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Editorial

Nitric oxide (NO), a signaling molecule with a killer soul

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This edition of Cell Death and Differentiation contains a series of reviews that discuss the role of nitric oxide (NO) in the modulation of cell survival and differentiation. These articles reflect the growing knowledge on the role of NO as an inhibitor or activator of cell death, and as modulator of cell differentiation and immune response.

NO is generated from the oxidation of L-arginine to L-citrulline, by a family NADPH-dependent enzymes, the NO synthases (NOS) (Figure 1). This family consists of three isoenzymes: the constitutively expressed neuronal NOS (bNOS), the endothelial NOS (eNOS), and the cytokine-inducible NOS (iNOS) (Figure 1). Among the molecules involved in cell signaling, NO is a diffusable gas with different chemical forms (NO^+, NO^-, $NO^\cdot$) and it has perhaps the most diverse degree of chemical reactivity. NO-mediated reactions seem to utilise a relatively common biochemical trigger (e.g., S-nitrosylation or nitration of proteins) (for a summary of NO chemical reactions see Figure 1). These mechanisms are responsible for the regulation of an impressive number of normal and abnormal functions in biological systems.[1,2] This complexity and diversity in biological actions of NO arises from a very complex production, storage and chemical reactions, as indicated in Figure 1. As neurotransmitter, NO regulates intestinal peristalsis, autonomic and neuroendocrine functions, and it plays a role in regulation of behavior. Neuronal differentiation and cell growth in general are also affected by NO. In contrast with its 'good' nature, NO can also cause relevant injury when its generation overwhelms defence mechanisms. For example, NO has been implicated in neuronal injury, such as that found in brain ischemia and Parkinson's disease and in killing of immune cells. In addition, NO can prevent the lethal action of other cytotoxic molecules, by interfering with the execution of cell death. NO is both a rapid physiological messenger and a cytotoxic mediator.

The targets and mechanisms of NO-mediated physiological actions are different from those involved in its cytotoxic effects.[3] For example, NO-elicited post-translational redox modification of protein thiols such as S-nitrosylation and disulfide formation, are reversible reactions which predominantly have regulatory functions in biological systems, including cytoprotection (Figure 2). On the contrary, irreversible modifications of cysteine residues to sulfenic and sulfinic acid are presumably associated with loss of protein function and cytotoxicity (Figure 2).

The role and mechanisms of NO-induced cell death, apoptosis or necrosis, has attracted growing interest over the past few years. NO in its various forms can elicit or suppress the natural cell death program, apoptosis. It has become clear that, depending on its concentration, the biological redox milieu, and the involvement/induction of intracellular defense mechanisms, NO can either suppress apoptosis and eventually stimulate proliferation or activate the cell death program.[4,5] The controversial actions of NO cannot be explained only by its different chemical forms and reactivity. Often the cell type is a relevant determinant of its effects on survival or death. For example, in cerebellar granule cells, which establish intricate synapses, NO can cause an excitotoxic loop, by stimulating glutamate release and apoptosis.[5] In this system, NO clearly does not block caspase activation that is involved in the execution of apoptosis.[6] NO also induces macrophage apoptosis, which is accompanied by accumulation of the tumour suppressor gene p53, changes in the expression of pro- and anti-apoptotic Bcl-2 family members, caspase3-like protease activation, and cytochrome c translocation.[7]

In contrast, as neatly described in the reviews by Dr. Stamler,[8] Dr. Dimmeler[9] and Dr. Billiar,[10] NO can block apoptosis by either inhibiting active caspases[11,12] or their activation.[13] Activation of caspases can be prevented by cGMP-dependent mechanisms, by nitrosylation of upstream signaling systems or, as recently shown, by inhibition of mitochondrial complexes, which in turn leads to ATP depletion.[4,5]

Protection from excitotoxic N-methyl-D-aspartate (NMDA)-mediated neuronal cell death is associated with down-regulation of excessive NMDA receptor activity following S-nitrosylation of the thiol groups of the receptor.[14] NO-mediated cell protection may also be achieved through the suppression of the superoxide/hydrogen peroxide-mediated cytotoxic effect by acting as a scavenger of reactive oxygen species or through the induction of protective proteins such as cyclooxygenase-2.[7] Upregulation of iNOS prevents apoptosis elicited in an *in vivo* model of tumour necrosis factor α-plus D-galactosamine-induced liver injury.[10] In this case the protective effects of NO would involve either the induction of the heat shock 70 response or the activation of the soluble guanylyl cyclase.

Unfortunately, it seems difficult to relate NO effects to a single biochemical reaction even in a given system. Caspase inhibition alone is not sufficient in some systems to fully rescue cells. Thus, NO has been shown to block cell death in some experimental systems, but more often it simply switches apoptosis into necrosis as initially proposed by us,[11] and indicated in Figure 3. NO may differently influence cell fate depending on its level of

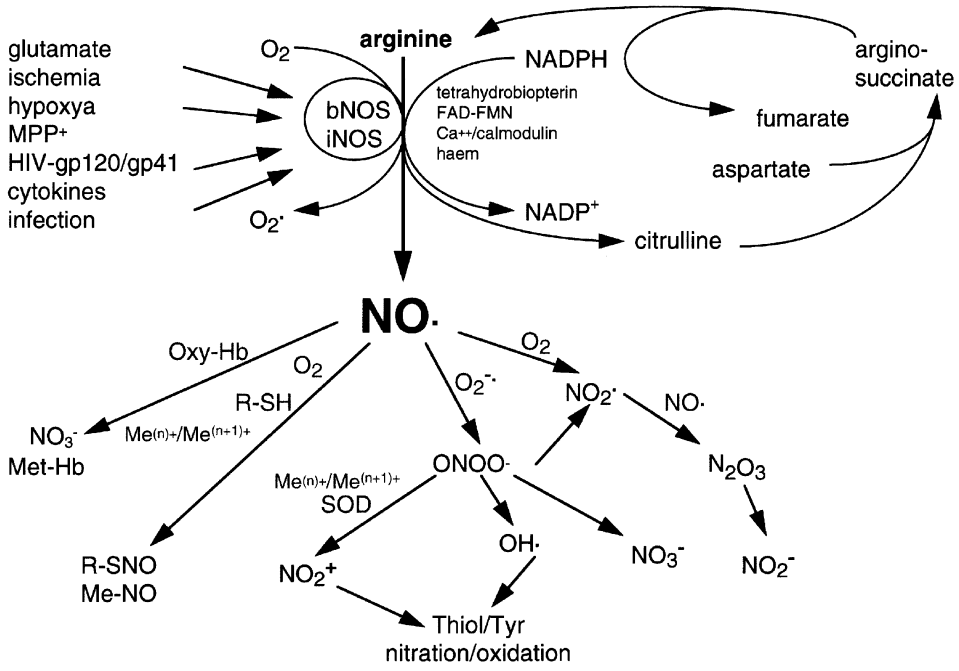

Figure 1 Schematic representation of the NO metabolism inside the cell. Both the synthesis (arrows before NO) and the chemical reactions exerted by NO (arrows after NO) are regulated in a highly complex manner at enzymatic and redox level, in order to produce a large variety of biological effects. The intracellular storage of NO, as well as a fine regulator of its bioavailability, is R-SNO where R-SH is glutathione (GSH). GSH binds directly NO as S-nitrosoglutathione (GSNO) or indirectly as dinitrosyl iron complex (DNIC), or more precisely diglutathionyl dinitroso iron ($Fe(NO)_2(RS)_2$). For more detailes see references[1,2]

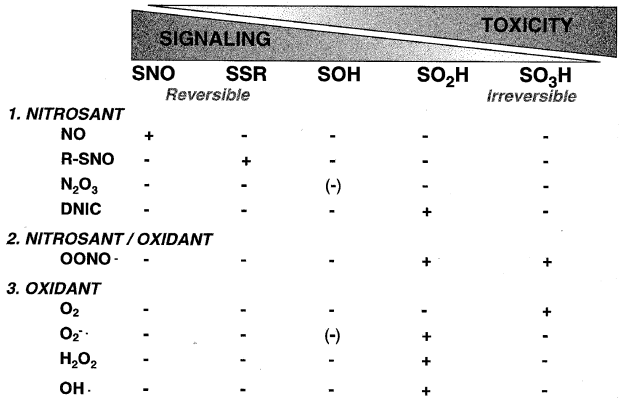

Figure 2 Simplified reactions of NO with thiols. The product of the reaction varies from S-nitrosylation (SNO), to sulfenic acid (SOH), to sulfinic acid (SO_2H), from reversible to irreversible adducts. While the figure elucidates the complexity of post-translational modifications of cysteines by redox-related species, it indicates a nitrosative/oxidative continuum where signaling might result in toxicity. While the reversible reactions involved in signaling seem more biologically relevant, exerting crucial physiological effects, the irreversible reactions might produce pathological phenomena. DNIC is dinitrosyl iron complex, e.g. diglutathionyl dinitroso iron ($Fe(NO)_2(RS)_2$). The scheme has been modified from Dr. Stamler[3]

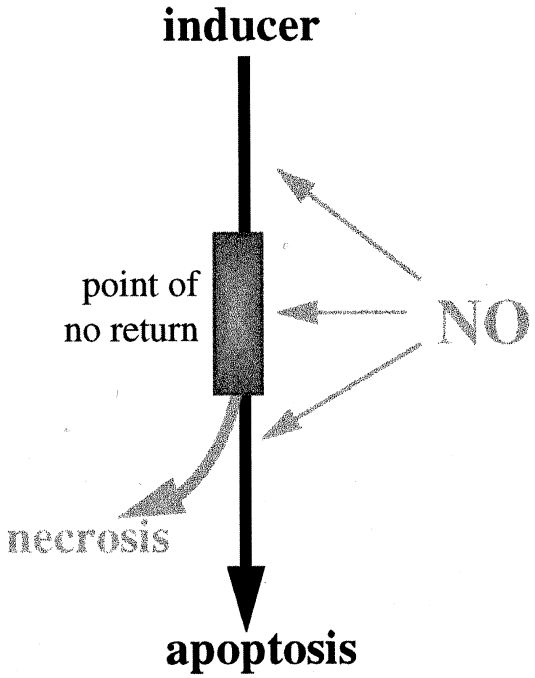

Figure 3 NO is able to interfere at different levels during the apoptotic pathway. Depending on the location of the major action, NO might result in inhibition of apoptosis (upstream of the point of no return), or in necrosis (downstream of the point of no return). Alternatively, the chemical reactions elicited by NO might also cooperate to apoptosis. Therefore, NO is in theory able to modulate apoptosis by increasing survival, enhancing apoptosis, or shifting death into necrosis. Therefore NO might act as a fine regulator of cell death

interaction with the apoptotic pathway. Indeed, NO may interfere with a cell which has received an apoptotic signal either upstream or downstream of the point of no return. The inactivation of the execution machinery in a cell which has still not been committed to die, might result in prevention of apoptosis and resistance to death. Alterna-

tively, the inhibition of apoptosis downstream of the point of no return, might lead to a change of the type of cell death from apoptosis towards necrosis.

It is finally apparent that modulation of differentiation and immune modulation, which eventually occurs at low physiological NO levels may have in common the nitration or nitrosylation of few key molecules. These include transcription factors and receptors and provide a neat mechanism to warrant at the same time regulated proliferation and differentiation while possible inhibiting cell death. Dr. Enikopulov nicely describes these effects of NO in his article.[15] NO plays an active role in the development of the visual system of *Drosophila*.[15] NO promotes differentiation of immature neurons eliciting growth arrest and is a modulator of nerve growth factor survival function. In *Drosophila*, NO acts as an antiproliferative agent during tissue differentiation and organ development; indeed, the inhibition of NOS results in surplus cell proliferation with excessive growth.

Some questions need to be kept in mind when reading these articles. First, it is unclear under which conditions NO can primarily nitrate or nitrosylate proteins *in vivo*. Recovery of nitrosylated enzymes has now become possible, but it does not tell us whether that particular nitrosylation in cells is the responsible of the biological effect attributed to NO. Second, the concomitant or alternative generation of superoxide radical may change the outcome by shifting reactivity of NO towards nitration. While these problems are under intense investigation by several laboratories, it is clear that the way forward is to elucidate the role and effects of NO in each single case without a pre-conceptual mechanism.

The possibility that the expression of NOS in different cell system and that enhanced NO generation can contribute to the protection or vulnerability of cells to toxicity is an open question to be addressed by future development in this area.

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