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Biochemical Mechanisms and Therapeutic Potential of the Pseudohalide Thiocyanate in Human Health

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Abstract

Thiocyanate (SCN^-) is an ubiquitous molecule in mammalian biology, reaching up to mM concentrations in extracellular fluids. Two-electron oxidation of SCN^- by H_2O_2 produces hypothiocyanous acid (HOSCN), a potent antimicrobial species. This reaction is catalyzed by chordate peroxidases (e.g., myeloperoxidase and lactoperoxidase), occurring in human secretory mucosa, including the oral cavity, airway and alimentary tract, and regulates resident and transient flora as part of innate immunity. Increasing SCN^- levels limits the concentrations of a family of 2-electron oxidants (H_2O_2 , hypohalous acids and haloamines) in favor of HOSCN formation, altering the oxidative impact on host tissue by substitution of repairable thiol and selenol oxidations instead of biomolecule degradation. This fine-tuning of inflammatory oxidation paradoxically associates with maintained host defense and decreased host injury during infections, due in part to phylogenetic differences in the thioredoxin reductase system between mammals and their pathogens. These differences could be exploited by pharmacologic use of SCN^- . Recent preclinical studies have identified antimicrobial and anti-inflammatory effects of SCN^- in pulmonary and cardiovascular animal models, with implications for treatment of infectious lung disease and atherogenesis. Further research is merited to expand on these findings and identify other diseases where SCN^- may be of use. High oral bioavailability and an increased knowledge of the biochemical effects of SCN^- on a subset of pro-inflammatory reactions suggest clinical utility.

Keywords

Hypothiocyanous acid; Hypochlorous acid; Peroxidases; Cystic fibrosis; Redox regulation

Introduction

Thiocyanate (SCN^- ; negative charge is localized on the sulfur atom) is a 58 Da pseudohalide ubiquitously found in the extracellular fluids of mammals, ranging from 0.1 μM to >2 mM in concentration depending on physiologic and environmental contexts. SCN^- is produced when a sulfur atom is sigma bound to the carbon of cyanide, which is the body's major

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Conflict of Interest

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the upper or lower airways, exhibit significant interpersonal variance in SCN⁻ concentration [11,17,28]. Airway secretions from research animals have more predictable concentrations of SCN⁻, perhaps owing to genetic similarity and regulated veterinary care [14,15,29]. In saliva, the most SCN⁻-enriched extracellular fluid in humans [23,30-32], SCN⁻ has a diurnal pattern with peak values occurring in early morning [23] and parotid saliva is more enriched with SCN⁻ than mixed [33]. SCN⁻ is also enriched in tears and gastric fluid [34,35]. These extracellular fluids also contain peroxidases, typically LPO or peroxidasin (also called vascular peroxidase) [11,36], and H₂O₂ (by way of superoxide dismutation) from locally expressed NADPH oxidases (NOX) and/or dual oxidases (DUOX) [37]. These factors are essential for the utilization of SCN⁻ in host defense processes by forming HOSCN. Extracellular fluids are also subject to inflammation during infection and other pro-inflammatory conditions, with invading leukocytes providing additional oxidase and peroxidase enzymes (i.e., NOX2, MPO and/or EPO) to increase HOSCN or HOX production [4,38].

SCN⁻ is eliminated in the kidneys with 90% reuptake from the glomerular filtrate [39], with the resting rate of SCN⁻ output in the urine reported at <10 µM [40,41]. A half-life of 1-5 days has been reported for plasma SCN⁻ in healthy subjects, while renal insufficiency increases mean half-life to 9 days [42]. Interestingly, C57BL6 mice appear to have much faster clearance of SCN⁻ from the plasma compared to reported human rates, with an apparent half-life of 4-5 hours following the administration of a large dose of SCN⁻ by nebulization [14].

Exogenous influences on physiologic SCN⁻

In healthy individuals, SCN⁻ is thought to originate from the diet or environment either as an intact molecule or resulting from the metabolism of cyanide by sulfurtransferases (e.g., rhodanese and mercaptopyruvate sulfurtransferase) [43]. Rhodanese-like enzyme activity (which catalyzes the formation of SCN⁻ from cyanide and a sulfur donor [1]) is chiefly located in the kidney, liver, brain, lung, muscle and stomach of humans [44] and is regulated by cysteine metabolism to form hydrogen sulfide and cysteine persulfides, as in the rhodanese catalytic cycle [45]. Tissue distribution of rhodanese activity varies among animals and may reflect the intrinsic diet of a species. For instance, herbivorous ruminants have increased alimentary rhodanese activity [46].

Dietary intake of SCN⁻ is primarily associated with glucoside and glucosinolate-containing plant foodstuffs, which are present in the human diet but can vary significantly in quantity and type consumed between ethnic or cultural groups [41,47]. In addition to cyanide, glucosinolates may also yield alkyl isothiocyanates and nitriles [48]. The health effects of cyanogens, which are metabolized into a milieu of biomolecules, should not be inferred as the direct effects of SCN⁻ without additional studies. However, dietary influence on SCN⁻ seems to have limited known health consequences. The average daily intake of SCN⁻ from diets rich in cyanogens is much less than the lowest observable adverse effect level of cyanogen exposure in animals [48]. Non-smoking vegans (who have heightened risk of iodine deficiency as well as increased diet-derived SCN⁻) excrete ~2-fold more SCN⁻ in urine compared to non-smoking vegetarians, but thyroid-stimulating hormone (TSH) and

free thyroxine (FT4) values do not significantly differ between the two groups and do not correlate with SCN^- even with adjustment for common confounders (e.g., age, gender, diet compliance) [41]. A study of mothers (6% smokers) and their breastfed infants found that SCN^- in breast milk, maternal urine, or infant urine was not predictive of infant TSH or FT4 values [40]. Urinary SCN^- was a significant predictor of serum FT4 in low iodine status non-pregnant women, but not pregnant women, non-pregnant women with normal iodine status or men [49]. Goitrous individuals given milk supplemented with 0.1 mg/L iodine and 328 μM SCN^- daily over four weeks maintained normal thyroxine, triiodothyronine and TSH values [50].

Several cyanide-containing drugs cause increases in plasma SCN^- . Smoking tobacco delivers as much as 320 μg cyanide per cigarette [51], significantly increasing plasma SCN^- (70-300 μM [24]) due to the increased detoxification of cyanide. Plasma SCN^- has not been studied in marijuana smokers, but these individuals are equally likely to have increased plasma SCN^- (marijuana smoke contains more cyanide than tobacco smoke, and smoking habits of marijuana users may result in increased smoke exposure [51]). Nitroprusside and cyanocobalamin also contribute to SCN^- concentration by releasing cyanide upon metabolism [52]. Nitroprusside treatment in particular can result in plasma SCN^- values approaching 1 mM at infusion rates above 1 $\mu\text{g kg}^{-1} \text{min}^{-1}$ [53]. However, SCN^- accumulation is not a major health concern in short-term treatment with nitroprusside of individuals with normal kidney function [39].

Exposure to cyanide and subsequent accumulation of plasma SCN^- may correlate with health effects that are not caused by SCN^- itself. In postmortem studies, elevated plasma SCN^- was used to distinguish smokers from non-smokers and correlated with atherogenic biomarkers such as oxidized low density lipoprotein (LDL), apolipoprotein E deposition and macrophage foam cells [54,55]. However, plasma SCN^- associates with decreased all-cause mortality in human myocardial infarction survivors [18] and protects against atherosclerosis when orally administered to pro-atherogenic rabbits [56]. Similarly, plasma SCN^- in smokers and non-smokers was not predictive of atherosclerotic coronary artery disease risk [57]. In another study plasma SCN^- associated with increased thiol oxidation when inflammatory factors were added to the plasma, but smokers and non-smokers had identical resting concentrations of plasma thiol [58]. Thus, unlike circulating MPO levels [18,59], increased plasma SCN^- does not seem to be predictive of cardiovascular disease and mortality but may actually be a protective factor.

Potassium and sodium salts of SCN^- are highly bioavailable orally and by inhalation [14,15,39]. The potassium salt of SCN^- was used as an antihypertensive in the early-to-mid 20th century, but later fell out of favor owing to the development of better treatments [10]. Post hoc toxicology from the time suggests most patients were tolerant of plasma SCN^- concentrations $\gg 10$ -fold higher than normal, but to achieve therapeutic effect physicians regularly gave doses resulting in steady-state plasma $\text{SCN}^- > 1.2 \text{ mM}$, which in some cases led to hypothyroidism, dermatitis or psychosis [60,61]. As this period in American medical history overlapped closely with the introduction of iodized salt, it is not clear how many patients that presented side effects were iodine deficient or sufficient. Because SCN^- was administered as its potassium salt, some side effects of treatment may have been a result of

hyperkalemia. Individuals with pre-existing renal impairment had the highest risk of negative side effects and made up at least half of the dozen individuals whose deaths were attributed to SCN^- in the contemporary literature [62]. The apparent toxicity threshold of ~ 1 mM steady-state plasma SCN^- from this body of literature is >2 -fold more than the concentration of SCN^- needed to saturate the oxidative output of peroxidases (Figure 2), suggesting inflammatory processes were not the cause of the observed toxicity case reports.

Whether endogenous stores of SCN^- are also synthesized from a natural metabolic precursor remains a matter of speculation, but the ubiquity of SCN^- in biologic systems and its exceptional concentration in many extracellular fluids suggest it is a possibility worth consideration. Cyanogen-enriched diets only account for $\sim 6 \mu\text{M}$ of plasma SCN^- , assuming 100% uptake of $16.3 \mu\text{mol}$ per day [48] in 2.75 L of whole body plasma, and none of the SCN^- transported to other bodily fluids. Salivary SCN^- from fasted individuals is $510 \pm 310 \mu\text{M}$ [33], making up a significant fraction of the concentrations reported from individuals without fasting in other studies [23] (however, differences in sample collection likely confound the comparison). Some bacteria generate cyanide from glycine [63], which would act as a metabolic precursor for endogenous SCN^- , but it is unclear whether similar pathways exist in eukaryotes or how cyanide would be compartmentalized and regulated to avoid deleterious effects.

Redox biochemistry of SCN^- and HOSCN

SCN^- shares chemical properties with chloride, bromide and iodide including a sub-zero pK_a [64], meaning it is ionized in all physiologic environments. The chemistry of SCN^- should not be confused with that of isothiocyanate, an unstable resonance structure of SCN^- limited to alkyl-conjugated species in nature (e.g. sulforaphane, iberin) [47,48]. SCN^- is relatively inert prior to oxidation, which is the major biochemical step necessary for its physiologically relevant effects. This process was discovered from the observation that a mixture of SCN^- , LPO and H_2O_2 has potent antimicrobial effects [2]. The product of this reaction has been repeatedly identified as HOSCN, a close chemical relative of HOX [3,65,66]. The first biological matrix shown to utilize SCN^- for antimicrobial activity in this way was milk [2], then saliva [67,68], airway epithelial lining fluid (ELF) [11], nasal lining fluid (NLF) [17], gastric fluid [34] and tears [35], and can be presumed to occur in other secretions without immune privilege. The extent to which HOSCN formation takes place inside of cells is unclear, though it is likely to occur in the neutrophil phagosome.

SCN^- is oxidized to form HOSCN at rates approaching the diffusion limit whether this occurs enzymatically or by the direct reduction of HOX (Figure 3). The initial step of both pathways is the reduction of H_2O_2 by chordate peroxidase heme, which occurs at a rate of $1\text{--}6 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ [7]. The second step in the enzymatic pathway is 2-electron oxidation of SCN^- to regenerate the heme. The second order rate constant for the oxidation of SCN^- by MPO compound I is $\sim 1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, while the same rates for EPO and LPO are 10-20-fold greater [7]. The direct oxidation of SCN^- by HOX follows oxidation of chloride or bromide by MPO or EPO [7], and thus is a three-step HOSCN generating process in parallel to the enzymatic pathway (Figure 3). The reaction of HOCl with SCN^- has a second order rate constant of $2.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ [69] while HOBr reacts more rapidly with a rate constant

of $2.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ [70], corresponding with the electrophilicity of these species [71]. Furthermore, SCN^- is the preferred 2-electron donor (assessed by specificity constant, k_{cat}/K_m^{-1}) of the compound I species of all chordate peroxidases and human peroxidase 1 [7-9,35,36]. The salivary peroxidase referred to in many publications is likely identical or nearly identical to LPO, as it is encoded by the same gene [72]. While MPO may oxidize chloride and bromide in addition to SCN^- , EPO is limited to bromide and SCN^- at physiologic pH and LPO will only oxidize SCN^- under physiologic conditions [7,73]. Although iodide is another potential electron donor for compound I of chordate peroxidases, it is relatively scarce in vivo ($\sim 100 \text{ nM}$ in plasma) and may only participate significantly in halogenation reactions in the thyroid [25,35].

HOSCN steady state fluxes in biological systems are ultimately limited by the concentration of H_2O_2 , chordate peroxidases and SCN^- . Given that H_2O_2 and chordate peroxidases are available and SCN^- is sufficiently concentrated, HOSCN will be the dominant oxidizing species in inflammatory milieu (Figure 2). However, the physiologic range of SCN^- in many compartments is limiting ($< 100 \text{ }\mu\text{M}$) so that HOSCN comprises $< 50\%$ of oxidants produced during inflammation [58]. Concentrations of SCN^- $> 400 \text{ }\mu\text{M}$ drive the totality of oxidant output by MPO to generate HOSCN in vitro [74,75].

The major physiologic target of HOSCN is cysteine thiol (RSH) [19], which is an ubiquitous component of peptides and proteins with large variations in abundance (e.g., glutathione (GSH) versus thioredoxin (Trx)) and chemical properties (e.g., free cysteine versus a catalytic residue influenced by protein context [76]). HOSCN reacts in its protonated form with ionized thiolates (RS^-), ultimately producing a sulenic acid (RSOH) or disulfide (RSSR) and transiently altering the function of affected catalytic residues [77]. The initial oxidation reaction proceeds through a sulfenyl SCN^- intermediate (RSSCN) which rapidly hydrolyzes at physiologic pH [78,79]. Formation of RSOH and RSSR is a regular feature of physiologic thiol reactions and in most cases is rapidly reversed by antioxidant enzymes, existing in a redox equilibrium ultimately regulated by NADPH:NADP^+ [22].

Chemical properties greatly distinguish HOSCN from HOX and H_2O_2 (Figure 4). HOSCN is a stronger acid than the HOX species with a pK_a of 4.85 [20], so the unreactive hypothiocyanite anion predominates in most physiologic fluids and limits rates of reaction (Figure 3). The decreased protonation and standard redox potential of HOSCN compared to HOX [80] make it a less reactive and more selective thiol oxidant than HOX (e.g., $10^{3-5} \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 for HOSCN vs. $10^{7-8} \text{ M}^{-1} \text{ s}^{-1}$ for HOX under similar conditions) [5,19,20,81]. Greater acidity of a thiol increases its rate of reaction with HOSCN by increasing the proportion of nucleophilic thiolate available, rather than impacting nucleophilicity directly [82]. The second order rate constants for the reaction of HOSCN with 5-thio-2-nitrobenzoic acid (TNB, $\text{pK}_a=4.38$, $k_2=3.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) is 15 times faster than with GSH ($\text{pK}_a=8.7$, $k_2=2.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$) at pH 7.4 [19,20]. In contrast, HOCl reacts at a similar rate with thiols ranging from $\text{pK}_a=4-9$ [83], although the rates are so fast that many are lower limits requiring re-evaluation with more sensitive methods [81]. The rate and selectivity of HOSCN reaction with thiols are both enhanced by mildly acidic pH (e.g., $k_{\text{TNB}}/k_{\text{GSH}}=15$ at pH 7.4, 52 at pH 6.7 and 193 at pH 6.0) [19], while HOX are less influenced by changes below physiologic pH owing to higher pK_a values [80,84]. In addition, HOSCN does not

react with methionine under physiologic conditions [19], while the rate constants of reaction of HOCl and HOBr with methionine are similar to that with cysteine [84,85].

HOSCN reacts 1-2 orders of magnitude more rapidly with a selenol (RSeH) than an otherwise identical thiol [21]. Selenols are present in proteins that include Sec in their primary structure (i.e., selenoproteins) [86], differing from cysteine in the identity of the nucleophilic atom (selenium instead of sulfur). Compared to thiols, selenols are not only more acidic (Sec $pK_a=5.24$ [87]) but also have much greater intrinsic nucleophilicity [88]. By contrast, acidity can limit thiol reactivity [76]. The enhanced nucleophilicity and acidity of Sec compared to cysteine [89,90] is a logical rationale for enhanced rates of reaction of HOSCN with selenols (e.g., HOSCN reacts 68 times faster with γ -Glu-Sec-Gly than GSH) [21]. Selenols probably undergo thiol-like redox chemistry upon reaction with HOSCN, the protonated oxidant reacting with an ionized selenolate (RSe^-) to yield a selenenyl SCN^- ($RSeSCN$) intermediate before hydrolyzing to selenenic acid ($RSeOH$), forming a diselenide ($RSeSeR$) or a mixed selenosulfide ($RSeSR$). The oxidized species would then either be reduced back to a selenol or further oxidized [91].

Though present in all kingdoms of life, selenoproteins are most abundant in high order metazoans such as mammals [86]. Mammalian TrxR is a selenoprotein with homology to GR and a C-terminal redox motif containing Sec that assists in catalysis [92]. This is in striking contrast to bacteria, plants and single celled eukaryotes, which diverge significantly in protein structure from metazoan TrxR and lack Sec expression in the protein [92]. TrxR is the primary redox regulator of Trx in almost all living organisms, which in turn regulates pathways controlling DNA synthesis, compartmental H_2O_2 flux, gene transcription and cell viability [93]. However, metazoan TrxR also acts on targets beside Trx [94]. E.g., HOSCN is catalytically reduced by direct reaction with mammalian TrxR, which is a cytoprotective mechanism, while bacterial TrxR is potently inhibited by HOSCN [75]. Expression of Sec by mammalian TrxR significantly enhances the removal of HOSCN [75] and improves its resistance to oxidative inactivation [94], suggesting Sec expression may have been selected to confer host-specific resistance to oxidative stress during infection [75].

Other reactions have also been reported for HOSCN from in vitro experimentation, though most of these are not favorable in biological systems owing to low rate constants and/or decreased abundance of the targets compared with thiols and selenols. Aqueous HOSCN ca. 200 μM has a half-life of 2-3 hours at room temperature [65] and undergoes accelerated decomposition at acidic pH [66]. When lacking a reaction partner, aqueous HOSCN slowly disproportionates into SCN^- and a mixture of sulfuric and sulfurous acid, cyanide and cyanate (OCN^-) [95]. HOSCN also reacts with cyanide to form SCN^- and OCN^- [96]. Thiocyanogen (SCN_2) is a condensation product in equilibrium with HOSCN and SCN^- but is difficult to isolate at physiologic pH and it is unclear what role this molecule plays, if any, in the effects of SCN^- on human health [71]. Oxidation of tryptophan by HOSCN has been reported [97], though the concentration of HOSCN required to observe this effect in a plasma matrix (~ 2.5 mM) is far above the reported physiologic range of this oxidant or the precursor SCN^- [30]. Further investigation indicated tryptophan is not significantly oxidized even by large excesses of HOSCN at physiologic pH [98]. Indirect evidence suggests ascorbate in buffered saline may react with HOSCN or modulate its generation by MPO

[97,99]; the former scheme supports a radical species arising from 1-electron oxidation of SCN^- by peroxidases in balanced salt solutions, though 1-electron oxidation of SCN^- (eq. 1), measured at pH=1-2 [100], is much less thermodynamically favorable than 2-electron oxidation (eq. 2), measured at pH=0 [80].

Half cell	Standard reduction potential (pH=0)	
$\text{SCN}^\bullet + \text{e}^- \rightarrow \text{SCN}^-$	E° 1620-1680 mV	(1)
$\text{HOSCN} + 2 \text{e}^- + \text{H}^+ \rightarrow \text{SCN}^- + \text{H}_2\text{O}$	$E^\circ = 820 \text{ mV}$	(2)

Alternatively, HOSCN has been proposed as a potential 1-electron reductant of peroxidase compound I that may compete with the 2-electron oxidation of SCN^- , evidenced indirectly by inhibition of compound II accumulation upon the addition of GSH [73].

The observation that mammalian cells are highly tolerant of HOSCN, which otherwise functions as a bacteriostatic (see below), led Wang and Slungaard to speculate that HOSCN “exploits subtle, perhaps sulfhydryl-based metabolic differences between host and parasite” to selectively target pathogens [101]. The chief thiol redox regulators in mammalian cells are TrxR, which maintains $\text{Trx}-(\text{SH})_2:\text{Trx}-\text{SS}$, and GR, which maintains $\text{GSH}:\text{GSSG}$. Mammalian TrxR evolved as a selenoprotein mutation of GR and significantly diverged from the ancestral TrxR protein family found in the overwhelming majority of human pathogens [92]. Furthermore, mammalian TrxR catalytically reduces HOSCN at biologically relevant rates, GR is capable of the same but much less efficient and bacterial TrxR is metabolically incompetent in this reaction [75]. Pharmacologic inhibition of mammalian TrxR with auranofin sensitized bronchial epithelia to HOSCN-mediated cell death [75]. The GSH-GR pathway has been posited as a regulator of HOSCN metabolism as well [35], which is supported by the great abundance of intracellular GSH compared to other HOSCN targets. Estimating the half-life of HOSCN in the presence of its targets requires accurate knowledge of both the reaction rate constants and abundance of the reactants. However, these remain uncertain for many factors likely to regulate HOSCN. The cysteine:cystine redox couple may also regulate HOSCN in vivo, particularly in plasma, though it is maintained at a significantly higher redox potential than that of GSH and Trx in most compartments [22].

The GR and TrxR nodes are not at thermodynamic equilibrium and may independently regulate redox signals of HOSCN [22]. The intracellular GSH level of alveolar macrophages has been reported at $1.26 \pm 0.15 \text{ mM}$ [102], estimating 200 g L^{-1} protein per cell volume [103], while in human umbilical vein endothelial cells (HUVEC) intracellular GSH has been reported at $800 \pm 80 \text{ }\mu\text{M}$ [104], calculated using the same protein per cell volume concentration. Based on the second order rate constant of HOSCN for reaction with GSH of $2.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ [19] and estimating $[\text{GSH}] = 0.001 \text{ M}$, the GSH-dependent half-life of intracellular HOSCN would be 40 ms, while in extracellular fluids where $[\text{GSH}] = 50\text{-}500 \text{ }\mu\text{M}$ the half-life approaches 1 second. HOSCN should react much more rapidly with Trx and peroxiredoxins (Prx) than GSH (based on their pK_a values compared to GSH [105] and, in the case of Prx, the relative similarity of HOSCN to H_2O_2 [106]). To the best of our

knowledge at the time of this writing, the kinetics of these reactions has not been published. However, HOSCN is directly metabolized by mammalian TrxR and GR, and the k_{cat} of HOSCN turnover with TrxR is >7-fold faster than with GR [75]. The second order rate constant of HOSCN reacting with Sec ($1.24 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$) [21] may serve as a surrogate for a single reaction of HOSCN with reduced mammalian TrxR, and comports with the mean specificity constant of mammalian TrxR for HOSCN ($1.86 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, calculated from data in [75]). For comparison, the specificity constant of GR for HOSCN under the same conditions is $5.00 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ [75], which approaches the value of typical thiol rate constants [19]. While TrxR (and likely Trx and several Prxs) outpace the absolute rate constants of reaction of HOSCN with GSH and GR by orders of magnitude, GSH is more abundant in the cytosol by approximately the same factor (e.g., cytosolic Trx has been reported at 5-11 μM [107]). This suggests TrxR and GR nodes will reduce roughly equal amounts of HOSCN inside the cell. Because these nodes are not in equilibrium [22], each may independently inhibit the effects of HOSCN on parallel redox signals. In agreement with this concept, use of the TrxR inhibitor auranofin sensitized human bronchial epithelia to HOSCN-mediated viability loss [75] even though GSH was likely to be competing with TrxR for HOSCN metabolism.

In spite of the aforementioned antioxidant systems, large concentrations of HOSCN exist at steady state equilibrium in human extracellular fluids such as saliva (60 μM , compared to 10 μM H_2O_2) [30], suggesting that the demand for this oxidant has led cells to generate a steady reserve. The relative paucity of antioxidant systems outside the cell versus in and the tendency of HOSCN to be generated by secreted enzymes supports the hypothesis of this oxidant as a front-line defender against pathogens first and foremost. However, some HOSCN is bound to escape extracellular fluid and react with cells, potentially acting as a signaling molecule for sensitive redox signaling pathways. Additional kinetic and abundance data are needed to enhance understanding of the ultimate fate of HOSCN and its effects on cells (see below).

Antimicrobial properties of HOSCN

HOSCN is a major component of innate immune response to pathogens [2]. Bacteria seem to be much more sensitive to the effects of HOSCN than mammalian cells. For example, a 120 μM hr^{-1} flux of HOSCN was toxic to clinical isolates of *P. aeruginosa* but 16HBE cells showed no toxicity [75]. However, an identical flux of HOCl caused viability loss in bacteria and 16HBE, while the same amount of H_2O_2 was not enough to cause viability loss in either cell type [75]. HOSCN ranges from 10- to 500-fold more potent than H_2O_2 in inhibiting the growth and metabolism of oral streptococci with a minimum effective concentration of 1-10 μM [2,68,108]. Antibacterial effects of HOSCN have been demonstrated against multiple gram-negative and gram-positive species [2,68,109]. Because HOSCN predominantly exists in an unreactive ionized state in physiologic fluids it may utilize porins and other hydrophilic channels to penetrate bacterial cells [110]. The reported targets of HOSCN in *Streptococcus* are glycolytic enzymes including hexokinase, glucose-6-phosphate dehydrogenase and aldolase as well as inhibition of oxygen uptake [2]. HOSCN blocks uptake and induces cellular leakage of glucose, amino acids and K^+ in *E. coli* and *S. lactis*, likely by targeting membrane-bound transport proteins for these nutrients [109]. Similarly,

HOSCN oxidizes glucose uptake transporters preventing glycolytic metabolism in *S. agalactiae* [111]. HOSCN inhibits *Helicobacter pylori* viability and urease activity, which the bacteria requires to alkalinize gastric juice for stomach colonization [112]. Salivary HOSCN has been reported to inhibit acid production by glucose-stimulated plaque [31] and reduce growth of both aerobic and anaerobic periodontopathic bacteria [113-115]. In a potentially complementary mechanism to its bactericidal effect, HOSCN also enhances the expression of endothelial cell adhesion molecules (eCAMs; see below) [116].

Bacterial TrxR is a newly identified target of HOSCN ($IC_{50}=2.75\ \mu M$), in striking contrast to mammalian TrxR (no inhibition at $[HOSCN]=50\ \mu M$) [75]. The TrxR-Trx system transfers electrons to ribonucleotide reductases crucial for DNA synthesis and repair in most living organisms [117], so the inhibition of bacterial TrxR by HOSCN may inhibit these processes leading to growth arrest. Metazoan divergence in TrxR from that of other organisms may have provided a selective advantage against single-celled pathogens by enabling host resistance against oxidative stress [75], resulting in its replacement of the original TrxR gene in early animals [92]. Interestingly, chordate peroxidases are also quite divergent from those of prokaryotes [118].

Ceruloplasmin has been found to play an important role in modulating the oxidizing activity of MPO [119] and may also affect its antimicrobial activity. Recent evidence suggests that MPO inhibition by ceruloplasmin is specific to chloride and bromide, with no inhibition detected when SCN^- was added to complete MPO compound I turnover or killing *E. coli* [120]. This study also demonstrated a synergistic effect on the killing of *E. coli* cells when MPO- H_2O_2 - SCN^- and lactoferrin were administered together rather than separately, which was not affected by the inclusion of ceruloplasmin. The synergistic effect of lactoferrin was also true for MPO- H_2O_2 -chloride but was inhibited by ceruloplasmin [120].

Multiple reports exist of NAD(P)H-dependent HOSCN resistance in oral and lactic streptococci associated with a purifiable inhibition “reversal factor” [2,121]. Similar HOSCN reductase activity was reported in a subset of antibiotic-resistant late isolate clinical strains of *P. aeruginosa* [75]. These adaptations allow bacteria to resist HOSCN-mediated oxidative stress in a similar fashion to the resistance conferred by TrxR in mammals [75], providing a selective advantage to such bacteria in SCN^- -rich extracellular fluids such as the human oral cavity. The acquisition of this advantage in bacteria could go either way towards the benefit or detriment of the mammalian host, with HOSCN-resistant commensal strains robustly occupying a niche in the host microbiome and keeping out potentially deleterious transient flora while HOSCN-resistant pathogens may pose an increased risk to human health. Perhaps for that reason it is not surprising this trait was identified in a few decade-long human colonizing *P. aeruginosa* strains [75].

In addition to bacteria, HOSCN has been shown to inhibit the growth of fungi and viruses. *Candida albicans* viability is inhibited by enzymatic exposure to HOSCN and ablated with acute exposure [122,123]. HOSCN blocks viral infection of human gingival fibroblasts by herpes simplex virus, respiratory syncytial virus [124]. Similarly, enzymatically-generated HOSCN ablates HIV replication in lymphocyte culture [125]. HOSCN may also be as potent as HOBr and HOCl in killing parasitic schistomula [126].

The SCN^- -peroxidase- H_2O_2 system plays an important role in the oral cavity, evidenced by HOSCN concentrations in human saliva ranging from 58-65 μM [30]. However, interest in the same host defense mechanism in the airway has grown since the role of LPO in airway bacterial clearance was discovered [127]. Normal concentrations of SCN^- and LPO actively inhibit airway-localizing bacteria [11], utilizing a source of H_2O_2 that was later identified as epithelial DUOX2 under the regulation of infectious stimuli [128]. The discovery that CFTR is an active efflux channel for SCN^- in human lung epithelia [26] highlighted the importance of this antimicrobial system in the airway and led researchers to ask whether failure of this system was a component of CF disease etiology that could be corrected with intervention (see below).

Effects of SCN^- on mammalian cells and biological systems

When SCN^- is oxidized by chordate peroxidases or HOX, other molecules potentially more harmful to mammalian cells are avoided (Figure 3). The favorable interaction of SCN^- with compound I of MPO and EPO [8,9] protects cells by generating a weaker, less reactive oxidant in place of HOX (i.e., HOSCN, $\text{pK}_a=4.85$, $E'^\circ=+560$ mV; HOCl, $\text{pK}_a=7.53$, $E'^\circ=+1280$ mV; HOBr, $\text{pK}_a=8.8$, $E'^\circ=+1130$ mV [20,80]) (Figure 4). In addition to scavenging HOCl and HOBr, SCN^- also reduces chloramines, a range of amino acid metabolites generated by HOCl [69,70,129]. Surprisingly, HOSCN may also reduce some chloramines [129]. Chloramine formation can progress to deleterious redox cascades or form nitrogen-centered radicals that lead to protein fragmentation [130]. SCN^- would presumably reduce bromamines as well. Thus, “trading” HOX for HOSCN in this manner changes the relationship of the ultimate oxidant with the biological system and alters outcomes. E.g., the replacement of HOX with HOSCN significantly limits the oxidation of methionine, histidine, tryptophan, lysine, tyrosine, terminal amines, peptide backbone amides, disulfides, nucleotides, poly-unsaturated fatty acids and Fe^{2+} [4,84,85,131-134]. SCN^- functions under the standard definition of an antioxidant by decreasing the reduction potential of the ultimate oxidant ($E'^\circ_{\text{HOSCN}} < E'^\circ_{\text{HOX}}$ [80]), inhibiting a cascade of irreversible, injurious reactions (Figure 3). In addition to the reactions already mentioned, SCN^- also inhibits urate radical formation by MPO (sparing ascorbate, GSH, and nitric oxide (NO)) [135]; EPO-catalyzed nitration of tyrosine and bovine serum albumin [9]; peroxidatic consumption of NO in physiologic fluids [136]; and LDL apoprotein oxidation by HOCl (although SCN^- also participated in MPO-catalyzed LDL oxidation) [99]. Formation of HOSCN is also a means of H_2O_2 removal in mammalian tissues [30,72], in agreement with observations that H_2O_2 is significantly more toxic to mammalian cells than HOSCN [14,74]. Ultimately, the formation of HOSCN at the expense of H_2O_2 /HOX channels more oxidizing equivalents toward the reversible oxidation of cysteine/Sec and away from irreversibly oxidizable targets [58,134].

Evidence for SCN^- -mediated protection from HOCl, HOBr and H_2O_2 in whole cells has been reported by many researchers. Accumulation of HOSCN in ex vivo neutrophils, eosinophils and macrophages is non-toxic [137]. SCN^- inhibited cell death of ex vivo rat aortic endothelia exposed to EPO, H_2O_2 and bromide [138]. Similarly, SCN^- protects HL-60 cells from MPO-mediated apoptosis in the presence of physiologic levels of chloride, while bromide was not protective [139]. Cell death caused by MPO, H_2O_2 and chloride was inhibited by the addition of 100-400 μM SCN^- in lung, nervous, pancreatic and endothelial

cells and also protected cells from H₂O₂ [74]. Addition of SCN⁻ to endothelial cell cultures inhibited fibronectin oxidation by HOCl and loss of cellular adhesion [140]. Human bronchial epithelia were protected against cell death caused by enzymatically generated HOCl or H₂O₂ by SCN⁻ [14] and SCN⁻ abolished HOCl-mediated necrosis in J774A.1 murine macrophage-like cells [15]. Emerging evidence suggests SCN⁻ also limits the release of neutrophil and eosinophil extracellular traps (ETs) [141]. ETs are strands of chromatin containing granulocytic antimicrobial factors released during a special type of cell death called ETosis, which is implicated in many inflammatory and auto-immune disorders [142].

The apparent lack of toxicity of HOSCN in mammalian cells, particularly when compared to bacteria, is mechanistically supported by the recent discovery that mammalian TrxR rapidly metabolizes this oxidant, while bacterial TrxR is inhibited [75]. This metabolic pathway and the selectivity of SCN⁻ for HOX and chordate peroxidase compound I (see above) suggests the evolution of a coordinated system in mammals utilizing SCN⁻ as a “funnel” to convert a range of oxidants to a species that can be safely metabolized while serving basic watchdog functions in cell signaling and host defense (Figure 5).

As a more selective oxidant, HOSCN could alter cell signaling and metabolism in different ways than HOX provided it escapes the clutches of the TrxR and GR antioxidant systems. HOSCN can inhibit GSH *S*-transferase pi (GSTP1), GAPDH and ATPase activity in erythrocytes suspended at 2% hematocrit and may be a more potent ATPase inhibitor than HOCl or HOBr, though this result might have differed with intact cells instead of isolated membranes [126]. The cysteine residues of constitutively inactive GSTP1-1 in human saliva are kept oxidized by HOSCN [143]. NF- κ B signaling was strongly implicated in the eCAM and tissue factor (TF)-inducing effects of HOSCN in serum-treated HUVECs. Isolated protein tyrosine phosphatase 1B is more sensitive to HOSCN-mediated inhibition than HOCl or HOBr but this was not assessed in whole cell experiments [144]. Uncoupling of endothelial nitric oxide synthase (eNOS), which generates NO critical to vascular tone, was observed in human coronary artery endothelial cells (HCAECs) exposed to HOSCN or HOCl, but only HOCl caused significant inhibition in isolated rat aortic rings [145]. As complexity increases in biological systems from isolated proteins to subcellular fractions, intact cells, tissues, organs and ultimately whole organisms it is increasingly less likely that HOSCN generated in situ will reach any one of these particular targets, as demonstrated in multiple studies wherein whole cells or tissues were not as sensitive to HOSCN as isolated proteins. However, the observations that HOSCN transduces eCAM and TF expression in whole cells submerged in 10% serum are particularly compelling [116,146] and it is now known that HOSCN is an important negative regulator of at least one salivary enzyme [143].

Although many findings in whole cells indicate protective effects of SCN⁻, acute exposures to bolus doses of HOSCN causes cytotoxicity in J774A.1 murine macrophage-like cells, HUVECs and HCAECs [147-149]. These observations suggest HOSCN can injure mammalian cells in certain contexts by oxidizing sensitive thiol targets, though the studies are discordant regarding the mechanism of cell death. However, prolonged HOSCN exposure enhances HUVEC viability [148] and HCAEC fare better when exposed to HOSCN compared with HOCl [149]. J774A.1 cells were very sensitive to high dose HOSCN-mediated apoptosis and, strikingly, resistant to HOCl [147]; however, in another

study these cells were sensitive to HOCl and protected by SCN⁻ [15]. Interestingly, these cells are also more resistant to H₂O₂-mediated cytotoxicity and have less TrxR activity than other macrophage-like cell lines (JD Chandler and BJ Day, unpublished data).

SCN⁻ is also linked to the production of OCN⁻, which is formed by additional oxidation of HOSCN by H₂O₂ or its disproportionation [3,126]. OCN⁻ *N*-carbamylates amino groups such as that of the lysine side chain, and incubating macrophages with *N*-carbamylated LDL significantly increased foam cell evolution [57]. However, the physiological relevance of SCN⁻-mediated *N*-carbamylation is difficult to assess for multiple reasons: chiefly, most HOSCN will be rapidly reduced by abundant thiol targets before being further oxidized in vivo; OCN⁻ is also derived from urea in vivo; OCN⁻ formation from HOSCN requires excess H₂O₂ [57,126], which is atypical even under inflammatory conditions (e.g., ~100 mol SCN⁻ per mol of H₂O₂ in the oral cavity [30]); and other MPO substrates (i.e., bromide and chloride) also contribute to a fraction of *N*-carbamylation in vitro [57]. Furthermore, plasma SCN⁻ is not predictive of atherosclerotic coronary artery disease risk and may be protective [18,57].

Noted discrepancies in whole cell response to HOSCN remind that different experimental methods can greatly influence outcomes and that “The dose makes the poison” (Paracelsus), highlighting the importance of comparing HOSCN to other oxidants at equal, biologically relevant concentrations to obtain the most meaningful results. Furthermore, while exogenous oxidant exposure is valid and necessary to elucidate cellular responses this should not lead to a “thermonuclear model” expose-to-effect approach, instead mimicking in vivo fluxes to observe physiologically relevant results [150].

Clinical and in vivo research on the health effects of SCN⁻

The major rationale for the use of SCN⁻ in lung disease came from studies of CF cell culture and animal models. CFTR is a member of the multi-drug resistance protein family that apically transports chloride and SCN⁻ as well as other small molecules [26,151-154]. CF airways are rapidly colonized by common bacterial strains easily resisted by healthy individuals, requiring antibiotic intervention [155]. *P. aeruginosa* is the most prevalent infectious agent in this population and is associated with lung function decline. CF patients also suffer from sustained airway neutrophilia [156], resulting in an excess of purulent phlegm and pro-inflammatory factors [157]. The combination of infection and inflammation ultimately drives >80% of CF patients' morbidity and mortality [155]. Nebulized hypertonic saline (HS) therapy improves lung function and decreases pulmonary exacerbations in CF patients, presumably by increasing airway surface hydration and decreasing mucus viscosity [158-160]. HS also increases SCN⁻ and GSH mobilization into the ELF, which may provide an under-appreciated contribution to its beneficial effects [29]. However, HS creates an irritating osmotic stress for airway epithelia [161] and years of HS therapy may have unanticipated consequences.

Observing the impairment of CF cultures in the transport SCN⁻ and killing of co-cultured bacteria contributed greatly to the rationale for SCN⁻ as a therapy CF therapy [12,13,26]. Furthermore, the CF airway concentration of SCN⁻ is deficient (either in resting

concentration or in the rate of transport) in mouse and pig models and in human CF saliva [17,29,32]. However, bronchoalveolar lavage fluid (BALF) from children and NLF from adults did not significantly differ between CF and healthy individuals [17,28]. The adult CF study did not control for *P. aeruginosa* infection status, but this would have severely limited the CF sample size (74% were infected). In the study of CF children, SCN^- did not significantly differ between the groups even when controlling for infection status or patient symptoms, although there was a trend toward higher BALF SCN^- in symptomatic individuals [28]. SCN^- did not associate with markers of inflammation (e.g., MPO). Without dilution factor correction for the neat airway ELF caused by lavage sampling [162] it is difficult to compare results between this and other studies of neat or urea-corrected ELF/NLF values. Other transporters like CaCC and pendrin might compensate for CFTR deficiency [27], though this still leaves the possibility of inhibited transport as observed in CF pigs [28]. Such a rate limitation may be problematic during infection, when increased SCN^- efflux could offset increased oxidation [14]. Furthermore, the use of SCN^- in treating lung infections could extend well beyond CF into diseases such as pneumonia and acute respiratory distress syndrome, limiting irreversible oxidative lung damage which contributes greatly to clinical severity.

Endogenous SCN^- in NLF and plasma been studied for its potential association with lung function and cardiovascular disease, respectively [17,18]. In CF patients, increasing NLF SCN^- correlates with higher 1-second forced expiratory volume (FEV_1 , as % predicted; $r=0.7457$, $p<0.0001$) [17]. Furthermore, clinical values of NLF SCN^- associate with *S. aureus* killing by cultured airway epithelia. The cardiovascular system has also been an area of focus for research on the effects of SCN^- . Because cyanide exposure from tobacco smoke results in higher plasma SCN^- , it has been used to identify cadavers as smokers or non-smokers so that many negative outcomes smoking may correlate with SCN^- [54,55]. However, in a 12-year study of myocardial infarction survivors (both smokers and non-smokers), every 1 μM increase in SCN^- resulted in a 0.9% decrease in mortality [18]. In another study plasma SCN^- was not predictive of atherosclerotic risk and there was a trend toward negative correlation with major adverse cardiac event risk [57]. Furthermore, SCN^- does not associate with plasma thiol values in smokers and non-smokers even though more thiols are oxidized in diluted plasma with higher SCN^- when MPO and H_2O_2 are added [18,58]. Plasma SCN^- inversely correlates with 3-chlorotyrosine (3-CT) and dityrosine, which are biomarkers of HOX formation, in plasma proteins from smokers and non-smokers [134]. These studies suggest SCN^- is not directly associated with cardiovascular injury but may actually be a protective factor against disease progression and worse outcomes.

There is a rather large body of pharmacologic and toxicologic data from the early 20th century when physicians used oral potassium SCN^- to treat hypertension (reviewed more extensively in [10]). Most striking are the consistently high values of SCN^- in patient plasma, which were regularly maintained in a purported therapeutic range of 1.2-2.1 mM. In the few placebo-controlled trials from the contemporary literature, orally administered SCN^- was only modestly more effective than placebo at lowering blood pressure [163,164]. Few if any clinical studies utilizing exogenous SCN^- with therapeutic intent are known to have taken place since these; however, a non-randomized non-placebo controlled trial concerning the potential deleterious effect of exogenous SCN^- on thyroid function in a susceptible

population of Sudanese individuals has been published [50]. Following a modest increase in plasma SCN^- , the study was negative in showing ill effects. Co-administered 0.1 mg L^{-1} iodine suppressed pituitary TSH secretion, and no changes in thyroid hormones were observed.

Animal studies have greatly added to the understanding of SCN^- as a potential therapeutic. Nebulized 0.5% (w/v) sodium SCN^- was assessed in mouse models of infection and spontaneous inflammation [14,15]. ELF SCN^- values increase from approximately $100 \mu\text{M}$ to over 2 mM and cleared over approximately 12-hours [14]. SCN^- -treated wild type mice rapidly regained lost body weight, cleared more bacteria from their lungs and had fewer airway neutrophils compared to vehicle-treated mice [14]. These results were repeated in pro-inflammatory epithelial sodium channel subunit β (βENaC) transgenic mice and wild type littermates in studies designed to assess the antibacterial potential of SCN^- in an exacerbated, pro-inflammatory airway model [15]. βENaC transgenic mice exhibit similar airway inflammation to that of CF patients, including neutrophilia and elevated glutathione sulfonamide (GSA; a HOX biomarker) [15,165,166]. However, these deleterious traits were decreased by SCN^- treatment [15]. Together these studies suggest that nebulized SCN^- could be used as a therapy in infectious and inflammatory lung diseases. The striking effect of SCN^- on bacterial clearance is interesting because in the absence of SCN^- other HOX would have been formed to kill bacteria instead. The effect of SCN^- may be to preserve the host defense response by inhibiting leukocyte necrosis so that in the long term, neutrophils clear bacteria more efficiently and chemokine signaling from the lung is attenuated. It is also possible that the conversion of HOX to a more thiol-selective species by SCN^- effectively increases the rate of glutathionylation of Fas receptor, an important component of airway bacterial clearance in mice [167]. SCN^- may also enhance the induction of cellular adhesion molecules that help direct phagocytic migration from the bloodstream [116,146]. The respective contributions of SCN^- to anti-inflammatory effects in the airway merits continued study, as SCN^- may be useful in non-infectious lung diseases characterized by uncontrolled inflammation in addition to infectious diseases [15].

Animal models have also revealed beneficial effects of SCN^- supplementation for cardiovascular diseases. SCN^- dose-dependently decreased cholesterol deposition in the aorta and kidneys of thyroidectomized rabbits given 0, 20 or 60 mg kg^{-1} potassium SCN^- and 300 mg kg^{-1} cholesterol daily [56]. Giving 10 mM sodium SCN^- in drinking water to LDL receptor-knockout, MPO-463G transgenic mice (an established proatherogenic model [168]) increased plasma SCN^- >2-fold over baseline and decreased aortic arch plaque area by 26% compared to control [16]. Together these studies suggest SCN^- may protect against adverse cardiovascular outcomes, in agreement with recent clinical research [18].

Some research supports pro-inflammatory effects of HOSCN in vivo. When C57BL/6 mice were exposed to an intraperitoneal injection of $150 \mu\text{M}$ HOSCN, peritoneal exudate neutrophils increased >4-fold in number, approximately half as many as were induced to migrate by an injection of $2 \mu\text{g kg}^{-1}$ TNF- α [116]. This supports the findings by the same group that HOSCN potently induces E-selectin, ICAM-1, VCAM-1 and tissue factor in 10% serum-submerged HUVECs by activating NF- κB signaling, unlike HOCl and HOBr [116,146]. NF- κB signaling activation by HOSCN to express eCAMs could be a novel

mechanism by which the vasculature further directs migrating neutrophils and monocytes to inflamed tissue and may paradoxically explain the effectiveness of nebulized SCN^- therapy in mice with lung infection [14,15] (see above), specifically amplifying inflammation at sites of infection.

Quantifying the efficacy of SCN^- in whole organisms would be greatly enhanced by a sensitive biomarker of HOX, which is the major therapeutic target (directly or preventatively) of exogenous SCN^- . 3-Chlorotyrosine (3-CT) and 3-bromotyrosine levels can be difficult to measure due to the relative scarcity of these species, but 3-CT inversely correlates with SCN^- in stimulated samples [134]. GSA is a stable cyclic metabolite of GSH formed by the condensation of cysteine thiol and the amino moiety of glutamate following reaction of GSH with 3 mol of HOX [169]. Importantly, GSA is only formed by HOX and not HOSCN [169] and is decreased in a CF lung disease mouse model following SCN^- treatment [15]. Thus, HOX biomarkers may also serve as reporters of the therapeutic effect of SCN^- in clinical samples or in vivo models.

Overview

SCN^- is an ubiquitous molecule in mammals that regulates outcomes of infection and inflammation. In inflammatory milieu, SCN^- is rapidly oxidized to HOSCN by reducing HOX or compound I of chordate peroxidases. If there is sufficient H_2O_2 from respiratory burst or other sources to drive peroxidases, SCN^- is the main limiting factor in HOSCN formation, displacing HOX and HOX metabolites. Conversion and displacement of HOX to a less oxidizing molecule makes SCN^- an antioxidant in this system, yet HOSCN inhibits bacteria, viruses and fungi by oxidizing critical thiols. Mammalian cells exhibit significant tolerance of HOSCN, which is metabolized by mammalian TrxR but not bacterial TrxR [75]. Exogenous SCN^- therapy may work by exploiting this recently discovered metabolic specialization to combat infection and/or inflammation.

Recent advances in animal and clinical studies demonstrate that SCN^- may be an effective therapy in CF and other infectious or inflammatory diseases [14-18]. SCN^- is not converted to the reactive HOSCN except during inflammatory conditions and thus may act as a pro-drug, and humans tolerate large increases in plasma SCN^- as demonstrated by smokers and the extreme doses given to hypertensive patients in the 20th century. However, toxicity at extreme plasma levels is apparent, particularly hypothyroidism in cases of iodine deficiency [10]. Nebulization requires less systemic absorption to achieve a therapeutic dose (approximately 400 μM) [14,15], which may improve safety. SCN^- may also aid in the search for new antibiotics: the thiol-based mechanism by which HOSCN targets bacteria is unlike mechanisms of current drugs, to which resistance is becoming increasingly common.

Maintaining the host defense features of inflammation while limiting oxidative injury is a novel therapeutic niche for SCN^- therapy compared to other drugs, which typically address only inflammation or pathogen survival/replication. This bivalent mechanism theoretically gives SCN^- more flexible utility compared to other drugs. Attempts to quench the oxidative imbalance in CF with exogenous doses of GSH and N-acetyl cysteine have failed to produce significant clinical improvements [170,171]. Similarly, inhibiting MPO activity [172] could

increase susceptibility to infection or pulmonary exacerbation [173]. However, a combined approach (exogenously bolstering SCN^- with limited MPO inhibition) may be conceivable to significantly limit oxidative injury in inflammatory diseases, supported by clinical data that low MPO and high SCN^- are cooperating protective factors against mortality in myocardial infarction survivors [18]. Ultimately, there is a growing body of evidence that SCN^- should continue to be investigated as a therapeutic in infectious and inflammatory diseases. SCN^- profoundly alters the oxidative output of chordate peroxidases, with differential effects on mammalian and bacterial cells underpinning its major therapeutic mechanism. More studies are needed to evaluate the anti-inflammatory effects of SCN^- and isolate them from its anti-microbial effects, as these will be key to understanding the true potential of SCN^- in pro-inflammatory diseases.

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List of abbreviations

BALF	Bronchoalveolar lavage fluid
CaCC	Calcium-dependent chloride channel
3-CT	3-chlorotyrosine
OCN⁻	Cyanate
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
RSeSeR	Diselenide
RSSR	Disulfide
DUOX	Dual oxidase
eCAM	Endothelial cell adhesion molecule
eNOS	Endothelial nitric oxide synthase
EPO	Eosinophil peroxidase
ELF	Epithelial lining fluid
βENaC	Epithelial sodium channel subunit β
ETs	Extracellular traps
FT4	Free thyroxine
FEV	Forced expiratory volume
GSH	Glutathione

GR	Glutathione reductase
GSTP1	Glutathione S-transferase pi
GSA	Glutathione sulfonamide
HCAEC	Human coronary artery endothelial cell
HUVEC	Human umbilical vein endothelial cell
HS	Hypertonic saline
HOB_r	Hypobromous acid
HOCl	Hypochlorous acid
HOX	Hypohalous acid
HOSCN	Hypothiocyanous acid
LPO	Lactoperoxidase
LDL	Low density lipoprotein
LDLR	Low density lipoprotein receptor
MPO	Myeloperoxidase
NOX	NADPH oxidase
NLF	Nasal lining fluid
NO	Nitric oxide
Prx	Peroxiredoxin
RSeOH	Selenenic acid
RSeSCN	Selenenyl thiocyanate
Sec	Selenocysteine
RSeH	Selenol
RSe⁻	Selenolate
RSeSR	Selenosulfide
NIS	Sodium-iodide symporter
RSOH	Sulfenic acid
RSSCN	Sulfenyl thiocyanate
SCN⁻	Thiocyanate
SCN₂	Thiocyanogen

RSH	Thiol
RS⁻	Thiolate
TNB	5-thio-2-nitrobenzoic acid
Trx	Thioredoxin
TrxR	Thioredoxin reductase
TSH	Thyroid-stimulating hormone
TF	Tissue factor

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Figure 1.

SCN^- and HOSCN interface with innate immunity and antioxidant defense systems. Processes of the innate immune system such as respiratory burst produce H_2O_2 , which is reduced by chordate peroxidases such as MPO. The resulting compound I form of the enzyme can be reduced to the native state by a 2-electron donor such as chloride or bromide (X^-) or SCN^- . In the case of reduction by a halide, the HOX molecule can subsequently be reduced by SCN^- as well. HOSCN is targeted by antioxidant systems of mammalian cells for safe removal of the oxidant by a reducing equivalent of NADPH , but it can also act as an antimicrobial agent.

Figure 2.

SCN^- is the primary determinant of HOSCN formation during inflammation. This graph qualitatively depicts the effect of increasing concentration of SCN^- on the output of oxidants by MPO assuming a set level of H_2O_2 , chloride and bromide. When no SCN^- is present, HOCl is the major 2-electron oxidant produced. As SCN^- is increased, HOCl and HOBr are replaced by HOSCN. A high enough concentration of SCN^- can drive a near-total replacement of the other oxidants with HOSCN.

Figure 3.

SCN⁻ alters biochemical outcomes of peroxidase activity. HOX produced by chordate peroxidases during inflammation significantly target thiols, selenols, methionine and terminal amino groups (among others). Targeting of amino acids and other biological molecules causes irreversible oxidation and macromolecule degradation, with significant potential for cytotoxicity. However, 2-electron thiol, selenol and methionine oxidations are generally reversible through a host of enzymatic mechanisms, and are known to regulate cell signaling pathways that are often designed to adapt cell behavior to a variety of stressors. By replacing HOX with HOSCN, oxidative equivalents of H₂O₂ only target thiols and selenols which can be repaired following oxidation and/or alter cell signaling, while macromolecule degradation from the irreversible oxidation of biomolecules is avoided.

Figure 4.

HOSCN is chemically distinct from HOX and H₂O₂. Redox potential is a strong predictor of the thermodynamic favorability of a reaction of an oxidant with a reductant and protonation is necessary for many of the reactions of 2-electron oxidants, such as with thiols. Standard biologic redox potentials and protonation at pH 7.4 are plotted. Dashed lines indicate the redox potential of the Fe³⁺/Fe²⁺ half-cell and 50% protonation, respectively. HOSCN alone falls into the lower left quadrant, “A”, for oxidants <50% protonated at physiologic pH and below the Fe³⁺/Fe²⁺ half-cell. All other oxidants fall into the upper right quadrant, “B”, where >50% of the molecules are protonated at physiologic pH and the half-cell redox potentials are greater than that of Fe³⁺/Fe²⁺. This analysis is predictive of HOSCN having significant thermodynamic and kinetic constraints of reaction less burdensome on the other HOX species, and comports with published kinetics of HOSCN in comparison with HOCl and HOBr. Note that H₂O₂ has the significant kinetic constraint of a poor leaving group, which is not indicated by these features.

Figure 5.

Antioxidant properties of SCN^- channel oxidizing equivalents of H_2O_2 into the well-tolerated HOSCN . H_2O_2 and 2-electron oxidants derived from it are depicted on a line qualitatively representing a range of half-cell redox potentials. By directly reducing both chordate peroxidase heme compound I, generated by the reduction of H_2O_2 , and alternative products of its 2-electron reduction (e.g., HOCl , HOBr and a variety of RNHX), SCN^- acts as an antioxidant and decreases the redox potential of the resulting oxidizing species, HOSCN . This maintains oxidative pressure from the host on pathogens while limiting the oxidant to a species regulated by the chief thiol redox nodes, TrxR and GR .