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The role of hypothiocyanous acid (HOSCN) in biological systems

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Abstract
Hypohalous acids (HOX), produced by peroxidase-catalysed reactions of halide and pseudohalide ions with H_2O_2, play an important role in the human immune system. However, there is compelling evidence that these oxidants also mediate host tissue damage and contribute to the progression of a number of inflammatory diseases. Although it is well established that significant amounts of hypothiocyanous acid (HOSCN) are formed under physiological conditions, the reactions of this oxidant with host biological systems are relatively poorly characterized. It is generally accepted that HOSCN is a mild oxidant that reacts selectively with thiols. However, it is becoming increasingly recognized that this selectivity can result in the induction of significant cellular damage, which may contribute to disease. This review will outline the formation and reactivity of HOSCN and the role of this oxidant in biological systems.

Keywords: *Hypothiocyanous acid, hypothiocyanite, thiocyanate, peroxidase, protein oxidation*

Introduction

Hypothiocyanous acid (HOSCN) is produced from thiocyanate (SCN^-) in biological systems via the action of peroxidase enzymes, where it plays an important role in mammalian defence mechanisms, owing to its antibacterial properties [1–3]. SCN^- is present in millimolar levels in biological fluids such as saliva, milk and tears, with lower concentrations of SCN^- (10–100 μM) present in plasma from dietary sources [2,4]. Smokers have elevated levels of SCN^- (up to 500 μM) due to the detoxification reaction between cyanide (CN^-) and thiosulphates [5]. In general, SCN^- is the most readily oxidized (pseudo) halide by haem peroxidases, owing to the lower standard reduction potential for the two-electron HOSCN/SCN^- redox couple of 0.56 V, compared to 0.78 V, 1.13 V and 1.28 V for I^-, Br^- and Cl^-, respectively, at pH 7 [6]. SCN^- is the main substrate for lactoperoxidase (LPO) and the closely related salivary peroxidase (SPO) [2], the preferred substrate for myeloperoxidase (MPO) [7] and eosinophil peroxidase (EPO) [8,9] and is also readily oxidized by gastric peroxidase (GPO) [10] and thyroid peroxidase (TPO) [11].

The halogenation and peroxidase cycles of MPO, EPO and LPO

The formation of HOSCN (and other hypohalous acids) occurs via the stepwise reduction of H_2O_2 by the peroxidase. First, the native, ferric form [E-Fe(III)] of the peroxidase reacts rapidly with H_2O_2 to generate the ferryl radical cation, compound I [E-Fe(IV) = $O^{\bullet+\pi}$] (Figure 1A). Compound I is capable of oxidizing SCN^- (or halide ions) very rapidly via a two-electron reaction, which regenerates the ground state, ferric form of the peroxidase and HOSCN (or corresponding hypohalous acid); this is known as the 'halogenation cycle' (Figure 1B). The apparent second-order rate constants for the reactions between compound I of MPO, EPO and LPO with SCN^- are again significantly higher than for Cl^- or Br^- (e.g. for MPO, 9.6×10^6 M^{-1} s^{-1} for SCN^-, 2.5×10^4 M^{-1} s^{-1} for Cl^- and 1.1×10^6 M^{-1} s^{-1} for Br^-) [6]. In addition, compound I can also react with

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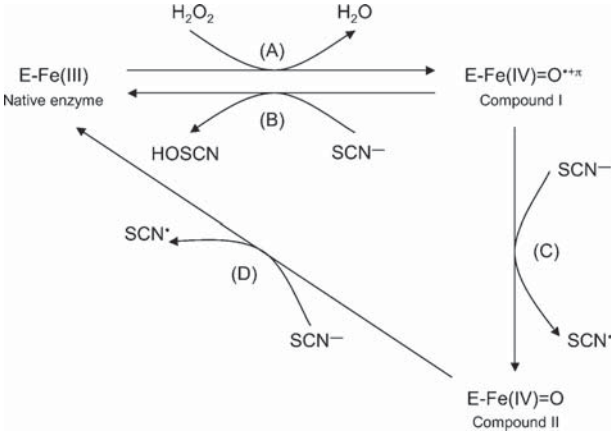

Figure 1. Generation and reactions of redox intermediates typically formed in reactions of SCN^- with human haem-containing peroxidases.

different organic and inorganic substrates, including SCN^-, by two successive sequential one-electron transitions, which generates radical species, such as $SCN^{\bullet}$, and the ground state, ferric enzyme, via an intermediate known as compound II [E-Fe(IV)=O] (the 'peroxidase cycle'; Figure 1C and D).

The partitioning between the halogenation and peroxidase pathways is dependent on the rate of two-electron compared to one-electron oxidation and the concentration of SCN^- [12,13]. The rate of the two-electron reduction of compound I to the native enzyme by SCN^- is very fast ($k_2 = 2 \times 10^8 M^{-1} s^{-1}$, 9.6×10^6 M^{-1} s^{-1} and 1×10^8 M^{-1} s^{-1} for LPO, MPO and EPO, respectively [14–16]), but there are no data regarding the rate of the one-electron oxidation reactions. The standard reduction potential for one-electron oxidation of $SCN^-/SCN^{\bullet}$ (1.65 V, pH 1 [17]) is much higher than the corresponding two-electron oxidation (0.82 V, pH 0 [6]) and the reduction potential of compound I/compound II redox couple (1.35 V for MPO [18] and 1.14 V for LPO [19]). This suggests that the two-electron reaction to the native enzyme will be more favourable than the one-electron reaction forming compound II with SCN^-. For comparison, the standard reduction potential of a good peroxidase substrate, such as Tyr to phenoxyl radical, is 1.07 V [20]. However, it has also been reported that SCN^- binds close enough to the catalytic site (haem pocket) of MPO, EPO and LPO to influence the reduction potential and binding affinity of small molecules [12].

Role of MPO and EPO in disease

Although it is well established that SCN^- is the major substrate for LPO and SPO [1–3], it has only been demonstrated recently that that the oxidation of SCN^- by other peroxidases, including MPO and EPO, may also be biologically relevant [7,9]. Thus, it has been estimated that ca. 50% of the H_2O_2 consumed by MPO oxidizes SCN^- under physiological halide ion concentrations (100–140 mM Cl^-, 20–100 μM Br^-, < 1 μM I^-, ≤ 120 μM SCN^-), with most of the remaining H_2O_2 (ca. 45%) used to oxidize Cl^- to hypochlorous acid (HOCl), based on the specificity constants of 1:60:730 for Cl^-, Br^- and SCN^-, respectively [7]. This is in agreement with data that SCN^- is the best electron donor for MPO compound I ($k_{SCN^-}:k_{Cl^-} = 385:1$) [14] and the standard reduction potentials for the two-electron HOX/X^- redox couple [6]. Similarly, the 2.8-fold preference of EPO for SCN^- compared to Br^- has led researchers to postulate that both hypobromous acid (HOBr) and HOSCN will be produced under physiological conditions, for example in the airways of asthmatics [8,21].

There is a large body of evidence showing that excessive or misplaced production of HOCl and HOBr by MPO and EPO, respectively, results in tissue damage that contributes to the progression of disease [21–23]. The evidence supporting a detrimental role of MPO in cardiovascular disease is particularly compelling, with enzymatically active MPO protein detected in all grades of human atherosclerotic lesions [24]. Similarly, the intensity of staining by a monoclonal antibody (HOP-1/2D10G9) specific for HOCl-damaged proteins and the level of the HOCl biomarker 3-chlorotyrosine (Cl-Tyr) are both elevated in human lesions [25–28]. In addition, MPO levels are recognized as both a major risk factor for coronary artery disease [29] and a powerful predictor of health outcomes in people presenting with chest pain [30], in patients with acute coronary syndromes [31] and after a myocardial infarction [32]. MPO is also associated with the development of inflammatory cancers, lung damage, neurodegenerative diseases, kidney disease, rheumatoid arthritis and a number of other non-infectious diseases [22,23,33]. Similarly, the detection of 3-bromotyrosine provides evidence for a critical role of HOBr and EPO in the oxidative damage observed in asthma, allergic reactions and other malignancies [21,34,35].

In contrast, the role of HOSCN in disease is not well understood, which may be associated with the lack of specific biomarkers for HOSCN-mediated damage. However, recent evidence suggests that HOSCN induces significant cellular dysfunction [36,37] and, in some cases, is more damaging than either HOCl or HOBr [38]. Similarly, a role of SCN^--derived oxidants in cardiovascular disease has been suggested by the detection of elevated levels of carbamylated proteins, formed by reactions mediated by cyanate (OCN^-) produced on decomposition of HOSCN, in atherosclerotic plaques [39]. This article will review the chemistry and biochemistry of HOSCN and discuss the role of these species in human disease.

Formation, stability and properties of HOSCN

The oxidation product responsible for the antibacterial activity of the LPO/H_2O_2/SCN^- system is often attributed to HOSCN/$OSCN^-$ [4,40–43]. NMR studies have demonstrated that the formation of HOSCN/$OSCN^-$ by LPO is strongly pH-dependent, with the maximal rate of formation observed at pH $\leq$ 6 [13]. Similarly, the kinetics of peroxidation of SCN^- to $OSCN^-$ are also highly dependent on the concentrations of both SCN^- and H_2O_2 [44,45]. The proposed mechanism involves initial formation of thiocyanogen [$(SCN)_2$, equation 1], which is rapidly hydrolysed to HOSCN (equation 2), as opposed to the direct formation of HOSCN via the classic halogenation cycle (Figure 1A and B). $(SCN)_2$ may be produced via reaction of SCN^+ with SCN^- or via reactions mediated by $SCN^\bullet$ generated by reaction of SCN^- with compound I (Figure 1C and D). The pK_a of HOSCN is 5.3 [46], therefore a mixture of both the protonated form and anion $OSCN^-$ will exist at physiological pH (equation 3). From here on, HOSCN will be used to designate this physiological mixture.

$$H_2O_2 + 2SCN^- \rightarrow 2H_2O + (SCN)_2 \quad (1)$$

$$(SCN)_2 + H_2O \rightarrow HOSCN + H^+ + SCN^- \quad (2)$$

$$HOSCN \rightleftharpoons OSCN^- + H^+ \quad (3)$$

The formation of HOSCN in LPO-mediated reactions has been confirmed in a number of NMR studies [13,47,48]. When SCN^- is present in excess compared to H_2O_2, a short-lived intermediate with a chemical shift of 127.4 was observed at pH 7 by ^{13}C NMR [48]. This species was attributed to a dithiocyanate ether anion, formed via the rapid association of $(SCN)_2$ with H_2O (equation 4). The putative ether anion decomposed to give SCN^- and a species with a chemical shift of 128.5, which was assigned to $OSCN^-$ (equation 5) [48]. However, this assignment has been disputed on the basis of recent ^{13}C NMR data, which attributes species with chemical shifts of 127.4 and 128.5 to $OSCN^-$ and OCN^-, respectively [47,49].

$$(SCN)_2 + H_2O \rightarrow NCS\text{-}O\text{-}SCN^{2-} + 2H^+ \quad (4)$$

$$NCS\text{-}O\text{-}SCN^{2-} \rightarrow OSCN^- + SCN^- \quad (5)$$

HOSCN and $OSCN^-$ decompose readily at physiological pH, particularly in the presence of excess H_2O_2 compared to SCN^-, which forms oxyacids, including cyanosulphurous acid (HO_2SCN) and cyanosulphuric acid (HO_3SCN) [4,13,42,43,50], via further reactions of HOSCN (equations 6 and 7) or reaction of HOSCN with H_2O_2 (equation 8).

Although direct evidence for these species in biological systems is lacking, owing to their instability

$$2\ HOSCN \rightarrow HO_2SCN + H^+ + SCN^- \quad (6)$$

$$HOSCN + HO_2SCN \rightarrow HO_3SCN + SCN^- + H^+ \quad (7)$$

$$H_2O_2 + HOSCN \rightarrow HO_2SCN + H_2O \quad (8)$$

in aqueous solution [13,46], the formation of OCN^- by MPO and EPO and the resulting protein carbamylation observed in cardiovascular disease for example suggests that they may play a role in mediating biological damage [39]. Thus, OCN^- accounts for ca. 10% and 50% of the SCN^- consumed by MPO and EPO, respectively [39,49], with the formation of this species attributed to reaction of HO_2SCN with H_2O_2 (equation 9) [4,49]. The formation of OCN^- may also occur via the hydrolysis of NC-SCN, which is formed on reaction of HCN with HOSCN [4]. NMR studies have also provided evidence to support the formation of cyanide (CN^-) on decomposition of $OSCN^-$, which may occur via decomposition of HO_3SCN (equation 10) [13,48].

$$H_2O_2 + HO_2SCN \rightarrow OCN^- + H_2SO_3 + H^+ \quad (9)$$

$$HO_3SCN + H_2O \rightarrow CN^- + H_2SO_4 + H^+ \quad (10)$$

With MPO and EPO, evidence was obtained to support the generation of $OSCN^-$ and additional short-lived, more reactive, oxidizing products, owing to the ability of SCN^- and $OSCN^-$ to act as peroxidase substrates (Figure 1C and D) [8]. Thus, radical species, such as $SCN^\bullet$, $OSCN^{-\bullet}$ or $(SCN)_2^{-\bullet}$ may also be important products of the peroxidase catalysed reaction of H_2O_2 and SCN^- [8,51,52]. Evidence for the formation of thiocarbamate-*S*-oxide [$H_2NC(=O)\text{-}S\text{-}O^-$], via direct hydrolysis of $OSCN^-$ under alkaline conditions has also been reported (equation 11) [53]. The formation and biological activity of this species *in vivo* remains to be established, though it has been demonstrated that thiocarbamate-S-oxide reacts with thiols, albeit slower than HOSCN [54].

$$OSCN^- + H_2O \rightarrow H_2NC(=O)SO^- \quad (11)$$

HOSCN may also be generated *in vivo* via the reaction of SCN^- with HOCl and HOBr [55,56]. This reaction is fast, particularly for HOBr (k_2 2.3×10^9 $M^{-1}\ s^{-1}$), which has led to the suggestion that SCN^- is the major scavenger of this oxidant under biological conditions [56]. The reaction of SCN^- with HOCl or HOBr at high pH (typically pH 13) is also a convenient chemical method for the generation of $OSCN^-$ [47]. Other chemical methods to generate HOSCN/$OSCN^-$ generally involve the hydrolysis of $(SCN)_2$ prepared in carbon tetrachloride via reaction of lead thiocyanate with bromine [47,48,57]. It is also possible to produce $OSCN^-$ via the decomposition of *N*-thiocyanatosuccinimide (NTS), which can be

isolated after reaction of *N*-bromosuccinimide with NaSCN in dichloromethane [58].

The concentration of HOSCN/$OSCN^-$ can be quantified by the UV spectrum in each case, with $OSCN^-$ and HOSCN absorbing at 220 nm (extinction coefficient 3870 M^{-1} cm^{-1}) and 240 nm (extinction coefficient 95 M^{-1} cm^{-1}), respectively [42]. Typically $OSCN^-$ is quantified at 235 nm, using an extinction coefficient in the range of 1.29–1.84 $\times$ 10^3 M^{-1} cm^{-1} [47]. Recently, it was reported that $OSCN^-$ has an additional absorbance maximum at 376 nm (extinction coefficient 26.5 M^{-1} cm^{-1}) [47]. Although the extinction coefficient is much lower than at 235 nm, it may be a useful wavelength to quantify $OSCN^-$, particularly with a long path length cell, owing to less interference with excess SCN^- or the presence of H_2O_2, which also absorb strongly in the 220–240 nm region [47].

Reactivity of HOSCN with biological molecules

Low-molecular mass thiols (R-SH)

Low-molecular mass thiols such as reduced glutathione (GSH) are important targets for HOSCN in studies performed with different cell types [38,49,59]. This results in the formation of unstable sulphenyl thiocyanate (RS-SCN) derivatives (equation 12) [4,38,49,59–62]. The stabilities of RS-SCN adducts are pH-dependent and under highly acidic conditions it is possible to isolate sulphenyl thiocyanates formed on GSH, Cys and penicillamine [60,62]. At physiological pH, these species react readily with other thiol molecules to form disulphides, which may occur via the formation of other reactive sulphur species, such as sulphenic acids or thiosulphinate esters [61–63] (equations 13 and 14). Thus, oxidized glutathione (GSSG) is the only product observed on treatment of GSH with HOSCN [60,64].

$$\text{R-SH} + \text{HOSCN} \rightarrow \text{RS-SCN} + \text{H}_2\text{O} \quad (12)$$

$$\text{RS-SCN} + \text{H}_2\text{O} \rightarrow \text{RS-OH} + \text{SCN}^- + \text{H}^+ \quad (13)$$

$$\text{RS-SCN} + \text{R}^1\text{-SH} \rightarrow \text{RS-SR}^1 + \text{SCN}^- + \text{H}^+ \quad (14)$$

Evidence has also been presented for the formation of mixed Cys dimers, though these experiments were performed under acidic conditions [62]. This is potentially significant as mixed disulphides, for example those formed on glutathionylation of proteins, play an important role in the regulation of many proteins, enzymes and signalling pathways [65]. The rate of reaction of HOSCN with low-molecular mass thiols is pH-dependent and increases at lower pH values, consistent with HOSCN rather than $OSCN^-$ mediating the reaction [66]. In addition, the rate constants for HOSCN with low-molecular mass thiols are inversely related to the pK_a of the thiol group, with values obtained in the range of 7.3 $\times$ 10^3 M^{-1} s^{-1} for N-acetyl-Cys to 3.8 $\times$ 10^5 M^{-1} s^{-1} for 5-thio-2-nitrobenzoic acid (TNB), as determined by competition kinetics using the rate constant for reaction with TNB as a reference, at pH 7.4 [66]. Similarly, the rate of reaction of HOSCN with GSH is 2.5 $\times$ 10^4 M^{-1} s^{-1} at pH 7.4. This suggests that it is likely that HOSCN will display some selectivity for particular thiols under biological conditions.

Proteins

Thiols. HOSCN reacts preferentially with protein thiol groups in studies with isolated proteins [4,50,57,67] and biological fluids such as plasma [67]. This generates protein-derived, RS-SCN derivatives (equation 12), as evidenced by the reversible incorporation of ^{14}C from HOS^{14}CN [50,57,67]. These protein RS-SCN derivatives are postulated to decompose to form sulphenic acid intermediates (equation 13), although direct evidence for the formation of these species on proteins is lacking [4,50]. With small proteins, RS-SCN derivatives typically react with a further thiol molecule to form a disulphide bond, resulting in the consumption of two thiol groups per molecule of HOSCN (equation 14) [4,50]. However, in many cases, reaction of HOSCN with protein thiols occurs with a 1:1 stoichiometry, owing to steric constraints preventing disulphide bond formation [50,67]. The rates of reaction of HOSCN with protein thiols are in the range of 1–7 $\times$ 10^4 M^{-1} s^{-1} [66], which are 2–3 orders of magnitude slower than the analogous reactions with HOCl or HOBr (ca. 3 $\times 10^7$ M^{-1} s^{-1} [68]). However, the relative lack of reactivity of HOSCN compared to HOCl and HOBr with other biological targets suggests that HOSCN is likely to play a major role in the oxidation of protein thiols mediated by peroxidases under inflammatory conditions *in vivo*.

Other protein residues. It is often reported that HOSCN lacks reactivity with other non-thiol, protein residues [4]. However, Aune et al. [57] have reported the stable incorporation of SCN^- into proteins in the presence of LPO/H_2O_2/SCN^- under conditions where the peroxide is present in excess compared to protein thiols. Modification of Trp, His and Tyr was observed, which was attributed to 'halogenation' type reactions mediated by $(SCN)_2$, rather than HOSCN [57]. However, Tyr is known to bind to the active site of LPO, which may have influenced the nature of the products formed [57]. The formation of stable protein-derived products containing ^{14}C in experiments with HOS^{14}CN in the absence of LPO has also been reported, though the site of adduct formation was not determined [67]. HOSCN also reacts with protein-bound Trp residues, in experiments with various proteins (including albumin, trypsin inhibitor, lysozyme and myoglobin) [67]. LC-MS studies with HOSCN-treated myoglobin

revealed the formation of products consistent with the addition of two oxygen atoms to Trp_7, Trp_{14} or both Trp residues [67]. However, no evidence for reaction was obtained in experiments with Trp or small Trp-containing peptides [67]. The reason for this difference is not certain, but it may be associated with the tertiary structure of the protein or attributable to the formation of reactive protein-derived intermediates, such as amino thiocyanate (RNH-SCN) derivatives (equation 15) [67].

$$RNH_2 + HOSCN \rightarrow RNH\text{-}SCN + H_2O \quad (15)$$

Although it is well known that HOCl and HOBr react readily with amines and amides to form *N*-chloro (RN-Cl) and *N*-bromo (RN-Br) species, respectively [22,69], evidence for the formation of the analogous RN-SCN intermediates on proteins is lacking. This is attributed to the rapid hydrolysis of RN-SCN intermediates at physiological pH (equation 16). Exceptions to this behaviour are observed with sulphonamides ($R\text{-}SO_2\text{-}NH_2$) and heterocyclic aromatic imines, such as imidazole and His, which react with HOSCN to form the corresponding thiocyanatosulphonamides ($R\text{-}SO_2\text{-}NH\text{-}SCN$) and thiocyanatimines (ring RNH-SCN), respectively [46]. These N-SCN derivatives retain the oxidizing ability of HOSCN and react rapidly with free thiols, such as TNB, resulting in the formation of the dimer, 5,5'-dithio-2-nitrobenzoic acid (DTNB) [46]. However, high-molecular mass, TNB-reactive material, is observed on treatment of poly-lysine with HOSCN at pH 7.4, suggesting that it is possible to form Lys-derived N-SCN adducts at physiological pH [67].

$$RNH\text{-}SCN + H_2O \rightleftharpoons RNH_2 + HOSCN \quad (16)$$

$$RNH_2 + H^+ + OCN^- \rightarrow RNHC(=O)NH_2 \quad (17)$$

Protein carbamylation occurs on reaction of OCN^-, formed on decomposition of HOSCN (e.g. equation 10), with amino, thiol, carboxyl, imidazole and phenolic hydroxyl functional groups (equation 17) [70]. However, only the modification of amino groups occurs in a non-reversible manner at physiological pH [70,71]. The formation of carbamylated Lys (homocitrulline) has been used as a biomarker for SCN^--derived oxidants *in vivo* [39]. However, OCN^- can also be produced by other pathways. For example, OCN^- can be generated via a two-electron reaction of CN^- with MPO (but not EPO or LPO) compound I [72,73], during reaction of HOCl with CN^- [74] and under conditions of uremia [75–77]. Carbamylation reactions are also observed on reaction of *N*-chloramines with SCN^- [72].

Lipids and DNA

There are relatively few studies that have examined the reactivity of HOSCN with other biological molecules including lipids and DNA. The presence of SCN^- promoted the MPO-dependent peroxidation of plasma lipids, as assessed by quantification of elevated levels of cholesteryl ester hydroperoxides and hydroxides using LC-MS, suggesting a role of SCN^--derived oxidants in mediating lipid damage [78]. Similarly, the formation of elevated levels of conjugated dienes and lipid hydroperoxides was observed on exposure of isolated low-density lipoprotein (LDL) to $MPO/H_2O_2/SCN^-$ [51]. The observation of lipid peroxidation in reactions involving MPO and SCN^- suggests the formation of radical species such as $SCN^{\bullet}$, $OSCN^{-\bullet}$ or $(SCN)_2^{-\bullet}$. This is supported by the observation that ascorbic acid, a potent radical scavenger, was highly effective at inhibiting the SCN^--dependent lipid peroxidation observed [51], although ascorbic acid may also act as a one-electron substrate for MPO compound I. However, no evidence was obtained for the reaction of HOSCN with the double bonds of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), in contrast to the formation of chloro- and bromo-hydrins observed in the corresponding experiments with HOCl and HOBr, respectively [79]. Similarly, no changes in the UV spectra of either isolated nucleosides or DNA were observed on reaction with HOSCN [80,81]. In addition, HOSCN was not found to be mutagenic in multiple bacterial strains, suggesting that reaction with the genetic material within cells is not competitive compared to other cellular targets [80].

Reactivity of HOSCN with cellular systems

HOSCN is often referred to as a relatively mild oxidant, that is innocuous to mammalian cells. Similarly, the presence of SCN^- and formation of HOSCN by peroxidases has been reported to be an important detoxification mechanism, responsible for the removal of potentially more damaging oxidants, such as H_2O_2 or HOCl [10,82–84]. However, HOSCN can induce the lysis of erythrocytes [49,59], act as a virucidal agent [85], bring about growth arrest or inhibit cell division in a number of bacterial cells [86], inhibit glycolysis and respiration [86,87] and decrease glucose uptake [88], which is described in detail in the next section.

Bacterial cells

The ability of HOSCN produced by the $LPO/H_2O_2/SCN^-$ system to inhibit bacterial growth, particularly in saliva and milk, is well documented [3,89–94]. The antimicrobial action of $HOSCN/OSCN^-$ is greater at

low pH, consistent with greater membrane penetration and reactivity of HOSCN rather than the charged $OSCN^-$ [93,95]. The inhibitory effect of HOSCN on bacterial growth is attributed to the oxidation of glycolytic enzymes, containing essential thiol groups (e.g. glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hexokinase, glucose-6-phosphate dehydrogenase and aldolase) resulting in the inhibition of glycolysis [86,87,90,96,97]. However, treatment of bacterial cells with HOSCN also results in decreased glucose transport [96] and inhibition of respiration [86,98]. The decreased transport of glucose and other essential nutrients may be associated with structural damage to the bacterial cell membrane or GLUT transporters [96,99]. HOSCN from LPO/H_2O_2/SCN^- also inhibits both the growth and urease activity of the gram negative bacterium *Helicobacter pylori* [100]. The inactivation of urease is attributed to the oxidation of functionally important thiols and is significant as urease activity is important for the colonization of this bacterium in the stomach [100]. The GPO/H_2O_2/SCN^- system may also be important in the inhibition of bacterial cell growth in the stomach, owing to the ability of the stomach to concentrate SCN^- to mM levels [10].

Although the peroxidation of SCN^- yields oxidation products that inhibit the growth and metabolism of bacteria, in many cases no significant loss in cell viability has been noted [82]. Similarly, bacteria treated with HOSCN, followed by washing to remove residual oxidant, regenerate their thiol groups and recover their ability to respire, suggesting that the antimicrobial activity of HOSCN is reversible [86]. However SCN^- has also been shown to potentiate the H_2O_2-induced cytotoxicity observed in the presence of LPO [92,101]. This effect is attributed to the formation of other reactive species, such as HO_2SCN, via reaction of HOSCN with H_2O_2 (equation 8) [92]. In addition, evidence for irreversible inhibition of respiration was obtained in studies where bacteria were treated with HOSCN for extended periods of time, owing to further reaction of R-SOH and RS-SCN intermediates resulting in non-reversible thiol modifications [86]. Similarly, the HOSCN-induced inactivation of hexokinase occurred in a time-dependent manner, with the loss in thiols and subsequent covalent incorporation of SCN^- correlating with loss in activity [102].

Mammalian cells

The effects of HOSCN on mammalian cells have not been studied as widely as bacterial cells, although it is becoming increasingly recognized that this oxidant can induce significant cellular dysfunction via the selective targeting of critical thiol residues on proteins [9,36–38,49]. As with bacterial cells, HOSCN can readily inactivate key thiol-dependent enzymes (e.g. GAPDH, glutathione *S*-transferases (GST), membrane ATPases, caspase 3) [9,38] and deplete low-molecular mass thiols, such as GSH, in various mammalian cell types [9,38,59]. Moreover, the extent of protein thiol loss [38] and enzyme inactivation [49] is significantly greater with HOSCN than either HOCl or HOBr. HOSCN induces apoptosis (and necrosis) on addition to murine macrophage cells (J774A.1) with greater efficacy and at lower concentrations than either HOCl or HOBr, by a caspase-independent pathway, suggesting that generation of this oxidant at sites of inflammation is likely to be a detrimental process [38]. However, the presence of physiological concentrations of SCN^- resulted in the protection of HL-60 cells from apoptosis induced by MPO/H_2O_2/Cl^-, an effect ascribed to the formation of HOSCN rather than HOCl [103]. Similarly, SCN^- protects eosinophils from both spontaneous and agonist-induced apoptosis [104]. The reason for this discrepancy is not certain, but may be associated with detoxification of the HOSCN by media components, as the latter studies were performed in the presence of media containing serum and/or BSA [103,104], rather than balanced salt solutions [38].

HOSCN is also a uniquely potent (up to 100-fold) transcriptional inducer of endothelial cell expression of tissue factor and monocyte adhesion molecules (ICAM-1, VCAM-1 and E-selectin) via a process mediated by NF-κB activation [36,37]. This suggests that HOSCN may play an important role in the development of a pro-inflammatory endothelial cell phenotype. The induction of endothelial cell expression of ICAM-1, VCAM-1 and E-selectin by HOSCN is particularly significant, as these molecules promote monocyte adhesion and migration, critical processes in atherosclerotic lesion development [105]. HOSCN also inhibits cellular protein tyrosine phosphatases (PTP), via reaction with the active site thiol, which leads to the hyper-phosphorylation of cellular proteins and influences cell signalling and gene expression via the kinase pathway (Lane et al., unpublished data).

Biological significance of HOSCN

It is well established that the LPO/H_2O_2/SCN^- system and generation of HOSCN play an important role in preventing not only microbial growth (see above), but also by acting as potent anti-viral and anti-fungal agents [85,106]. However, the potential detrimental role of HOSCN in disease is not well understood, in contrast to the other peroxidase-derived oxidants, HOCl and HOBr [21–23]. This is mainly due to a lack of specific biomarkers for HOSCN-mediated damage, which makes assessing the role of this oxidant *in vivo* difficult.

Beneficial effects

Dental hygiene. The role of peroxidases in promoting dental health in the oral cavity has been studied extensively [107–109]. Saliva contains significantly higher levels of SCN^- (0.5–3 mM [110]) compared to most other biological fluids, with levels of 20–60 μM HOSCN contained in resting human saliva [111]. The inhibitory effect of HOSCN on the growth and metabolism of bacteria (described above) is beneficial in terms of dental health, by preventing bacterial sugar and acid formation, leading to a decrease in plaque and caries [92,97,112]. Indeed, the inhibitory effect of HOSCN on oral bacteria led to the development of a toothpaste containing the LPO/H_2O_2/SCN^- system, which elevated salivary levels of HOSCN ca. 5-fold (to 100–300 μM) [113]. However, certain types of oral bacteria, including *Streptococcus sanguis,* are resistant to the deleterious effects of HOSCN, due to the presence of a NADH:-hypothiocyanite oxidoreductase, which converts $OSCN^-$ into SCN^- [114,115]. Human saliva also possesses anti-viral and anti-fungal properties, with evidence that HOSCN can inactivate the human immunodeficiency virus [116] and herpes simplex virus type 1 (HSV-1) [85,117,118] and inhibit oral fungi such as *Candida albicans* [106,119]. The inactivation of HSV-1 by HOSCN may be important in the prevention of oral lesions associated with this virus [85,118].

Milk and food preservation. There is significant interest in the use of the LPO/H_2O_2/SCN^- system in the preservation of food products, particularly milk and dairy products [1–3]. The ability of HOSCN produced via the LPO system, to inhibit many types of bacteria that would otherwise compromise milk quality, have been investigated widely [3]. Thus, supplementing raw milk with the LPO system has utility for the prevention of microbial growth and milk spoilage, particularly in the absence of reliable refrigeration [120,121]. Similarly, the combination of pasteurization and the presence of the activated LPO system increases the shelf-life of even refrigerated milk, by a significant amount compared to non-treated milk [122]. However, in some cases, the formation of HOSCN by an activated LPO system in milk can compromise the quality of manufactured fermented milk products, such as yogurt and cheese [3]. The LPO/H_2O_2/SCN^- system may also be a useful way to preserve the nutrient quality of heat-sensitive, dairy-containing foods, such as dips, desserts, spreads and salad dressings, by reducing the thermal resistance of the bacteria and allowing subsequent heat-treatment at lower temperatures [123].

Airway protection. There is evidence for the presence of peroxidases in various types of cells associated with the respiratory tract [124]. The surface fluid of the airway contains a number of bactericidal agents including defensins (antimicrobial peptides), lysozyme, lactoferrin, immunoglobulins, complement factors and lipopolysaccharide-binding protein that act either directly on the bacteria or by facilitating the action of phagocytic cells contained within the airway [125]. LPO is also a major component of airway surface fluid, with inhibition of this peroxidase resulting in decreased bacterial clearance from the airway [126]. This suggests that the production of HOSCN by LPO may be an important defensive mechanism in the airway [126–128]. Thus, SCN^- is actively transported by lung epithelial cells by different mechanisms involving a series of anion channels, transporters and membrane proteins [129,130]. This results in SCN^- levels of ca. 400 μM in airway secretions compared to 20–100 μM found in plasma [128]. In addition, airway epithelial cells utilize dual oxidases (DUOX) to produce H_2O_2 [131]. This results in the presence of sufficient concentrations of H_2O_2 and SCN^- to form HOSCN via LPO-catalysed reactions [126–128]. There is also evidence that the increased susceptibility of patients with cystic fibrosis (CF) to suffer from chronic respiratory infections may be associated with reduced production of HOSCN, owing to the impaired ability of the CF lungs to transport SCN^- [132,133]; this highlights the importance of HOSCN in airway defence.

Detrimental effects

There is a growing body of indirect evidence that the production of HOSCN by MPO may be involved in atherosclerosis. A significant correlation exists between the magnitude of deposits of oxidized LDL and fatty streaks in the aortae of young people and serum SCN^- levels [134], consistent with SCN^--derived oxidants being a key driver of LDL-modification and lipid deposition. Similarly, smokers, who have high plasma SCN^- levels, have a greater numbers of lipid-laden macrophages (foam cells) compared to non-smokers [135]. OCN^- production via MPO-catalysed oxidation of SCN^- has been implicated as the major pathway responsible for protein carbamylation in human atherosclerotic lesions, with higher levels detected than normal arterial tissue [39]. Elevated levels of carbamylated proteins in plasma independently predict increased adverse cardiovascular events, including the development of coronary artery disease, future myocardial infarction, stroke and death [39]. However, evidence of protein carbamylation is not necessarily specific for the presence of SCN^--derived oxidants, as described earlier.

Similarly, the oxidation of SCN^- by TPO and formation of HOSCN initiates thyroid cell necrosis [11].

This has important implications and may help to explain the elevated prevalence of thyroid destruction leading to endemic myxoedematous cretinism in Central Africa, where SCN^- overload is common. The high levels of SCN^- are a consequence of the consumption of cassava roots containing the cyanogenic glucoside linamarin, which is readily metabolized to SCN^- [11].

Summary

Although historically HOSCN is referred to as a mild, innocuous oxidant, whose bacteriostatic properties make it an important defensive component of saliva, milk and the airway, increasing evidence shows that it may also be responsible for the initiation of host tissue damage. The ability of HOSCN to induce cell death via apoptosis and necrosis and the expression of tissue factor and monocyte adhesion molecules shows that this oxidant has significant detrimental effects on mammalian cells [11,36–38]. In particular, the induction of ICAM-1, VCAM-1 and E-selectin is likely to play a critical role in atherosclerotic lesion development and supports the recent studies that provide *in vivo* evidence for the involvement of SCN^--derived oxidants in coronary artery disease [39]. Thus, although it has been reported that under certain situations the formation of HOSCN may be a detoxification mechanism [10,56,82–84], the potentially damaging effects of this oxidant should not be underestimated and clearly warrant further investigation.

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