MINIREVIEW

Nitric Oxide Donors (44565)

TADAHIKO YAMAMOTO AND RICHARD J. BING[1]

Department of Experimental Cardiology, Huntington Medical Research Institutes, Pasadena, California 91101

Abstract. **Nitric oxide (NO) donors are pharmacologically active substances that release NO *in vivo* or *in vitro*. NO has a variety of functions such as the release of prostanoids, inhibition of platelet aggregation, effect on angiogenesis, and production of oxygen free radicals. This report discusses the chemical and pharmacological characteristics of NO donors, their effect on platelet function and cyclooxygenase, their cardiac action including myocardial infarction, and release of superoxide anions. This review stresses NO tolerance and the effect of NO donors on angiogenesis in myocardial infarction and in solid tumors.** [P.S.E.B.M. 2000, Vol 225:200–206]

Nitric oxide (NO) donors are pharmacologically active substances that, *in vivo* or *in vitro*, release NO. The discovery of nitric oxide as an endothelium-derived relaxing factor has established a pharmacological basis for the role of NO donors (1, 2). It is apparent that the activity of NO, a volatile free radical gas, is not confined to vasodilatation alone. Rather NO has a variety of functions, such as release of prostanoids in infarcted myocardium (3), inhibition of platelet aggregation and leukocyte adhesion (4), preservation of vascular impermeability (5), an effect on angiogenesis (6), and production of oxygen free radicals (7).

Chemical and Pharmacological Characteristics

Figure 1 describes the structure of NO donors. All of them have a nitrate functionality within the molecule, and a nitroso functional group is present in all of these compounds. Glyceryl trinitrate (GTN; nitroglycerin) is the nitrate ester of glycerol. In sodium nitroprusside (SNP) a molecule of nitric oxide is coordinated to iron metal forming the square bipyramidal complex. 3-Morpholinosydnonimine (SIN-1) is a zwitterionic compound formed by combination of a morpholine and a sydnonimine. S-nitroso-N-acetyl-penicillamine (SNAP) is an N-acetylated amino acid derivative with a nitrosothiol functional group. Diethylenetriamine/NO (DETA/NO) is a compound of nitric oxide covalently linked to diethylenetriamine. NCX 4016 is an m-nitroxymethyl phenyl ester of acetyl salicyclic acid.

The amount and duration of NO release by the respective NO donors determines their pharmacological properties. *In vivo*, some compounds act rapidly, and the amount of NO released is relatively small. In others, such as NCX 4016 (NO aspirin), the effect is slow and lasts for hours (8). The route of administration (oral and parenteral) and the duration of release of NO also differ. NO is connected with a specific molecular target; by binding to iron in the heme group of guanylate cyclase, it produces cyclic guanosine monophosphate (cGMP), which activates a cascade of cellular processes (9).

GTN. GTN releases NO into the headspace of subcellular fractions of bovine coronary smooth muscle cells (10). To be pharmacologically active, NO donors belonging to the group of organic nitrates involve biochemical conversion (biotransformation) of the nitrate group to NO (11). Studies have shown that GTN is metabolized in cultured smooth muscle cells by a glutathione-dependent pathway. Specifically, the glutathione-S-transferase and glutathione are involved in GTN metabolism by smooth muscle cells (12). It was first proposed that the biotransformation of

[1]To whom requests for reprints should be addressed at the Department of Experimental Cardiology, Huntington Medical Research Institutes, 99 N. El Molino Avenue, Pasadena, CA 91101. E-mail: cardio@hmri.org

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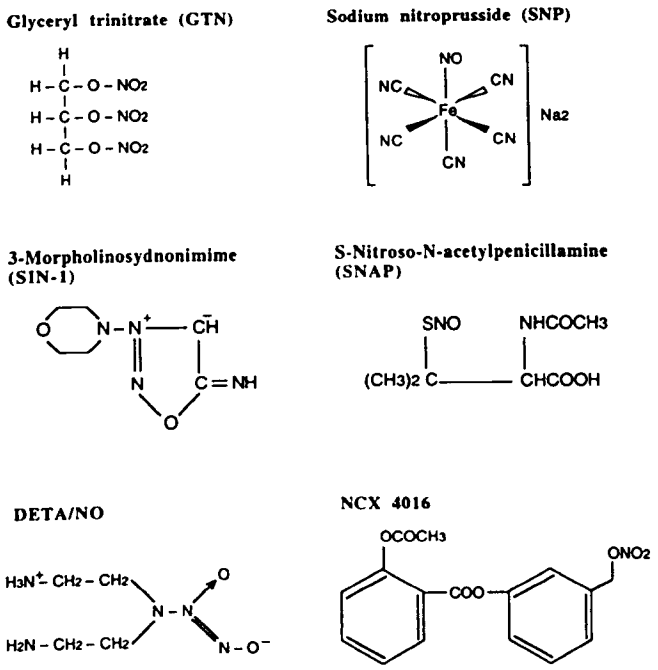

Figure 1. Chemical structures.

GTN involves a multistep process through the formation of S-nitrosothiols (2). It is likely that the release of NO from GTN is primarily enzymatic. Chung and Fung (10) have provided evidence of release of NO by GTN *via* an enzyme system attached to the cellular surface membrane.

SIN-1. SIN-1, the enzymatic hepatic metabolite of molsidomine, produces NO. By means of physicochemical and enzymatic methods, Feelisch *et al.* (13) demonstrated that, in addition to nitrite and nitrate, superoxide anions are generated by SIN-1 during the liberation of NO, thus changing the extent of stimulation of soluble guanylate cyclase. SIN-1 is metabolized by enzymatic hydrolysis and decarboxylation. This metabolite is then spontaneously converted to SIN-1C by cleavage of NO, forming SIN-1 A as an intermediate metabolite (14).

SNP. The nitroprusside dianion is a complex of ferrous ion with five cyanide anions (CN^-) and a nitrosonium ion (NO^+). NO production by SNP requires the presence of vascular tissue (15, 16). Interaction of SNP with a reducing agent such as a thiol leads to the formation of NO (15). Light is an essential condition in the release of NO from solutions of SNP, and vascular tissues promote the release of NO. When protected from light, SNP does not release NO, and the release depends on the intensity of light (17). Nonenzymatic 1-electron reduction does release NO. It is likely that the release of NO by vascular tissue, including cell membranes and smooth muscle cell membranes, serves this reducing function. NO formation is accompanied by cyanide (CN^-) release and can be inhibited by exogenous cyanide (15). Cyanide is rapidly metabolized by the liver to thiocyanate that is released by the kidney. Furthermore, released NO is such a powerful vasodilator that SNP is effective at doses that do not produce toxic amounts of cyanide (17).

SNAP. SNAP belongs to the class of S-nitrosothiols. In common with other NO donors (S-nitroso-glutathione, S-nitroso-albumin, and S-nitrosocaptopril), S-nitrosothiols act by releasing NO (2, 18–20). These substances are important intermediates in the metabolism of organic nitrate and endogenously derived NO (18). The long half-lives of naturally occurring S-nitrosothiols in blood are the result of their low reactivity for plasma components and lack of reactivity for red blood cell membranes (19). The formation of NO from SNAP involves both tissue-independent and tissue-dependent processes. For example, bovine vascular smooth muscle exhibits substantial catalytic activity for NO generation from SNAP (16, 21).

DETA/NO. DETA/NO is a 1-substituted diazen-1-ium-1,2-diolates, containing a $[N(O)NO]^-$ group, generally referred to as diazenium diolates (22). It has a half-life of 20 hr and releases NO without prior biotransformation (23).

NO Aspirins. A new group of NO donors, attached to an aspirin moiety, has been synthesized recently: NCX 4016 (2 acetoxybenzoate 2-(1-nitroxy-methyl)-phenyl ester) and NCX 4215 (2 acetoxybenzoate 2-(2-nitroxy)-butyl ester). The former possesses a second benzene ring to which the lateral chain containing the NO group is bound (24). These compounds were originally designed to protect the gastric mucosa against the effect of aspirin. NO-generating compounds have been shown to reduce the severity of mucosal injury, probably because of NO and prostacyclin production. Prostacyclin protects gastric mucosa by preserving blood flow and increasing the synthesis of mucus (25, 26). NO aspirins possess antiaggregating effects because of inhibition of cyclooxygenase (COX) by aspirin and formation of soluble guanylate cyclase by NO (27).

Platelet Function and Effect on Cyclooxygenase

As to be expected from its antiaggregation action, NO in therapeutic doses inhibits platelet function in patients with acute ischemic syndromes (28). It inhibits platelet aggregation through stimulation of guanylate cyclase. NO donors also activate the enzyme COX, leading to the production of prostanoids, including prostacyclin and thromboxane A2 (29, 30).

SIN-1 inhibits intracoronary platelet aggregation *in vitro* (31). Continuous infusion of SIN-1 inhibits intracoronary platelet thrombus formation (32). SIN-1 also releases prostacyclin and inhibits platelet aggregation (4). GTN stimulates coronary vascular prostaglandin formation at concentrations that are in the range of therapeutic plasma levels (33). The antiaggregating activity of SNAP is stable for 60 min when incubated at 37°C (34).

The addition of an NO-producing moiety to aspirin profoundly influences the pharmacodynamics of aspirin. Wallace *et al.* (35) carried out a comparison of aspirin (100 mg/kg) and equimolar doses of NCX 4215 (166 mg/kg). Whereas both compounds result in the appearance of

plasma salicylates, the increase after aspirin in plasma was more rapid, more prolonged, and three-fold higher. NCX 4016, as compared with aspirin, markedly increased cGMP concentration in the medium (27). NCX 4016 was more effective than NCX 4215 in inhibiting thromboxane A2 production (36).

Cardiac Action of NO

NO has important functions on the cardiovascular system. NO has infarct-sparing effects through its preconditioning activity (37) and reduces myocardial ischemia (38). The cardioprotective effect of NO likely acts by generating free radicals and activating protein kinase C and the signal transduction pathway associated with ischemic preconditioning (39). Ventricular myocytes from rat hearts decrease contractile response to β-adrenergic agonists following exposure to NO (40). NO protects against ventricular fibrillation (41). cGMP formed from NO reduces lactate accumulation in the hypoxic ventricular muscle (42) and, through cGMP, inhibits anaerobic cardiac metabolism by blocking phosphofructokinase (43).

GTN has a relaxant effect on all types of vessels. Coronary arteries have been found to be more sensitive to GTN than peripheral arteries. It has been shown that in commonly used concentrations, GTN is a powerful dilator of large coronary arteries, but produces only a relatively small and brief effect on coronary flow. This suggests that GTN has little effect on the smaller coronary vessels. Harrison and Bates (17) mention potential explanations for the failure to increase coronary flow, such as autoregulatory influences, absence of guanylate cyclase in the vascular smooth muscle, and inability of the smaller coronary microvessels to form an active vasodilator from GTN. Bing *et al.* (44, 45) found a different response of coronary arteries to GTN dependent on the presence of atherosclerotic changes. They demonstrated no increase in coronary blood flow after administration of GTN in patients with coronary heart disease. However, in patients without coronary artery disease, administration of GTN increased coronary flow.

The hemodynamic effect of SIN-1 consists of a decrease in left-ventricular systolic, mean arterial, and end-diastolic pressures, stroke volume, and systemic vascular resistance. Heart rate and diastolic coronary blood flow were increased (46). SNP is an effective afterload reducing agent on both right and left ventricles because of the fall in peripheral and pulmonary vascular resistance. SNAP *in vivo* is a more potent vasodilator than GTN, acting to the same degree on the arterial and venous beds (47). However, Tseng *et al.* (48) found that in arterial conductant vessels, GTN is a more potent vasodilator than SNP.

DETA/NO is cytostatic, has antiplatelet effects, and causes marked vasodilation (49, 50). DETA/NO also possesses significant negative inotropic effects (diminution in dP/dT and left ventricular pressure). Its negative inotropic effect and its intravenous administration preclude its use as an antianginal agent. It possesses pronounced antiproliferative activity as a result of inhibition of ribonucleotide reductase and reaction with aconitase (49, 50). This antiproliferative activity has suggested its use in the prevention of restenosis following coronary angioplasty (23).

NO Donors and Myocardial Infarction

Production of NO and formation of prostaglandins are increased in infarcted myocardium (51). The inducible form of NO synthase (iNOS) by activated macrophages releases NO (52, 53). An L-arginine-dependent pathway in macrophage monolayers synthesizes L-citrulline and nitrite, which, when coupled to an effector mechanism, inhibit DNA synthesis and mitochondrial respiration (54). iNOS is inhibited by dexamethasone (55), higher doses (150 mg/kg) of aspirin (51), arginine analogs such as N^G-nitro-L-arginine, and other specific inhibitors (56).

Both 6-keto prostaglandin F1α and thromboxane B_2 (the stable products of prostacyclin and thromboxane A_2) increase in infarcted myocardium together with NO, indicating the close relationship between NO and prostanoids (51). The action of NCX 4016 on the infarcted heart is the result of both COX inhibition and NO production (3). Whereas aspirin alone lowers the concentrations of prostanoids, NCX 4016 activates COX and thus the formation of prostanoids from arachidonic acid (Fig. 2). Thus, it partially counteracts the effects of aspirin. At the same time, NCX 4016 significantly increases the oxidation products of NO in

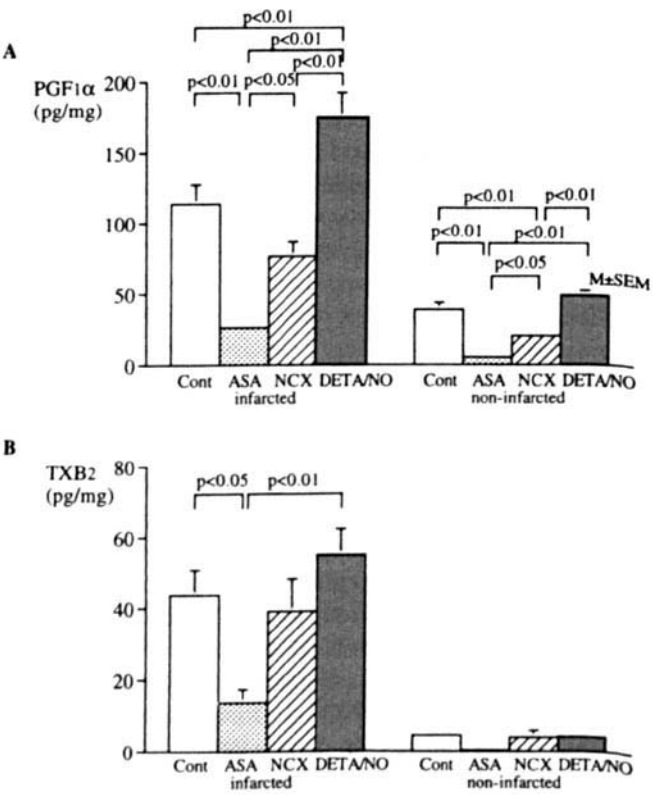

Figure 2. The effect of aspirin (ASA), NCX 4016 (NCX), and DETA/NO on (A) 6-keto prostaglandin F1α (PGF1α) and (B) thromboxane B_2 (TXB_2) in infarcted and noninfarcted portions. All of the values in the infarcted region significantly elevated as compared with the noninfarcted region ($P < 0.01$). Cont: control.

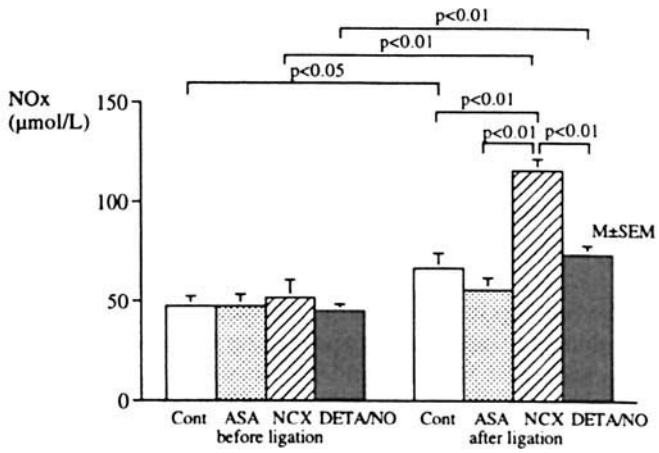

Figure 3. Changes in plasma nitrite and nitrate (NO_x) concentrations in arterial blood following ligation of a coronary artery. A significant change in NO_x concentration in arterial blood was seen following administration of NCX 4016. A slight increase was noted in NO_x concentration following DETA/NO administration. Cont: control.

plasma beyond that encountered after myocardial infarction alone (Fig. 3). DETA/NO administered intravenously (1mg/kg/day) significantly increases myocardial 6-keto prostaglandin F1α production in the infarcted myocardium (Fig.2) (3). However, it has no effect on thromboxane B_2 production or on myocardial activation of iNOS. Only a small increase in the oxidation products of NO in plasma following administration of DETA/NO is noted (Fig. 3).

NO Tolerance

Tolerance to GTN is a well-known clinical phenomenon. Efficiency of GTN diminishes after 18–24 hr (17). Tolerance has been ascribed to depletion of sulfhydryl groups, which are necessary co-factors for the biochemical conversion of the nitrate group to NO (57). In contrast to GTN, SIN-1 causes no nitrate tolerance and is not cross-tolerant to GTN (58). It does not require enzymatic bioactivation; however, vascular sulfhydryl groups are not involved in the development of GTN tolerance (59, 60). It is apparent that the underlying mechanisms responsible for nitrate tolerance are multifactorial and may include desensitization of the target enzyme guanylate cyclase or a decrease in GTN biotransformation (61, 62). Physiological (systemic) mechanisms may also play a role in nitrate tolerance such as changes in plasma neurohormonal levels and receptor density (63).

Recently, it was discovered that the formation of superoxide radicals induced by organic nitrate correlates with the development of nitrate tolerance (64). It was further postulated that increased flux of superoxide is a mediator of nitrate tolerance. Superoxides inactivate NO released from either exogenous or endogenous sources (65, 66); therefore, it appears logical to re-establish sensitivity to NO by dietary supplements of various compounds that intercept free radicals (67–70). The development of nitrate tolerance is prevented by supplementation with vitamin C, an antioxidant (71, 72). Vitamin E has also been successful in the prevention of the development of nitrate tolerance (73). Nitrate tolerance can also be prevented by a long-term angiotensin-converting enzyme inhibition; this is probably the result of an endothelium-dependent mechanism involving mainly an enhanced NO availability (74, 75). The beneficial effects of an angiotensin II receptor antagonist on nitrate tolerance may be due to the fact that components of the renin-angiotensin system are important in the development of vascular tolerance (74).

Release of Superoxide Anions by NO Donors

NO donors form peroxynitrite ($ONOO^-$) from endogenous superoxide and liberated NO (76). It was documented that two NO donors, SNP and SNAP, through spontaneous release of NO, are involved in the formation of superoxide anions (76). Other workers described the formation of $ONOO^-$ and subsequent $^{\cdot}OH$ from the simultaneous generation of NO and O_2^- during aqueous decomposition of SIN-1 (7). Eiserich *et al.* (77) found that nitrite, a major end product of NO metabolism, readily promotes tyrosine nitration through formation of nitryl chloride and nitrogen dioxide by reaction with the inflammatory mediators hypochlorous acid or myeloperoxidase. They also showed that activated human polymorphonuclear neutrophils convert nitrite into nitryl chloride and nitrogen dioxide through myeloperoxidase-dependent pathways.

The tissue damaging effect of peroxynitrite may be related to apoptosis (78). Peroxynitrite is a damaging oxidant, produced *in vivo,* that may account for the cytotoxicity ascribed to NO. In the isolated perfused heart, $ONOO^-$ produces coronary vasodilation. It may also be responsible for general and permanent tissue damage (79). It has been suggested that the toxic effects of peroxynitrite are counteracted by the production of NO in a dose-dependent manner, primarily through its conversion to S-nitrosothiols or other NO donors.

Peroxynitrite may play a role in the pathogenesis and progression of atherosclerosis. NO synthase has a dual role dependent on the concentration of NO released (80). In small concentrations, NO and probably peroxynitrite favor an antiatherosclerotic environment. In contrast, during hyperlipidemia and atherosclerosis, NO may contribute to the formation of oxidative stress by reduction of tetrahydrobiopterin, which could favor the development of toxic concentrations of peroxynitrite in atherosclerotic plaques. On the other hand, impairment of endothelial function by atherosclerosis and restoration of endothelium-dependent relaxation are related to altered NO function (81).

The role of free radicals in vascular responses of atherosclerotic vessels was demonstrated by Cathcart *et al.* (82), who showed that free radicals produced by polymorphonuclear leukocytes lead to oxidation of lipids, which are toxic to endothelial cells. The involvement of peroxynitrite in the pathogenesis and progression of atherosclerosis represents additional proof of the role of free radicals. Which of

the multiple factors are responsible in the altered response of atherosclerotic vessels to vasodilators cannot be stated, but it is likely that a combination of interconnected functional and morphological changes alter the response of endothelium or smooth muscle of atherosclerotic vessels to vasoactive substances.

Nitrotyrosine also has serious tissue-damaging effects. It is formed from NO and tyrosine, and the addition of peroxynitrite to tyrosine results in up to 8% yield of nitrotyrosine (83, 84). Nitrotyrosine residues are found within the endocardium, myocardium, and coronary vasculature of patients with inflammatory cardiac diseases (85). This may indicate the presence of a NO-dependent oxidant, possibly peroxynitrite.

NO Donors and Angiogenesis

NO and NO donors have assumed considerable importance in the initiation and promotion of angiogenesis. In angiogenesis, endothelial processes (angioblasts) assemble in a primitive network (vasculogenesis) that expands and remodels (angiogenesis) (86). Angiogenesis initiates with vasodilation. Vascular endothelial growth factor (VEGF) increases vascular permeability, furthering angiogenesis. A number of molecules are involved in angiogenesis, such as angiopoietin 1 and 2, proteinases of the plasminogen activator, chymases, angiostatin, endostatin, COX-2, and others. Aside from VEGF, fibroblast- and platelet-derived growth factors also play a role. Apparently, NO also promotes pathological angiogenesis (86).

Considerable evidence suggests that angiogenesis and NO are related. Much information is derived from a study of NO production by solid tumors. In solid tumors, angiogenesis can be blocked by inhibition of iNOS formation (87, 88) Tumor levels of NO synthase activity and cGMP are significantly higher in tumor tissue, and metastases are more angiogenic than specimens of nonmetastatic tumors. It is also believed that angiogenesis in tumors is an independent predictor of metastases.

In solid tumors, the production of NO and its effect on angiogenesis have been documented in numerous publications (89, 90). Whereas in solid tumors production of NO is undesirable because it promotes growth and spreading of the tumor, in myocardial infarction, NO is advantageous because of the initiation of new vascular growth. The production of cytokines by inflammatory cells with subsequent activation of iNOS and COX occurs both in infarcted myocardium and in solid tumors (91). Nitrosation and oxidative stress are interrelated; NO may have multiple and divergent effects (92). The production of NO may also be responsible for tumor reduction through apoptosis (93); therefore, it is not surprising that chemically derived NO from SNP significantly inhibits growth of allografted pancreatic tumor cells. In addition, prostanoids are found both in infarcted myocardium and solid tumors. In colon cancer, prostanoid production has become of particular importance, since activation of COX-2 leads to increased production of angiogenic factors that induce angiogenesis in endothelial cells (94).

The relationship between NO and angiogenesis has been confirmed repeatedly in infarcted myocardium. NO donors and growth factors induce angiogenesis *in vivo* and cause proliferation of endothelial cells in cell cultures (6, 95, 96). VEGF acts on endothelial cells through NO synthase activation and cGMP production (95). NO production is necessary for the growth-promoting effect of VEGF. These findings have considerable implication for the use of NO donors as angiogenesis-promoting factors in ischemic myocardium.

Recently, a new compound, B-NOD, has been introduced with NO release for 8 hours, no fall in blood pressure and which can be orally administered (97).

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