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Review

Role of nitric oxide in inflammatory diseases

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Abstract. Nitric oxide (NO) is a signaling molecule that plays a key role in the pathogenesis of inflammation. It gives an anti-inflammatory effect under normal physiological conditions. On the other hand, NO is considered as a pro-inflammatory mediator that induces inflammation due to over production in abnormal situations. NO is synthesized and released into the endothelial cells by the help of NOSs that convert arginine into citrulline producing NO in the process. Oxygen and NADPH are necessary co-factors in such conversion. NO is believed to induce vasodilatation in cardiovascular system and furthermore, it involves in immune responses by cytokine-activated macrophages, which release NO in high concentrations. In addition, NO is a potent neurotransmitter at the neuron synapses and contributes to the regulation of apoptosis. NO is involved in the pathogenesis of inflammatory disorders of the joint, gut and lungs. Therefore, NO inhibitors represent important therapeutic advance in the management of inflammatory diseases. Selective NO biosynthesis inhibitors and synthetic arginine analogues are proved to be used for the treatment of NO-induced inflammation. Finally, the undesired effects of NO are due to its impaired production, including in short: vasoconstriction, inflammation and tissue damage.

Key words: Nitric oxide; Asthma; Rheumatoid arthritis; Inflammatory bowel diseases

Introduction

Nitric oxide (NO) is a member of the labile radical entities known as reactive oxygen species (ROS) and contains 1 nitrogen atom covalently bonded to an oxygen atom with one unpaired electron. It is particularly reactive with oxygen and heme-iron containing groups which reduce NO to more stable nitrate compounds (Ignarro, 1989) For this reason, the bioavailability of NO in certain tissues (notably blood-rich in haemoglobin and muscle rich in myoglobin) is extremely low and the biological actions are restricted temporally and spatially close to its site of synthesis (Ignarro et al., 1993). Paradoxically, NO is also lipid soluble, making it highly membrane permeant (Subczynski et al., 1996). Therefore, many of its most well described actions involve its diffusion between cells to act as a paracrine-signaling molecule.

The NO's role was firstly discovered by several groups of scientists who were attempting to identify the agent as to be responsible for promoting blood vessel relaxation and regulating vascular tone. This agent was termed endothelium-derived relaxing factor (EDRF), and was initially assumed to be a protein like most other signaling molecules. The discovery that EDRF was in fact nitric oxide has led to explosion of interest in this field and resulted in many thousands of publications over the last few years.

NO has now been demonstrated to be a versatile molecule that plays its actions in a variety of biological processes including immune defenses, inflammation and neurotransmission.

NO is a fairly short-lived molecule (with a half life of 6 s) produced from enzymes known as nitric oxide synthases (NOSs) and provides its actions through the $_{L}$-arginine substrate that is transported into the cells (Moncada et al., 1991).

Since it is a small molecule, NO is able to penetrate rapidly across cell membranes and diffuse through distances of more than several microns. This means that NO can be formed or synthesized in a variety of tissues and as a consequence it is capable of affecting a number of important biological processes and has been implicated in several disease (Gonon et al., 2004).

This paper is going to concern more on specific disease conditions related to joint, gut and asthmatic inflammatory disorders.

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Formation of NO

To begin with, nitric oxide (NO) is a paracrine mediator that is released by endothelial cells and by certain neurons. Because NO is rapidly oxidized, its biological lifetime is only several seconds. For this reason, NO affects only cells in the immediate vicinity of the cell that produces it.

The formation of NO is catalyzed by nitric oxide synthases (NOS), which are dimeric flavoproteins, contain tetrahydrobioprotein and have homology with cytochrome P450 and is most likely to be in the cardiovascular and central nervous systems. These enzymes convert arginine into citrulline, producing NO in the process. Oxygen and NADPH (nicotinamide adenine dinucleotide Phosphate with extra hydrogen) are necessary co-factors. There are three isoforms of NOS named according to their activity or the tissue type in which they were first described. The isoforms of NOS are neuronal NOS (or nNOS), constitutive endothelial NOS (or eNOS) and inducible NOS (or iNOS). These enzymes are also sometimes referred to by number, so that nNOS is known as NOS1, iNOS is known as NOS2 and eNOS is NOS3. Despite the names of these enzymes, all three isoforms can be found in a variety of tissues and cell types (Kolb-Bachofen et al., 2006).

2.1. General mechanism of NO formation by NOSs

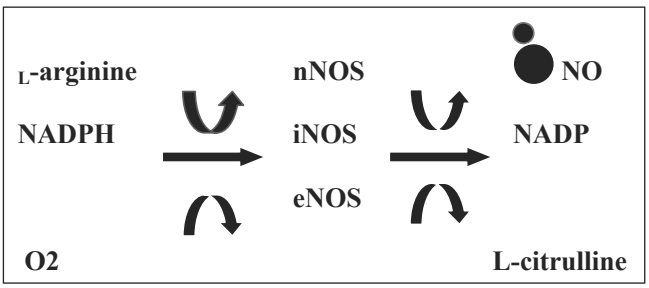

Figure 1 shows the nitrogen atom of NO is derived from the terminal guanido group of L-arginine. Details of the reaction mechanism are controversial, but it is known that NOS enzymes are functionally 'bimodal' in that they combine oxygenase and reductase activities associated with distinct structural domains. The oxygenase domain contains haem, while the reductase domain binds calcium-calmodulin, FMN (flavin mono-nucleotide), FAD (flavin adenine di-nucleotide) and NADPH. By analogy with cytochrome P450, it is believed that the flavins accept electrons from NADPH and transfer them to the haem iron, which binds to oxygen and catalyses the stepwise oxidation of $_{L}$-arginine (essential alpha-amino acid) to NO and citrulline (alpha-amino acid).

General structure of the NOS

The functional NOS protein is a dimer formed of two identical sub-units. There are three distinct domains in each NOS sub-unit: a reductase domain, a calmodulin-binding domain and an oxygenase domain (Liu and Gross, 1996).

1. The reductase domain: This domain contains the calcium-calmodulin, FMN, FAD moieties and NADPH. It acts to transfer electrons from NADPH to the oxygenase domain. It should be noted that the reductase domain transfers electrons to the oxygenase domain of the *opposite* sub-unit of the dimer, and not to the domain on the same sub-unit (Liu and Gross, 1996).
2. Calmodulin binding: The binding of calmodulin is required for the activity of all the NOS isoforms. It detects changes in intracellular calcium levels, although its precise function is slightly different in each of the three isoforms (Phil Dash, 2001).
3. The oxygenase domain: This domain contains the binding sites for tetrahydrobiopterin, haem (heme) and arginine. The oxygenase domain catalyses the conversion of arginine into citrulline and NO (Knowles and Moncada, 1994).

Two of the enzymes (nNOS and eNOS) are constitutively expressed in mammalian cells and synthesize NO in response to increases in intracellular calcium levels. In some cases, however, they are able to increase NO release indirectly, in response to stimuli such as shear stress. The shearing forces act on the luminal surface of the vascular endothelium and increase the flow velocity of the calcium atoms, which in turn, increase the activity of nNOS and eNOS (Suschek et al., 2004)..

iNOS activity is independent of the level of calcium in the cell; however its activity – like all of the NOS isoforms – is dependent on the binding of calmodulin. Increases in cellular calcium leads to increases in levels of calmodulin and the increased binding of calmodulin to eNOS and nNOS leads to a transient increase in NO production by these enzymes. By contrast iNOS is able to bind tightly to calmodulin even at very low cellular concentration of calcium. Consequently, iNOS activity isn't able to respond to changes in calcium levels in the cell. As a result the production of NO by iNOS lasts much longer than from the other isoforms of NOS, and tends to produce much higher concentrations of NO in the cell (Koppenol and Traynham, 1996).

3. Physiological roles of NO

Since the discovery that nitric oxide is able to induce vasodilation in the cardiovascular system, a large number of other roles have been described for NO. It is also known to play a role in the immune system, the nervous system and in programmed cell death (apoptosis) (Kolb and Kolb-Bachofen, 1998).

In the Cardiovascular system (CVS)

NO formed in the endothelium from the amino acid precursor L-arginine by the activity of the constitutive endothelial NOS isoenzymes has been shown to play an important role in the regulation of local vasomotor tone and other vascular roles and is thought, on the other hand to be due to transcriptional diversity (Ignarro et al., 1993). Based on such mechanisms, NO roles in the CVS are vasodilatation (ligand mediated and flow dependent), inhibition of vasoconstrictor influences (e. g., inhibits angiotensin II and sympathetic vasoconstriction), inhibition of platelet adhesion to the vascular endothelium (anti-thrombotic), inhibition of leukocyte

adhesion to vascular endothelium (anti-inflammatory), anti-proliferative (e. g., inhibits smooth muscle hyperplasia following vascular injury) (Hocher et al., 2004). COX-inhibiting NO donors (CINODs) have been suggested as potentially beneficial drugs on cardiovascular and renal abnormalities ((Muscara and Wallace, 2006).

Release of NO as an important inflammatory mediator

In inflammatory reactions, pro-inflammatory cytokines lead to expression of the inducible NO synthase in monocyte/macrophages, neutrophil granulocytes and many other cells; in the case of bacterial infection, endotoxin is another strong inducer of expression. In consequence, large amounts of NO are synthesized, exceeding the physiological NO production by up to 1000-fold (Forstermann et al., 1994; Knowles and Moncada, 1994; Weinberg et al., 1995; Cook and Cattell, 1996).

NO is secreted by neutrophils and macrophages in the following sequence:

With the onset of inflammation, circulating neutrophils begin to move out of the blood across the endothelium of venules to enter the inflamed area. This multistage process is known as chemotaxis. It involves a variety of protein and carbohydrate adhesion molecules on both endothelial cell and the neutrophil, and is regulated by messenger molecules released by cells in the injured area, including the endothelial cells. These messengers are collectively termed chemoattractants (also termed chemotaxins or chemotactic factors) (Scales et al., 1988).

In the first stage, the neutrophil is loosely tethered to the endothelial cells via a particular class of adhesion molecules; this event is associated with rolling of the neutrophil along the vessel surface. In essence, this initial reversible event permits the neutrophil to be exposed to chemoattractants being released in the injured area. These chemoattractants act on the neutrophil to induce the rapid appearance of another class of adhesion molecules in its plasma membrane-molecules that bind tightly to their matching molecules in the endothelial cells. In the next stage, via still other adhesion molecules, a narrow projection of the neutrophil is inserted into the space between two endothelial cells, and the entire neutrophil squeezes through the endothelial wall and into the interstitial fluid. In this way, huge numbers of neutrophils migrate into the inflamed area and move toward the microbes (Scales et al., 1988).

Movement of leukocytes from the blood cells into the damaged area is not limited to neutrophils. Monocytes follow later, and once in the tissue they undergo anatomical and functional changes that transform them to macrophages (Scales et al., 1988).

Killing by Phagocytes

The initial step in phagocytosis is contact between the surfaces of the phagocyte and microbe. One of the major triggers for phagocytosis during this contact is the interaction of phagocyte receptors with certain carbohydrates or lipids in the microbial cell walls. Contact is not itself always sufficient to trigger engulfment, however, particularly with those bacteria that are surrounded by a thick, gelatinous capsule. Chemical factors produced by the body can bind the phagocyte tightly to the microbe and markedly enhance phagocytosis. Any substance that does this is known as an opsonin, from the Greek word that means 'to prepare for eating' (Scales et al., 1988). As the phagocyte engulfs the microbe, the internal, microbe-containing sac formed in this step is called a phagosome. A layer of plasma membrane separates the microbe from the phagocyte's cytosol. The phagosome membrane then makes contact with one of the phagocyte's lysosomes, which is filled with a variety of hydrolytic enzymes. The membranes of the phagosome and lysosome fuse, and the combined vesicles are now called the phagolysosome. Inside the phagolysosome, the microbe's macromolecules are broken down by the lysosomal enzymes. In addition, other enzymes in the phagolysosome membrane produce NO as well as hydrogen peroxide and other oxygen derivatives, all of which are extremely destructive to the microbe's macromolecules (Scales et al., 1988).

Such *intercellular* destruction is not the only way phagocytes can kill microbes. The phagocytes also release anti-microbial substances into the extracellular fluid, where these chemicals fight and destroy the microbes without prior phagocytosis. These chemicals can also damage normal tissue (Scales et al., 1988).

Some of these substances such as NO secreted into the extracellular fluid also function as *inflammatory mediators.* Thus, positive feedback occurs such that when phagocytes enter the area and encounter microbes, inflammatory mediators, including chemokines, are released that brings in more phagocytes (Scales et al., 1988).

The overproduction of NO as an inflammatory mediator can lead to tissue destruction such as in inflammatory autoimmune diseases. Thus, depending on the concentration of NO, it can process pro- or anti-inflammatory effects. As a result NO is called 'double-edged sword' or 'Jekll and Hide' (Pfeilschifter et al., 1996).

4.4. Role of NO in inflammation-mediated Neurodegeneration

Several studies provided evidences of the close association between inflammation in the brain and the pathogenesis of several degenerative neurologic disorders, including Parkinson's disease, Alzheimer's diseases, multiple sclerosis, amyotrophic lateral sclerosis, AIDS and dementia. The brain inflammation is mainly caused by activation of glial cells, especially in microglia that in turn produce a variety of pro-inflammatory and neurotoxic factors, including cytokines, fatty acid metabolites, free radicals-such as NO and superoxide. Excessive production of NO, as a consequence of NOS induction in activated glia, has been attributed to be involved in neurodegeneration and neuroprotection (Moncada and Higgs, 2006a).

Possible pro-inflammatory pharmacological effects of NOS
NO is to be produced by specific mechanisms (as mentioned earlier) with the help of NOSs. The two isoforms (iNOS and eNOS) involved in inflammation produce NO that acts as an

inflammatory mediator. On the other hand, the neuronal enzyme contributes only in the production of NO in the central nervous system to act as a neurotransmitter. Thus, our focus will be on iNOS and eNOS, which have pro-inflammatory effects (Moncada and Higgs, 2006b).

Once there is a bacterial invasion (for example), the bacteria will release endotoxins (part of the bacterial cell wall). And when the immune cells are exposed to these bacterial endotoxins or pro-inflammatory cytokines, they (immune cells) start to produce iNOS, which in turn results in an increase in cellular NO that contributes to inflammation and host defenses. Furthermore, the scientists suggested that the endotoxins activate the eNOS in macrophages that is essential in triggering the induction of iNOS (Corraliza and Moncada, 2002).

NO aids host defenses by killing the invading organism through inhibition of metabolic enzymes and destruction of DNA. However, overproduction of NO by iNOS can lead to septic shock (sepsis, a systemic bacterial infection) that causes host-damage. In sepsis, this is manifested predominantly as a profound hypotension, inadequate tissue perfusion and organ failure, which often result in death. Sepsis and other forms of host-damage (due to overproduction of NO) can be managed by many drugs that can inhibit NO synthesis or action by several mechanisms (Kolb-Bachofen et al., 2006).

6. Involvement of NO in joint, gut and lung inflammatory disorders

It is mainly the inducible form of NO synthase (iNOS) that is involved in inflammatory reactions. Virtually, all inflammatory cells express the inducible form of the enzyme in response to cytokine stimulation. NOS is also present in the bronchial epithelium of asthmatic subjects, in mucosa of the colon in patients with ulcerative colitis and in synoviocytes in inflammatory joint diseases. Inhibitors of iNOS are under investigation for treatment of inflammatory conditions (Hocher et al, 2000).

6.1. NO in joint inflammatory disorders

NO is not only a marker, but also a pro-inflammatory mediator of arthritis.

Increased serum concentration of nitrate, indicating enhanced NO production in serum and synovial fluid of the inflamed joints in patients with rheumatoid arthritis (RA), osteoarthritis (OA) and ankylosing spondylitis (Farrell et al., 1992; Kanno et al., 1992; Bode-Boger et al., 1996). Urinary nitrate (NO present in the kidney as nitrate) and c-GMP excretion are influenced in parallel when the constitutive NO synthase is activated (Stichtenoth et al., 1995; Van der Vliet et al., 1994). The urinary nitrate excretion was decreased significantly by therapy with prednisolone or non-steroidal anti-inflammatory drugs (NSAIDs) (Stichtenoth et al., 1995).

Further evidence is given by the measurement of elevated nitrotyrosine concentrations in serum and synovial fluid from patients with RA. Nitrotyrosine is formed by reaction of peroxynitrate with tyrosine and is an index of NO-dependent oxidative damage (Van der Vliet et al., 1994). Statins have been reported to possess a number of so-called pleiotropic (vasculoprotective actions that include improvement of endothelial function, increased nitric oxide (NO) bioavailability, antioxidant properties, stabilization of atherosclerotic plaques, regulation of progenitor cells, inhibition of inflammatory responses and immunomodulatory actions) actions. The anti-inflammatory effects of statins may have clinical impact in a number of non-vascular conditions including multiple sclerosis and rheumatoid arthritis (Matthias Endres., 2006).

6.1.b. Cellular origin and actions of NO in arthritis

Nearly, all mammalian cells can express the inducible NO synthase after stimulation by cytokines, which are enhanced in inflammatory joint diseases as mentioned above. In humans, the following extra- and intra-articular sources of inflammatory NO production were identified: synovial fibroblasts, synoviocyts, endothelial cells, monocytes/macrophages in blood stream and synovial membrane, osteoblasts and chondrocytes. In patients with active RA, blood mononuclear cells had increased NO synthase activity due to expression of the inducible isoenzymes; the NO synthase activity correlated with the tender and swollen joint count (Weinberg et al., 1994).

Physiological NO production inhibits bone resorption by osteoclasts and it may have some acute protective effects in cartilage breakdown. On the other hand, the high amounts of NO produced by inflamed synovium lead to enhanced bone resorption, diminished bone proliferation and may induce chondrocyte apoptosis (Ralston et al., 1993).

All of these effects contribute to joint damage, thus NO must be considered as an important effector molecule of disease progression.

6.2. NO in gut inflammatory disorders

The role of NO as a pro-inflammatory mediator is proven for other chronic inflammatory diseases, such as chronic inflammatory bowel diseases. An increased NO production by the inducible NO synthase was found. Cellular sources of this NO production were mucosal neutrophils in the acute phase, and monocytes/macrophages and lymphocytes in the chronic phase (Miller and Clark, 1994).

In a study of patients with ulcerative colitis or Crohn's disease, there is a demonstration of enhanced activity of NO synthase in the inflamed mucosa. This activity was calcium independent, suggesting expression of the iNOS. As in RA, in ulcerative colitis there will be an increased urinary nitrate/nitrate excretion as compared to healthy individuals. And after treatment with hydrocortisone by which the disease was inactivated, urinary nitrate/nitrate excretion was normalized (Weinberg et al., 1994; Ralston et al., 1993; Miller and Clark, 1994). Since the iNOS is the most important isoenzyme in the production of NO in gut inflammatory disorders, the majority of studies have shown improvement in experimental bowel diseases with iNOS inhibition.

6.2.a. Cellular effects of NO

NO can have potent effects on leukocyte adherence and chemotaxis. NO production leads to decreased expression

of adhesion molecules on neutrophils and endothelial cells. These effects can result in changes in leukocyte adhesion and recruitment of postcapillary venules in vivo (Banick et al., 1997; Crisham et al., 1998).

NO can down-regulate macrophage cytokine production. However, it is important to consider that iNOS-derived NO from macrophages is also critical component of mucosal defense against luminal pathogens, such as *Helicobacter pylori*. Furthermore, NO can modulate neutrophil and monocyte chemotaxis induced by a variety of factors (Gobert et al., 2002; Sato et al., 2000).

Under conditions of oxidative stress, NO can scavenge free radicals and thus prevent cellular injury. However, iNOS activity is associated with inhibition of proliferation, increased apoptosis and cytotoxicity. High levels of NO are associated with mutagenesis and other forms of DNA damage (Liu and Hotchkiss, 1995). NO can also act to alter the function of iron-sulfur-containing enzymes and disrupt mitochondrial respiration (Kurose et al., 1995).

6.2.b. Tissue effects of NO: NO role in gastrointestinal Secretion, Permeability and Mucosal Blood Flow

In Gastro-intestinal Secretion. Mucus and epithelial cell fluid secretion are important in host defense in the intestine against microbes, toxins and irritants such as bile salts. NO has been shown to play an important role in both of these epithelial cell functions. NO induces gastric mucus and electrolyte secretion via activation of soluble guanylate cyclase, and this NO production appears to be due to activation of cholinergic receptors. However, prolonged over-expression of iNOS has been linked to decreased intestinal electrolyte transport (Weinberg et al., 1995; Cook and Cattell, 1996).

In permeability. In addition to effects on transcellular transport, NO has also been linked to alterations in paracellular permeability and barrier function. Interferon (INF) gamma has been well shown to cause alterations in permeability and several reports have linked this effect to induction of NO production (Sugi et al., 2001). The exact mechanism of how NO may alter the tight junctional complex or have other effects remains to be determined.

In Mucosal Blood Flow. NO is a potent vasodilator, an effect that is well documented in sepsis. This effect is also of great importance in the gastrointestinal mucosa. The increase in mucosal blood flow that can occur in response to injury from a variety of causative factors can have obvious effects in that there is resulting buffering of acid, dilution of toxins and stimulation of angiogenesis, all of which are critical in mucosal protection (Lippe and Holzer, 1992).

6.3. NO in lung inflammatory disorders

6.3.a. Introduction

Inflammatory diseases of the respiratory tract are commonly associated with elevated production of NO and increased indices of NO-dependent oxidative stress. Although NO is known to have anti-microbial, anti-inflammatory and antioxidant properties, various studies support its involvement to lung injuries in several diseases. Such studies are also often presumed that NO dependent oxidations are due to the formation of the oxidant peroxynitrate, although alternative mechanisms involving the phagocyte-derived heme proteins myeloperoxidase and eosinophil peroxidase might be operative during conditions of inflammation (Moncada et al., 1991).

6.3.b. Roles, cellular origin and generation of NO in the respiratory tract

Since its discovery as a biological messenger molecule more than 10 years ago, NO is now well recognized for its roles and actions in diverse biological processes, including vasodilatation, bronchodilation, neurotransmission, tumor surveillance, anti-microbial defenses and regulation of inflammatory-immune process (Moncada et al., 1991; Weinberger et al., 1999).

In the respiratory tract, NO is generated by the three distinct isoforms of NO synthase (nNOS, iNOS and eNOS) that are present to different extents in numerous cell types, including airway and alveolar epithelial cells, neuronal cells, macrophages, neutrophils, mast cells, and endothelial and smooth muscle cells (Gaston et al., 1994).

In contrast with the other two NOS isoforms (nNOS and eNOS), which are expressed constitutively and activated by mediator-induced or stress-induced cell activation, iNOS activity is primarily regulated transcriptionally and is commonly induced by bacterial products and pro-inflammatory cytokines. As such, inflammatory diseases of the respiratory tract, such as asthma, acute respiratory distress syndrome (ARDS) and bronchiectasis, are commonly characterized by an increased expression of iNOS within respiratory epithelial and inflammatory-immune cells, and a markedly elevated local production of NO, as an additional host defense mechanism against bacterial or viral infection. The drawback of such excessive NO production is its accelerated metabolism to a family of potentially harmful reactive nitrogen species (RNS), including peroxynitrate and nitrogen dioxide, especially in the presence of phagocyte-generated oxidants. The formation of such RNS is thought to be the prime reason why NO can be considered as a pro-inflammatory mediator (contribute to the etiology of inflammatory lung diseases) (Gaston et al., 1994; Grisham et al., 1999).

Reynaert et al. in 2005 discussed the presence of high levels of nitric oxide in the expired breath of asthmatic patients and proposed the possible therapeutic benefits of NO inhibitors in the treatment of these patients. NO containing steroid moiety in its structure may provide useful anti-inflammatory drugs along with bronchodilating property.(Tallet et al., 2002).

Possible future prospects of NO-related therapy for inflammatory conditions

NO has a double-edged role endogenously. It is an essential physiological signaling molecule mediating various cell functions, but on the other hand, it induces cytotoxic and mutagenic effects when present in excess (under oxidative stress condition). Thus, in this objective the concern will be on suppressing the overproduction of NO in which it causes inflammatory tissue damage.

Such suppression will occur through inhibition of the $_{L}$-arginine/nitric oxide pathway by different mechanisms using several agents including:

1. Selective NO biosynthesis inhibitors which inhibit the inducible (but not constitutive) NOS.
2. Synthetic arginine analogues, which compete with arginine and are useful experimental tools.

Selective NO biosynthesis inhibitors

The selective inhibition of enhanced NO synthesis is a new, so far exclusively experimental therapeutic strategy in the treatment of chronic inflammatory, non-infectious diseases (Di Rosa et al., 1990; Radomski et al., 1990).

Some established drugs for the therapy of these diseases inhibit activity or expression of the inducible NO synthase, which may contribute to their anti-inflammatory effects (Radomski et al., 1990).

Glucocorticoids inhibit expression of the inducible NO synthase, but have no effects on the activity of both inducible and constitutive NO synthases (Di Rosa et al., 1990; Radomski et al., 1990). The mechanism of action is complex and includes inhibition of transcription and translation, as well as reduced enzyme stability (Kunz et al., 1994).

Cyclosporin derivatives inhibit NO synthase expression. This could be explained by their actions on IL secretion and by direct effects on gene transcription (Muhl et al., 1993). Similar effects on the expression of inducible NO synthase are described for non-steroidal anti-inflammatory drugs (Kepka-Lenhart et al., 1996). However, the mechanism and clinical implications of these findings remain unclear.

In addition, salicylates are scavengers of NO. 5-Aminosalicylic acid was found to reduce both NO production and disease activity in inflammatory diseases especially in adjuvant arthritis (Grisham and Miles, 1994; Stichtenoth et al., 1997).

Specific and selective inhibition of the inducible NOS is so far possible only in animal experiments to some extent. For use in humans, only highly selective and non-toxic substances are suitable, since the pro-inflammatory NO production by the inducible NO synthesis, but not the homeostatic NO synthesis by the constitutive enzymes, must be inhibited. The latter inhibition can lead to vasoconstriction and platelet aggregation, both of which would augment the inflammatory tissue damage (Miller and Clark, 1994).

A number of substances for selective inhibition of pathological NO over production are now under development.

Synthetic arginine analogues

Drugs can inhibit NO synthesis or action by several mechanisms. Currently, the most useful drugs are arginine analogues, which compete with arginine for NOS and in some cases, also compete with the carrier that transports arginine into endothelial cells. Several such compounds, e. g. N^{G}-monomethyl-L-arginine (L-NMMA) and N^{G}-nitro-L-arginine methyl ester (L-NAME), have proved to be of great value as experimental tools. The use of L-NMMA is being investigated in disorders where there is overproduction of NO (e. g. inflammation and neurodegenerative diseases). Disappointingly, L-NMMA increases mortality in one such condition (sepsis) (Grisham and Miles, 1994; Stichtenoth and Frölich, 1998).

Besides selective inhibitors of the inducible NOS activity and synthetic arginine analogues, several other targets of pharmacological intervention have emerged: inhibition of enzyme transcription and translation, cofactor and substrate supply (Miller and Clark, 1994).

Undesired effects of NO

The undesired effects of NO are due to over or impaired production of such mediator and the affected endothelium becomes, as a result, damaged or dysfunctional. The following effects can result in vasoconstriction (e.g., coronary vasospasm, elevated systemic vascular resistance, hypertension), platelet aggregation and adhesion, which can lead to thrombosis, up-regulation of leukocyte and endothelial adhesion molecules leading to enhanced inflammation, vascular stenosis or restenosis as occurs following balloon angioplasty and stent placement and increased inflammation and tissue damage mediated by reactive oxygen species such as superoxide anion and hydroxyl radical (Malmström and Weitzberg, 2004).

Conclusion

In conclusion, NO plays important roles in the pathophysiology of inflammatory disorders. These roles are crucial and controversial at the same time. Therefore, further studies are required to understand the whole picture of such mediator. However, various established non-steroidal anti-inflammatory drugs having NO releasing properties have been under intense clinical evaluations in the treatment of pain and inflammatory disorders (Marshall et al., 2006; Fiorucci et al., 2004; Hoogstraate et al., 2003) Furthermore, these combinations may have gastrointestinal protective properties.(Wallace, 2006; Ellis et al., 2005; Brzozowski et al., 2000; Ukawa et al., 1998).

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