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THIOCYANATE: A potentially useful therapeutic agent with host defense and antioxidant properties☆

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Abstract

Thiocyanate (SCN) functions in host defense as part of the secreted lactoperoxidase (LPO) microbicidal pathway. SCN is the preferred substrate for LPO-driven catalytic reduction of hydrogen peroxide (H_2O_2) forming hypothiocyanous acid (HOSCN). HOSCN is selectively generated by many peroxidase enzymes that can utilize SCN including: eosinophil peroxidase (EPO), gastric peroxidase (GPO), myeloperoxidase (MPO), salivary peroxidase (SPO), and thyroid peroxidase (TPO). These enzymes generate HOSCN through a two-electron halogenation reaction. HOSCN is a potent microbicidal agent that kills or nullifies invading pathogens but is better tolerated by host tissue. Some controversy exists as to whether physiologic levels of HOSCN are non-toxic to host tissue, but the disagreement appears to be based on results of enzymatic generation (yielding moderate steady-state exposure) versus direct high level acute exposure in mammalian cell lines. This apparent duality is also true of other endogenous oxidants such as hydrogen peroxide and relates to the difference between physiologically relevant oxidant production versus supra-physiologic bolus dosing approaches. SCN has antioxidant properties that include the ability to protect cells against oxidizing agents such as hypochlorous acid (HOCl) and repair protein chloramines. SCN is an important endogenous molecule that has the potential to interact in complex and elegant ways with its host environment and foreign organisms. SCN's diverse properties as both host defense and antioxidant agent make it a potentially useful therapeutic.

Keywords

Cystic fibrosis; Hypothiocyanite; Hypochlorite; Lactoperoxidase; Myeloperoxidase

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1. Biology of thiocyanate

1.1. Origin and distribution of SCN

SCN is a small, strongly acidic [1] pseudohalide thiolate (Fig. 1) that is ubiquitously found in the extracellular fluids of mammals, including plasma, saliva, airway epithelial lining fluid (ELF), nasal lining fluid (NLF), milk, tears, and gastric juices at a wide range of concentrations (0.01–3 mM) [2-4]. SCN enters the body from the diet (such as cruciferous vegetables) [5] or is synthesized from cyanide by sulfurtransferase enzymes including mitochondrial rhodanese and cytosolic mercaptopyruvate sulfurtransferase [6]. SCN has been studied both in host defense and as a detoxification product of cyanide.

SCN is thought to originate primarily from the diet. Daily intake of SCN varies between ethnic and cultural groups based on differences in diet, including those of glucosidic cyanogen-rich plants such as cassava, yam, maize, sugar cane, sorghum, and linseed [5,7]. SCN is also a known product of glucosinolate metabolism in addition to N-conjugated thiocyanates and the structurally related isothiocyanates (e.g., sulforaphane) [5]. The effects of cyanogens cannot be inferred as the direct effects of SCN because most cyanogens readily break down into a milieu of biomolecules, including cyanide, isothiocyanates, and nitriles [5]. The ubiquity of cyanogens in plant matter make it the most obvious dietary source of SCN and provide a rationale for the distribution of rhodanese activity across species, particularly ruminants where some segments of the alimentary tract may exceed the liver in sulfurtransferase activity [8]. Currently it is unknown whether SCN may also be synthesized from an endogenous source of cyanide, but the ubiquity of SCN in biologic systems and its ability to be rapidly concentrated in extracellular fluids [9] suggest this possibility. Interestingly, lung epithelial cells have measurable levels of apical and intracellular SCN when grown in media that is devoid of any detectable SCN source [9]. Some bacteria, including *Pseudomonas aeruginosa* (*Pa*), have been shown to generate cyanide from glycine [10], but it is unclear if similar pathways exist in eukaryotes.

Extracellular fluids are abundant sources of SCN (Table 1). Plasma values of SCN typically range between 5 and 50 μM in human non-smokers [11,12] and much higher in smokers [13]. In contrast to the plasma, sampling of the ELF from the human airways has produced undiluted SCN values many fold higher with a mean value of 460 μM [12,14], while NLF has been reported at similar concentrations with wide interpersonal variance [19]. A study in young children reported a dilute bronchoalveolar lavage fluid (BALF) SCN mean value of 280 nM, which would roughly predict an ELF SCN level around 30 μM [15]. BALF corrected with the urea dilution factor (expressed as ELF) measured 100 μM in C57BL/6 mice [9]. Sampling of undiluted airway secretions produced a mean value of 160 μM in sheep [16]. Most of these findings suggest that airway SCN is concentrated from the plasma pool via the active transport of the basolateral sodium-iodide symporter (NIS) and apical anion channels such as the cystic fibrosis transmembrane conductance regulator (CFTR) [3,9,17,18] and cytokine-regulated channels SLC26A4 (pendrin, an electroneutral halide-exchange channel) and TMEM16A (Ca^{2+}-dependent Cl^- channel (CaCC), an active transporter of halides) [19,20].

The saliva and oral cavity have even higher SCN levels than the airway, owing to their heavy demand for a complex and potent mixture of antimicrobial defenses [21]. The oral cavity ranges from 0.5 to 3 mM SCN [22] and utilizes the same active transport system as found in the airway [3] making saliva the most SCN-rich matrix known in the body. The high concentration of SCN in the oral cavity underscores its importance, where it inhibits colonization of many bacterial species as a substrate for peroxidase activity and HOSCN formation [21,23]. In human breast milk the median value of SCN has been reported at 5.6 μg/L (100 nM) [24], although another report found breast milk to have comparable levels to

cow milk reported at 0.1–10 ppm (1.7–170 μM) [25]. SCN has been observed in human tears at about 150 μM [26], which is considered bacteriostatic with LPO and H_2O_2 [16]. SCN has also been reported in the alimentary tract several fold higher than the plasma (ca. 250–300 μM) where it is utilized by GPO in a bactericidal mechanism [4]. Qualitative observation has also located SCN in semen [25].

1.2. Elimination of SCN

Elimination of SCN occurs in the kidneys at a half-life of 3 days in healthy individuals. This is due to a 90% reuptake rate of SCN from glomeruli filtrates [27,28]. Mean relative volume of distribution (V_d) in healthy subjects is 0.25 L/kg [28]. Renal insufficiency increases the volume of distribution of SCN to 0.36 L/kg and extends the half-life to 7–9 days, with the elimination constant inversely proportional to renal creatinine clearance [27,28]. Renal insufficient patients exposed to SCN through cyanogenic drugs such as high infusion rates of nitroprusside are prone to accumulating high concentrations in the plasma and require monitoring for toxicity [27,28].

1.3. Consistent measurement of SCN in biological samples

Oxidation and covalent binding can interfere with the accuracy of reported SCN levels if steps are not taken to preserve SCN in biological matrices. It is likely that some discrepancies in the literature on SCN levels in extracellular fluid relate to this issue. A detailed method is beyond the scope of this review; however a simple step is worth mention. Use of trichloroacetic acid (TCA) ca. 3% (w/v) and precipitation of protein can prevent SCN from reacting with matrix compounds and will ablate significant loss of the analyte prior to assay [29]. SCN treated with TCA for preservation and cryostorage is well-suited for analysis with either spectrophotometric [9], HPLC/electrochemical [15], or GC/MS [30] detection methods.

1.4. Host defense by the peroxidase-SCN-H_2O_2 system

Attention was first brought to the role of SCN in host defense when it was discovered that it participates with peroxidases in the catalytic reduction in H_2O_2, yielding antimicrobial activity [31]. The product of this halogenation-like reaction was later identified to be HOSCN [2] (Fig. 2). The pK_a of HOSCN has been reported between 4.85 and 5.3 [2,32], suggesting the conjugate base hypothiocyanite ($OSCN^-$) predominates in most physiologic fluids. The term “HOSCN”is used to refer to the acid and conjugate base at their pH-dependent equilibrium unless otherwise noted. Although milk was the first biological matrix shown to utilize this activity, it is now known that saliva, ELF, NLF, gastric juices and tears can also support the antibacterial properties of the peroxidase-SCN-H_2O_2 system [2,3,12,26].

HOSCN reacts selectively with sulfhydryl groups resulting in the oxidation of proteins and thiol-based antioxidants [32,33]. Reaction of these sulfhydryl groups with HOSCN produces sulfenyl thiocyanates (RS-SCN) [32,34] which in turn may form disulfides (RSSR) and sulfenic acids (RSOH) that can then be repaired through enzymatic mechanisms [35]. HOSCN has been reported to deplete antioxidants such as glutathione (GSH) from cells, perhaps as a result of export from the cytosol or protein conjugation of glutathione (PSSG) rather than irreversible oxidation [35]. HOSCN may also react with nucleophilic selenols such as selenocysteine [36]. In vitro data also suggest some capacity of HOSCN to react with nitrogen atoms [34], although the physiologic relevance of the resulting thiocyanatimines and thiocyanatosulfonamides is unclear. There is a curious lack of in vivo evidence for the sustained formation of thiocyanogen (SCN_2) from the condensation of HOSCN by SCN, in spite of the fact that SCN is an acidic thiolate in all physiologic compartments. This may be a result of the rapid hydrolysis of SCN_2 in physiologic matrices

[37] (Fig. 2). This is also in spite of reports of SCN-catalyzed decomposition of HOSCN consistent with the formation of SCN_2 [35].

The antibacterial activity of HOSCN is often attributed to its ability to cross the bacterial cell wall before oxidizing critical metabolic elements [33]; this may be a result of HOSCN's thiol-selective tendency and because it reacts in its protonated form [32]. HOSCN preferentially targets acidic thiols such as reduced Ellman's reagent (2-nitro-5-thiobenzoate, TNB^-) or an acidic cysteine moiety associated with an enzyme [32]. The reported bacterial targets of HOSCN are critical cysteines found in glycolytic enzymes including glyceraldehyde-3-phosphate dehydrogenase, hexokinase, glucose-6-phosphate dehydrogenase, and aldolase, leading to the hypothesis that HOSCN's effect on bacterial growth is glycolysis-mediated [33]. However, glucose transport and respiration may also be targets of HOSCN, and it has been reported to inhibit the activity of urease, which is critical to the ability of *Helicobacter pylori* to alkalinize gastric juice in order to colonize the stomach [4]. HOSCN may also transduce the expression of cellular adhesion molecules regulated by NF-κB in a mechanism of selective inflammatory amplification at sites of phagocytic activity, such as infected tissues [38].

The bulk of reporting on the antibacterial action of the SCN-peroxidase-H_2O_2 system in vivo has come from the oral cavity. The oral cavity has a large pool of SCN, steady sources of H_2O_2, and mildly acidic conditions that optimize LPO activity. HOSCN is readily detected in the saliva at resting levels ranging 10–70 μM [39]. This constant source of HOSCN has been reported to inhibit acid production by glucose-stimulated plaque [40] and reduce growth of periodontopathic bacteria [41]. The LPO-SCN-glucose oxidase (GOX)-containing toothpaste Biotène has been reported to significantly increase SCN and reduce cariogenic bacteria load in 3–5-year olds treated for caries after 4 weeks of brushing compared to controls [42]. The commensal bacteria *Streptococcus sanguinis*, which is a normal component of the healthy human oral cavity flora, is among the strains of *Streptococcus* that have developed high expression of NADH-hypothiocyanite oxidoreductase that reduces $OSCN^-$ back to SCN. This adaptation allows the resident bacterium to resist oxidative stress by HOSCN, which benefits the host by preventing vacancy in the oral environment that could be taken advantage of by transient microbes [43]. It is tempting to speculate whether secretory epithelium have similar protective defenses against HOSCN. HOSCN has been shown to effectively inhibit the growth of oral fungi such as *Candida albicans* [44] and has also been reported to inhibit viral infection of gingival cells at physiologic concentrations of HOSCN. HOSCN inhibited herpes simplex virus, respiratory syncytial virus, and echovirus [45], implicating an antiviral host defense role that may relate to the regulation of pendrin by IFN-γ [20].

Interest in the role of HOSCN on microbicidal activity in the airway has grown since the importance of LPO in airway bacterial clearance was first reported [16] (Fig. 3). SCN and LPO were observed to be concentrated and active enough to support antibacterial activity in the airway and airway-localizing bacteria tested sensitive to the SCN-LPO-H_2O_2 system's effects [14]. Airway epithelia dual oxidase 2 (Duox2) was later identified as the probable source of hydrogen peroxide needed for LPO activity under the regulation of infection-mediated stimuli [46], albeit having its own potent heme peroxidase activity when expressed on the epithelia surface [47]. The importance of this antibacterial activity was later underscored by the discovery that CFTR is a major transporter of SCN and may account for the poor bacterial clearance observed in CF subjects that have dysfunctional CFTR activity [3]. Later studies confirmed the importance of CFTR in SCN-mediated host defense in vitro and did not identify cellular toxicity, suggesting a powerful host defense mechanism was missing in CF [17,18]. However, the discovery that pendrin and CaCC also transport SCN to the airway surface under the direction of cytokines [19] and reports of SCN concentrations

similar to control values in CF patients [12,15] has since called this hypothesis into question. Nevertheless, the correlation of improved lung function with SCN and results from IV administration of SCN in newborn CF pigs [12] support the idea of host defense and antioxidant effects for SCN in CF. It is possible that a deficiency or dysregulation of SCN exists in CF that may later be counterbalanced by disease progression and the changed expression of ion transport proteins.

Intriguingly, multiple *Pseudomonas* strains have been reported that produce methyl thiocyanate (CH_3SCN) that was measureable in the headspace of bacterial cultures and the breath condensates of infected children [48]. It is unclear why *Pa* would make this agent but its structure suggests that strains of *Pa* may have adapted to neutralize SCN as an antimicrobial agent, perhaps even turning it against its host considering the potent toxicity of CH_3SCN (rat oral LD_{50} = 60 mg kg^{-1} [49], compare to 764 mg kg^{-1} for sodium SCN [50]).

1.5. Novel antioxidant effects of SCN

Although HOSCN formation is considered beneficial for its antibacterial properties, SCN's selective interaction with peroxidases, replacing Cl^-, Br^-, and I^- [51], may also buffer cells against injurious oxidative damage by their hypohalous acid products such as HOCl and HOBr in what is considered one of its major antioxidant functions (Figs. 2 and 3). Accumulation of HOSCN in ex vivo neutrophils, eosinophils and macrophages was reported to be non-toxic [52]. SCN was reported to protect HL-60 cells from H_2O_2-mediated apoptosis in the presence of physiologic levels of Cl^- and was proposed to be cytoprotective by effectively competing for Cl^- as a preferred substrate of MPO [53]. SCN was shown to completely ablate toxicity associated with the MPO-Cl^--H_2O_2 system at concentrations of 100–400 μM in lung, nervous, pancreatic and endothelial cells and similarly detoxify GOX-driven H_2O_2 build up through the activity of LPO [54]. SCN has also been reported to prevent eosinophils from undergoing spontaneous and agonist-induced apoptosis [33]. Indeed, the apparent lack of toxicity SCN during utilization by these enzyme systems may implicate an evolutionary rationale for the substrate selectivity of SCN over the true halides. This is illustrated by the formation of equimolar amounts of HOCl and HOSCN at a respective 10:1 ratio of Cl^- to SCN in the presence of MPO [51,54]. HUVEC cells exposed to HOSCN for 24 h were more viable than untreated cells [38]. SCN also reacts directly with HOCl to ablate cytotoxicity and similarly can repair chloramines after chlorination by HOCl and prevent their accumulation in tissue [52,53,55] (Fig. 2). Under acidic conditions, SCN may even act to stabilize HOSCN by condensation and formation of SCN_2 [32] (Fig. 2), although evidence for this in vivo is still lacking. SCN has also been shown to block urate radical formation by stimulated neutrophils in plasma, sparing ascorbate, GSH, and nitric oxide [56].

Interestingly, SCN has been reported to evolve molecular oxygen from H_2O_2 in saliva samples with relatively little formation of HOSCN detected at slightly alkaline pH [57]. The significance of this reaction under the mildly acidic conditions of the saliva, where HOSCN formation is encouraged by the ubiquity of microbes, is unclear but sets up a potential role for SCN as a pseudocatalase substrate that does not always produce an oxidant when reducing H_2O_2.

1.6. Potential roles of SCN in cytotoxicity

In spite of SCN's association with cyanide, it has long been considered relatively non-toxic with an oral LD_{50} in rats of 764 mg kg^{-1} for the sodium salt [50]. Similarly the peroxidase-SCN-H_2O_2 system is often regarded as a benign alternative to other peroxidase products, particularly HOCl and HOBr. However, some researchers using acute bolus doses of HOSCN have observed toxicity in multiple cell lines [35,58]. Lloyd et al. first reported acute

cytotoxicity of HOSCN in the murine alveolar macrophage cell line J774 [58]. HOSCN has also been assayed for cytotoxicity in human umbilical vein endothelial cells (HUVEC) [35]. In this system, HOSCN was reported to inhibit caspase-3 cleavage and apoptosis at concentrations as low as 10 μM. HUVEC cells were positive for both annexin V and PI at high HOSCN concentrations (300 μM exposure for 3 h without serum or media) suggesting a necrotic mechanism of cell death predominates when cells are treated with HOSCN in HBSS.

Our lab has observed a wide variance in the bolus dose toxicity of HOSCN in cultured cells, particularly between macrophages and epithelia (unpublished data). However, we have not been able to reproduce this toxicity using a peroxidase-driven system in any cell type, which is in agreement with reports from several other groups who have studied cells both in balanced salt solution and in culture medium [52-54]. There are a number of possible reasons for this apparent discrepancy between bolus dosing of superphysiologic levels of HOSCN and HOSCN generated by SCN-coupled peroxidase systems. One reason may relate to alternative pathways taken by the SCN coupled peroxidase system or the inhibition of peroxidases by SCN oxidation [57,59]. However a simpler explanation is that a superphysiologic bolus dose given to isolated cells can overwhelm basic countermeasures of cells against HOSCN, preventing conditioning and adaptation that could occur during prolonged exposure to physiologically relevant concentrations of HOSCN. Indeed, HUVEC cell monolayers treated with HOSCN for 24 h were more viable with less apoptosis than untreated cells [38]. Either way, the SCN-coupled peroxidase system would seem to be a more physiologic model of HOSCN exposure.

HOSCN does appear to induce changes in cell signaling that may include anti-apoptotic and pro-inflammatory mechanisms [35,38], but there remains a significant lack of direct evidence for SCN and HOSCN formation in disease. On the contrary, published human data correlates SCN concentration with improved lung function in healthy and cystic fibrosis individuals [12] and there may be similar effects in other diseases where SCN acts as an antioxidant and host defense factor. The diseases that may be associated with increases in SCN (and potentially HOSCN) involve exposure to other toxic agents and mixtures including cyanide, tobacco smoke, and cyanogenic glucosides that can directly contribute to pathogenesis. However, the direct toxic effects of SCN used as a pharmaceutical agent have been assessed previously and are described in the next section.

2. Clinical history and outcomes of thiocyanate administration

2.1. Early use in essential hypertension

In 1903, Pauli introduced SCN as a therapeutic agent for the treatment of hypertension [60] and it was investigated more thoroughly as an antihypertensive when a quick and reliable method to measure plasma levels of SCN was introduced in the 1940s [61]. Values originally reported as mg/100 dL(mg percent) will be reported here as the converted molar value. Reports of SCN's efficacy as an antihypertensive agent varied amongst authors during this time, with many enthusiastic supporters and several skeptics. By the mid-20th Century SCN again fell out of favor with physicians, ultimately being replaced by more potent anti-hypertensives.

2.2. SCN dosing and efficacy in essential hypertension

SCN treated patients were commonly observed with plasma SCN values of 400–2000 μM, with additional case reports of far higher concentrations in the literature, up to 7 mM [62,63]. Oral administration of SCN produces nearly 100% absorption in healthy subjects [28]. Doses of SCN (as a potassium salt) were generally given orally between 400 and 1000 mg per day and therapy could last from weeks to several years [60,64]. SCN levels were

recommended to be maintained between 1400 and 2000 μM to sustain therapy and avoid toxicity. In a study designed to control for placebo effect in senior adults, nine months of 300–900 mg SCN per day produced a reduction of at least 15/10 (systolic/diastolic, mm Hg) in basal blood pressure and was found significant when compared within each patient to a placebo treatment [65]. In the same study, patients receiving up to 600 mg per day had a median plasma level of 1500–2250 μM SCN [65]. However in another study the authors did not find significant difference in blood pressure reduction from placebo except at plasma levels that were regarded as unsafe [63]. Cardiac pathology also correlated inversely with therapeutic efficacy of SCN so that hypertensive subjects with the least chronic disease symptoms were the most likely to experience benefits [65].

2.3. Toxicity of SCN administration for essential hypertension

The toxicity of SCN was noted with excessive or uncontrolled dosing or in cases of suicide [62]. A variety of symptoms have been reported in cases of SCN-associated adverse events, with the caveats that many of the reports came from patients whose plasma level of SCN was not monitored prior to toxicity, who may have already had sensitizing health problems (renal impairment, disorders of the central nervous system, and/or marked age), or who had an intention to commit suicide using a high dose of the drug [62]. Because of the methods put forth to measure SCN from the blood, toxicology reports from the time tend to include descriptions of the very high exposures of SCN the patients experienced, up to 7 mM in the plasma [66]. No reports of SCN-related toxicity were made at plasma levels lower than 500 μM. Serious complications were frequently associated with plasma values of SCN well above 1 mM.

Symptoms of SCN toxicity tended to be related to the nervous system [62,66], the thyroid (although goiters appeared only infrequently [60,67]), the kidneys [62,66], and the skin [64] in patients at or above 1 mM SCN in the plasma [60]. Patients experiencing nervous symptoms presented a number of psychoses and motor-impairments including clonic contractions, aphasia, difficulty walking or standing, zoopsia, confusion, tremors, and anxiety [62,64,66], which were often worsened by pre-existing brain damage and the co-administration of bromide sedatives which produced similar psychotic effects [62,66]. In many cases these nervous symptoms could be reversed [64,66], but in severe cases of toxicity they often persisted [66]. Symptoms of hypothyroidism were less common than may be expected with the contemporary knowledge that SCN competes with I^- for the NIS [3], perhaps owing to high dietary I^- [67]. Skin rash and pustules were also occasionally reported, occurring on the back and elbow and subsiding with the cessation of treatment [64]. In general, these symptoms were more likely to occur or to be more severe if there were pre-existing conditions such as nervous disorders or renal impairment, but high levels of plasma SCN, especially if left unchecked at length, presented marked toxicity even in patients without these conditions.

2.4. Other SCN exposure studies

SCN was also studied as a metabolite of cyanogenic drugs such as nitroprusside. These studies were largely negative in finding toxicity of SCN as a drug metabolite, where it tended to be at lower concentrations (<1 mM) [27,28]. More recently groups of researchers have identified cohorts of elevated plasma SCN levels as a result of diet or behavior (i.e., smoking) and studied its potential impact on health. In addition, disease states have been discovered that may result in a deficiency of SCN that have identified it as a potential pharmacologic agent once more.

2.4.1. SCN exposure from dietary sources and smoking—There are two major groups with high probabilities for heightened SCN exposure: cultural groups that promote a

diet rich in cyanogenic plants and smokers. Both groups pose similar problems in terms of studying toxicity as they do not ingest pure SCN, but rather one or more parent compounds that are metabolized to SCN (these are the cyanogenic glucosides and glucosinolates and hydrogen cyanide and acetonitrile, respectively).

Dietary exposure to SCN correlates with vegetable-rich diets. In a study focused on the average intake of SCN by Koreans, who consume a large quantity vegetables of the Brassicaceae family, the average daily intake of SCN was reported to be 16.3 μmol per person per day, far below levels of glucosinolate required to induce adverse events in animal studies [5], suggesting this population is not at risk of hypothyroidism and other toxicologic endpoints of SCN. Another study reported that goitrous Sudanese individuals given 250 mL milk containing 0.1 mg/L iodine and 328 μM SCN daily had their plasma SCN increased by 30 μM over four weeks and thyroxine, triiodothyronine, and TSH were maintained in the normal range [68]. In a study of vegetarian and vegan individuals it was reported that urinary iodine excretion was lower in vegans than vegetarians (median 78.5 μg/L and 147.0 μg/L, respectively), whereas median SCN excretion was higher in vegans than in vegetarians (630 μg/L and 341 μg/L, respectively) [7]. However, TSH and free T_4 were not significantly different between the two groups and did not correlate with SCN even when multiple variables were adjusted, leading the authors to conclude that even in these groups at risk of low iodine, SCN is not associated with thyroid dysfunction [7].

SCN levels are known to go up in smokers [69] and SCN has even been used as a biomarker to check whether smokers have quit smoking. Since SCN can be utilized by peroxidases to generate HOSCN, this has led some to hypothesize that elevated SCN will predispose individuals to inflammation and disease [13,69]. Although in vivo models that are stimulated to mimic inflammation elevations in SCN do appear to enhance inflammatory responses, in vivo correlation between SCN and inflammatory disease has not materialized [13,69]. Smokers are also difficult to study due to the multiple mechanisms of toxicity caused by tobacco smoke. These results indicate that SCN may be associated with inflammatory mechanisms ex vivo but the in vivo risk posed by elevated SCN is less clear.

2.5. Rationale for SCN treatment in cystic fibrosis

SCN as a pharmaceutical agent has been dormant for over half a century in Western medicine. Interest in SCN has renewed since the discovery that it may be dysregulated in CF and other diseases associated with defects in host defense [3,12,17,18]. It is unclear at the present time if SCN administered orally or by nebulization might be able to correct this imbalance, but the correlation of increased SCN with better lung function [12] is suggestive that there may indeed be a rational basis for SCN therapy in CF.

2.5.1. Role of CFTR and other anion channels in airway concentration of SCN

—The possible connection of SCN with cystic fibrosis (CF) was first noted when Fragoso et al. observed that apical transport of SCN by primary airway epithelial cells (AECs) was blocked by the CFTR inhibitors glibenclamide and diphenylamine-2-carboxylic acid and was stimulated by cyclic AMP (cAMP) and forskolin [3]. Moskwa et al. later showed that AECs with the ΔF508 CFTR mutation were less efficient at exporting SCN from their apical surface and did not respond to cAMP [17]. Transfection of functional CFTR was able to restore this function and bacterial killing was dependent on the apical transport mechanism [17]. Conner et al. then published similar results in AECs that showed stimulation and inhibition of CFTR modulated SCN efflux in wild type cells but did not impact the much lower rate of CF AEC efflux and also reproduced the result of bacterial killing derived from basolateral SCN [18]. These studies contributed to the hypothesis that SCN may be a

missing component of the ELF in CF and may be responsible for regulating multiple symptoms of the disease, including chronic bacterial infection and inflammation (Fig. 3).

Minarowski et al. reported a significant 21% decrease of SCN levels in the saliva of CF patients versus healthy controls and healthy smokers [70]. In gut-corrected CF knockout mice, SCN was observed to be heavily depleted in the ELF compared to wild type controls and human airway CFTR-deficient IB3 cells were hypersensitive to HOCl toxicity and did not export as much SCN onto their apical surfaces as CFTR-corrected C38 cells [9]. However in a study of young children with CF, SCN values from diluted BALF were reported higher in CF patients than in controls and both groups' SCN levels were lower than in other studies [15]. In another study of undiluted NLF sampled from the nasal airway, CF patients and healthy controls were found to have the same mean value of 400 μM SCN and the same ratio of airway SCN to plasma SCN (approximately 30) [12]. However in the same study, newborn CF mutant and wild type pigs were assayed for SCN 2 h after IV administration and the correlation between plasma and airway SCN was lower in the CF pigs than in the wild type [12]. The authors also found lung function of CF patients correlated positively with SCN [12].

The use of uninfected, non-symptomatic controls may bias comparisons with CF patients that may express different amounts of the SCN transport complex (CFTR, CaCC, pendrin, and possibly others), potentially voiding the isolation of CFTR as a variable. In the Lorentzen study, only 9% of CF patients were not using antibiotics versus 100% for the controls, and 74% of the CF patients had *Pa* in their sputum compared to no detectable *Pa* in the sputum of controls [12]. However CF and wild type pigs were used within 12 h of birth and were not likely to be significantly infected. Interestingly, in these animals there was a decrease in SCN concentration in the airway of CF individuals compared to serum 2 h after an IV dose which suggests the absence of CFTR limited the rate of transport [12]. Lung function also correlated positively with SCN in CF patients [12]. In the Thomson study of young children, infection status and its relationship with SCN was considered within the CF patients but was not reported for controls, although within the CF group no difference in SCN based on infection status was reported [15]. Infection status and respiratory symptoms may work in tandem to produce heightened SCN efflux in the lung. Pedemonte et al. reported that IL-4 stimulates cells to upregulate expression of CaCC and pendrin, anion channels capable of increasing apical efflux of SCN [19]. In fact, IL-4, IL-13, and IFN-γ can each regulate the expression of pendrin in times of allergic response or infection [20]. In CF, patients are often chronically infected with gram-negative bacteria including *Pa*. In cases of infection, inflammation, and other CF symptoms, CaCC and pendrin may be upregulated in response and compensate for the lack of active SCN transport by CFTR (Fig. 3), although the putative cell-signaling molecule capable of this mechanism in CF has not yet been identified.

2.5.2. Future directions for SCN as a therapeutic intervention—Given the wealth of historical data on orally administered SCN in humans for the treatment of essential hypertension, there is abundant pharmacokinetic and clinical safety data to proceed directly into human clinical studies. Some studies in animals would be needed to determine if the doses used in the essential hypertension intervention are relevant to improved host defense in lung infection models and ultimately improve clinical outcomes in CF patients. A number of issues need to be resolved on whether the oral absorption of SCN is altered in CF subjects and whether the oral route will deliver an efficacious level of SCN in the ELF to improve host defense, diminish lung oxidative stress, and improve lung function in CF subjects. If one cannot achieve these goals with oral administration, then SCN could be given by inhalation to bypass these potential obstacles. Beyond CF, there are many other potential human lung disease applications for SCN therapy that involve impaired lung host defense

and/or dysregulated lung inflammation including acute respiratory distress syndrome, pneumonia, asthma, chronic obstructive pulmonary disease and granulomatous lung disease.

3. Conclusions

SCN is ubiquitous throughout the extracellular fluids of the human body and functions in both host defense and as an antioxidant (Fig. 3). SCN's ubiquity underscores its importance in each of these roles. SCN's host defense capacity is unique in inhibiting the metabolism of pathogens by targeting strongly acidic thiols, an entirely different mechanism of action than the current array of approved antibiotics. SCN's antioxidant capacity to scavenge cytotoxic oxidizing agents, such as HOCl and RNHCl, spares host tissue while generating more of the host defense factor HOSCN in turn. Peroxidases strongly select for SCN over other electron donors preventing the formation of additional oxidizing agents and radicals. Certain diseases of dysregulated anion transport, most notably CF, may result in SCN deficiency or maladaptive stress responses that could benefit from SCN supplementation to improve bacterial clearance, diminish oxidative stress, and improve lung function. The clinical history of SCN and contemporary observations in high exposure groups demonstrate that it is well tolerated and should be considered a strong candidate for therapeutic use.

Abbreviations

SCN	thiocyanate
LPO	lactoperoxidase
HOSCN	hypothiocyanous acid
EPO	eosinophil peroxidase
GPO	gastric peroxidase
MPO	myeloperoxidase
SPO	salivary peroxidase
TPO	thyroid peroxidase
HOCl	hypochlorous acid
ELF	epithelial lining fluid
NLF	nasal lining fluid
Pa	pseudomonas aeruginosa
BALF	bronchoalveolar lavage fluid
NIS	sodium-iodide symporter
CFTR	cystic fibrosis transmembrane conductance regulator
CaCC	calcium-dependent chloride channel
TCA	trichloroacetic aid
H_2O_2	hydrogen peroxide
$^{-}$OSCN	hypothiocyanite
RSSCN	sulfenyl thiocyanates
RSOH	sulfenic acids
RSSR	disulfides

GSH	glutathione
GSSG	glutathione disulfide
TBN	2-nitro-5thiobenzoate
SCN_2	thiocyanaogen
GOX	glucose oxidase
IFN-γ	interferon gamma
Duox	dual oxidase
HOBr	hypobromous acid
HUVEC	human umbilical vein endothelial cells
AEC	alveolar epithelial cells
CF	cystic fibrosis
IL	interleukin.

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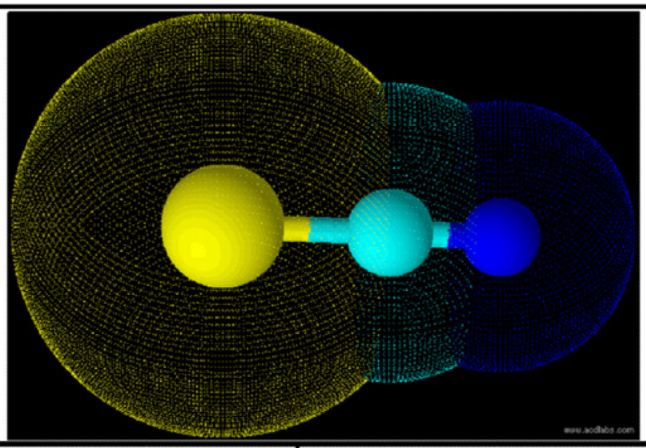

Properties	SCN
Molecular weight	57 g/mol
Thiol pKa	2
Sources	Dietary, Thioltransferases
Functions	Host defense, Antioxidant

Fig. 1.
Physical and chemical properties of SCN.

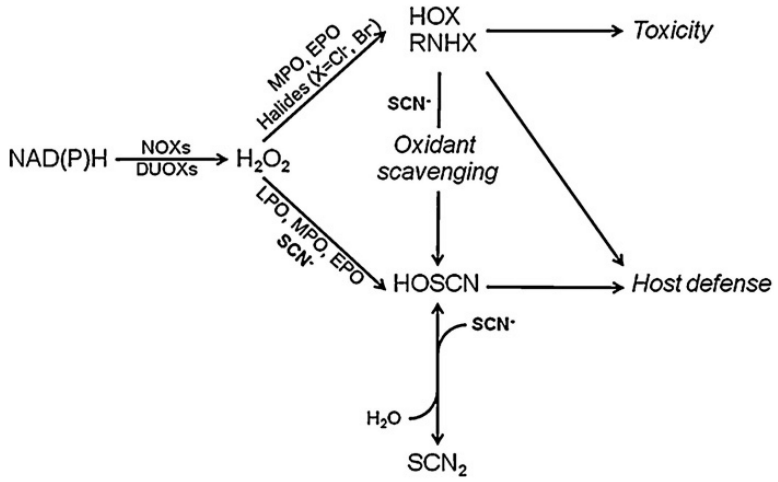

Fig. 2.

Chemical and enzymatic pathways driving the host defense and antioxidant properties of thiocyanate (SCN). Halide peroxidases such as myeloperoxidase (MPO), lactoperoxidase (LPO) and eosinophil peroxidase (EPO) utilize hydrogen peroxide (H_2O_2) from NAD(P)H oxidases (NOX) and dual oxidases (DUOX) to form HOSCN and hypohalous acids (HOX) that include hypochlorous acid (HOCl), hypobromous acid (HOBr) and hypothiocyanous acid (HOSCN). HOX readily reacts with amino groups to form haloamines (RNHX) and readily oxidizes cellular macromolecules leading to toxicity. SCN can directly scavenge HOX and repair RNHX and in the process generate HOSCN which still has host defense properties and is better tolerated by host tissue than HOX.

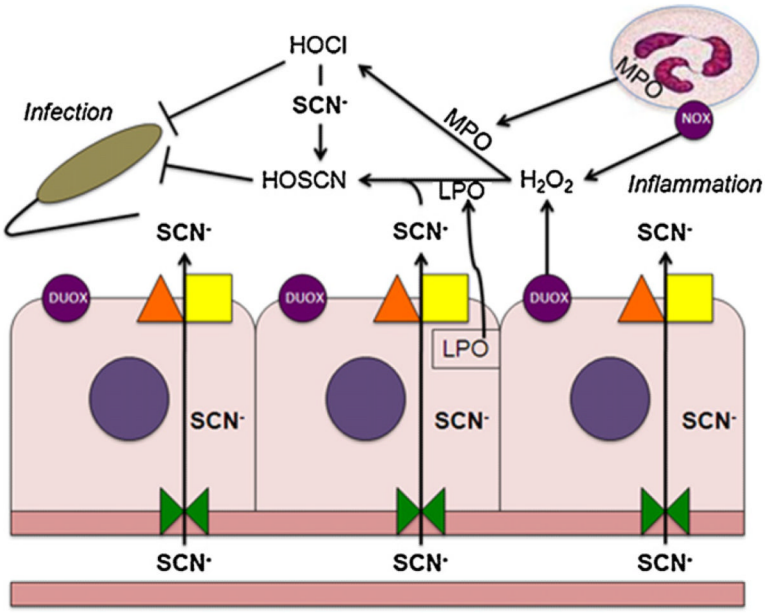

Fig. 3.

Transport of thiocyanate (SCN) by secretory epithelium and its protective roles in the lumen during infection and inflammation. SCN enters secretory epithelia from the blood stream by sodium-iodide symporter (NIS, double-triangle) and is exported into the lumen by cystic fibrosis transmembrane conductance regulator (CFTR, triangle) and/or calcium-dependent chloride channel (CaCC) and pendrin (square). Peroxidases arrive in the lumen from different sources, including the epithelia, which secrete lactoperoxidase (LPO), and inflammatory leukocytes such as neutrophils, which release myeloperoxidase (MPO) upon activation. Epithelia and inflammatory leukocytes also provide H_2O_2 to drive peroxidase activity with dual oxidase (DUOX) and NAD(P)H oxidase (NOX) depicted as circles. The peroxidases drive the formation of thiol-selective hypothiocyanous acid (HOSCN) and the more promiscuous hypochlorous acid (HOCl). SCN directly reduces HOCl to form HOSCN and spares potential toxicity resulting from HOCl.

Table 1

Reported thiocyanate (SCN) levels in human extracellular fluids.

Compartment	SCN (μM)	Peroxidase	References
Saliva	500–3000	SPO, MPO[a]	[22], [70], [26]
Nasal airway fluid	100–1200	LPO, MPO[a], EPO[a]	[12]
Lung airway fluid	30–650	LPO, MPO[a], EPO[a]	[15], [14]
Gastric fluid	250–300	GPO	[4]
Tears	150	LPO	[26]
Plasma	5–50	MPO[a], EPO[a]	[27], [12]
Milk	0.1–4	LPO	[24], [25]
Semen	Qualitative report	Not reported	[25]

[a]During inflammation.