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Proton Secretion in Freshly Excised Sinonasal Mucosa from Asthma and Sinusitis Patients

Do-Yeon Cho, M.D.^{*,#}, Muhammad Hajighasemi[#], Peter H. Hwang, M.D.^{*}, Beate Illek, PhD.[#], and Horst Fischer, PhD.[#]

^{*}Division of Rhinology, Department of Otolaryngology – Head and Neck Surgery, Stanford University School of Medicine, Stanford, California 94305-5739

[#]Children's Hospital Oakland Research Institute (CHORI), 5700 Martin Luther King Jr. Way, Oakland, California 94609

Abstract

Introduction—One major function of the airways is to inactivate and remove pathogens from inhaled air and thereby prevent infection. The secretion of protons by the airway epithelium presents an integral part of the innate host defense mechanism and involves the proton channel (hydrogen voltage-gated channel 1, HVCN1). The purpose of this study was to measure the effect of airway inflammation on acid secretion in asthmatic and non-asthmatic patients with chronic rhinosinusitis by using freshly excised human sinonasal tissue.

Methods—Nasal or sinus mucosa from subjects with three different conditions (normal, chronic rhinosinusitis (CRS), CRS with asthma) was harvested during sinus surgery. The rate of proton (H^+) secretion by each tissue was measured using the pH-stat titration technique in an Ussing type chamber. The contribution of the HVCN1 proton channel to acid secretion was identified using $ZnCl_2$ as a blocker.

Results—Nasal epithelia isolated from subjects with a diagnosis of both chronic rhinosinusitis (CRS) and asthma had a mucosal equilibrium pH = 6.95 ($n = 5$), which was significantly lower than the equilibrium pH found in nasal epithelia from normal ($n = 5$, 7.35 ± 0.21) or from subjects with CRS without asthma ($n = 5$, 7.33 ± 0.15). When alkalinizing the mucosal pH to 8.0, nasal epithelia from CRS with asthma ($n = 5$) secreted H^+ at a rate of $135.1 \pm 46.2 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$. This rate was significantly higher compared to normal ($73.3 \pm 39.3 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, $n = 8$) or CRS without asthma ($51.4 \pm 27.7 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, $n = 7$). Mucosal addition of $ZnCl_2$ blocked H^+ secretion by 70% in normal, 52.6% in CRS without asthma, and by 50.8% in CRS with asthma.

Conclusion—Freshly excised human nasal and sinus epithelia secrete acid across the apical cell membrane. Sinonasal tissue isolated from asthmatic CRS patients showed lower mucosal pH values and higher rates of H^+ secretion than CRS and normal subjects, and ~50–70% of acid secretion was mediated by the HVCN1 H^+ channel in all groups. Excessive acid secretion might contribute to epithelial injury in CRS patients with asthma.

Corresponding Author Horst Fischer PhD, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, TEL: 510-450-7696, FAX: 510-450-7910, hfischer@chori.org.

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The study protocol has been approved by Stanford University and CHORI Institutional Review Board.

Keywords

proton secretion; pH stat; Ussing chamber; mucosa; asthma; sinusitis; airway surface liquid (ASL); acid

Introduction

Innate host defense mechanisms of the airways continuously protect against inhaled pathogens.¹ The mucosa of the airway surface epithelium is covered with a thin layer of fluid called airway surface liquid (ASL).² The composition of the ASL affects its physiological functions, the most important of which are removal of inhaled infectious particles and antimicrobial activity.³ It is believed that ASL is normally acidic relative to blood pH and studies of ASL pH have measured values in vivo of ~6.6 in the airways of normal humans using a bronchoscopically-directed pH electrode and ~7.1 in the surgically exposed trachea of normal mice using a pH-sensitive fluorescent dye.^{4, 5} These studies also suggest that the airway epithelium expresses cellular processes that are able to monitor and regulate ASL pH. ASL pH is altered by disease and airway inflammation, and acidification of the ASL is promoted by airway inflammation.^{4, 6} Previously it was shown that the airway epithelium secretes increased amounts of acid into the ASL during lung inflammation due to the activation of apical proton channels.² Excessive acidification of the luminal pH is predicted to have effects on the innate immune responses and likely suppresses microbe clearance from the respiratory tract by hindering the mucociliary escalator and by reducing phagocytic activity.⁷

Rhinosinusitis is one of the most common comorbidities in patients with asthma and the coexistence of these conditions has been recognized. In patients with asthma, inflammation in the nose and sinuses share features of disease of the distal airways. Although common inflammatory mediators are implicated in the upper and lower airway under the unified airway hypothesis, it has proven difficult to differentiate pathways that distinguish sinonasal inflammation in asthmatics from nonasthmatics.⁸ Hunt et al. reported that in asthma, the pH of condensed exhaled breath was markedly low, which suggested increased rates of acid secretion by the airway epithelium.⁹ However, little is known about nasal airway pH or acid secretion in chronic rhinosinusitis (CRS) patients. The purpose of this study was to measure the release of acid by freshly excised sinonasal epithelial tissue and determine whether acid secretion is altered in CRS patients with and without asthma.

Methods

Study Subjects

This study was approved by the institutional review boards at Stanford University and Children's Hospital Oakland Research Institute. Normal sinonasal mucosae were obtained intraoperatively from patients undergoing endoscopic sinus surgery (ESS) for pituitary tumor, benign sinonasal tumor, or lacrimal obstruction. Diseased sinonasal mucosa was also taken from medically refractory CRS patients with or without asthma during endoscopic sinus surgery (ESS). All patients fulfilled diagnostic criteria for CRS developed by the Task Force for Defining Adult Chronic Rhinosinusitis and endorsed by the American Academy of Otolaryngology, and had failed medical management.¹⁰ Tissues from asthmatics were from patients that had been diagnosed in accordance with the American Thoracic Society at least 12 months before surgery.¹¹ Specimens from nasal septum and turbinates were categorized as nasal tissue and those from uncinate process and ethmoid sinus were categorized as sinus tissue. In total, six groups of sinonasal specimens were collected: 1) normal nasal epithelia, 2) normal sinus epithelia, 3) nasal epithelia from CRS without asthma, 4) sinus epithelia

from CRS without asthma, 5) nasal epithelia from CRS with asthma, and 6) sinus epithelia from CRS with asthma.

Specimen Processing

After surgical removal, tissue specimens were stored immediately in ice cold LHC-9 media (Invitrogen, USA) and transferred to the laboratory for investigation. Proton secretion was measured using the pH stat titration method in an Ussing chamber (Physiologic Instruments Inc. San Diego, CA).¹² Figure 1 shows a schematic of the measuring setup. A thin layer of epithelial tissue was dissected from the surgical specimen and mounted on sliders with apertures of 0.040 to 0.71cm², and inserted between Ussing-type hemichambers. Tissues were bathed serosally with HEPES-buffered solution and mucosally with buffer-free solution (5 ml each). The bathing solutions were circulated by a gas-lift system at 37.°C. The buffer-free solution on the mucosal (luminal) side contained (in mM) 140 NaCl, 2 KCl, 15 glucose, 2 CaCl₂, and 1 MgCl₂ and was gassed with nitrogen to prevent air CO₂ from entering the solution. The HEPES-buffered solution on the serosal side contained (in mM) 140 NaCl, 2 KCl, 5 glucose, 10 HEPES, 2 CaCl₂, and 1 MgCl₂ and was gassed with oxygen. The pH of the mucosal solution was initially allowed to equilibrate for ~15 min and the final pH was recorded as the equilibrium pH. Then the mucosal pH was continuously titrated to a target pH of 8.0 by a pH stat titration apparatus (TitraLab 856, Radiometer Analytical SAS, Lyon, France) using 1 mM NaOH as a titrant. An alkaline mucosal pH was used to increase the driving force for protons and amplify the measured signal. From the amount of base added at constant pH, the acid secretion of the tissues was determined and is given in nmole·min⁻¹·cm⁻². At the end of the experiment, ZnCl₂ (10μM, known as a proton channel (hydrogen voltage-gated channel 1 (HVCN1)) blocker) was added to the mucosal side to block proton channels.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 15.0) and p<0.05 was considered significant. Two-way and one-way ANOVA, Mann-Whitney rank sum test, or Kruskal-Wallis one-way analysis of variance of ranks, followed by the Dunn multiple comparison tests were used as appropriate. Data are given as individual measurements or as mean ± SE.

Results

Equilibrium pH

Individual and average values of the equilibrium pH from sinus and nasal epithelia are plotted in Figure 2. Equilibrium pH values were 7.35 ± 0.21 for normal nasal epithelia (n = 5); pH 7.28 ± 0.21 for normal sinus epithelia (n = 2); pH 7.33 ± 0.15 for nasal epithelia from CRS without asthma (n = 5); pH 7.44 ± 0.10 for sinus epithelia from CRS without asthma (n = 6); pH 6.95 ± 0.34 for nasal epithelia from CRS with asthma (n = 5); and pH 7.33 ± 0.24 for sinus epithelia from CRS with asthma (n = 4) (Figure 2). The tissue types (nasal vs.sinus) or disease status (normal vs. CRS without asthma vs. CRS with asthma) did not show significant effects on measurements (two-way ANOVA, p = 0.181). However, within the nasal epithelial group, the equilibrium pH of nasal epithelia from CRS with asthma was significantly lower compared to nasal epithelia from normal and CRS without asthma (p = 0.037) (Figure 2). In the sinus epithelial group, there were no statistical differences among equilibrium pH values for the three different disease groups (normal, CRS without asthma, CRS with asthma).

Proton secretion across freshly excised human nasal epithelia

At a mucosal pH of 8.0, all tested epithelia acidified the mucosal medium (Figure 3). The rate of acid secretion was $73.3 \pm 39.3 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ in normal nasal epithelia ($n = 8$) and $51.4 \pm 27.7 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ in nasal epithelia from CRS without asthma ($n = 7$). Nasal epithelia from CRS with asthma ($n = 5$) secreted protons at a rate of $135.1 \pm 46.2 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$. This rate was significantly higher compared to normal and CRS without asthma ($p = 0.005$).

Proton Channel Blocker Experiment

We tested the effect of proton channel inhibitor on acid secretion across the freshly excised sinonasal epithelia. Exposure of the mucosal surface to ZnCl_2 ($10 \mu\text{M}$, a HVCN1 proton channel blocker) significantly reduced proton secretion across all nasal tissues (Figure 4). Mucosal addition of ZnCl_2 resulted in block of 71.0%, 52.6%, and 50.8%, respectively, of acid secretion in normal, CRS without asthma, and CRS with asthma, indicating a role of proton channels (HVCN1) in acid secretion by these tissues. The magnitude of ZnCl_2 block was higher in normal nasal epithelia compared to nasal epithelia from CRS with and without asthma ($p = 0.043$).

Discussion

To our knowledge this is the first study that measured acid secretion of freshly excised sinonasal human tissues. We report both the equilibrium pH and the rates of acid secretion in normal controls and CRS patients. Our main finding is that nasal (but not sinus) epithelia from CRS patients with asthma had both a significantly reduced mucosal equilibrium pH and an increased proton secretion compared to nasal tissues from normals or CRS patients. Both outcome measures are indications of an increase rate of acid release by the airway epithelium: the equilibrium pH is a measure for acid release in absence of an external pH gradient, while the measured rate of acid secretion at a mucosal pH of 8 is used to amplify the signal to more accurately measure the rates.

Airway epithelial cells express multiple mechanisms to secrete protons across the apical membrane into the ASL. For example, an ATP-driven apical mechanism has been identified that secretes H^+ against a pH gradient into an acidic ASL.^{13, 14} In addition, passive gradient-driven H^+ release governed by apical H^+ channels has been described in cultured human tracheal epithelia.² H^+ channels are considered as part of the airway NADPH oxidase defense system, where H channels function to release intracellular H^+ that is released by NADPH oxidases.¹⁵ Schwarzer et al. proposed a model of acid release in the mucosal membrane of airway surface epithelial cells (Figure 5).¹⁶ In this model the airway NADPH oxidase (Duox1 and Duox2) releases intracellular H^+ from NADPH and acidify intracellular pH, and intracellular protons exit across a Zn^{2+} -sensitive apical proton channel HVCN1.

Recently, Harper et al. demonstrated that the airway NADPH oxidase Duox1 and Duox2 were upregulated by the inflammatory cytokines IL-4, IL-13, and IFN- γ .¹⁷ In addition, airway acidification in asthmatics has been observed in exhaled breath condensate, whose pH was over two pH units lower than normal controls.⁹ Similarly, our data from freshly excised nasal tissue showed lower values of equilibrium pH and higher acid secretion in CRS patients with asthma. This observation suggests that it may be important to consider airway pH when interpreting the pathophysiology of asthmatic and allergic diseases in patients with CRS.⁹ Measurement of nasal pH may prove clinically useful as an outcome measure in CRS and may correlate with some of the differences in clinical severity notable among asthmatics with CRS. Further, we speculate that therapies directed at normalizing

nasal airway pH may help to prevent the cascade of events that leads to sinonasal diseases or exacerbation of airway inflammation.

The quantitative contribution of individual H^+ secretory mechanisms may be different under experimental circumstances. Schwarzer et al. noted that the H^+ channel contributed to 49% of total H^+ secretion as determined from blocker experiments in primary cultures of human tracheal epithelial cells.¹⁶ In our experiments using freshly excised human nasal epithelial tissues, 70.4% of total H^+ secretion was due to H^+ channels in normal nasal epithelia, 52.6% in CRS, and 50.8% in CRS with asthma. The rate of proton secretion in Zn^{2+} -exposed nasal epithelia from CRS with asthma was equal to the rate in normal nasal epithelia without the blocker. Based on these data, Zn^{2+} -sensitive H^+ channels (HVCN1) contribute more to the total H^+ secretion in normal nasal epithelia, compared to contribution in nasal epithelia from CRS with or without asthma suggesting that CRS and asthma up-regulate the contribution of H^+ secretory mechanisms other than H^+ channels.

Conclusions

Surgical specimens from freshly excised nasal mucosa secreted H^+ across the mucosal membrane into an alkaline ASL by a mechanism that was in part mediated by the Zn^{2+} -sensitive H^+ channel (HVCN1). Our data suggest that inflammatory processes in CRS with asthma may lead to enhanced H^+ secretion by the nasal airway epithelium and acidify the ASL. It is possible that ASL acidification is predicted to negatively impact the innate immune system and is a significant step during airway inflammation and infection in CRS with asthma.

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Figure 1.

Schematic drawing of pH stat titration method in an Ussing chamber. A thin layer of epithelial tissue, dissected from the surgical specimen, was bathed serosally with HEPES-buffered solution and mucosally with buffer-free solution (5 ml each). Solutions were gassed with oxygen (serosal) and nitrogen (mucosal). The pH of the mucosal solution was continuously measured, maintained, and recorded by a continuous pH stat titration apparatus (TitraLab 856, Radiometer Analytical SAS, Lyon, France).

Figure 2.

Distribution of equilibrium pH of freshly excised sinonasal epithelia. Individual equilibrium pH values are plotted and average values are shown as horizontal bars. Nasal epithelia from CRS with asthma had significantly lower equilibrium pH of 6.95, compared to nasal epithelia from normal and CRS without asthma ($p = 0.037$). Sinus epithelia had a tendency for more alkaline equilibrium pH values compared to nasal epithelia in both groups of CRS with and without asthma ($p > 0.05$). *: denotes a significant difference ($p < 0.05$). Solid line: mean. ●: Nasal epithelia. ○: Sinus epithelia.

Figure 3.

Proton secretion across freshly excised human nasal epithelia. Rates of proton secretions were plotted from individual experiments and average values are shown as horizontal bars. At a mucosal pH of 8.0, all tested epithelia acidified the mucosal medium. Nasal epithelia from CRS with asthma secreted protons at an average rate of 135.1 nmol·min⁻¹·cm⁻². This rate was significantly higher compared to normal and CRS without asthma ($p = 0.005$). Nasal epithelia from CRS without asthma secreted less protons than normal controls ($p > 0.05$). Solid line: mean. *: denotes a significant difference ($p < 0.05$).

Figure 4.

Inhibition of proton secretion by using the proton channel inhibitor ZnCl_2 . Rates of proton secretions were plotted from individual experiments and average values are shown as horizontal bars. Mucosal addition of ZnCl_2 blocked 71.0% (normal), 52.6%, (CRS without asthma) and 50.8% (CRS with asthma) indicating a role of mucosal Zn^{2+} -sensitive proton channels (HVCN1) in proton secretion by these tissues. Magnitude of ZnCl_2 sensitive proton secretion was higher in normal nasal epithelia, compared to nasal epithelia from CRS with and without asthma ($p < 0.05$). Solid line: mean.

Figure 5.

Model of acid release in the mucosal membrane of airway surface epithelial cells. In this model, a Duox-based NADPH oxidase releases intracellular H^+ from NADPH and acidifies intracellular pH. Intracellular protons exit across a Zn^{2+} -sensitive proton channel (HVCN1). In addition, airway cells release acid by way of a bafilomycin-sensitive H^+ ATPase and an ouabain-sensitive K^+/H^+ ATPase. The source of H^+ for the H^+ channel is NADPH, whereas the ATPases release H^+ from water resulting in an alkalization of intracellular pH in this model.