Nitric oxide (NO) a byproduct of the oxidative reaction catalysed by nitric oxide synthase (NOS) that converts L-arginine to citrulline, act as a pleiotropic mediator in various physiological and pathophysiological conditions. The gaseous free radical, nitric oxide (half life < 15 sec) is readily oxidised to NO₂ and NO₃⁻, and is highly reactive to free thiols and transition metal ions. Nitric oxide synthase has three major isoforms, neuronal (nNOS) and endothelial (eNOS) being constitutive, inducible (iNOS) is expressed only following the induction by the inflammatory mediators such as lipopolysaccharide (LPS), interleukins (IL-1, IL-11) and tumour necrosis factor (TNF-α).

Cells that contain isoforms of NOS, the agents that activate and inhibit them, and the molecules with which their products interact, trigger a diverse of physiological effects in mammals. In addition to its role in regulating vessel tone, blood vessel dilatation, neurotransmission, modulation of the hair cycle, and penile erections, NO plays a vital role in host defence and immunity, including the modulation of inflammatory responses. The expression of iNOS is regulated by the balance of cytokines in the microenvironment; for example, transforming growth factor p (TGFp), IL-4, and IL-10 inhibit iNOS expressions in macrophages. Interestingly, iNOS can be expressed in many cells when they are exposed to stimulatory cytokines, including cells that constitutively express eNOS. A key concept is that there are species and cell variability with regard to the regulation of iNOS expression. For example, in vitro, while iNOS is readily induced by IL-1β and TNF-α in murine macrophages, human monocytes and macrophages have been demonstrated to express iNOS in a variety of disease states, including rheumatoid arthritis (RA), malaria, and vasculitis, suggesting that in vivo when exposed to the requisite stimuli, these cells are capable of iNOS upregulation, where many pathological conditions are the manifestations of elevated NO. In such conditions suppression of iNOS is found to be effective, although many chemical inhibitors of iNOS are available but recently more efficient suppression of iNOS at post-transcriptional level by using DNAzymes specific to iNOS mRNA has been shown to be successful both in vitro and in vivo.

Recent insight has been provided by reports that interferon-γ (IFN γ) can induce iNOS messenger RNA (mRNA) and protein in human monocytes both in vivo (patients undergoing treatment for chronic hepatitis C) and in vitro.

Nitric oxide is toxic to bacteria, tumour cells in presence of cytokines and other human pathogens. The induction of iNOS in response to excessive cytokine production is a nonspecific event which will occur in a wide variety of cell types. Increased production of NO (and/or increased iNOS expression in tissues) has been implicated in sepsis.

Recently, a Korean herbal formula Kagamjuaguiew (KJE) has been found to mimic NO production in murine macrophage in conjunction with IFN-γ for potentiating tumour therapy. However, KJE is almost neutral in NO production by itself. Such compounds can have high pharmacological values and more safe as the compounds are natural.

Numerous studies have established that endogenously generated or exogenously supplied NO is potentially toxic in macrophages, pancreatic β-cells, chondrocytes, and thymocytes. In contrast, it has also been reported that NO inhibits neuronal cell death induced by withdrawal of neurotrophic factors from culture medium, and that NO inhibits apoptosis in human B cell lines or in endothelial cells treated with TNF-α. Thus, the role of NO in cell death is controversial, and the precise mechanisms of its effects are unclear. Recently NO has been implicated as a key modulator in
induction of apoptosis in tumour cells by many complex mechanisms that are yet to be resolved. Many lines of evidences have come out where NO has proved beneficial in anti-tumour therapies inducing cytotoxicity directly or indirectly. Photodynamic therapy (PDT) is a promising therapeutic modality used for the cancer treatment. The principle is based on the formation of singlet oxygen and other activated oxygen metabolites that result in apoptotic tumour cell death. The search for the chemical agents, therefore, which are able to enhance the anti-tumour activity of radiation therapy and induce the tumour cell apoptosis, is of great importance. The use of pharmacologic agents such as donors of nitric oxide (NO) or modulators of NOS can be one of the approaches to improve the therapeutic efficiency of PDT.

Nitric oxide synthases play a crucial role in controlling blood flow, memory formation, and the immune response. These proteins can be structurally divided into oxygenase and reductase domains. The reductase domain shares a high degree of sequence homology with P450 reductase, which is thought to be the major enzyme responsible for the one-electron reduction of foreign compounds, including bioreductive anti-tumour agents currently undergoing clinical trials. In view of the structural similarities between NOS and P450 reductase, these have been investigated for the capacity of NOS to reduce the hypoxic cytotoxin tirapazamine, the anti-tumour agent doxorubicin, and also the redox cycling compound menadione. Study suggests that the NOS could play a key role in the therapeutic effects of tirapazamine, particularly because NOS activity is markedly increased in several human tumours. In addition, the presence of NOS in the heart indicates that these enzymes may contribute to the cardiotoxicity of redox cycling drugs, such as doxorubicin.

NO plays a central role in angiogenesis as a mediator of signaling by vascular endothelial growth factor and other angiogenic factors. Low concentrations of NO produced in response to angiogenic factors stimulate angiogenesis, whereas higher concentrations typical of inflammatory responses inhibit angiogenesis. The proangiogenic activity of NO is mediated by activation of soluble guanylyl cyclase. Roberts et al. have identified components downstream of NO as the primary target of the endogenous angiogenesis inhibitor thrombospondin-1 and have shown that circulating levels of thrombospondin-1 are sufficient to limit angiogenic responses by antagonizing NO signalling. This provides new insights into the significance of the widespread loss of thrombospondin-1 expression during tumour progression.

Our understanding of cell-cell communication is dominated by a single paradigm: signaling is accomplished by molecules that bind covalently to specific receptors through complementarity of shape. The most surprising insight to arise from NO research is that there exists a fundamentally different form of intercellular signaling. In this new system, the messenger molecule reacts with its targets covalently on the basis of their redox potential. The latter system may prove to be as ubiquitous and physiologically important as the former. Covalent bonding, redox active intercellular messengers are not limited to reactive intermediates of the oxidation of nitrogen. It has been known for years that mammalian cells have a widespread ability to generate reactive intermediates of the reduction of oxygen. It has only recently been appreciated that reactive oxygen intermediates have broad potential to act as secretagogues, enzyme activators and regulators of transcription along with their more familiar roles as enzyme inactivators, antisepsics, cytokotins, and mutagens. Close biochemical and biological parallels between reactive nitrogen intermediates prompt the hypothesis that NO may share the ability of reactive oxygen intermediates to activate proteases, protein tyrosine kinases, protein kinase C, fos, jun, and NF-kB. This could result in differentiative responses in NO producing or neighbouring cells, including the induction of cytokines or growth factors. Support for this hypothesis is provided by Magrinat et al. that NO gas and NO donors can induce mRNA for tumour necrosis factor in leukaemic cells and promote their morphologic differentiation. The ability to induce cytokine synthesis and other differentiative responses may underlie the presumptive role of NO in remodelling of synaptic connections. A major challenge for physiology is to understand what confers specificity and diversity on intercellular signaling by covalently reactive messengers. The tempo and extent of release of these molecules are likely to be among the most critical variables. In this view, the high chemical reactivity of NO and its short half-life represent “two different sides of the same biologic coin”. Evolution appears to have detected advantages on both sides, giving rise to nitric oxide’s dichotomous functions. In host defence, specially anti-tumour and antimicrobial activities, a large, sustained flux of NO that can be immunologically induced in many and perhaps most nucleated cells may
represent a useful weapon precisely because NO is tiny, membrane-permeant, and broadly reactive. Against such a toxin, it may be difficult to evolve to a resistant state, based either on lack of uptake or on dispensability of intracellular targets. Thus, in its high-output mode, production of NO may represent a primitive, broad-spectrum, antimicrobial, antiviral and anti-tumour defence. On the other side of the coin, for rapid regulation it may be advantageous to resort to brief puffs of small amounts of an agonist-induced, Ca^{2+} regulated mediator with a short half-life. Evolution may also have found a use for the edge of this coin, middle-level mediator with a short half-life. To utilize the capacity of this major systems of intercellular communication will turn out to be connected. To utilize the capacity of this versatile tiny molecule for anticancer therapy we have to explore a lot to understand its potential yet more thoroughly.

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