Drugs of the Future 2002, 27(8): 777-784

CCC: 0377-8282/2002

Review Article

Lipophilic *S*-nitrosothiols: a means of targeted delivery of nitric oxide to areas of endothelial injury?

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Introduction

Endothelium-derived nitric oxide (NO) is recognized to be a key local mediator of vascular relaxation that also protects against atherosclerosis and thrombosis through inhibition of vascular smooth muscle mitogenesis, monocyte adhesion and platelet adhesion and aggregation (1). These effects have largely been attributed to increased intracellular cGMP levels through NO-mediated activation of soluble guanylate cyclase (sGC) in target cells (2). Reduced NO bioavailability as a result of damage to the endothelium, dysfunction in NO synthesis and/or inactivation by oxygen-derived free radicals is a crucial factor in both the initiation and progression of atherosclerosis and restenosis (3), and is also implicated in thrombosis. Delivery of supplementary NO to regions of the vasculature where its availability from endogenous sources is deficient is an attractive therapeutic option (4). In order for such a strategy to succeed without causing potentially detrimental hypotension, however, donor drugs must be selective for areas of vascular injury and, preferably, should demonstrate selectivity for activity in platelets and inflammatory cells over vascular smooth muscle. Organic nitrates are NO donor drugs that are used extensively in the symptomatic treatment of angina (5). The therapeutic benefits of nitrates in this arena are derived from their ability to selectively dilate capacitance veins and collateral coronary vessels, thus reducing cardiac workload while improving oxygen supply to ischemic areas of the heart (6). The venoselective nature of nitrates, their relative inactivity in platelets (7) and the need for interrupted therapy to avoid tolerance, indicate that these drugs are unlikely to meet the criteria that are theoretically optimal for the prevention of atherosclerosis and thrombosis.

New nitric oxide (NO) donors

Diazeniumdiolates, sydnonimines and *S*-nitrosothiols are prominent examples of novel NO donors that might have additional benefits over organic nitrates (8). All are apparently resistant to tolerance but *S*-nitrosothiols probably represent the most promising because they are believed to be platelet selective (9). Furthermore, the existence of endogenous low- and high-molecular-weight *S*-nitrosothiols (10-14) indicates that the functional group at least is nontoxic at physiologically relevant concentrations.

S-Nitrosothiols

A number of endogenous and synthetic *S*-nitrosothiols (general formula RSNO) have been identified. All decompose thermally on dissolution (15) to generate NO and the thiyl radical ($RS^{\cdot}$) which spontaneously dimerizes to form the relevant disulfide (RS-SR) (16). However, the rate at which this reaction proceeds is ultimately determined by the chemical nature of the parent thiol and varies greatly between different compounds. Furthermore, the reaction is highly sensitive to catalysis by trace Cu^{+} ions (17), and is accelerated by Cu^{2+}-containing enzymes (*e.g.*, Cu-Zn superoxide dismutase; SOD) (18, 19), ascorbate (20) and superoxide (O_2^{-}) (21). Clearly, all of these reactions are biologically relevant, suggesting that *S*-nitrosothiols are likely to decompose rapidly on contact with blood, extracellular fluid or cells, raising questions about their suitability as potential NO donor drugs. Moreover, the existence of *S*-nitrosothiol donor drugs in the cellular environment is further compromised through transfer of the NO^{+} (22, 23) moiety to endogenous thiols including cysteine (Cys) residues in plasma albumin, hemoglobin and protein disulfide

isomerases (PDI) (24), as well as the low-molecular-weight thiols, cysteine and glutathione. However, it is now generally accepted that these "transnitrosation" reactions are a useful means of retaining, protecting and transferring NO between cellular compartments, and might represent an important sGC-independent means of altering cell function.

Much of the biological and clinical research in this field to date has centered on the *S*-nitrosated adducts of endogenous thiols, including glutathione (*S*-nitrosoglutathione; GSNO) Cys (*S*-nitrosocysteine; Cys-NO), as well as proteins with available Cys residues (hemoglobin and albumin). Of these, Cys-NO is the least stable, largely on account of an exquisite sensitivity to Cu^{+}-mediated catalysis. *S*-Nitrosated albumin is generally regarded to be the most abundant and stable endogenous *S*-nitrosothiol *in vivo*. The obvious advantage of these compounds from a therapeutic perspective is that they have all been measured in biological systems and their decomposition products are naturally occurring and unlikely to be toxic. Crucially, these endogenous *S*-nitrosothiols appear to be platelet selective (9), raising the possibility of antiplatelet effects without potentially detrimental global vasodilatation and hypotension. They have all been shown to have NO-mediated effects *in vitro* and clinical studies have shown that GSNO has the potential benefits of preventing thrombosis following angioplasty (25) and in reducing formation of emboli (26, 27). Similarly, *S*-nitrosoalbumin has been shown to be a powerful inhibitor of platelet adhesion and, importantly, appears to have selectivity for areas of endothelial damage, probably on account of the naturally adhesive nature of the protein (28). Artificial surfaces coated with *S*-nitrosoalbumin have since been shown to protect against thrombus formation immediately after implantation, (28) promoting speculation that *S*-nitrosoalbumin might represent a suitable coating for stents.

A recent advance in *S*-nitrosothiol development is the *S*-nitrosation of existing nonsteroidal antiinflammatory drugs (NSAIDs) like aspirin and diclofenac (29). Nitrosated versions of NSAID-NO hybrids have evolved from the nitrooxylated prodrug, nitro-aspirin (NCX-4016) (30). This compound apparently retains the beneficial effects of aspirin, while offering the added benefit of NO-mediated protection against ulceration of the gastric mucosa. A potential limitation of nitroxy-ester hybrids, however, is that they are likely to require metabolism to facilitate NO release and might be expected to be prone to vascular tolerance. *S*-Nitrosothiol adducts, on the other hand, could offer the benefits of nitro-aspirin without the threat of tolerance. Early results suggest that NO-hybrid compounds are more effective than the parent NSAID in a number of cardiovascular applications (31-33) and their potential therapeutic benefits certainly merit further investigation.

S-Nitroso-*N*-acetylpenicillamine (SNAP)

N-Acetylpenicillamine is closely related to Cys, but the *S*-nitrosated adduct (SNAP) is more stable than that of Cys. SNAP has long been used as an NO donor *in vitro* and it is well-characterized as a compound with powerful antiplatelet and vasodilator properties. SNAP has also been shown to be resistant to vascular tolerance and cross-tolerance with organic nitrates (34). However, it too is susceptible to Cu^{+}-mediated decomposition, prompting chemists to design more stable analogues. It was soon recognized that the addition of bulky side-chains and groups to *N*-acetylpenicillamine stabilized the molecule by preventing Cu^{+}-catalysis through steric hindrance (35). Thus, dipeptide (36), glycosylated (35) and *N*-substituted derivatives of SNAP (Fig. 1) (37) all displayed increased stability *in vitro* compared to the parent compound. Interestingly, the vasodilator activity of these derivatives proved relatively unaffected, despite their reduced tendency to release NO, suggesting either that release of free NO is not necessarily a prerequisite for biological activity or that a copper-independent common biological pathway exists to release NO from *S*-nitrosothiols, irrespective of their structure.

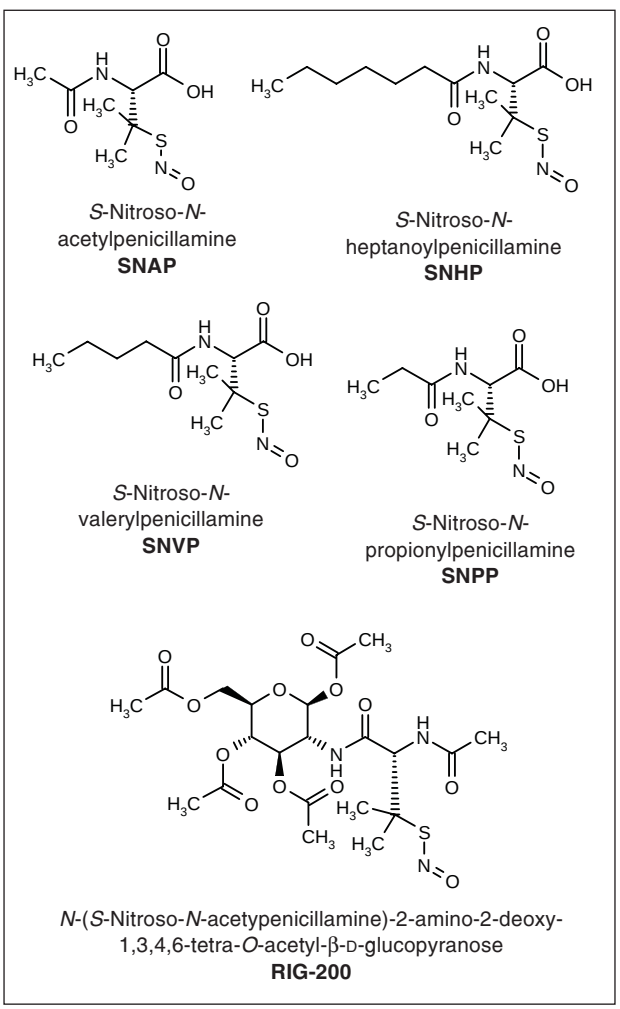

Fig. 1. Chemical structures of SNAP and its lipophilic analogues.

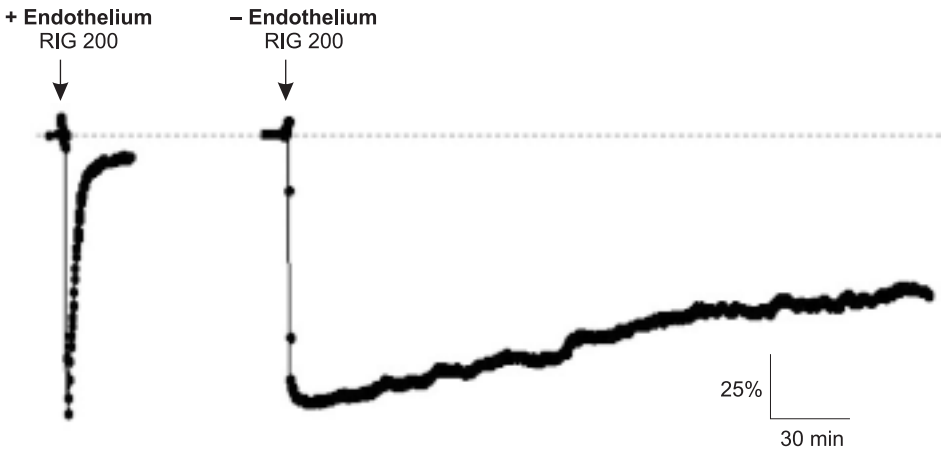

Fig. 2. Vasodilator responses to bolus injections (10 nmol; arrows) of RIG-200 in endothelium-intact and endothelium-denuded isolated perfused rat femoral arteries. The sustained vasodilatation in denuded vessels was fully reversed by the NO scavenger, hemoglobin (10 μM, not shown). (From Megson, I.L. et al. *Prolonged effect of a novel S-nitrosated glyco-amino acid in endothelium-denuded rat femoral arteries: Potential as a slow release nitric oxide donor drug.* Br J Pharmacol 1997, 122: 1617-24. Reproduced with permission from Nature Publishing Group.)

Tissue selectivity of SNAP analogues

The increased stability of SNAP-related *S*-nitrosothiols was recognized to be a significant benefit over the parent compound in terms of handling and reproducibility of vascular responses. However, these modest benefits were overshadowed by an interesting and potentially revolutionary property that was inherent in a number of the SNAP analogues investigated. Bolus microinjections of RIG-200 (SNAP linked to acetylated glucosamine) into the perfusate of isolated perfused, precontracted rat femoral arteries were found to induce unusually protracted (> 4 h) vasodilatation in endothelium-denuded vessels; equivalent responses to RIG-200 in intact arteries lasted for only 5-10 min (Fig. 2) (35). Similar microinjections of SNAP itself recovered rapidly, irrespective of the endothelial integrity. Importantly, the sustained effects of RIG-200 were reversed by the NO scavenger, hemoglobin, ruling out a cytotoxic effect and implying that the phenomenon was entirely NO-mediated. The subsequent development of a family of *N*-substituted analogues of SNAP helped to determine that the sustained activity of SNAP-related *S*-nitrosothiols in endothelium-denuded blood vessels is closely related to their lipid solubility (37). Thus, hydrophilic SNAP fails to demonstrate sustained effects in denuded vessels while its more lipophilic propyl, valeryl and heptonyl counterparts show an increasing selectivity for endothelial denudation (Fig. 3).

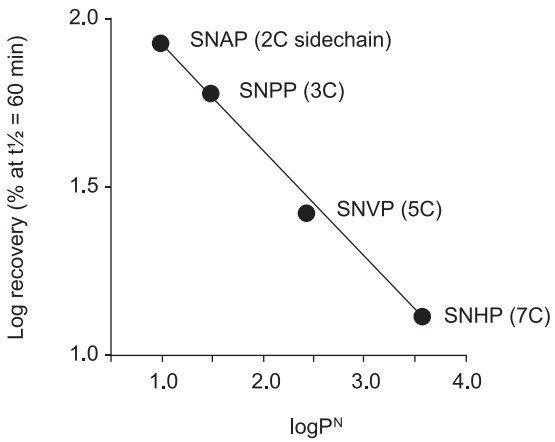

Fig. 3. Relationship between lipophilicity (log P^N) and % recovery of responses to *N*-substituted analogues of SNAP (10 nmol) in isolated, perfused endothelium-denuded rat femoral arteries, measured 1 h after bolus injection. (From Megson, I.L. et al. *N-Substituted analogues of S-nitroso-N-acetyl-D,L-penicillamine: Chemical stability and prolonged nitric oxide mediated vasodilatation in isolated rat femoral arteries.* Br J Pharmacol 1999, 126: 639-48. Reproduced with permission from Nature Publishing Group.)

These results led us to hypothesize that the sustained effects of lipophilic *S*-nitrosothiols are due to their retention in areas where the endothelium has been removed. We intimate that endothelial cells ordinarily represent an effective barrier to the retention of *S*-nitrosothiols in vessels, resulting in transient vasodilatation. Removal of the endothelium, however, exposes lipid-rich subendothelial structures capable of retaining lipophilic compounds that subsequently decompose slowly to facilitate sustained vasodilatation via activation of nearby smooth muscle cell sGC (Fig. 4). The close proximity of the primary target for NO makes for efficient delivery and is likely to result in significant dilatation in response to very small amounts of retained NO donor. This hypothesis is consistent with all our data to date but remains to be confirmed.

Irrespective of the precise mechanism involved, the implications for the therapeutic potential of NO donors

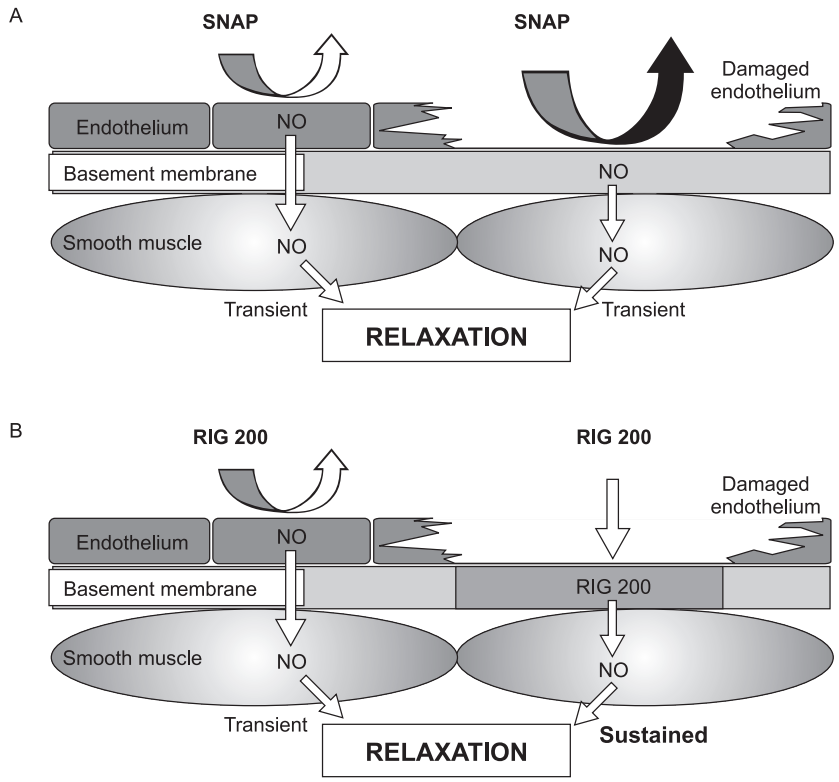

Fig. 4. Hypothesis to explain sustained vasodilatation in response to lipophilic *S*-nitrosothiols in endothelium-denuded arteries. A) Hydrophilic compounds like SNAP are rapidly washed out of blood vessels on cessation of administration, producing transient vasodilatation, irrespective of endothelial integrity. B) Lipophilic *S*-nitrosothiols like RIG-200 are readily washed out of the lumen of endothelium intact vessels but removal of the endothelium exposes lipid-rich elements of the subendothelium (e.g., the basement membrane) that retain the drug for several hours after washout. The drug continues to generate locally active NO long after cessation of drug administration.

that specifically target areas of endothelial damage are striking because such drugs would, for the first time, offer the potential of NO delivery to treat large vessel disease without necessarily causing detrimental vasodilatation and hypotension. More recent experiments have built on the earlier findings and confirmed sustained effects of RIG-200 in isolated human internal mammary arteries and saphenous veins (38), suggesting that agents like RIG-200 might be useful in preventing arterial vasospasm that is occasionally associated with bypass grafting. Experiments in dorsal hand veins *in vivo* also showed that RIG-200 caused significantly more sustained dilatation in vessels denuded of endothelium by water irrigation than in endothelium intact veins (Fig. 5) (39). Dilatation in response to sodium nitroprusside (SNP), however, was not affected by endothelial denudation, again indicating that sustained effects are a feature that is specific to lipophilic *S*-nitrosothiols. It is now imperative that the potential benefit of specific NO delivery to areas of vascular injury be investigated in an atherosclerotic model with a view to its prophylactic use in the prevention of coronary artery disease.

Potential benefits of *S*-nitrosothiols

Platelet selectivity

On account of the multifaceted nature of NO in the cardiovascular arena, one would anticipate that NO donor drugs might fail to offer the necessary specificity for some therapeutic applications. By way of example, use of NO donors as platelet inhibitors could be limited by the accompanying vasodilator action, resulting in undesirable hypotension. Similarly, drug-derived NO concentrations would have to be moderated to ensure cardiovascular protection without induction of NO-mediated proinflammatory effects. However, it is now well established that the selectivity profile of NO donors varies greatly between drug classes, a feature that is likely to reflect different NO-related species that are generated. *S*-Nitrosothiols are now recognized to be relatively selective for platelets over blood vessels (9) and for arteries over veins (40). In contrast, existing organic nitrates exhibit selectivity for veins over arteries, with relatively weak antiplatelet effects. Such divergence in activity profile is of great

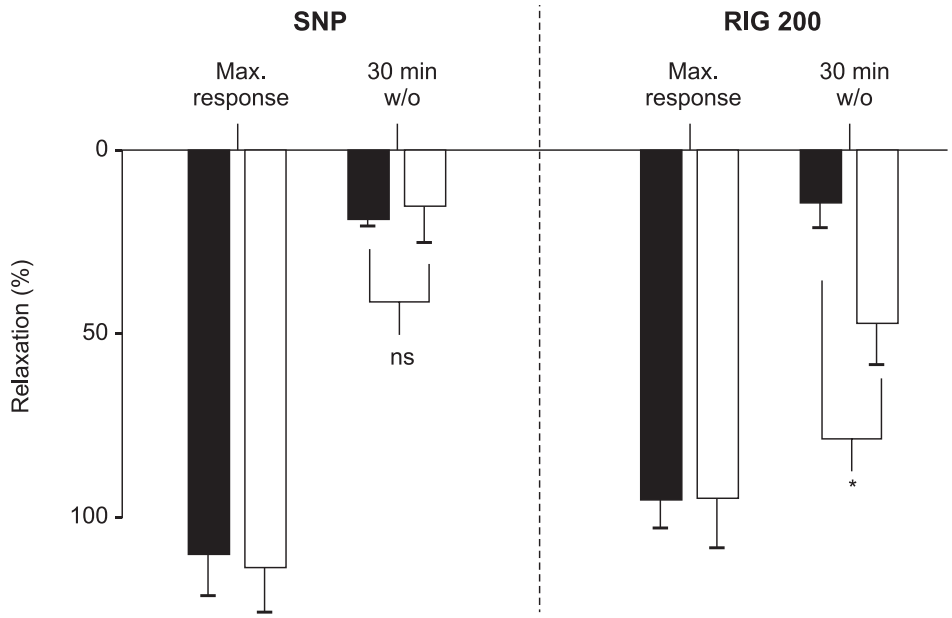

Fig. 5. Venodilator responses to equivalent infusions (6 nmol/min) of sodium nitroprusside (SNP) and RIG-200 in precontracted, endothelium-intact (■ = + endothelium) and endothelium-denuded (□ = – endothelium) hand veins in humans *in vivo*. Maximum dilatation to neither drug was significantly affected by endothelial denudation and the response remaining at 30 min after SNP washout was also unaffected. However, endothelial denudation significantly increased the dilatation remaining at 30 min after washout of RIG-200. (Redrawn from data presented in Sogo, N. et al. *A novel S-nitrosothiol (RIG200) causes prolonged relaxation in dorsal hand veins with damaged endothelium*. Clin Pharmacol Ther 2000, 68: 75-81.)

interest, both from the standpoint of mechanism of action and for potential therapeutic applications.

The relative lack of platelet selectivity shown by organic nitrates is most easily explained by the absence of the necessary metabolic pathway for NO release from the nitroxy ester moieties (41). Nitrates must first be metabolized in the vascular wall, and the resultant NO is primarily sequestered by smooth muscle sGC, while the overspill is susceptible to inactivation by oxygen-derived free radicals and red blood cell hemoglobin before it reaches blood-borne platelets. *S*-Nitrosothiols, however, are powerful inhibitors of platelet activation, even in the absence of vascular tissue (42). Given the wide variety of chemical and cellular mechanisms available to release NO from *S*-nitrosothiols, it is easy to envisage that generation of free NO is the primary means of inhibiting platelet aggregation via activation of sGC. However, our recent experiments have shown that activation of this enzyme is not the only means by which *S*-nitrosothiols inhibit platelet aggregation since they remain at least partially effective in the face of sGC inhibition (43). Furthermore, the sGC-independent element of inhibition is more marked with the lipophilic *S*-nitrosothiol, RIG-200, than with GSNO and is more prominent in ADP-activated platelets than in those activated by collagen. The results are all the more interesting when compared to NO derived from “pure” NO donor drugs: the spontaneous NO donor, diethylamine diazeniumdiolate (DEA/NO) also exhibits sGC-independent effects, but the relative importance in ADP- and collagen-induced platelet aggregation is the reverse of that seen with *S*-nitrosothiols. SNP, on the other hand, requires cellular metabolism to generate NO and its effects are exclusively sGC-mediated. Our interim conclusions from these results are that a number of sGC-independent pathways are available to both extracellular NO and *S*-nitrosothiols (43). The most likely mediator of such effects is transnitrosation of critical thiol residues in proteins/receptors on the platelet surface but research is ongoing to fully elucidate the mechanisms involved. Interestingly, this dual modality is not shared by vascular smooth muscle, where *S*-nitrosothiol-mediated vasodilatation is exclusively sGC-dependent, perhaps indicating the sGC-independent effects in platelets are responsible for the platelet selectivity of these agents over vascular tissue (44).

The antiplatelet activity of *S*-nitrosothiols, combined with the apparent selectivity of lipophilic compounds in areas of endothelial damage, represents a unique opportunity to prevent the inflammatory and thrombotic events that are important in atheroscerotic and restenotic processes. Localization of these beneficial effects would be ensured through rapid scavenging of NO by red blood cell hemoglobin distal from the site of injury (45), thus preventing any systemic vasodilator or antiplatelet effects and eliminating the possibility of hypotension or prolonged bleeding. An obvious tangible application is in balloon angioplasty, where compounds like RIG-200 or

SNVP could offer benefits as an adjunct to stenting to slow disease progression.

Lack of tolerance

A major limitation of organic nitrates is induction of tolerance with prolonged or high-dose administration (5). The mechanism underlying tolerance is complex and highly controversial but is generally accepted to involve dysfunction of the mechanism required to reduce nitroxy ester moieties to NO (5). The relative ease of NO generation from *S*-nitrosothiols, the multiple chemical and cellular mechanisms available for the process, and the ability of *S*-nitrosothiols to activate cellular processes through transnitrosation without the need for NO generation, suggests that these agents might not be susceptible to tolerance. Indeed, our studies (46) with GSNO, together with the lipophilic compounds, RIG-200 and SNVP, indicate that *S*-nitrosothiols are continually active in isolated arteries for a period in excess of 20 h; responses to GTN were lost within 2 h of exposure (46). Furthermore, *S*-nitrosothiols were still fully active in arteries made tolerant to GTN, indicating that there was no cross-tolerance between the donors (46). It is important to note that these experiments were carried out *in vitro*, where the mechanism of tolerance has been suggested to be different from that *in vivo* (47). However, SNAP has been shown to be resistant to tolerance *in vivo* as well (34). It is clear, therefore, that *S*-nitrosothiols are not prone to tolerance and that they represent a viable alternative to organic nitrates, particularly in situations where prolonged administration is required.

Lack of toxicity?

The existence of endogenous *S*-nitrosothiols at physiologically relevant concentrations indicates that the functional group of these compounds at least is nontoxic. Clearly, GSNO, Cys-NO and *S*-nitrosated albumin are unlikely to have toxic side effects on account of the endogenous nature of all the by-products. It is perhaps surprising, however, that a recent study suggests that a glycosylated adduct of SNAP (glu-SNAP) has been shown to be cytotoxic in tumor cells at concentrations as low as 2 μM (48). This feature was not shared by the parent molecule, SNAP, and the authors suggest that the effect is dependent on glucose transport. To date, it is unclear whether similar effects are found in healthy cells or whether acetylated glucosamine-conjugated SNAP (RIG-200) exhibits a cytotoxic action. Certainly, cytotoxic effects were not apparent with RIG-200 in our vascular experiments despite exposure for a period of several hours, but the tumor results might suggest that *N*-substituted analogues of SNAP that do not contain glucose-related species (*e.g.*, SNVP) might prove less toxic than RIG-200 in the cardiovascular arena.

Delivery routes

Due to the experimental nature of the drugs investigated in this group so far, little is known of the potential availability of *S*-nitrosothiols via traditional drug delivery routes. The issue is not a consideration for interventional cardiological techniques where intra-arterial drug administration is facilitated via the balloon catheter. Similarly, drug can easily be delivered to conduit arteries and veins for bypass surgery between mobilization and implantation. However, sustained drug delivery as a preventative measure for chronic vascular disease would benefit greatly from an oral preparation of such compounds. Theoretically, it is anticipated that *S*-nitrosothiols would be stabilized in the acid environment of the stomach but it is likely that current drug preparations are unlikely to survive intact in the bloodstream. A more likely scenario is that NO^+ is transferred to blood-borne endogenous thiols (*e.g.*, albumin), perhaps losing the targeting potential. However, it is possible that transdermal delivery might offer an alternative. *S*-Nitroso-acetylglucose has been shown to cause vasodilatation of skin microvessels following topical application (49) but it is unclear whether the intact drug is responsible or whether an NO-related species is transferred across the skin to cause vasodilatation. Nevertheless, these data raise the possibility of transdermal delivery for *S*-nitrosothiols. Fortunately, the chemical characteristics of compounds like SNVP and RIG-200 are theoretically favorable for this delivery route; they are relatively small (RMM approximately 300-500) and the octanol/water partition coefficients are within the optimal range to facilitate transfer across the corpus corneum.

Conclusions

S-Nitrosothiols clearly represent a viable class of NO donors that offer potential advantages over existing nitrates. Lipophilic *S*-nitrosothiols that are selective for areas of endothelial damage represent a particularly attractive subclass of agents that could be therapeutically beneficial in preventing spasm in arteries used in coronary bypass grafting and thrombosis immediately following balloon angioplasty. *S*-Nitrosothiol coated stents and prophylactic use of lipophilic compounds to target early endothelial damage that predisposes to atherosclerosis are possible future extensions to these applications. However, the routes of delivery and the durability of the compounds *in vivo* remain the major limitations for existing *S*-nitrosothiols and might limit their therapeutic applications to acute antithrombotic therapy during surgery or interventional cardiology procedures. Their potential use as preventative medicines in atherosclerosis is likely to depend on the development of oral or transdermal preparations. In addition to the cardiovascular system, one might envisage use of similar *S*-nitrosothiols in the treatment of asthma and other respiratory disorders, while

high doses applied topically might also be of benefit in the treatment of chronic skin lesions and infections.

References

1. Moncada, S., Palmer, R.M.J., Higgs, E.A. *Nitric oxide: Physiology, pathophysiology and pharmacology.* Pharmacol Rev 1991, 43: 109-42.

2. Waldman, S., Murad, F. *cGMP synthesis and function.* Pharmacol Rev 1987, 39: 163-96.

3. Abrams, J. *Role of endothelial dysfunction in coronary artery disease.* Am J Cardiol 1997, 79: 2-9.

4. Bult, H., Herman, A.G., Matthys, K.E. *Antiatherosclerotic activity of drugs in relation to nitric oxide function.* Eur J Pharmacol 1999, 375: 157-76.

5. Bennett, B.M., McDonald, B.J., Nigam, R., Simon, W.C. *Biotransformation of organic nitrates and vascular smooth muscle cell function.* Trends Pharmacol Sci 1994, 15: 245-9.

6. Abrams, J. *Pharmacology of nitroglycerin and long-acting nitrates.* Am J Cardiol 1985, 56: 12A-18A.

7. Megson, I.L.,Webb, D.J. *Nitrate resistance in platelets from patients with stable angina pectoris.* Circulation 2000, 102: e87.

8. Megson, I.L., Webb, D.J. *Nitric oxide donor drugs: Current status and future trends.* Exp Opin Invest Drugs 2002, 11: 587-601.

9. De Belder, A.J., MacAllister, R., Radomski, M.W., Moncada, S., Vallance, P. *Effects of S-nitroso-glutathione in the human forearm circulation: Evidence for selective inhibition of platelet activation.* Cardiovasc Res 1994, 28: 691-4.

10. Scharfstein, J.S., Keaney, J.F., Slivka, A. et al. *In vivo transfer of nitric oxide between a plasma protein-bound reservoir and low molecular weight thiols.* J Clin Invest 1994, 94: 1432-9.

11. Stamler, J.S., Jaraki, O., Osborne, J. et al. *Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin.* Proc Natl Acad Sci USA 1992, 89: 7674-7.

12. Stamler, J.S., Simon, D.I., Osborne, J. et al. *S-Nitrosylation of proteins with nitric oxide: Synthesis and characterization of biologically active compounds.* Proc Natl Acad Sci USA 1992, 89: 444-8.

13. Gow, A.J., Stamler, J.S. *Reactions between nitric oxide and haemoglobin under physiological conditions.* Nature 1998, 391: 169-73.

14. Jia, L., Bonaventura, C., Bonaventura, J., Stamler, J.S. *S-Nitrosohaemoglobin: A dynamic activity of blood involved in vascular control.* Nature 1996, 380: 221-6.

15. Bainbrigge, N., Butler, A.R., Gorbitz, C.H. *The thermal stability of S-nitrosothiols: Experimental studies and ab initio calculations on model compounds.* J Chem Soc Perkin Trans 2 1997, 351-3.

16. Williams, D.L.H. *S-Nitrosation and the reactions of S-nitroso compounds.* Chem Soc Rev 1985, 14: 171-96.

17. Dicks, A.P., Swift, H.R., Williams, D.L.H., Butler, A.R., Al-Sa'doni, H.H., Cox, B.G. *Identification of Cu^{+} as the effective reagent in nitric oxide formation from S-nitrosothiols (RSNO).* J Chem Soc Perkin Trans 2 1996, 481-7.

18. Jourd'heuil, D., Laroux, F.S., Kang, D., Miles, A.M., Wink, D.A., Grisham, M.B. *Stability of S-nitrosothiols in presence of copper, zinc-superoxide dismutase.* Meth Enzymol 1999, 301: 220-7.

19. Jourd'heuil, D., Laroux, F.S., Miles, A.M., Wink, D.A., Grisham, M.B. *Effect of superoxide dismutase on the stability of S-nitrosothiols.* Arch Biochem Biophys 1999, 361: 323-30.

20. Holmes, A.J., Williams, D.L. *Reaction of S-nitrosothiols with ascorbate: clear evidence of two reactions.* Chem Commun 1998, 1711-2.

21. Jourd'heuil, D., Mai, C.T., Laroux, F.S., Wink, D.A., Grisham, M.B. *The reaction of S-nitrosoglutathione with superoxide.* Biochem Biophy Res Commun 1998, 246: 525-30.

22. Askew, S.C., Butler, A.R., Flitney, F.W., Kemp, G.D., Megson, I.L. *Chemical mechanism underlying the vasodilator and platelet anti-aggregating properties of S-nitroso-N-acetyl-D,L-penicillamine and S-nitrosoglutathione.* Bioorg Med Chem 1995, 3: 1-9.

23. Meyer, D.J., Kramer, H., Ozer, N., Coles, B., Ketterer, B. *Kinetics and equilibria of S-nitrosothiol-thiol exchange between glutathione, cysteine, penicillamines and serum albumin.* FEBS Lett 1994, 345: 177-8.

24. Zai, A., Rudd, A., Scribner, A.W., Loscalzo, J. *Cell-surface protein disulfide isomerase catalyzes transnitrosation and regulates intracellular transfer of nitric oxide.* J Clin Invest 1999, 103: 393-9.

25. Langford, E.J., Brown, A.S., Wainwright, R.J. et al. *Inhibition of platelet activity by S-nitrosoglutathione during coronary angioplasty.* Lancet 1994, 344: 1458-60.

26. Molloy, J., Martin, J.F., Baskerville, P.A., Fraser, S.C.A., Markus, H.S. *S-nitrosoglutathione reduces the rate of embolization in humans. Circulation.* 1998, 98: 1372-5.

27. Kaposzta, Z., Martin, J.F., Markus, H.S. *Switching off embolization from symptomatic carotid plaque using S-nitrosoglutathione.* Circulation 2002, 105: 1480-4.

28. Maalej, N., Albrecht, R., Loscalzo, J., Folts, J.D. *The potent platelet inhibitory effects of S-nitrosated albumin coating of artificial surfaces.* J Am Coll Cardiol 1999, 33: 1408-14.

29. Bandarage, U.K., Chen, L., Fang, X. et al. *Nitrosothiol esters of diclofenac: Synthesis and pharmacoligical characterization as gastrointestinal-sparing prodrugs.* J Med Chem 2000, 43: 4005-16.

30. Del Soldato, P., Sorrentino, R., Pinto, A. *NO-aspirins: A class of new antiinflammatory and antithrombotic agents.* Trends Pharmacol Sci 1999, 20: 319-23.

31. Momi, S., Emerson, M., Paul, W. et al. *Prevention of pulmonary thromboembolism by NCX 4016, a nitric oxide-releasing aspirin.* Eur J Pharmacol 2000, 397: 177-85.

32. Fredduzzi, S., Mariucci, G., Tantucci, M., Del Soldato, P., Ambrosini, M.V. *Nitro-aspirin (NCX4016) reduces brain damage induced by focal cerebral ischemia in the rat.* Neurosci Lett 2001, 302: 121-4.

33. Wainwright, C.L., Miller, A.M., Work, L.M., Del Soldato, P. *NCX4016 (NO-aspirin) reduces infarct size and suppresses arrhythmias following myocardial ischaemia/reperfusion in pigs.* Br J Pharmacol 2002, 135: 1882-8.

34. Bauer, J.A., Fung, H.L. *Differential hemodynamic effects and tolerance properties of nitroglycerin and an S-nitrosothiol in experimental heart failure.* J Pharmacol Exp Ther 1991, 256: 249-54.

35. Megson, I.L., Greig, I.R., Gray, G.A., Webb, D.J., Butler, A.R. *Prolonged effect of a novel S-nitrosated glyco-amino acid in endothelium-denuded rat femoral arteries: Potential as a slow release nitric oxide donor drug.* Br J Pharmacol 1997, 122: 1617-24.

36. Butler, A.R., Al-Sa'doni, H.H., Megson, I.L., Flitney, F.W. *Synthesis, decomposition and vasodilator action of some new S-nitrosated dipeptides.* Nitric Oxide 1998, 2: 193-202.

37. Megson, I.L., Morton, S., Greig, I.R. et al. *N-Substituted analogues of S-nitroso-N-acetyl-D,L-penicillamine: Chemical stability and prolonged nitric oxide mediated vasodilatation in isolated rat femoral arteries.* Br J Pharmacol 1999, 126: 639-48.

38. Sogo, N., Campanella, C., Webb, D.J., Megson, I.L. *S-Nitrosothiols cause prolonged, nitric oxide-mediated relaxation in human saphenous vein and internal mammary artery: Therapeutic potential in bypass surgery.* Br J Pharmacol 2000, 131: 1236-44.

39. Sogo, N., Wilkinson, I.B., MacCallum, H. et al. *A novel S-nitrosothiol (RIG200) causes prolonged relaxation in dorsal hand veins with damaged endothelium.* Clin Pharmacol Ther 2000, 68: 75-81.

40. MacAllister, R.J., Calver, A.L., Riezebos, J., Collier, J., Vallance, P. *Relative potency of nitrovasodilators on human blood vessels: An insight into the targetting of nitric oxide delivery.* J Pharmacol Exp Ther 1995, 273: 1529-37.

41. Weber, A.-A., Neuhaus, T., Seul, C. et al. *Biotransformation of glyceryl trinitrate by blood platelets as compared to vascular smooth muscle cells.* Eur J Pharmacol 1996, 309: 209-13.

42. Radomski, M.W., Rees, D.D., Dutra, A., Moncada, S. *S-Nitroso-glutathione inhibits platelet aggregation in vitro and in vivo.* Br J Pharmacol 1992, 107: 745-9.

43. Sogo, N., Magid, K.S., Shaw, C.A., Webb, D.J., Megson, I.L. *Inhibition of human platelet aggregation by nitric oxide donor drugs: Relative contribution of cGMP-independent mechanisms.* Biochem Biophys Res Commun 2000, 279: 412-9.

44. Wanstall, J.C., Jeffery, T.K., Gambino, A., Lovren, F., Triggle, C.R. *Vascular smooth muscle relaxation mediated by nitric oxide donors: A comparison with acetylcholine, nitric oxide and nitroxyl ion.* Br J Pharmacol 2001, 134: 463-72.

45. Megson, I.L., Sogo, N., Mazzei, F.A., Butler, A.R., Walton, J.C., Webb, D.J. *Inhibition of human platelet aggregation by a novel S-nitrosothiol is abolished by haemoglobin and red blood cells in vitro: Implications for anti-thrombotic therapy.* Br J Pharmacol 2000, 131: 1391-8.

46. Miller, M.R., Roseberry, M.J., Mazzei, F.A., Butler, A.R., Webb, D.J., Megson, I.L. *S-Nitrosothiols do not induce tolerance and are not cross-tolerant in rat femoral arteries.* Eur J Pharmacol 2000, 408: 335-43.

47. Munzel, T., Hink, U., Yigit, H., Macharzina, R., Harrison, D.G., Mulsch, A. *Role of superoxide dismutase in in vivo and in vitro nitrate tolerance.* Br J Pharmacol 1999, 127: 1224-230.

48. Cantuaria, G., Magalhaes, A., Angioli, R. et al. *Antitumour activity of a novel glyco-nitric oxide conjugate in ovarian carcinoma.* Cancer 2000, 88: 381-8.

49. Khan, F., Greig, I.R., Newton, D.J., Butler, A.R., Belch, J.J.F. *Skin blood flow after transdermal S-nitrosothio-acetylglucose.* Lancet 1997, 350: 410-1.