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Commentary

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.

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

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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; RS-SCN, 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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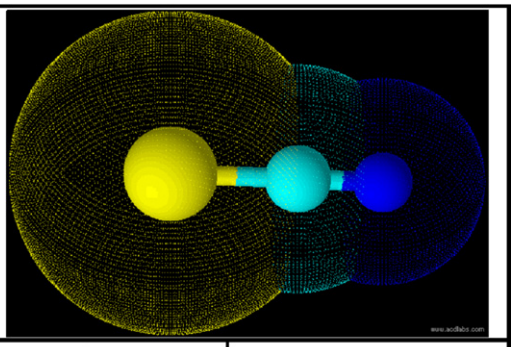

Properties	SCN
Molecular weight	57 g/mol
Thiol pKa	2
Sources	Dietary, Thioltransferases
Functions	Host defense, Antioxidant

Fig. 1. Physical and chemical properties of SCN.

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 electro-neutral halide-exchange channel) and TMEM16A (Ca^{2+}-dependent Cl^- channel (CaCC), an active transporter of halides) [19,20].

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.

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