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Therapeutic Uses of Nitric Oxide-donating Drugs in the Treatment of Cardiovascular Diseases

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Abstract: Nitric oxide biology has been extensively studied and several aspect of its roles in physiological and pathophysiology clarified. Nitric oxide exerts critical and diverse functions in the cardiovascular system. Impairment of NO production and/or function plays a key role in a number of cardiovascular disease processes, including atherosclerosis, thrombosis, restenosis, cerebral vasospasm and hypertension. Replacement of endogenous NO with exogenous drug-derived NO has been a common medical practice for many years, and these pharmacological compounds present an attractive option in the treatment of cardiovascular disease. Nitric oxide donors either donate NO directly or are pro-drugs that require enzymatic metabolism to generate bioactive NO. Direct NO donors such as organic nitrates have been used for many years in cardiovascular therapeutics but they exhibit limitations such as the development of nitrate tolerance and their inability to improve patient outcome among others. Cofactor-dependent and enzymatically active pro-drugs such as molsidomine, s-nitrosothiols, diazeniumdiolates and mesoionic oxatriazoles and bifunctional NO-donors such as NO-aspirin are potential alternatives to conventional nitrates. This review focuses on the chemical and pharmacological characteristics of conventional and novel NO donors, and their therapeutic potential in the treatment of a variety of cardiovascular disorders.

Key words: Nitric oxide, cardiovascular, donors, conventional, novel

INTRODUCTION

Physiology and pathophysiology of NO in cardiovascular system: Approximately twenty five years after its discovery, nitric oxide (NO) has fully reached an established position in physiology, medicine and therapeutics. NO is an inorganic free radical which is one of the smallest known biologically active messenger molecule. It is a colourless gas with good water solubility. The chemical properties of NO are crucial to its biological roles, both as a transcellular signal in the cardiovascular and nervous systems and as a cytotoxic antipathogenic agent released during the inflammatory response.

Endogenous nitric oxide is synthesized from the amino acid L-arginine by three isoforms of the enzyme NO synthase (NOS) (Moncada *et al*., 1991). The endothelial (eNOS) and the neuronal (nNOS) isoforms that synthesize NO for transcellular signalling are constitutively expressed and tightly regulated by a number of cofactors (Griffiths and Stuehr, 1995). These NOS isoforms typically synthesize small amounts of NO and require activation by Ca^{2+}-calmodulin (Abu-Soud and Stuehr, 1993), making them sensitive to agents and processes that increase intracellular calcium levels. The NO generated diffuses to neighbouring target cells where it acts primarily through activation of soluble guanylyl cyclase (sGC) to generate cGMP from GTP, and bring about the cellular response through a reduction in intracellular calcium levels (Waldman and Murad, 1987). Inducible NOS (iNOS) is not expressed constitutively but is induced in response to pathogenic challenge (Steuhr and Marletta, 1987) or in cardiovascular pathologies like atherosclerosis and heart failure (Curtis and Pabla, 1997). When iNOS is up regulated, the high NO output results in local concentrations of NO that reach cytotoxic levels (Moncada *et al*., 1991).

Nitric oxide exerts critical and diverse functions in the cardiovascular system. In the healthy cardiovascular system, NO synthesized in the endothelium influences a number of cellular processes central to vascular proliferative diseases including protection against thrombosis and atherogenesis through inhibition of monocyte and platelet adhesion, platelet aggregation and smooth muscle proliferation (Cooke and Dzau, 1997).

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Dysfunction in NO synthesis has been implicated as a major contributory factor in development of a wide range of cardiovascular diseases including atherosclerosis, hypertension, heart failure, coronary heart disease, arterial thrombotic disorders, and stroke (Ignarro *et al*., 1999). The detrimental effects of reduced NO synthesis, as a result of enzyme dysfunction or endothelial damage, are often exacerbated in cardiovascular disease by increased generation of oxygen free radicals which rapidly inactivate NO forming cytotoxic peroxynitrite (Gryglewski *et al*., 1986).

The replacement or augmentation of endogenous NO by exogenous NO-donating drugs to correct the insufficiency in a variety of clinical conditions has provided the foundation for a broad field of pharmaco-therapeutics in cardiovascular medicine. Replacements of endogenous NO with exogenous drug-derived NO have been a common medical practice for many years. Organic nitrate and nitrite esters have been widely used to treat angina pectoris, myocardial infarction and congestive heart failure for more than a century. However, treatment with conventional nitrate preparations is limited by their therapeutic half-life, systemic absorption with potentially adverse haemodynamic effects and drug tolerance. To overcome these limitations, novel NO donors that offer selective effects, a prolonged half-life, and a reduced incidence of drug tolerance have been developed. The recent developments of novel classes of NO donors might facilitate a move away from conventional nitrates to potentially superior drugs which could help to prevent progression of cardiovascular disease and improve outcome. The major objective of this review is to examine conventional and novel NO-donating compounds that are used in the treatment of cardiovascular conditions.

Enzymatically-activated NO prodrugs: Classic nitrovasodilators and newer compounds such as nicorandil and nitroaspirins belong to this group of NO donors. The classic nitrovasodilators, glyceryl trinitrate (GTN), amyl nitrite, isosorbide dinitrate (ISDN), and isosorbide 5-mononitrate have been used to treat cardiovascular diseases (Gruetter *et al*., 1981). These drugs represents the prototypical form of NO-replacement therapy and their principal action is vasorelaxation, mediated by guanylyl cyclase activation and by direct inhibition of nonspecific cation channels in vascular smooth muscle cells (VSMCs).

Nitrate tolerance, defined as loss of therapeutic effect during continuous administration, is the major therapeutic limitation of nitrate therapy. Nitrate tolerance is associated with increased angiotensin II-dependent vascular production of superoxide anion from NADPH-linked oxidase and eNOS (Munzel *et al*., 1995). The superoxide anion generated by these enzymes reacts with NO derived from the NO donor to form peroxynitrite, as indicated by the findings of increased urinary 3-nitrotyrosine in nitrate-tolerant patients (Skatchkov *et al*., 1997). Tissue thiol depletion is also a likely cause of nitrate tolerance as it provides a satisfactory explanation for the partial reversal of tolerance by delivery of exogenous thiols such as N-acetylcysteine (NAC) (Horowitz *et al*., 1983). Nitrate tolerance can be partially reversed by antioxidant species such as vitamin C (Watanabe *et al*., 1998), vitamin E (Watanabe *et al*., 1997) and low molecular weight thiols, L-arginine, tetrahydrobiopterin, hydralazine, ACE inhibitors, and folate which have been successfully used to reverse or prevent tolerance (Loscalzo, 2001). Other limitations of nitrate therapy include severe headaches and flushing in some patients, particularly when delivered in formulations with rapid onset. They can also cause postural hypotension and are not recommended as an adjunct to other vasodilator drugs or in patients with low blood pressure (Parker, 1987). Sinitrodil belongs to the benzoxazinones, a class of organic nitrates. The major potential advantage of sinitrodil over conventional nitrates is its selectivity for large arteries, imparting coronary vascular selectivity over GTN and ISDN (Monzani *et al*., 1999) However, the mechanism of sinitrodil has not been fully characterized and it is not known whether it engenders tolerance or is cross-tolerant with conventional nitrates.

The hybridization of NO donor moieties with currently available cardiovascular drugs is an innovative recent development in NO donor research. Nitro-aspirins are nitrate ester compounds and include 2-acetoxy-benzoate 2-(2-nitroxy-methyl)-phenyl ester (NCX - 4016) and 2-acetoxy-benzoate 2-(2-nitroxy)-butyl ester (NCX-4215). NCX-4016 is chemically related to aspirin and is able to release NO and to inhibit cyclo-oxygenase (COX) (Llechi *et al*., 1996). NCX-4016 is a stable compound that requires enzymatic hydrolysis to liberate NO, and the kinematics of this metabolic processing lead to durable production of NO released at a constant rate from the site of metabolism (Wallace and Cirino, 1994).

The biological activity of NCX-4016 has been evaluated in different experimental models to characterize its anti-inflammatory and anti-thrombotic effects. NCX-4016 has been shown *in vitro* to prevent the release of thromboxane (TXB_2) (Llechi *et al*., 1996) from activated human platelets through irreversible inhibition of platelets, and reduce the degree of restenosis after balloon angioplasty in hypercholesterolemic mice which was associated with reduced VSMC proliferation and macrophage infiltration at the site of arterial injury

(Napoli *et al*., 2001). Furthermore, NCX-4016 reduced infarct size in experimental myocardial ischaemia (Wainwright *et al*., 2002), attenuated the development of atherosclerosis in hypercholesterolemic mice (Rossoni *et al*., 2000) and dose-dependently inhibits the release of TBX_2 and cytokines from stimulated human monocytes (Minuz *et al*., 2001). This suggests that NCX-4016 may have a favourable profile of activities in clinical setting of athero-thrombosis. However, cross-tolerance between NCX-4016 and GTN have been observed in a cultured kidney epithelial cell line, and prolonged treatment with NCX-4016 caused down-regulation of the cellular cyclic GMP response, suggesting that tolerance may occur during therapy with NCX-4016 (Grosser and Schroder, 2000). Captopril reacts with NO to form S-nitrosocaptopril (SNOCap), an S-nitroso derivative and a hybrid compound of ACE and NO (Loscalzo *et al*., 1989). S-nitrosocaptopril is a unique compound with properties of both a direct nitrovasodilator and an ACE inhibitor and is not cross-tolerant with GTN (Loscalzo *et al*., 1989).

Direct NO donors: These pharmacological compounds possess either a nitroso or nitrosyl functional group and in contrast to organic nitrates, they spontaneously released NOx (defined as a range of redox forms of nitrogen monoxides generated by the donor molecules). Direct NO donors include sodium nitroprusside, sodium trioxodinitrate and NONOate or diazeniumdiolate such as diethyltriamine-NO and spermine NONOate. In sodium nitroprusside (SNP), NO is coordinated as a nitrosyl group ligand to iron in a square bipyramidal complex and is released spontaneously at physiological pH from the parent compound (Ignarro *et al*., 1999). Sodium nitroprusside has been used clinically as a vasodilator, principally in the treatment of hypertension and heart failure. Tissue dependent NO generation from SNP is thought to be mediated by membrane-bound proteins (Kowaluk *et al*., 1992), although some studies suggest that SNP may also exert its effects through cGMP-independent mechanisms (Otsuka *et al*., 1988). SNP is a markedly more potent inhibitor of platelet aggregation than nitrates (Chirkov *et al*., 1999), but it is not used therapeutically as an antiplatelet agent. The use of this nitrovasodilator is limited by the need to administer it parenterally, by tolerance, and by the potential for the development of thiocyanate toxicity with prolonged administration (in rhodanase-deficient individuals) (Vesey and Cole, 1985). Despite these limitations, SNP is often the NO drug of choice in clinical studies and in the laboratory, particularly when a reference cGMP-mediated response is required.

In response to the clinical demand for new NO donors, diazeniumdiolates or NONOates [N(O)NO] were also developed. Diazeniumdiolates are a unique class of nitric oxide-based vasodilators that are formed when NO reacts with polyamine (Morley and Keefer, 1993). Polyamine NONOates are a class of soluble NO donors produced by combining NO with compounds such as putreanine, spermine, and spermidine. Although generally stable in solid form, they decompose spontaneously in solution to generate up to two NO radicals per molecule, capable of stimulating sGC in a number of biological systems (Ying and Dusting, 1997; Du *et al*., 1998; Billi *et al*., 1997). The predictable rate of NO generation offered by NONOates and the range of compounds available are distinct advantages over most other donor drugs.

Preclinical studies have already identified a number of potential applications for NONOates, particularly in cardiovascular diseases. Spermine diazeniumdiolate (spermine-NONOate) incorporated into a silastic periarterial collar has been shown to prevent formation of neointima in rat carotid artery (Yin and Dusting, 1997), while intracarotid infusion of diethylamine-NONOate (DEA-NO) has been shown to both reverse and prevent vasospasm in a primate model of subarachnoid haemorrhage (Pluta *et al*., 1997). Diethylamine-NONOate has been shown to be associated with better preservation of coronary artery flow and cardiac function in the donor rat heart following transplantation (Du *et al*., 1998). Intratracheal instillation of another NONOate, 2-(dimethylamino) ethylputreamine (DMAEP-NO), has been shown to selectively dilate the pulmonary vasculature with minimal systemic haemodynamic effects. A related compound, ethylputreanine NO (EP/NO, an ester NONOate with an uncharged end group) and SNP had both systemic and pulmonary effects (Billi *et al*., 1997). Aerosolization of dimethylamino-ethylputreamine/NO (Jacobs *et al*., 2000) selectively reduces pulmonary artery pressure and pulmonary vascular resistance, and improves oxygenation in porcine models of pulmonary hypertension and oleic acid induced acute lung injury.

Cofactor-dependent NO prodrugs: These heterocyclic NO-releasing compounds include those of the oxatriazolium class (sydnonimines) and the furoxan class and require cofactors to facilitate NO release. The sydnonimines require oxidants such as molecular oxygen, and the furoxans require thiols. The NO donor, 3-morpholino-sydnonimine (SIN-1) is the vasoactive metabolite of the anti-anginal prodrug molsidomine (N-ethoxycarbonyl-3-morpholinosydnonimine). Molsidomine is bioactivated in the liver to SIN-1 *in vivo*, and subsequent transformation of SIN-1 to SIN-1A in the blood via hydroxylation reaction. Molecular oxygen initiates conversion of SIN-1A to SIN-1C, resulting in the

release of NO (Noack and Feelisch, 1989). Soluble guanylyl cyclase is the most important physiological target of SIN-1; SIN-1 which has been shown to stimulate sGC activity by an NO-mediated pathway in blood vessels (Plane *et al*., 1998). The activation of sGC by SIN-1 involves the formation of S-nitrosoglutathione, a thionitrite that activates sGC via trace metal-catalyzed release of NO (Schrammel *et al*., 1998).

Nitrovasodilation is a prominent therapeutic strategy in the treatment of coronary artery disease. The pharmacological profile of sydnonomines is similar to that of organic nitrates, although the onset of action is slower and the preload-reducing effect is more pronounced with sydnonimines. They are therapeutic alternatives to organic nitrates in stable angina, coronary vasospasm and congestive heart failure (Lehmann *et al*., 1995), with the obvious benefit of avoiding tolerance. Unlike organic nitrates, molsidomine and SIN-1 appear to have significant antiplatelet activity at therapeutic doses (Drummer *et al*., 1991). SIN-1 might also help to prevent atherosclerosis by inhibiting the oxidation of low density lipoprotein (Rikitake *et al*., 1998) and smooth muscle cell proliferation (Groves *et al*., 1995). However, large-scale clinical trials have failed to reach firm conclusions as to the effect of short-term treatment of molsidomine and organic nitrates on survival following myocardial infarction (The ESPRIM trial, 1994). The therapeutic potential of molsidomine and its metabolites is limited by cogeneration of superoxide, and chronic treatment with SIN-1 increases fatty streak development in a rabbit model of atherosclerosis (De Meyer *et al*., 1997). Furthermore, although SIN-1 is considered resistant to tolerance, its short duration of action necessitates an increase dose frequency which might prove inconvenient in a clinical setting (Lehmann *et al*., 1998).

In response to clinical demand for new NO donors, substituted mesoionic oxatriazoles (MOTA) were developed. Mesoionic 3-aryl substituted oxatriazole-5-imine derivatives are structurally related to sydnonimidines and include mesoionic oxatriazole imines (GEA 3162, GEA 5024) and their derivatives, sulphonylamides (GEA 3175, GEA 3268), amides (GEA 3233, GEA 3232). The biological activity of some of these compounds (e.g., GEA 3162) might be mediated through spontaneous NO generation on dissolution, while others (GEA 3175) are seemingly stable *in vitro* but still retain biological activity (Kankaanranta *et al*., 1996). Furthermore, enzymatic degradation of the sulfonamide moiety has to take place before NO is released (Karup *et al*., 1994).

Mesoionic 3-aryl substituted oxatriazole-5-imine derivatives demonstrate a wide range of NO-mediated actions including vasodilation, inhibition of platelet aggregation (Plane *et al*., 1998), inhibition of smooth muscle proliferation (Lahteenmaki *et al*., 1998), and prevention of oxidation of lipoproteins (Malo-Ranta *et al*., 1994). They synergize with prostacyclin in their anti-thrombolytic actions and suppress the release of histamine and leukotriene B_4 and prevent degradation of granulocytes (Corell *et al*., 1994). GEA compounds are particularly effective NO donors in eliciting dilation of bronchioles, with GEA-3175 significantly more effective than SIN-1 and SNAP (Hernandez *et al*., 1998). By manipulating the chemical structures of MOTA, it may be possible to obtain selectivity towards vasorelaxant, antiplatelet, thrombolytic or tracheorelaxant properties. These compounds may also prove more beneficial than conventional organic nitrates because they do not engender tolerance, and are better than SIN-1 because there is no apparent cogeneration of oxygen (Holm *et al*., 1998).

S-nitrosothiols general formula (RS-N = O) constitute another class of NO donor drugs which are formed by S-nitrosation of thiols or cysteine residues of proteins. S-nitrosothiols such as S-nitrosocysteine (SNOCys), S-nitroso-N-acetylpenicillamine (SNAP), S-nitrososglutathione (GSNO) and S-nitroso-N-acetylcysteine (SNAC) can decompose spontaneously in solution to their disulphide form, generating NO in the process (Williams, 1985) and present a potential alternative to current NO donors, particularly as they do not appear to engender vascular tolerance (Megson *et al*., 1999). The beneficial effects of S-nitrosothiols are largely mediated by GSNO, however their therapeutic potential is limited by the rapid and unpredictable nature of their decomposition due to the catalytic effect of trace Cu(I) ions (Williams, 1985).

S-nitrosoglutathione (GSNO) has been shown to have several potential cardiovascular benefits. GSNO is a powerful *in vitro* inhibitor of platelet activation (Radomski *et al*., 1992), prevent platelet expression of P-selectin and of glycoprotein IIb/IIIa following coronary balloon angioplasty (Langford *et al*. 1994) and is of significant benefit in patients with acute myocardial infarction and unstable angina (Langford *et al*., 1996). Furthermore, GSNO is a platelet-selective antithrombotic agent (Salas *et al*., 1998) with arterioselective vasodilator properties (DeBelder *et al*., 1994) and has been shown to reduce the rate of embolization in humans (Molly *et al*., 1998). Research has also shown that GSNO is beneficial to patients with pre-eclampsia (Lees *et al*., 1996) and reduced arterial blood pressure in anaesthetized dogs, making it a potential antihypertensive agent (McGrowder *et al*., 1999).

SNAP is one of the most commonly used S-nitrosothiols in experimental setting, however NO released from SNAP is, however, unpredictable largely due to its sensitivity to Cu (I) ions (Williams, 1985). It has been shown to have powerful vasodilator and antiplatelet activity in the cardiovascular system (Ignarro *et al*., 1981) and was found to significantly reduce mean arterial blood pressure in animals (McGrowder *et al*., 2001). However, its use as an antiplatelet agent is limited by its intense vasodilatory, and hence hypotensive effects. Analogs of SNAP, such as RIG-200 and SNVP which are Cu(I) resistant have significant potential in the treatment of cardiovascular disease. RIG-200 consist of SNAP linked to acetylated glucosamine and causes prolonged vasodilation in isolated arteries with damaged endothelium (Megson *et al*., 1997) and SNVP (the valeryl analog) have prolonged nitric oxide mediated *in vitro* vasodilation in rat femoral arteries (Megson *et al*., 1999).

The NO donor (+)-(E)-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide (FK409) is a structurally unique compound which has been discovered from the fermentation products of *Streptomyces griseosporeus*, with vasodilator and antiplatelet activity (Hino *et al*., 1989). Unlike nitrates, FK409 is very unstable, with a half-life of approximately 45 min at 37°C in phosphate buffer (Fukuyama *et al*., 1997) although the rate of NO release can be accelerated by endogenous thiols (Fukuyama *et al*., 1995). Furthermore, although FK409 releases NO rapidly in plasma (Kita *et al*., 1994), it may have limited clinical use. The NO-releasing rate of ((+)-N-4-ethyl-3-[(Z)-hydroxyimino-5-nitro-3-hexen-1-yl]-3-pyridinecarboxamide; FR144420), a derivative of FK409 in which the amide group of FK409 was substituted for methylene amide moiety, was lower than that of KF409. FR144420 also decompose and release NO spontaneously at 37°C in sodium phosphate buffer but at a slower rate than FK409 (Kita *et al*., 1995a). Furthermore, FR144420 significantly decreased mean blood pressure for a longer duration after intravenous administration in rats, compared with FK409 (Kita *et al*., 1996). Both FR144420 and FK409 have been shown to be more powerful inhibitors of platelet aggregation than nitrates (Kita *et al*., 1995b), SIN-1 and SNP (Kita *et al*., 1995a) and in contrast with organic nitrates their continuous exposure would not be expected to lead to depletion of the sulfhydryl groups (Isono *et al*., 1994). The release of NO from FK409 and FR144420 could show beneficial effects in the treatment of cardiovascular diseases such as angina (Feelisch and Noack, 1991) and atherosclerosis (Barrett *et al*., 1989).

CONCLUSIONS

The establishment of the role of NO in various physiological and pathological conditions has opened up the possibility of designing new drugs that are capable of delivering NO into blood streams in a sustained and controlled manner. The strategy has been to identify novel molecules with an improved pharmacological activity either in terms of enhanced therapeutic efficacy or reduced side effects. NO-donating drugs have proven useful therapeutic options in the treatment of cardiovascular diseases. The beneficial effects of organic nitrates are mediated by NO and, despite their limitations they remain the most commonly used drugs in cardiovascular medicine. S-nitrosothiols have great potential as therapeutic agents as they have been shown to improve the indices of maternal well-being of pre-eclamptic patients, prevent restenosis post-angioplasty, and are useful alternatives to organic nitrates, as they do not lead to the development of tolerance. Few human studies have been done with S-nitrosothiols and so there is need for large-scale trials that will evaluate their full therapeutic potential and their future use in clinical practice. There is evidence from animal studies which supports the potential of the novel NO-donor drugs described in this review. However, it is difficult to draw conclusions from the few existing human studies with regards to the effects of NO released from these drugs in cardiovascular diseases due to a lack of well-controlled protocols. Furthermore, due to the ubiquitous nature of NO, most of these drugs affect more than one physiological function. There is a clear requirement for the development of NO donor drugs that will target specific tissues and thus improve outcome.

With the wide range of potential therapeutic effect of novel NO donors, it is possible that in the future the use of these drugs may be viable therapeutic options in broad-based treatment of cardiovascular diseases. Further work involving well-controlled clinical trials is required to unravel the complexities of their mechanism of action and the search for new drugs being developed through the exploitation of the basic and clinical knowledge linked to nitric oxide is continuing.

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