving myocardium. Thus isothioureas may be potential tools for studying the role of NOS isoenzymes and iNOS in various pathophysiological conditions.

CLINICAL USE OF OTHER NOS INHIBITORS

In addition to above described iNOS inhibitors, several other agents of different structures have been reported to inhibit NOS activity. For instance, methylene blue, an inhibitor of soluble guanylate cyclase also inhibits NOS activity, improves hemodynamic in intact rabbits with septic shock. Mercaptoethylguanidine is a selective inhibitor of iNOS and peroxynitrite scavenger, it protects against the associated extravasation and bone destruction[42]. Tirapazamine, a new anticancer drug exploiting hypoxia in solid tumors, can inhibit ecNOS activity and retard the growth of tumor cells[43]. Methylguanidine and guanidine have inhibitory activity on both the neuronal constitutive and lung inducible isoforms of NOS. The lack of selectivity of methylguanidine and guanidine in inhibiting both NOS enzymes could account for some pathological manifestations like neurological disorders, host defense impairment, and probably hypertension, that often occur in patients with uremia or chronic renal failure[44].

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诱导型一氧化氮合酶抑制剂的特性：临床治疗展望

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