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Concentration of the antibacterial precursor thiocyanate in cystic fibrosis airway secretions

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Abstract

A recently discovered enzyme system produces antibacterial hypothiocyanite (OSCN⁻) in the airway lumen by oxidizing the secreted precursor thiocyanate (SCN⁻). Airway epithelial cultures have been shown to secrete SCN⁻ in a CFTR-dependent manner. Thus, reduced SCN⁻ availability in the airway might contribute to the pathogenesis of cystic fibrosis (CF), a disease caused by mutations in the *CFTR* gene and characterized by an airway host defense defect. We tested this hypothesis by analyzing the SCN⁻ concentration in the nasal airway surface liquid (ASL) of CF patients and non-CF subjects, and in the tracheobronchial ASL of CFTR-ΔF508 homozygous pigs and control littermates. In the nasal ASL, the SCN⁻ concentration was ~30-fold higher than in

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serum independently of the CFTR mutation status of the human subject. In the tracheobronchial ASL of CF pigs, the SCN^- concentration was somewhat reduced. Among human subjects, SCN^- concentrations in the ASL varied from person to person independent of CFTR expression, and CF patients with high SCN^- levels had better lung function than those with low SCN^- levels. Thus, although CFTR can contribute to SCN^- transport, it is not indispensable for the high SCN^- concentration in ASL. The correlation between lung function and SCN^- concentration in CF patients may reflect a beneficial role for SCN^- .

Keywords

thiocyanate; dual oxidase; lactoperoxidase; cystic fibrosis; airway surface liquid

INTRODUCTION

Most inhaled bacteria become entrapped in a mucus layer that covers the conducting airways. This mucus layer is constantly cleared from the healthy respiratory tract by the concerted movement of airway cilia. During the mucociliary clearance process, bacterial growth and survival is limited by the antimicrobial proteins of the airway surface liquid (ASL). Recent studies suggest that in addition to the antimicrobial proteins of airway secretions an oxidative host defense mechanism of airway epithelia may also contribute to the antibacterial activity in the ASL [1–3].

Airway epithelial cells express two plasma membrane-embedded cytochromes—Dual oxidase 1 (Duox1) and Duox2—that generate H_2O_2 on the extracellular side of the apical membrane [4–7]. Within the extracellular space, H_2O_2 is metabolized by the secretory protein lactoperoxidase (LPO) [8,9], which uses H_2O_2 to oxidize the physiological ASL component thiocyanate (SCN^-) to the potent antibacterial molecule hypothiocyanite (OSCN^-). Cultured airway epithelia produce sufficient H_2O_2 to support OSCN^- generation at levels toxic to bacteria [10–12]. Furthermore, inhibiting LPO activity *in vivo* hinders bacterial clearance from the lower airways [1].

Although the airway epithelium does not synthesize SCN^- , the concentration of SCN^- in the ASL (~460 μM) is far higher than that in the serum (5–50 μM) [3]. Cell-culture experiments indicated that SCN^- is imported into the airway epithelium basolaterally, via the $\text{Na}^+\text{-I}^-$ symporter NIS [13]. SCN^- subsequently leaves the cells apically, through the CFTR anion channel [10,11,14], which is permeable to SCN^- as well as to chloride (Cl^-) and bicarbonate. Mutations in the gene encoding CFTR lead to cystic fibrosis (CF) disease, which in the airway is characterized by recurrent and chronic infections [15]. Notably, primary cultures of CF airway epithelia are defective for OSCN^- -dependent bacterial killing, due to a reduction in SCN^- secretion [10,11]. These findings are consistent with the notion that insufficient SCN^- secretion in CF airways might contribute to the pathogenesis of CF lung disease. However, the SCN^- concentration in the ASL of CF patients has not been determined.

We used a recently developed porcine model of CF to evaluate the effect of CFTR inactivation on the SCN^- concentration in tracheobronchial secretions. We also evaluated SCN^- levels in the nasal secretions from CF patients and non-CF subjects. Contrary to our expectations, we found that the SCN^- concentration was similar in the nasal secretions of CF and non-CF subjects, whereas a moderate reduction in SCN^- concentration was detected in the tracheobronchial secretions of CF pigs as compared to control littermates. Furthermore, in humans CFTR-independent factors led to significant person-to-person

variability in ASL SCN^- concentrations, and CF patients with high SCN^- levels exhibited better lung function than those with low SCN^- .

MATERIALS AND METHODS

Human subjects

23 CF patients and 21 non-CF subjects participated in this study. Nasal ASL was collected from all participants. 14 CF subjects and 18 non-CF subjects also provided blood samples. Both CF and non-CF volunteers were non-smokers and experienced no symptoms of upper airway infection or allergic rhinitis during the three weeks prior to recruitment. For all recruited patients, the diagnosis of CF had been previously confirmed by genotyping. The pulmonary function of CF patients was evaluated based on the spirometric measurement of forced expiratory volume in one second (FEV_1). Spirometry was done according to the American Thoracic Society guidelines [16]. Additional subject information is summarized in Table 1. This study was approved by the Institutional Review Board of the University of Iowa.

CFTR mutant and control pigs

Production of heterozygous CFTR- ΔF508 pigs was previously reported [17]. These animals were intercrossed to generate homozygous CFTR- ΔF508 pigs and wild-type littermates. The lung phenotypes of homozygous CFTR- ΔF508 pigs and CFTR-null pigs [18–20] are indistinguishable (unpublished observation). Newborn pigs were genotyped immediately, and homozygous CFTR- ΔF508 pigs ($n=6$) and wild-type pigs ($n=14$) were used for this study within 12 hours of birth. This study was approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Blood and ASL collection

Venous blood of human subjects was collected from an arm vein. Blood of CFTR- ΔF508 homozygous and wild-type pigs was collected under propofol anesthesia. Prior to the analysis of anion composition, the serum fraction was filtered (3 kDa cut-off Ultracel filter, Millipore) to remove the majority of serum proteins.

Nasal ASL was harvested from human subjects using microsampling probes (Olympus BC-402C) [21,22]. Prior to sample collection, nostrils were kept closed with a diver's clip for 5 min to minimize evaporation. The probes were then introduced deep into the nose and held gently to the nasal turbinates. After 30–60 seconds, probes were removed from the nose and placed onto filters in microcentrifuge tubes (Costar Spin-X filter). Undiluted ASL was recovered from the probes by centrifugation.

Lower airway secretions were collected from pigs under propofol anesthesia. The tracheas of pigs were surgically exposed and opened horizontally using electro-cauterization. Microsampling probes were introduced into the respiratory tract through the surgical opening, and were held gently to the surface of the trachea and bronchi at multiple points. Our initial experiments indicated that the volume of ASL collected from the lower airways was not always sufficient for analysis when dry probes were used, and that the efficiency of collection could be improved by pre-wetting the probes with 2 μL isosmotic mannitol solution containing 300 μM Evans blue dye. After ASL collection was completed, fluid was extracted from the probes by centrifugation, and the ASL content of the harvested fluid was calculated based on the extent to which Evans blue dye was diluted. The dilution factor was determined by measuring the optical density of the collected fluid at 600 nm, using NanoDrop ND-1000 spectrophotometer.

Ion-exchange chromatography

Ultrapure water was used to dilute the serum (4-fold) and ASL samples (50- and 100-fold) prior to measuring ion concentration using a Metrohm Advanced Ion Chromatography system (MIC-2, Metrohm USA, Inc.) and a Metrosep A Supp 5–150 column. The mobile phase was composed of 1 mM sodium carbonate and 3.2 mM sodium bicarbonate. Anions were detected based on changes in conductivity, and the conductivity detector was calibrated with standard solutions.

Bacterial killing assay

Well-differentiated primary cultures of human airway epithelia were obtained from the *In Vitro* Models and Cell Culture Core at the University of Iowa [23]. These cultures were maintained at air-liquid interface and incubated in the absence of antibiotics for 5 days prior to the bacterial killing assays. The bacterial killing activity of airway epithelial cultures was measured as previously described [10]. In brief, mid-log phase liquid cultures of *Staphylococcus aureus* (strain Xen8.1; Xenogen Corporation, Hokinton, MA) were pelleted and resuspended in PBS. Bacterial density was estimated by measuring optical density at 550 nm. Approximately 3,000 and 1,000 colony forming units (CFU) bacteria were inoculated onto the apical surface of airway epithelial cultures in PBS (60 μ L inoculum/1 cm² surface area) supplemented with LPO (7 μ g/mL), SCN[−] (0–700 μ M), and HEPES (10 mM, pH=6.6). Epithelial H₂O₂ production was maximized with the apical addition of ATP (100 μ M) [6,24,25]. Following a 3-hour incubation at 37°C, liquid was collected from the apical surface. Epithelial cultures were then lysed with 1% saponin in distilled water, and lysates were pooled with the previously collected apical fluid. The number of surviving bacteria was determined using quantitative liquid culture, as described previously [26].

Hypothiocyanite measurement

OSCN[−] production by primary airway epithelial cultures was assessed by detecting the oxidation of 2-nitro-5-thiobenzoate (TNB), as previously described [27]. Briefly, ATP (100 μ M) stimulated airway epithelial cultures were incubated in the presence of LPO (7 μ g/mL) and SCN[−] (0–700 μ M) in PBS (supplemented with 10 mM HEPES, pH=6.6) for 40 minutes at 37°C. Following incubation, samples were removed and catalase added to remove excess H₂O₂. TNB (350 μ M) was added to the samples, and the molar concentration of OSCN[−] was calculated based on the loss of absorbance at 412 nm (ϵ =14,000 M^{−1}cm^{−1}).

Statistical analysis

Data are reported as mean $\pm$ SEM. All statistical analyses were carried out using the GraphPad Prism 4.03 software. Ion concentrations in CF and non-CF samples were compared using two-tailed Mann-Whitney tests. Correlations between variables were studied using ordinary least-square linear regressions. Pearson's correlation coefficients (r) were calculated as measures of linear association. The equality of regression line slopes to zero was evaluated using two-tailed Student's *t* test. Equality of regression curves was tested using an F-test.

RESULTS

SCN[−] concentrations in upper-airway secretions from CF patients and non-CF subjects are similar

In order to evaluate the impact of CFTR deficiency on the SCN[−] concentration in nasal ASL, we collected upper airway secretions from CF and non-CF subjects and measured the SCN[−] content of the collected samples. We used a previously developed microsampling probe [21,22] for the collection of nasal ASL. Samples were recovered from the probe by

centrifugation and analyzed by anion-exchange chromatography. This method consistently detected anions in the ASL at the retention times characteristic of fluoride (F^-), Cl^- , nitrate (NO_3^-), hydrogen phosphate (HPO_4^{2-}), sulfate (SO_4^{2-}), and SCN^- (Fig. 1A, calibration data are presented in the online data supplement Fig. S1). The identity of the SCN^- peak was verified by the LPO-catalyzed oxidation of SCN^- to $OSCN^-$ *ex vivo* (Fig. 1A inset).

The analysis of nasal ASL samples demonstrated that SCN^- concentrations varied significantly from person to person in both subject groups (Fig. 1B). Moreover, the mean SCN^- concentration in the nasal secretions of CF patients did not differ significantly from that in the nasal secretions of non-CF subjects (Fig. 1B). This was also the case for F^- , HPO_4^{2-} , and SO_4^{2-} (data not shown). In contrast, the Cl^- concentration was significantly elevated in the CF samples (Supplemental Fig. S2), in agreement with a recent study [28]. Similarly, the NO_3^- concentration tended to be elevated in the ASL of CF patients compared to that of non-CF subjects (Supplemental Fig. S3).

The similarity in the SCN^- concentrations found in the upper airway secretions of CF and non-CF subjects indicated that the SCN^- concentration in nasal ASL did not depend on CFTR activity. However, it remained possible that the SCN^- concentration gradient between the ASL and serum was lower in CF patients than in non-CF subjects. Therefore, we determined the SCN^- concentration not only in the ASL but also in the serum of CF and non-CF subjects. Our results revealed a linear correlation between the serum and ASL SCN^- levels in both subject groups (Fig. 1C). Furthermore, our data showed that the SCN^- concentration was approximately 30-fold higher in the ASL than in the serum, regardless of the mutation status of CFTR (Fig. 1C). These data indicate that the lack of CFTR activity did not reduce the SCN^- concentration gradient between the serum and nasal ASL.

The SCN^- concentration in tracheobronchial secretions of newborn pigs is partly CFTR dependent

In order to evaluate the SCN^- concentration in tracheobronchial secretions, we used a recently developed porcine model of CF [17,18]. This animal model has two unique advantages for our study. The first advantage is that homozygous CFTR- $\Delta F508$ pigs can be studied immediately after birth, prior to the first incidence of airway infection. The lack of respiratory infection in the model system is important because inflammation itself could potentially alter ion transport across the airway mucosa. The second advantage of the pig model is related to the health risks of the ASL collection procedure. Our initial experiments in human subjects indicated that general anesthesia was necessary for the collection of undiluted tracheobronchial secretions free of contamination by saliva and local anesthetics (unpublished observation). The availability of CFTR mutant pigs make it possible to collect ASL from CF lower airways without exposing humans to risks associated with general anesthesia.

We collected ASL from the trachea and bronchi of homozygous CFTR- $\Delta F508$ pigs and wild-type littermates under propofol anesthesia within 12 hours of birth (nasal ASL could not be collected due to the presence of amniotic debris in the snout). After ASL collection, blood samples were drawn, and anion concentrations were determined using anion-exchange chromatography. We found that both the ASL and serum of newborn pigs contained much less SCN^- than did those of adult humans ($<20 \mu M$ SCN^- in ASL and $<2 \mu M$ SCN^- in serum; $n=4$ wild-type pigs). The low SCN^- concentration in the serum of newborn pigs may have been related to the age of the animals, because older pigs had higher SCN^- levels in the serum ($7.2 \pm 0.35 \mu M$ SCN^- ; 7-week old wild-type pigs, $n=3$). To replicate the wide range of serum SCN^- concentrations found in humans, we administered intravenously various doses of SCN^- to newborn pigs. 2 hours after SCN^- injection, ASL and serum were collected and analyzed by ion-exchange chromatography. We found that the ASL SCN^-

concentration increased dramatically in pigs after intravenous SCN^- administration (Fig. 2A and 2B). In both the homozygous CFTR- ΔF508 pigs and wild-type littermates, the SCN^- concentration of the ASL was higher than that of the serum (Fig. 2C). However, this difference in SCN^- levels between the ASL and serum was less pronounced in the CF group (Fig. 2C). These data indicate that SCN^- concentration increased in the tracheobronchial secretions of SCN^- -injected control pigs in a partly CFTR-dependent manner.

SCN^- concentration in upper-airway secretions correlates with lung function in CF patients

Having determined that in both CF subjects and CFTR mutant pigs, the ASL SCN^- concentration correlated strongly with serum SCN^- levels (Fig. 1C and 2C), we next investigated whether additional correlations could be found between the ASL SCN^- concentration and any other parameter that had been recorded for CF patients. The investigated parameters included age, gender, CFTR genotype, FEV_1 , body mass index, hospitalization status, the presence of *Pseudomonas aeruginosa* in sputum, the use and type of antibiotics, and the concentrations of F^- , Cl^- , NO_3^- , HPO_4^{2-} , and SO_4^{2-} in nasal ASL. Among these, only FEV_1 correlated with the concentration of SCN^- in nasal ASL (Fig. 3 and Supplemental Tables S2 and S3), and this correlation was positive, *i.e.* CF patients with high ASL SCN^- concentrations exhibited better lung function than those with low SCN^- (Fig. 3).

SCN^- concentrations in the physiological range affect the antibacterial activity of airway epithelial cultures

Although the bactericidal activity of Duox/LPO enzymes of airway epithelia is known to depend strictly on the presence of SCN^- [10–12], the SCN^- dose dependence of the bacterial killing process has not been established. Therefore, we examined whether individual differences in ASL SCN^- concentrations could affect OSCN⁻ production and OSCN⁻-mediated host defense. Well-differentiated primary cultures of human airway epithelia were stimulated by apical ATP (100 μM) to maximize Duox-dependent H_2O_2 production [6,24,25], and the rate of OSCN⁻ production was measured in the presence of LPO and various SCN^- concentrations using the colorimetric substrate TNB. The tested SCN^- concentrations (with the exception of zero) were selected from the physiological range in nasal ASL. The SCN^- -dependence of OSCN⁻ production was nearly hyperbolic with half-maximal reaction rate at $255 \pm 83 \mu\text{M}$ SCN^- (Fig. 4A), which agrees well with the *in vitro* established kinetic properties of LPO at pH 7 [29].

Next, we investigated the effect of various SCN^- concentrations on the ability of airway epithelia to kill a potential respiratory pathogen, *S. aureus*. Approximately 3,000 CFU of *S. aureus* were inoculated on the apical surface of human airway epithelial cultures in the presence of the physiological LPO concentration (7 $\mu\text{g/mL}$) and various concentrations of SCN^- (Fig. 4B). Duox activity was maximized with the P2Y agonist ATP (100 μM , apical side). In negative control cultures, the apical buffer contained glutathione (5 mM) or catalase (1,000 U/mL) in addition to LPO, SCN^- , and ATP. After a 3-hour incubation, we assessed the number of surviving bacteria by quantitative culture. Bacterial survival on the epithelial cultures mirrored the SCN^- dose dependence of OSCN⁻ production (Fig. 4B). Furthermore, both glutathione and catalase inhibited the antibacterial activity of airway epithelia, confirming that the SCN^- -dependent bacterial killing required H_2O_2 production (Fig. 4B). When a smaller inoculum (1,000 CFU *S. aureus*) was tested, the SCN^- dose dependence of bacterial killing was not altered (Supplemental Fig. S4). These data suggest that the observed person-to-person differences in ASL SCN^- concentration were in a range that is relevant for the H_2O_2 -dependent antibacterial activity of airway epithelia.

DISCUSSION

In this study, we show that the concentration of the antibacterial precursor molecule SCN^- is ~30-fold higher in the nasal ASL than in serum, independently of the CFTR mutation status of the human subject. In the tracheobronchial ASL of newborn CF pigs, the SCN^- concentration also exceeded the serum SCN^- level shortly after the intravenous injection of SCN^- ; however, the SCN^- concentration gradient that developed between serum and ASL was somewhat greater in the wild-type pigs than in CF littermates. Our results also show that CF patients who had high SCN^- concentrations in the ASL had better lung function than those with comparatively low SCN^- concentrations. However, it will be necessary to carry out large-cohort longitudinal studies to unequivocally establish a correlation between ASL SCN^- levels and lung function. We have also found that the person-to-person differences in ASL SCN^- concentrations were in a range that is relevant for the antibacterial activity of airway epithelia in a cell-culture setting.

A previous study found ~460 μM SCN^- in the tracheal secretions of non-CF adults [3]. We measured ~400 μM SCN^- in the nasal secretions of both CF and non-CF adult subjects (Fig. 1B). Thus, the SCN^- concentration gradient across the airway epithelium may be very similar in the nose and trachea of non-CF subjects. In contrast to the high SCN^- level found in adults, a recent study has shown that the bronchoalveolar lavage from young non-CF and CF children contain barely detectable concentrations of SCN^- [30]. Based on the assumption that the ASL was 100-fold diluted in these bronchoalveolar lavage samples, the SCN^- concentration was estimated to be 28–56 μM in the ASL of young children [30]. Thus, the SCN^- concentration in ASL may be substantially lower early in life than in adult subjects. This notion is also supported by our animal experiments that revealed much lower serum SCN^- levels in newborn pigs than in 7-week old animals (*i.e.* <2 μM SCN^- versus 7.2 ± 0.35 μM SCN^-).

What factors may lead to an increase in serum SCN^- concentration in healthy subjects? Animal cells do not synthesize SCN^- [31], and thus the SCN^- of body fluids is thought to be derived from vegetables and other plant foods. Cruciferous vegetables (broccoli, cabbage, etc.) are regarded as especially rich sources of SCN^- [32]. In addition, when cyanide-containing food is ingested (*e.g.* cassava), SCN^- is generated in the liver through the rhodanese-catalyzed detoxification of cyanide. Nevertheless, serum SCN^- levels do not fluctuate with food intake because NIS-expressing organs with substantial fluid volumes (*i.e.* stomach and salivary glands) serve as SCN^- reservoirs [31]. Thus, we speculate that long-term differences in the diet may account for at least some of the differences in serum SCN^- concentrations among human subjects.

Our analysis of the nasal ASL samples showed that the Cl^- concentration was elevated in the nasal secretions of CF subjects (Supplemental Fig. S2). A recent study also reported elevated Cl^- in the nasal ASL of CF patients [28]; however, elevated Cl^- levels were also found in the nasal ASL of non-CF patients with rhinitis [28]. Thus, increased Cl^- concentration in the ASL may be a general sign of airway inflammation [28]. Our ASL analysis also revealed that the NO_3^- concentration tended to be elevated in the nasal secretions of CF subjects (Supplemental Fig. S3). Previous studies reported either increased [33–35] or normal NO_3^- concentrations [36, 37] in the airway secretions of CF subjects. In contrast, Thomas Kelley and colleagues measured reduced NO_3^- levels in the lung homogenates of CFTR mutant mice and found reduced iNOS expression in the airway epithelia of these animals [38]. Subsequent human subject studies reported reduced iNOS expression in the inflamed, but not in the non-inflamed, airway epithelium of CF patients [36, 37, 39]. A mechanism by which reduced iNOS expression might be accompanied by unaltered or increased NO_3^- concentration in the ASL of human subjects has been proposed

by Anna Chapman and colleagues [36]. According to these authors, increased myeloperoxidase (MPO) and oxidant levels in the inflamed CF airway may enhance the metabolism of nitrogen monoxide to NO_3^- [36].

Our data revealed that CFTR was not indispensable for the generation of a SCN^- concentration gradient between the nasal ASL and serum in adult human subjects. In contrast, the *ex vivo*-differentiated airway epithelium requires CFTR for SCN^- secretion when the cells are maintained under standard culture condition [10,11,14]. These data suggest that the cultured airway epithelium lacks the CFTR-independent SCN^- transporter of the airway mucosa. Interestingly, the anion transporter pendrin is capable of exporting SCN^- from cells [14], and the expression of this transporter in the airway epithelium is upregulated dramatically by inflammatory cytokines both *ex vivo* and *in vivo* [14,40,41]. Therefore, pendrin is a strong candidate for mediating CFTR-independent SCN^- secretion, especially in the inflamed airways.

SCN^- concentration in upper-airway secretions correlated with lung function in CF patients. Although correlation does not necessarily imply a causal relationship between higher SCN^- levels in ASL and better lung function, there are at least two mechanisms through which SCN^- could favorably influence pulmonary function in CF patients. The first mechanism involves the intrinsic antibacterial activity of OSCN^- . In the CF lung, several innate immune mechanisms are thought to be dysfunctional, including mucociliary clearance, antibacterial proteins and peptides, and neutrophil granulocytes [42]. In this context, low OSCN^- production and the consequently low OSCN^- -dependent bacterial killing activity might serve to undermine one more constituent of an already impaired antimicrobial arsenal. The second, but not mutually exclusive, mechanism is based on the antioxidant properties of SCN^- [43–45]. During bacterial infection of the respiratory tract, activated neutrophil granulocytes are recruited into the airway lumen, where they produce hypochlorous acid (HOCl) by the combined effects of the phagocyte NADPH oxidase and the neutrophil granule protein MPO [30,46]. Because HOCl is more toxic to eukaryotic cells than is OSCN^- [47,48], any reactions that increase the OSCN^- :HOCl ratio will likely reduce oxidative damage of the host. SCN^- can divert the net HOCl-generating activity of neutrophils towards OSCN^- production in two ways [43–45]. First, SCN^- inhibits the production of HOCl by competing with Cl^- for MPO and H_2O_2 . Second, SCN^- reacts with HOCl, which leads to the consumption of HOCl and the production of OSCN^- . As a result of these reactions, SCN^- might benefit the CF airways not only because SCN^- enhances Duox/LPO-dependent bacterial killing, but also because SCN^- may prevent tissue damage inflicted by HOCl. Future studies are required to determine if either the antibacterial or antioxidant functions of SCN^- support lung function in CF patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ASL	airway surface liquid
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CFU	colony forming unit
Duox	Dual oxidase
FEV₁	forced expiratory volume in one second
H₂O₂	hydrogen peroxide
HOCl	hypochlorous acid
LPO	lactoperoxidase
MPO	myeloperoxidase
NIS	Na ⁺ -I ⁻ symporter
OSCN⁻	hypothiocyanite
SCN⁻	thiocyanate

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Fig. 1. Anion composition analysis of nasal ASL in CF and non-CF subjects

(A) A representative chromatogram of human ASL sample. ASL was collected from the nasal mucosa and diluted 50-fold prior to chromatography. The F⁻ (I), Cl⁻ (II), NO₃⁻ (III), HPO₄²⁻ (IV), SO₄²⁻ (V), and SCN⁻ (VI) peaks are indicated with Roman numerals. The identity of the SCN⁻ peak was verified by oxidizing the SCN⁻ content of diluted ASL using an excess of LPO and H₂O₂ (inset). The detector response is shown in arbitrary units (AU). (B) SCN⁻ concentrations in the nasal ASL of CF and non-CF subjects, as determined by ion-exchange chromatography (n=23 CF, 21 non-CF; Mann-Whitney test, p=0.89). (C) Correlation between SCN⁻ concentrations in serum and nasal ASL of non-CF (▲) and CF (□) subjects. Each data point represents one subject. Solid and dotted lines indicate the best-fit linear regression lines to non-CF and CF data, respectively (non-CF: r=0.60, **p=0.0084; CF: r=0.97, ***p<0.0001). The slopes of the two regression lines are not significantly different (F-test, p=0.31).

Fig. 2. SCN^- secretion in the lower airways of CF and control pigs

(A and B) Representative chromatograms of pig ASL samples. ASL samples were collected from the trachea and bronchi of (A) an untreated pig (representative of 4 newborn animals) and (B) a pig intravenously injected with 8 mg NaSCN/kg body weight (representative of 3 animals). The F^- (I), Cl^- (II), NO_3^- (III), HPO_4^{2-} (IV), SO_4^{2-} (V), and SCN^- (VI) peaks are indicated with Roman numerals. Note that SCN^- was not detected in the ASL of the untreated pig. The detector response is shown in arbitrary units (AU). (C) Relationship between SCN^- concentrations in the serum and tracheobronchial ASL of homozygous CFTR- ΔF508 (open symbols) and wild-type (closed symbols) pigs 2 hours after intravenous injection of NaSCN at the following doses (in mg/kg body weight): 0.25 (up triangle), 1 (circle), 2 (square), 4 (down triangle), and 8 (diamond). Each data point represents one animal. Dotted (CF) and solid (wild-type, WT) lines were generated by fitting the CF and non-CF data to the Michaelis-Menten equation. The two curves are significantly different (F-test, $*p=0.019$).

Fig. 3. The relationship between nasal ASL SCN⁻ concentration and FEV₁ in CF patients
 Each data point represents the ASL SCN⁻ concentration and FEV₁ of one subject. The Pearson's correlation test indicates association between the ASL SCN⁻ concentration and FEV₁ ($r=0.7457$, $***p<0.0001$).

Fig. 4. SCN⁻ concentration dependence of OSCN⁻ production and bacterial killing by airway epithelia

(A) OSCN⁻ production by primary cultures of human airway epithelia in the presence of indicated SCN⁻ concentrations, ATP (100 μ M), and LPO (7 μ g/ml) (n=3 for each data point). (B) Bacterial survival on the apical side of airway epithelia as a function of SCN⁻ concentrations. The apical surface of human airway epithelial cultures was inoculated with 3,000 CFU *S. aureus* in the absence (squares) or presence of apically added H₂O₂ scavengers (5 mM glutathione, circles; 1,000 U/mL catalase, triangles). The apical buffer of all epithelial cultures also contained the indicated SCN⁻ concentrations plus ATP (100 μ M) and LPO (7 μ g/ml); the number of surviving bacteria was evaluated after a 3-hour incubation using a quantitative bacterial culture method (n=5 for each data point).

Table 1

Study-subject information.

Characteristic	CF (n=23)	Non-CF (n=21)
Age in years (SD)	34.7 (9.7)	28.4 (7.1)
Age range in years	23–60	21–52
Gender ratio (M/F)	14/9	13/8
$\Delta F508$ homozygous	70%	0
$\Delta F508$ compound heterozygous	30%	0
Inpatients	48%	0
Outpatients	52%	0
Intravenous antibiotics	36%	0
Oral antibiotics	83%	0
Inhaled tobramycin and/or colistin	61%	0
No antibiotics	9%	100%
<i>P. aeruginosa</i> in sputum	74%	ND ^a

^a not determined