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REVIEW

S-nitrosothiols as selective antithrombotic agents – possible mechanisms

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S-nitrosothiols have a number of potential clinical applications, among which their use as antithrombotic agents has been emphasized. This is largely because of their well-documented platelet inhibitory effects, which show a degree of platelet selectivity, although the mechanism of this remains undefined. Recent progress in understanding how nitric oxide (NO)-related signalling is delivered into cells from stable S-nitrosothiol compounds has revealed a variety of pathways, in particular denitrosation by enzymes located at the cell surface, and transport of intact S-nitrosocysteine via the amino acid transporter system-L (L-AT). Differences in the role of these pathways in platelets and vascular cells may in part explain the reported platelet-selective action. In addition, emerging evidence that S-nitrosothiols regulate key targets on the exofacial surfaces of cells involved in the thrombotic process (for example, protein disulphide isomerase, integrins and tissue factor) suggests novel antithrombotic actions, which may not even require transmembrane delivery of NO.
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Abbreviations: csPDI, cell surface protein disulphide isomerase; cyclic GMP, guanosine 3′:5′-cyclic monophosphate; cysNO, S-nitrosocysteine; DAF-FM, 4-amino-5-methylamino-2′7′-difluorofluorescein; GSNO, S-nitrosoglutathione; L-AT, amino acid transporter system-L; PDI, protein disulphide isomerase; RSNO, S-nitrosothiol; sGC, soluble guanylate cyclase; tPA, tissue plasminogen activator

Introduction

Cardiovascular disease is the most common cause of death in developed countries and arterial thrombosis, following rupture of an atherosclerotic plaque, underlies most cases of myocardial infarction and stroke. Thrombus formation involves the rapid accumulation of blood platelets and fibrin into an occlusive mass within the blood vessel. Platelets adhere to collagen and von Willebrand factor exposed in the ruptured plaque, and their activation is up-regulated by locally generated thrombin, thromboxane and ADP, with subsequent surface display of integrin adhesion molecules (principally αIIbβ3) in their active conformation, allowing fibrinogen binding and platelet aggregation (Mackman, 2008). Thrombin-mediated fibrin formation follows triggering of the blood coagulation cascade by exposure (Steffel *et al.*, 2006) or de-encryption (Bach, 2006) of tissue factor, either located within the plaque itself or arriving with the cells accumulated by the thrombus. Following these initiating events, thrombin generation is amplified and propagated by assembly of coagulation enzyme complexes on the surface of activated platelets and other cells (Hoffman and Monroe, 2001) with resulting fibrin deposition.

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Endothelial dysfunction and loss of nitric oxide (NO) bioactivity

Intravascular platelet activation is evident in ischaemic syndromes affecting both the coronary (Gurbel *et al.*, 2004) and cerebral circulations (Badimon and Vilahur, 2007), and interactions between activated platelets and the vessel wall are thought to contribute not only to the final thrombotic events in atherosclerotic disease, but also in the initiation and progression of atheroma (Langer and Gawaz, 2008). Failure to control platelets in such circumstances is because of endothelial dysfunction, in particular the loss of NO activity (Vanhoutte *et al.*, 2009). Common conditions such as diabetes mellitus, hypertension and renal failure are characterized by chronic oxidative stress, which increases cardiovascular risk, in part by diminishing the availability of bioactive NO and thus permitting platelets to contribute to thrombosis (Freedman and Loscalzo, 2003). These considerations provide a rationale for NO supplementation using donor agents to limit thrombotic risk and a variety of compounds are available for this purpose (Miller and Megson, 2007). This review will focus on the antithrombotic potential of S-nitrosothiols (RSNOs), and particular attention will be paid to the mechanism by which these compounds deliver NO to platelets and other cells of the vascular compartment, since there has been progress in this area that may explain reports of selective anti-platelet action from certain RSNO molecules.

S-nitrosothiols

S-nitrosothiols are a class of compounds produced by the S-nitrosation of sulphydryl groups (usually cysteine thiols), and with the general formula R-SNO. They are sometimes referred to as thionitrites in the older chemical literature and their general chemistry and biological properties are documented in several review articles (Al-Sa'doni and Ferro, 2000; Hogg, 2000), as are prospects for their therapeutic use (Richardson and Benjamin, 2002). Their attractiveness as therapeutic agents is increased by the fact that they occur naturally in blood and tissues as endogenous metabolites of NO, suggesting that toxicity associated with their use might be low.

Following the identification of NO as the active component of nitrovasodilator drugs (Murad, 1999) and the molecule responsible for the endogenous activity known as endothelium derived relaxing factor (Palmer *et al.*, 1987), it was suggested that physiological RSNO formation might provide a means of stabilizing and extending the activity of NO (Myers *et al.*, 1990; Stamler *et al.*, 1992a). Despite the fact that NO itself is a poor nitrosating agent, plausible mechanisms exist for RSNO formation within the biological environment (Zhang and Hogg, 2005) and these compounds, principally in the form of S-nitrosoalbumin and S-nitrosohaemoglobin, but also as low molecular weight forms (S-nitrosocysteine, s-nitrosocysteinylglycine, S-nitrosoglutathione), are found to occur naturally in blood and tissues (Giustarini *et al.*, 2003). Circulating RSNO concentrations are a matter of dispute, largely through differences in methodology (Stamler, 2004); however, most reports put them in the low nanomolar range.

RSNOs are simple and cheap to synthesize in the laboratory, an advantage if they were to be employed as drugs. Among the suggested clinical uses of RSNOs, their potential as antithrombotic agents has often been highlighted. There is a substantial literature recording the platelet-inhibitory action of RSNOs, and a number of mechanisms have been identified. In addition, a smaller number of reports have suggested that other components of the haemostatic system (for example coagulation and fibrinolysis) may be influenced by these agents.

Antiplatelet actions of RSNOs

Very soon after the identification of NO as an endogenous mediator, it was shown that NO inhibited platelet function and that this inhibition coincided with stimulation of soluble guanylyl cyclase (sGC) and intra-platelet cyclic GMP accumulation (Radomski *et al.*, 1987). Nitrovasodilator drugs, including various RSNO compounds, had already been shown to suppress platelet aggregation via sGC stimulation (Mellion *et al.*, 1983), and further reports documented cyclic GMP-mediated inhibition of platelet adhesion, aggregation, granule secretion and fibrinogen binding by S-nitrosocysteine (cysNO) and S-nitrosoglutathione (GSNO) (Mendelsohn *et al.*, 1990; Lieberman *et al.*, 1991; Radomski *et al.*, 1992). Accumulation of the second messenger cyclic GMP influences platelet function by activation of protein kinase G, with consequent phosphorylation of numerous intracellular targets and inhibition of processes including calcium mobilization, integrin αIIbβ3 activation, cytoskeleton re-arrangement, granule secretion (Schwarz *et al.*, 2001), activity of thromboxane receptors (Wang *et al.*, 1998) and phosphoinositide 3-kinase (Pigazzi *et al.*, 1999). It should be noted that cyclic GMP-induced platelet inhibition can under some circumstances occur independently of NO (Riba *et al.*, 2008); however, the physiological importance of this mechanism, relative to endothelial NO release, is not yet clear.

Although sGC stimulation probably represents the primary mode of action of NO donor agents, including RSNOs, it has nevertheless become evident that platelet control is also exerted by cyclic GMP-independent mechanisms (Gordge *et al.*, 1998b; Pawloski *et al.*, 1998; Sogo *et al.*, 2000). A variety of molecular alterations have been proposed to mediate this process, including prevention of thromboxane synthesis (Tsikas *et al.*, 1999), nitration of α-actinin (Marcondes *et al.*, 2006), inhibition of the platelet $P2Y_{12}$ ADP receptor (or, more precisely its cellular signalling partners) (Kokkola *et al.*, 2005) and either S-nitrosylation (Walsh *et al.*, 2007) or altered phosphorylation (Oberprieler *et al.*, 2007) of the important platelet integrin αIIbβ3. There appears to be a requirement for extracellular generation of NO to occur before cyclic GMP-independent inhibition of calcium signalling and platelet aggregation can be brought about by NO donor compounds, including RSNOs (Crane *et al.*, 2005).

Other effects of RSNOs on the haemostasis process

Haemostasis involves the interaction of a variety of components, including platelets, vascular cells and proteins of the coagulation and fibrinolytic systems. However, relatively little work has been published on the direct influence of NO on coagulation and fibrinolysis. Clot formation via thrombin-induced polymerization of fibrin and its subsequent crosslinking by factor XIII is accelerated by nitrating, but not non-nitrating oxidants, suggesting that nitrosative stress may bring about a pro-thrombotic state (Vadseth *et al.*, 2004), although paradoxically others have reported inhibition of fibrin polymerization by peroxynitrite (a well-known nitrating agent) (Lupidi *et al.*, 1999). Conversely, exposure of fibrinogen to the RSNO compound GSNO suppresses fibrin polymerization (i.e. an antithrombotic effect) through what appears to be an allosteric interaction separate from covalent modification of the fibrinogen molecule (Akhter *et al.*, 2002; Geer *et al.*, 2008). RSNOs may further oppose thrombosis by inhibiting the action of transglutaminase enzymes, including coagulation factor XIII (Catani *et al.*, 1998; Lai *et al.*, 2001). The major vascular initiator of fibrinolysis is tissue plasminogen activator (tPA), and S-nitrosylation of tPA, as might be brought about via transnitrosation from RSNO molecules, confers antiplatelet properties on tPA without altering its fibrinolytic action (Stamler *et al.*, 1992b). A further intriguing, and possibly crucial aspect of coagulation control by RSNOs involves their involvement in switching tissue factor (the main physiological trigger for coagulation) into a coagulation inactive form (Ahamed *et al.*, 2006). This fits into an emerging paradigm in which tissue factor activity is regulated by a variety of post-translational modifications (Egorina *et al.*, 2008), in particular redox modification of an allosteric disulphide bond (Chen *et al.*, 2006). It should be noted, however, that the concept of redox regulation of tissue factor is contested (Pendurthi *et al.*, 2007).

Thus, via a variety of pathways beyond simple inhibition of platelet function, RSNO compounds show potential for antithrombotic action.

Antithrombotic action of RSNOs – *in vivo* studies

Both low molecular weight and protein forms of RSNO suppress platelet activation in animal models (Radomski *et al.*, 1992; Keaney *et al.*, 1993), and the anti-platelet action of GSNO coincided with improved tissue survival in a rat model of ischaemia/reperfusion injury (Kuo *et al.*, 2004). Novel antithrombotic RSNO molecules with chemical modifications conferring selectivity for areas of vascular injury (Miller *et al.*, 2003) or for platelets (Vilahur *et al.*, 2004) have been developed, and shown to be effective in rabbit and porcine models.

In human patients, GSNO limits platelet activation in severe pre-eclampsia (Lees *et al.*, 1996). In addition, a number of small clinical trials have documented a significant antithrombotic and/or anti-embolic effect of GSNO administration, following surgical procedures such as coronary artery bypass grafting (Salas *et al.*, 1998), carotid enderartectomy (Molloy *et al.*, 1998) and carotid angioplasty (Kaposzta *et al.*, 2002). An interesting property of GSNO identified during human *in vivo* studies is that it shows a degree of platelet selectivity, in that platelet inhibition could be demonstrated with doses of GSNO that failed to produce significant vasodilatation (De Belder *et al.*, 1994). The mechanism of this platelet selective behaviour was not fully defined, but the authors speculated that it might relate to different abilities of platelets and other vascular cell types to mediate enzymatic release of NO from GSNO.

Delivery of NO signalling by RSNO compounds

The tissue effects of RSNOs (cyclic GMP generation, vasodilatation, platelet aggregation inhibition) are not shown by the non-nitrosated parent thiol compounds (Mathews and Kerr, 1993), implying that RSNOs must act via transmission of NO-related signals. Nevertheless, in the early 1990s it was recognized that the rate of spontaneous NO release from different RSNOs failed to correlate with their corresponding potencies in bioassay systems therefore spontaneous NO liberation could not explain the biological actions of RSNOs (Kowaluk and Fung, 1990; Mathews and Kerr, 1993). This lack of correlation probably reflects the fact that NO release from RSNOs into solution almost always results from catalysis by copper (I) ions, whereas *in vivo* other pathways are involved. The mode of intracellular delivery of NO from RSNOs is more complex and RSNOs cannot be viewed as simple 'NO donors'.

Cellular metabolism of RSNOs

Cellular metabolism is one possible means of NO delivery and a number of studies have documented NO release from RSNOs mediated by intact platelets and other cell types (Simon *et al.*, 1993; Gordge *et al.*, 1998a; Liu *et al.*, 2001; Zeng *et al.*, 2001; Cornwell *et al.*, 2003; Shah *et al.*, 2003). In addition, RSNOs are substrates for a variety of enzymes including glutathione peroxidase (Freedman *et al.*, 1995), a copper (I)-dependent enzyme (Gordge *et al.*, 1996), γ-glutamyl transferase (Hogg *et al.*, 1997), thioredoxin reductase (Nikitovic and Holmgren, 1996), superoxide dismutase (Jourd'heuil *et al.*, 1999), protein disulphide isomerase (Sliskovic *et al.*, 2005), cytoplasmic metalloprotein (Mani *et al.*, 2006) and GSNO reductase (glutathione-dependent formaldehyde reductase, or alcohol dehydrogenase 3) (Liu *et al.*, 2001; 2004). This latter enzyme appears to play a crucial role in regulating nitrosative stress via adjustment of intracellular levels of S-nitrosylated proteins (Foster *et al.*, 2009; Staab *et al.*, 2009); however, there is no direct evidence yet for a role in the transfer of NO signalling from extracellular RSNOs.

Cell surface protein disulphide isomerase promotes NO delivery across the plasma membrane

Of the enzymes mentioned earlier, protein disulphide isomerase (PDI) has perhaps the best credentials as a mediator of RSNO signalling. PDI was originally characterized as a resident of the endoplasmic reticulum, assisting in the correct folding of nascent proteins (Gruber *et al.*, 2006). In recent years PDI has been documented at locations outside the ER, including the cell surface, cytosol and nucleus (Turano *et al.*, 2002). Cell surface isomerases, including PDI (csPDI), have attracted particular research interest through their involvement in infectious disease (Conant and Stephens, 2007), HIV entry into CD4 positive lymphocytes (Barbouche *et al.*, 2003), platelet aggregation (Burgess *et al.*, 2000; Lahav *et al.*, 2003; Jordan *et al.*, 2005; Robinson *et al.*, 2006; Manickam *et al.*, 2008), and control of tissue factor activity (Chen *et al.*, 2006; Versteeg and Ruf, 2007). Studies in mice have confirmed an *in vivo* role for csPDI in both fibrin generation and platelet thrombus formation (Cho *et al.*, 2008), thus csPDI has a direct bearing on haemostatic regulation.

A further line of research has shown that csPDI plays an important role in NO signalling, specifically the transfer of NO from extracellular membrane-impermeant RSNOs across the plasma membrane of target cells (Zai *et al.*, 1999; Ramachandran *et al.*, 2001; Bell *et al.*, 2007). The best-developed model described so far postulates that csPDI denitrosates RSNO molecules in the vicinity of the plasma membrane, releasing NO that then enters the membrane by virtue of its lipophilicity and combines there with oxygen to produce the nitrosating agent N_2O_3. When this, in turn, nitrosates target molecules on the cytoplasmic side of the plasma membrane the goal of NO internalization is achieved (Ramachandran *et al.*, 2001). Our own experimental studies have confirmed the role of csPDI in delivery into platelets of NO-related signalling from RSNOs. However, we also found that active csPDI was necessary for signal delivery from donors of nitroxyl (NO^-) and of NO (Bell *et al.*, 2007). csPDI-mediated denitrosation should not be required for entry of NO, and further work is therefore needed to reconcile these results. Redox mechanisms involving the vicinal thiols of the csPDI active site underlie the process of RSNO denitrosation (Sliskovic *et al.*, 2005). The published scheme shows plausibly how a single enzyme turnover brings about NO release, but the mechanism of active site thiol regeneration, required to continue RSNO signalling, is not yet defined. Several studies have documented thiol oxidation within csPDI and loss of enzyme activity as a result of the interaction with RSNO (Zai *et al.*, 1999; Root *et al.*, 2004; Shah *et al.*, 2007). Redox regeneration of csPDI may derive from both internal sources, via *trans*-membrane oxidoreductases such as NAD(P)H oxidase, and/or from reducing equivalents present in blood plasma. The relative importance of these various systems, and the effects of oxidative/nitrosative stress on csPDI-mediated processes, need to be known for a full understanding of csPDI pathophysiology.

Some RSNO molecules are delivered intact via membrane transporters

An alternative means of RSNO-mediated signalling is by cellular uptake of an intact RSNO molecule via a membrane transporter. Evidence has emerged from a number of different laboratories showing that the low molecular weight RSNO compounds cysNO and S-nitrosohomocysteine act as substrates for the widely-distributed amino acid transporter system-L (L-AT). This mechanism for transmembrane transport of cysNO could explain why stereoselective haemodynamic effects are seen following administration of L-cysNO and D-cysNO to rats (Davisson *et al.*, 1996), and experimental studies carried out *in vitro* have confirmed its presence in a number of cell types including erythrocytes (Sandmann *et al.*, 2005), endothelial cells (Broniowska *et al.*, 2006), vascular smooth muscle cells (Li and Whorton, 2007; Riego *et al.*, 2009), epithelial cells (Granillo *et al.*, 2008), and various transformed cell lines (Zhang and Hogg, 2004; Li and Whorton, 2005), although to date, there has been no direct demonstration of cysNO uptake via L-AT in platelets. In these published studies, other forms of RSNOs, including GSNO, S-nitroso-cysteinyl-glycine, S-nitroso-N-acetyl-penicillamine and S-nitrosoalbumin, failed to be transported via L-AT, nor could they mediate NO-related signalling in target cells unless extracellular cysteine was supplied. In the presence of extracellular cysteine, cysNO is formed from the inert RSNO by a process of transnitrosation, with subsequent uptake on the L-AT system and intracellular signal transmission. If only cystine is available, a cystine–cysteine shuttle mediated by the X_c^- aminoacid transport system can import cystine and subsequently release cysteine into the surrounding medium following intracellular reduction, thus providing substrate for transnitrosation and L-AT mediated uptake of cysNO (Zhang and Hogg, 2004; Li and Whorton, 2005). This mechanism has been shown to be relevant for a wide range of signalling events and also for the accumulation of intracellular RSNOs. Experiments have generally been performed using relatively high RSNO concentrations (20 μM upwards) and endpoints measured after RSNO exposure for at least 15 min. An interesting feature to emerge from these studies is that in general, cellular effects mediated by cysNO/L-AT are insensitive to the presence in the extracellular medium of NO scavengers, such as oxyhaemoglobin, thus excluding NO release from the mechanism (Zhang and Hogg, 2004; Zhu *et al.*, 2008). An exception is when cysNO-mediated stimulation of sGC is considered – this process is inhibited by oxyhaemoglobin, but only because intracellular reduction of cysNO to NO is required before a cyclic GMP response can occur (Riego *et al.*, 2009).

Different modes of RSNO delivery may explain their selective antithrombotic action

RSNOs are potent platelet inhibitors (see above) but it is not yet clear that their antiplatelet actions require prior conversion of RSNO to cysNO and transport into the platelet via the L-AT system. *In vitro* aggregation of washed platelet suspensions is inhibited by a range of RSNO molecules without the need for addition to the surrounding medium of cysteine or cystine (Radomski *et al.*, 1992; Mathews and Kerr, 1993; Simon *et al.*, 1993; Gordge *et al.*, 1998b), and unlike L-AT-mediated actions, platelet inhibition (both cyclic GMP-dependent and -independent) is inhibited by haemoglobin (Radomski *et al.*, 1992; Megson *et al.*, 2000; Crane *et al.*, 2005), implying that release of free NO must occur as part of the process. For protein RSNOs, such as S-nitrosoalbumin, to inhibit platelet aggregation, there does appear to be a requirement for prior transnitrosation to a low molecular weight thiol for the anti-platelet action to be realized; however, this function can be fulfilled as efficiently by glutathione or cysteinyl-glycine as by cysteine (Crane *et al.*, 2002). Studies using washed platelets have not included measurement of cysteine concentrations in the surrounding medium, and it is therefore possible that cysteine released by the platelets themselves mediates NO signal transfer via L-AT. However, this seems unlikely as the antiplatelet action of GSNO, for example, is evident at lower concentrations (10 μM or less) and within a more rapid timeframe (<2 min) than effects reported for L-AT-mediated signalling. Furthermore, if the inhibitory action of GSNO depended upon cysNO/L-AT then it might be expected to be more potent in platelet-rich plasma (where plasma cysteine/cystine is available) than in washed platelet suspensions, whereas in fact the reverse is true (Radomski *et al.*, 1992). In a recent publication that addressed the possible modes of intra-platelet transport of NO, neither cyclic GMP accumulation nor DAF-FM fluorescence in response to GSNO was significantly inhibited by the L-AT inhibitors BCH or L-leucine (Bell *et al.*, 2007). Therefore, despite a wealth of evidence for the importance of the cysNO/L-AT system in endothelial and smooth muscle cells of the vascular wall, platelets appear to respond to RSNOs in a

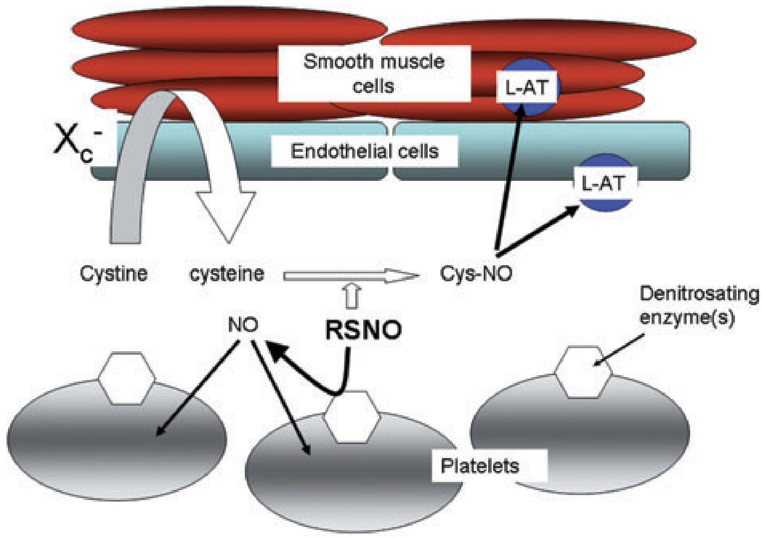

Figure 1 Different modes of nitric oxide (NO) delivery from S-nitrosothiols (RSNOs). Cells of the vascular wall import NO principally via uptake of S-nitrosocysteine (cysNO) on the amino acid transporter system L (L-AT), following an extracellular process of transnitrosation from RSNO to cysteine. A cystine–cysteine shuttle mediated by the X_c^- transporter may act as a supply of extracellular reduced cysteine. In contrast NO delivery into platelets relies on the activity of cell surface denitrosating enzymes, such as cell surface isomerases (csPDI). This scheme indicates the main routes of NO uptake, but does not exclude the possibility that alternative or additional routes are available for each cell type.

different way (Figure 1). If this anomalous behaviour of platelets, compared with other vascular cells, can be confirmed then a possible explanation for the reported platelet-selectivity of GSNO is suggested (De Belder *et al.*, 1994), as, at low concentrations, this molecule may have access to a direct antithrombotic action on platelets that is not available to endothelial or smooth muscle cells.

The susceptibility of GSNO's antiplatelet action to NO scavenging by haemoglobin suggests instead that it undergoes platelet-mediated metabolism, either by a (so far uncharacterized) copper-dependent surface enzyme (Gordge *et al.*, 1995; 1996), or by csPDI, which is known to be present on platelets (Essex *et al.*, 1995) and capable of releasing NO (Root *et al.*, 2004). If differences exist in csPDI expression between platelets and vascular cells, then it might be possible to exploit this to provide selective antithrombotic action. The inhibition of platelet csPDI that results from interaction with RSNO molecules (Shah *et al.*, 2007) is potentially a major antithrombotic mechanism, as there is abundant evidence that active csPDI is required for platelets to function efficiently during haemostasis (Essex, 2004).

Another possible reason why RSNOs might mediate selective antithrombotic effects is that a number of functionally important nitrosation targets exist on the *external* surface of platelets. These include csPDI itself but also the adhesion molecules glycoprotein 1b (Burgess *et al.*, 2000) and integrin αIIbβ3 (Yan and Smith, 2000; Walsh *et al.*, 2007). Allosteric disulphides on tissue factor and other haemostatically active extracellular proteins (Chen and Hogg, 2006) present further possible targets for alteration by plasma RSNOs. Thus although RSNO-mediated signalling to the vessel wall via intracellular thiol modification requires processing via the cysNO/L-AT system, the ability of GSNO (and other RSNOs) to modify important exofacial targets on platelets and other cells may confer selective antithrombotic action (Figure 2).

Conclusion

Possible mechanisms for selective antithrombotic action of RSNOs may be summarized as:

- Differences between platelets and cells of the vascular wall in expression of RSNO metabolizing enzymes, such as csPDI, and/or dependence on csPDI for signal transmission.
- Differences between platelets and cells of the vascular wall in expression of the L-AT system, or dependence on cysNO/L-AT as a mode of NO delivery.
- A heightened role in platelets, compared with cells of the vascular wall, for modification of target proteins on the *external* surface of the plasma membrane, thus allowing at least partial inhibitory effects to be achieved without the need for intracellular delivery of NO.
- RSNO-mediated regulation of blood coagulation, in particular tissue factor exposed at sites of vessel injury or on circulating monocytes or platelet microparticles. This might be either a direct effect or secondary to csPDI modification.

There are few experimental data directly comparing these different mechanisms between platelets and cells of the vascular wall, so it is difficult to grade the mechanisms in order of importance. Nevertheless, the evidence currently available suggests a scenario in which denitrosating enzymes on platelets permit low concentrations of RSNO to mediate antithrombotic NO signalling, whereas higher concentrations of RSNO are required for vasodilatory signalling via the cysNO/L-AT mechanism. A further impeding factor may be the need for RSNO to cross the endothelial monolayer to gain entry into vascular smooth muscle, both of which steps involve cysNO/L-AT. At present these ideas remain speculative, however, they do suggest lines of enquiry that might help

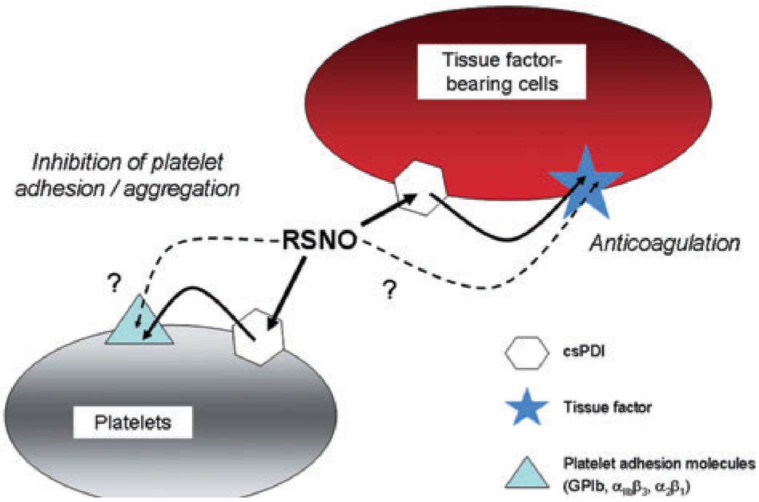

Figure 2 Cell surface targets for the antithrombotic action of S-nitrosothiols (RSNOs). Inhibition of the thrombotic process may be mediated without the need for intracellular nitric oxide (NO) entry, by inactivation of platelet surface adhesion molecules and/or of tissue factor exposed on the surface of the damaged vascular wall, on activated monocytes or circulating microparticles. These modifications occur indirectly via RSNO-induced inhibition of cell surface protein disulphide isomerase (csPDI), and also possibly via direct transnitrosation of the target molecule.

define and realize the antithrombotic potential of RSNO compounds.

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Statement of conflicts of interest

None.

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