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The design of nitric oxide donor drugs: s-nitrosothiol tDodSNO is a superior photoactivated donor in comparison to GSNO and SNAP

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ABSTRACT

We have recently developed *tert*-dodecane S-nitrosothiol (tDodSNO) as a photoactivated nitric oxide (NO) donor. We here compare the potency of tDodSNO to S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP), drugs which are also based upon the S-nitrosothiol functionality and have been extensively used for NO release studies. Photoactivation *in vitro*, at a clinically relevant light fluence rate (200 W/m^2), demonstrated that tDodSNO released much higher levels of NO than either GSNO or SNAP. When evaluated in an *ex vivo* aortic ring vasorelaxation assay, tDodSNO was also the only drug to exhibit a photodynamic response, with an 8 fold decrease in EC_{50} (8.1–1.0 μM) upon irradiation. While both GSNO and SNAP induced NO dependent vasorelaxation at lower concentrations than tDodSNO (EC_{50}'s of 158 and 38 nM respectively), this activity was due to their rapid metabolic decomposition, and could not be modulated by photoactivation. Additionally, tDodSNO's photodynamic response allowed vascular tone to be directly regulated by light intensity. Molecular modeling of drug properties suggested that these differences in activity could be attributed to a combination of an increase in tDodSNO's hydrophobicity, and substantial steric shielding of molecule's S-nitrosothiol group from solvent interactions. In conclusion, our study demonstrates that tDodSNO is currently the most effective known s-nitrosothiol for phototherapeutic applications.

1. Introduction

There have been extensive efforts to develop drugs capable of delivering nitric oxide (NO) to tissue, as NO releasing donors have the potential to provide treatments for multiple pathologies including: ischemic cardiovascular disease (Richardson and Benjamin, 2002), hypertension (Bath et al., 2001) and cancer (Huerta et al., 2008). At present clinically utilized NO based therapeutics require metabolic activation to release NO, restricting their applications due to the onset of hypotension, which can result in syncope (Thadani and Rodgers, 2006), and the rapid development of tolerance (Thadani, 1997). A promising strategy to improve upon these agents is photodynamic therapy (PDT), whereby NO release is controlled by an applied light stimulus (Ford, 2013). Several photolabile pharmacophores have been examined as potential NO donors, including: N-nitrosamines (Karaki et al., 2012; Namiki et al., 1999), S-nitrosothiols (SNTs, also known as thionitrites) (Sexton et al., 1994), caged diazeniumdiolates (Makings and Tsien, 1994) and transition metal-nitrosyl complexes (Madhani et al., 2006), as well as nanoparticle formulations (Deniz et al., 2012). Of these photoactives the SNTs are potentially the most useful for a range of PDT applications, as they can be excited by light in both the UV (340 nm) and visible (560–600 nm) regions of the spectrum (Szacilowski and Stasicka, 2001). However, the development of these molecules as viable agents for PDT has been hindered by their rapid metabolism and metal catalyzed decomposition (Singh et al., 1996).

To circumvent the limitations associated with current SNTs, we have recently developed a NO donor, *tert*-dodecane S-nitrosothiol (tDodSNO, Fig. 1), that exhibits improved NO release characteristics (Giles et al., 2012). The rationale for the development of tDodSNO was to increase its hydrophobicity in order to partition the molecule into cell membranes, shielding it from the cellular metabolic pathways, metal ion interactions, and trans-nitrosation reactions that typically degrade SNT based drugs (Al-Sa'doni and Ferro, 2004). Such a strategy has previously been followed with the long acting beta agonists used in asthma management (Lotvall, 2001), and we hypothesized that similarly hydrophobic SNTs would display augmented stability and hence controllable NO release. The current paper examines the effects of tDodSNO on

Abbreviations: DTPA, diethylenetriaminepentaacetic acid; GSNO, S-nitrosoglutathione; KPi, potassium phosphate; MbO_2, oxy-myoglobin; NO, nitric oxide (nitrogen monoxide); ODQ, 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one; PDT, photodynamic therapy; PE, phenylephrine; SNAP, S-nitroso-N-acetylpenicillamine; SNT, S-nitrosothiol; tDodSNO, *tert*-dodecane S-nitrosothiol

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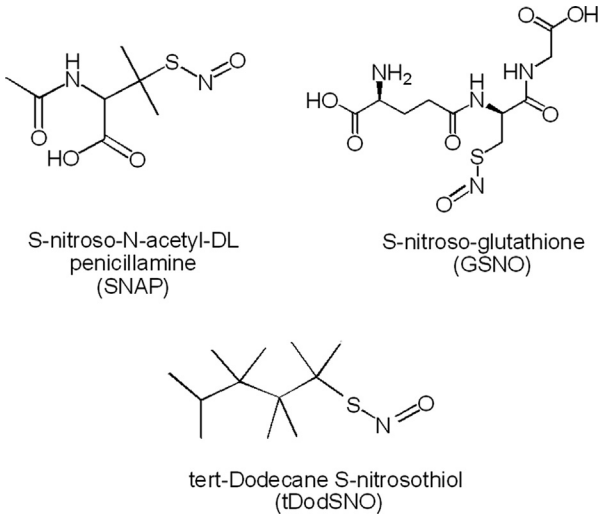

Fig. 1. Structures of SNTs.

vascular tone in comparison to the commonly employed SNTs S-nitrosoglutathione (GSNO, Fig. 1) and S-nitroso-N-acetylpenicillamine (SNAP, Fig. 1). Within vascular tissue, tDodSNO was the only molecule to act as a photoactive agent, indicating that tDodSNO is a superior candidate for PDT based applications.

2. Materials and methods

2.1. Materials

S-nitrosoglutathione (GSNO), horse heart myoglobin, phenylephrine (PE), indomethacin and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) were purchased from Sigma-Aldrich (Auckland, NZ). S-nitroso-N-acetylpenicillamine (SNAP) was purchased from Invitrogen (Auckland, NZ). tDodSNO was synthesized as previously described (Giles et al., 2012). All other chemicals were of analytical grade.

2.2. Molecular modeling

Quantum mechanical derivations of molecular structure were calculated *via* the MOPAC simulation program applying the AM1 semiempirical Hamiltonian force field (Chem3D Pro v11.0, CambridgeSoft Corporation, Cambridge, MA, USA).

2.3. Determination of SNT concentration

The concentration of stock solutions of SNTs was determined by their characteristic absorbance at $\lambda = 340$ nm. SNTs were either dissolved in DMSO (tDodSNO $\varepsilon_{340} = 675\ M^{-1}\ cm^{-1}$ (Giles et al., 2012) and SNAP $\varepsilon_{340} = 1168\ M^{-1}\ cm^{-1}$ (Megson et al., 1999)) or MilliQ water (GSNO $\varepsilon_{340} = 922\ M^{-1}\ cm^{-1}$ (Hart, 1985)).

2.4. Photoactivation

Irradiation was regulated using a 100 W halogen cold light source with variable power output (LG-PS2; Olympus, Auckland, New Zealand). Fluence rate was recorded using a P-9710 optometer fitted with an RW-3705 detector head (Gigahertz-Optik, Munich, Germany).

2.5. SNT NO release kinetics

The release of NO from SNTs was quantified using a free radical analyzer equipped with a polarographic NO sensing electrode (ISO-NOP, WPI, Sarasota, FL, USA). NO release measurements were conducted in a stirred, sealed incubation chamber, with no headspace, at 37 °C in KPi buffer (100 mM KPi, 2 mM DTPA, pH 7.4) as previously described (Giles et al., 2012). The electrode response was calibrated *via* the chemical generation of NO from sodium nitrite (Hummel et al., 2006; Zhang, 2004).

2.6. Oxy-myoglobin preparation

Commercially available horse heart myoglobin consists of a mixture of oxy (MbO_2) and met (met-Mb) myoglobin. This mixture was reduced to Mb *via* the addition of excess sodium dithionite in KPi buffer (pH 7.4, 100 mM KPi, 2 mM DTPA). To re-oxygenate and purify MbO_2, the solution was then passed through a PD10 desalting column (Sephadex G-25M, GE Healthcare, Auckland, NZ) (Zhang and Hogg, 2002). The MbO_2 concentration in the eluted solution was then determined at $\lambda = 542$ nm ($\varepsilon_{542} = 13,900\ M^{-1}\ cm^{-1}$) (Feelisch and Stamler, 1996).

2.7. Preparation of aortic rings

All animal experiments were conducted in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals, and the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (2-3 months old, 250–300 g) were obtained from the University of Otago Animal Resource Unit. The rats were housed at 25 °C under a controlled 12-h day/night cycle in standard rat cages and fed *ad libitum* on a standard rat diet before experimentation. The rats were killed by decapitation and the thoracic aortas immediately dissected into 4 mm wide transverse rings. The aortic rings were then washed in ice-cold Krebs Henseleit (KH) buffer (118 mM NaCl, 4.7 mM KCl, 1.66 mM $MgSO_4$, 24.88 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 11 mM glucose, 0.5 mM EDTA, 3 mM $CaCl_2$, pH 7.4) before use.

2.8. Vasorelaxation assay

Endothelium intact aortic rings were placed between two stainless-steel stirrups and equilibrated under a resting tension of 2 g in a 10 ml organ bath containing KH buffer at 37 °C, gassed with 95% O_2–5% CO_2 (Resende et al., 2004). Indomethacin (10 μM) was included in the buffer to exclude prostaglandins as potential modulators of vascular tone. Vascular tone was recorded using an isometric force transducer (FT03, Grass) coupled to a PowerLab 2/26 recorder (ADInstruments, Dunedin, New Zealand). During this interval, the aortic rings were repeatedly washed in KH buffer and the applied force readjusted until the resting tone was maintained. The rings were then pre-constricted with phenylephrine (PE, 0.1 μM) to generate a reproducible, maintained level of vascular tone, and concentration–responses to SNTs elicited from this PE-induced contraction. To confirm the involvement of NO-mediated pathways in the SNT vasorelaxant response, aortic responses to each of the SNTs were also studied in the presence of oxymyoglobin (MbO_2, 5 μM), a rapidly acting NO scavenger (Feelisch and Stamler, 1996; Zhang and Hogg, 2002), and excess 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μM), a selective inhibitor of soluble guanylate cyclase activity (Garthwaite et al., 1995; Moro et al., 1996).