NEW NITRIC OXIDE DONORS WITH THERAPEUTIC POTENTIAL

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NEW NITRIC OXIDE DONORS WITH THERAPEUTIC POTENTIAL (Abstract): Nitric oxide (NO), formerly known as the endothelium-derived relaxing factor, is a mediator with a key role in the body, both in the central nervous system and in periphery. NO is synthesized by several cell types, where it acts as an autocrine and paracrine signaling molecule. Harnessing the impressive therapeutic potential of nitric oxide (NO) remains an ongoing challenge. In order to overcome the limitations linked with the use of nitric oxide and specially to increase the release of the radical in the targeted area, promising therapeutic strategies have been implemented, based on specific technologies which create releasing agents and vehicles for nitric oxide. Organic nitrites are the most known NO donor drugs, used especially in the treatment of cardiovascular diseases. In recent years, technological advances have allowed obtaining variations synthetic derivatives (such as diazeniumdiolates, S-nitrosothiols), which can generate NO in a controlled mode in the body and to chemically stabilize it; these compounds were studied with promising results in various animal models of vasospasm and pulmonary hypertension. Another high value therapeutic path is represented by the development of hybrid drugs (new nonsteroidal anti-inflammatory NO donor agents), with practical applications in inflammatory disorders accompanied by pain. Also, there is increasing evidence of the existence of NO donors with important antioxidant and hepatoprotective effects. Keywords: NITRIC OXIDE, NO DONORS, DIAZENIUMDIO-LATES, S-NITROSOTHIOLS.

During the 1980s, the free radical nitric oxide (NO) was discovered to be a crucial signaling molecule, with a variety of pharmacological activities in the cardiovascular, nervous and immune systems. In 1992, the free radical nitric oxide was awarded the title ‘molecule of the year’ (1).

Formerly known as the endothelium-derived relaxing factor, nitric oxide (NO) has been intensively studied over the years, its importance in the body being fully recognized in 1998 when the Nobel Prize for medicine was awarded to a team of researchers for their studies on the biological effects of this molecule (2). Despite its structural simplicity, NO plays an active role in complex and varied biological processes. An important fact is that its sphere
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of influence is likely to extend to only ~100 µm from its origin. This property has important consequences: first, it means that NO must be rapidly synthesized on demand in response to stimuli and second, it is a local mediator that does not require complex metabolism for clearance; it is simply oxidized to nitrite/nitrate as it diffuses away from its source (3). Therefore, an important property of NO, which distinguishes it from other mediators of the central nervous system, is diffusivity. This is increased in both the aqueous and the lipid media, providing a three-dimensional spreading signal, regardless of the presence of membranes (4).

The most studied actions of NO are on the cardiovascular system, where it is continuously produced by the endothelial cells that line the lumen of blood vessels. Here, L-arginine is converted into NO by the endothelial isoform of the enzyme NO synthase, in response to mechanical and chemical stimuli that act by mobilizing intracellular calcium.

NO diffuses away from the cell of origin, passing easily through membranes of neighboring cells, bringing on a range of physiological effects. NO participates in maintaining and regulating blood pressure, due to its role in adjusting systemic vascular resistance (5, 6).

NO acts almost exclusively in vascular smooth muscle cells (3) via the soluble enzyme guanylate cyclase, stimulating cyclic guanosine-3′,5′-monophosphate (cGMP) production and elicit vasodilation via cGMP-dependent protein kinases. Also, NO has an impact on circulating platelets, where, in response to endothelium and platelet-derived NO, both cGMP-dependent and independent mechanisms play a part in its powerful inhibitory effect on aggregation and adhesion (7, 8).

Considering that a characteristic of many cardiovascular diseases is the reduced level of NO in the soluble guanylate cyclase pathway, an intake of exogenous NO would be a possible and attractive therapeutic choice (6, 9).

**NO donors in the cardiovascular disease**

The translation of therapeutic potential of NO to bedside has been slowed down by its short biological lifetime, instability during storage and potential toxicity. Taking these into account, new drugs have been obtained, with the role of molecular NO vehicles (also named NO donors), created for stabilizing this radical, until the moment when it needs to be released (10). Increased understanding of the essential role of nitric oxide (NO) in various physiological processes and diseases has stimulated the development of multiple pharmacologic strategies to release NO at the involved level of pathogenic pathways (11). These approaches include therapies that directly or indirectly release or “donate” NO and the use of existing drugs, such as antihypertensive agents (e.g. nebivolol) and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) which enhance NO bioactivity in the body (12).

Capitalizing on the biological actions of NO, these therapeutic strategies are providing new insights into the pathogenesis and treatment of various diseases, including hypertension, atherosclerosis, cancer, Alzheimer’s disease, colitis and asthma (3, 13).

The organic nitrites are the most commonly used NO donor drugs: glyceryl trinitrate (GTN; also, known as nitroglycerin) has been the most studied nitrate, used mainly in the relief of acute pain associated with angi-
na, whereas other slower release preparations, such as isosorbide mononitrate, are used for the treatment of chronic angina. The other clinically relevant NO donor in current use is sodium nitroprusside, known to provide rapid lowering of blood pressure in severe hypertensive crises (14, 15).

A great number of synthetic compounds (e.g., N-nitrosamines, S-nitrosothiols, nitrosohydroxylamines and nitrosyl metal complexes) have been developed to chemically stabilize and release NO in a controlled manner, and have been exploited in many biomedical applications (3, 16). Diazene diolates represent another class of NO donors to which increased attention has been paid recently for their ability to generate predictable amounts of NO (17). The biological activity of diazenium diolates was tested with promising results in various experimental models, such as vaso- spasm, pulmonary hypertension, platelet adhesion/aggregation (11, 3).

In addition, S-nitrosothiols are considered an excellent source of NO. S-nitrosothiols-based therapeutics can be considered more efficient than NO-based therapeutics, due to its more prolonged release of NO, as well as a more facile transnitrosating capability (16). These donors were proven to induce more prolonged vasodilation than the other NO donor types tested. Moreover, lipophilic S-nitrosothiols induce even longer vasodilation than the conventional S-nitrosothiols (11).

**NO donors with antioxidant and hepatoprotective effects**

From another point of view, there is an increase of evidence in support of the fact that NO reduces the arachidonic acid-induced oxidative stress. Also, it was demonstrated that antioxidants can prevent the L-N^G^-Nitroarginine Methylester (L-NAME) potentiation of arachidonic acid toxicity. Toxic effects to mitochondria induced by arachidonic acid were demonstrated by a decline in the mitochondrial membrane potential. L-NAME potentiated this decline in mitochondrial membrane potential, this being linked to its increase in arachidonic acid-induced oxidative stress (18).

NO donors decrease the decline in mitochondrial membrane potential, due to their decrease in arachidonic acid-induced oxidative stress (19). These results indicate that NO can exert hepatoprotective effects against CYP2E1-dependent toxicity, preventing arachidonic acid-induced oxidative stress (20). Published research data sets show that NO contributes to liver homeostasis, its production playing an important role in both cytoprotectant and cytotoxicity processes (21, 22).

Experimental data shows that NO seems to be beneficial to hepatocytes, having cytoprotective effects against inflammation and tissue damage and direct cytotoxic effects on invading microorganisms and tumor cells (23, 24).

**NO donors with a beneficial role in reducing inflammation and pain**

Another high value therapeutic path is represented by the development of NO donor hybrid drugs. These are the new nonsteroidal anti-inflammatory NO donor drugs (NO-NSAIDS) that keep the pharmacodynamic actions of the basic compound (analgesia, inhibition of inflammation) as well as have other benefits (assigned to NO release in body) such as the protection of gastric mucosa against the irritating action of NSAIDS (25).

These compounds are prepared by adding a radical such as nitrobutyl or nitro-
sothiol by short-chain ester linkage of an NO-releasing moiety to conventional NSAIDS, such as aspirin (NO-Aspirin), flurbiprofen (NO-flurbiprofen), naproxen (NO-naproxen), diclofenac (Nitrofenac), Ibuprofen (NO-ibuprofen) and indomethacin (NO-indomethacin) (25, 26). Experimental and clinical data sets show that the capacity of NO-NSAID to release NO appears to reduce the gastrointestinal toxicity compared with NSAIDs alone (27).

The various mechanisms that underline the protective effect of NO on the stomach include vasodilation of the gastric mucosa, inhibition of caspase enzymes activity and inhibition of leukocyte adhesion (25).

**CONCLUSIONS**

We concluded that multiple new NO donors (such as: diazeniumdiolates, S-nitrosothiols, nonsteroidal anti-inflammatory NO donor compounds) have been developed in the last years. They were proven to have beneficial pharmacodynamics effects in various experimental models of induced vasospasm, pulmonary hypertension, inflammatory states, acute and chronic liver diseases. As we have outlined in this material, these compounds appear to be promising agents that may be used in the treatment of cardiovascular maladies, inflammatory conditions, liver function disturbances, or of neoplastic diseases.

**REFERENCES**


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**NEWS**

**NEXT GENERATION SEQUENCING (NGS) AS A PROMISING TOOL IN FORENSIC GENETICS**

The biggest challenge for a forensic geneticist is to obtain sufficient information from trace samples, and thus being able to sequence every DNA molecule in a sample is a very intriguing idea. At the present, the forensic markers are typed using PCR-CE and there are individual assays for autosomal STRs, Y-chromosome STRs, X-chromosome STRs, indels, mtDNA SNPs, autosomal SNPs, Y-chromosome SNPs etc. The most important advantage of NGS is that all these assays could be combined into a single NGS assay. This will save time when dealing with cases where supplementary investigations are necessary and reduce the overall time needed for processing a sample. Another advantage is that the DNA fragments do not need to be separated by lengths in CE, so all the analyzed fragments can be designed to be as short as possible, increasing the chance of typing degraded DNA (Claus Børsting, Niels Morling. Next generation sequencing and its applications in forensic genetics. *Forensic Sci Int Genet* 2015; 18: 78-89. doi: 10.1016/j.fsigen.2015.02.002).

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