ORIGINAL ARTICLE

The effect of nasal irrigation formulation on the antimicrobial activity of nasal secretions

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Background: Saline-based irrigation solutions are evidence-based rhinological treatments; however, the formulation of these solutions could theoretically alter the function of innate antimicrobial peptides. The aim of this study was to determine if the antimicrobial activity of normal human nasal secretions in vivo is altered by commercially available large volume irrigation solutions.

Methods: Minimally manipulated sinonasal secretions were collected from patients with chronic rhinosinusitis (CRS; n = 10) and normal healthy volunteers (n = 20). In a subset of control patients (n = 10) secretions were collected prior to, and at 1 hour, 6 hours, and 24 hours after nasal irrigation with 4 commercial irrigation solutions. Lysozyme and lactoferrin levels were analyzed and the antimicrobial activity of secretions determined using a radial diffusion assay.

Results: The antimicrobial activity of nasal secretions was reduced in CRS patients compared to healthy volunteers ($p < 0.01$), but there was no significant difference in antimicrobial peptide concentrations. Isotonic nasal irrigation reduced lysozyme and lactoferrin levels, which returned to baseline levels by 6 hours; in addition to a sustained decrease in antimicrobial activity before returning to baseline at 24 hours. Low-salt solution stimulated peptide secretion by approximately 40% at 6 hours and 24 hours, but produced a transient decrease in antimicrobial activity, returning to baseline levels by 6 hours. Hypertonic solution initially decreased lysozyme and lactoferrin levels but maintained baseline levels of antimicrobial activity and increased peptide secretion by approximately 30% at 24 hours.

Conclusion: The formulation of nasal irrigation solutions significantly affects the measured levels and functionality of sinonasal antimicrobial peptides. © 2015 ARS-AAOA, LLC.

Key Words:
sinusitis; nasal lavage; antimicrobial cationic peptides; innate immunity; mucus; nasal mucosa; nasal spray

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C hronic rhinosinusitis (CRS) causes a significant reduction in patients' quality of life, and costs billions of healthcare dollars each year.[1,2] The etiology of CRS is incompletely understood, with current hypotheses including bacterial and/or fungal infection, biofilms, anatomical obstruction of sinus drainage, and dysregulated mucosal immunity.[3–6]

Nasal secretions contain cationic antimicrobial peptides such as lysozyme, lactoferrin, human β-defensin, and secretory leukocyte protease inhibitor.[7–9] These proteins are an important component of innate immunity against inhaled antigens and microorganisms with documented antimicrobial activity toward bacteria, fungi, and viruses. Lysozyme is the most abundant secreted innate immune defense protein from the paranasal sinuses[10]; it works both as an antibacterial agent via its enzymatic muramidase activity[11] and as a cationic protein.[12–14] Our previous work has demonstrated that lysozyme exhibits fungicidal activity,[15] supporting a role for lysozyme in paranasal sinus innate

immunity against both bacteria and fungi. Subsequent immunohistochemical studies found increased lysozyme protein present in the sinus mucosa of CRS patients,[16] suggesting that decreased functional activity rather then decreased protein levels may be an important factor in CRS pathophysiology.

The tonicity (osmotic activity) of airway surface liquid (ASL) is difficult to quantify,[17] but is generally assumed to be similar to plasma and extracellular fluid (ie, isotonic). However, there is some evidence to suggest that under normal conditions ASL is hypotonic[18,19] compared to plasma, with a pH of 5.5 to 6.5.[20–22] In contrast, in patients with airway inflammation, infection, or cystic fibrosis the ionic composition of ASL was found to approach or reach isotonicity,[18] with a pH of 7.2 to 8.3,[22] suggesting that changes to ion and water secretion occur during an inflammatory state.

There is also evidence in the literature that the local ionic state can affect the innate immune system; studies from cystic fibrosis patients with high ASL chloride concentration showed a lack of antimicrobial activity despite the presence of key antimicrobial peptides.[23] Increases in sodium chloride concentration are known to influence the antimicrobial activity of cationic antimicrobial peptides,[23–27] likely due to ions present in solution having an electrical charge, which can interact with both the cationic antimicrobial peptides and the anionic microbial cell wall.

Commercial irrigation solutions are generally formulated as either isotonic (154 mM) or hypertonic saline solutions. The ionic strength of these solutions has the potential to inhibit the antimicrobial activity of innate cationic antimicrobial peptides present in sinonasal secretions. A low-salt nasal irrigation formulation was recently released in Australia to reduce this potential effect.

There is now level 1 evidence that different large-volume nasal irrigation formulations can affect clinical outcomes. In a randomized controlled trial performed in our unit, postoperative irrigation with Ringer's lactate provided superior patient outcomes to normal or hypertonic saline.[28] This justifies further research looking at clinical outcomes for patients with CRS in both the preoperative and postoperative phase, following different irrigation frequency regimes with variable salt concentrations.

This study was designed to determine: (1) the antimicrobial activity of nasal secretions from CRS patients compared to normal healthy people; and (2) if the antimicrobial activity of normal nasal secretions was altered after nasal irrigation with 4 commercially available irrigation formulations (isotonic saline, hypertonic saline, lactated Ringer's, and "low salt" formulation).

Subjects and methods

This project was reviewed and approved by the Southern Adelaide Clinical Human Research Ethics Committee: Project ID 214.12.

Minimally manipulated sinonasal secretions

Sinonasal secretions were collected from normal healthy volunteers (n = 20) and patients diagnosed with CRS (no previous surgery, both with and without nasal polyps; n = 10) by European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) criteria.[2] Volunteers had no history of nasal disease and were recruited from hospital research advertisements, with 22-item Sino-Nasal Outcome Test (SNOT-22) scores of <8 and normal nasendoscopy. CRS participants were excluded if they had used antibiotics or topical steroids in the preceding 4 weeks. A commercial sinus secretion sponge[29] (polyurethane foam measuring 5 mm × 25 mm) was placed between the inferior turbinate and nasal septum for 10 minutes.

To investigate the effect of irrigation on innate peptide concentration and antimicrobial activity, secretions were collected from 10 of the healthy volunteers (selected from the initial 20 due to their time-availability for the second phase of the study; 6 females:4 males; mean age 37.5 years; range, 19.9 to 64.4 years). SNOT-22 mean = 2.3). Secretions were collected before (0 hours) and after (1 hour, 6 hours, and 24 hours) irrigation with a commercially available large volume solution (isotonic saline: 0.9% Na; hypertonic saline: 2.7% Na; lactated Ringer's: 0.9% Na; and a "low salt" formulation: isotonic but 0.0375% Na). All 4 irrigation solutions were provided in a randomized order to each participant, with a minimum of 48 hours between irrigations.

All secretions were kept on ice and stored at −20°C for later use. Secretions were extracted from the sponge by centrifugation and sonicated to disrupt mucoprotein aggregates and facilitate accurate handling. Secretions were irradiated at 200 Gy (20k rad; Adelaide Radiotherapy Centre; Siemens ONCOR; Siemens Australia, Bayswater, Victoria, Australia) to kill endogenous bacteria that would compromise the antimicrobial assay. The sonication and irradiation processes used had been shown not to effect antimicrobial peptide activity.[30]

Antimicrobial activity assay

A highly sensitive microbial assay was used to determine antimicrobial activity of sinonasal secretions. This assay, designed specifically for assessing cationic antimicrobial peptides,[31,32] utilizes a double layer technique that optimizes the composition of the underlay to support antimicrobial activity by keeping the ionic strength and divalent cation content low, with a conventional overlay to support microbial growth. The underlay gel contained metabolically-active microbes in 1% agarose and 0.03% trypticase soy broth (TSB) in 10 mM sodium phosphate (pH 7.4). A series of wells was punched out of the solid agar and 5 μL of purified peptide (0 to 100 μM recombinant human lysozyme, L1667; Sigma-Aldrich, St. Louis, MO) or nasal secretions was applied. Plates were incubated at 37°C for 3 hours to allow peptide diffusion before being covered with an overlay gel containing 6% TSB and

1% agarose. Antimicrobial activity was identified as a zone around the well absent of microbial growth after 18 to 24 hours of incubation. The surface of the overlay gel was washed with 10 mL disinfecting solution (5% acetic acid in 25% methanol) to remove any surface bacterial colonies.

The assay was used with *Esherichia coli* as the test organism (ATCC 25922; strain commonly utilized in antibiotic resistance tests). For quality control and normalization between plates a 5-μL aliquot of antibiotic was added to a well (*E. coli*: 2 μg/mL Ciprofloxacin).

The dose characteristics of this assay allow it to be used quantitatively.[32] However, traditional analysis of this assay by measuring the zone of clearance (defined as a zone around the sample well devoid of bacterial colonies) and presenting it as clearance units [(zone mm − well mm) × 10 Units] was not sensitive enough for our purposes. Although the pure antimicrobial peptides produced clear zones of inhibition, many of the nasal secretion samples demonstrated a zone of reduced growth surrounded by a "halo" of increased growth, with growth within the zone being variable from no colonies to a density consistent with the bacterial lawn. Therefore plates were photographed and electronic images were analyzed using ImageJ (v1.46r; NIH, Bethesda, MD; http://imagej.nih.gov/ij). Briefly, each plate image was converted to an 8-bit black and white image, black and white colors were inverted (ie, the absence of growth was represented by white), and then the image was scaled appropriately to represent the 90-mm plate. The area selected for analysis was defined as the area within the inner rim of the "halo" and the integrated density (IntDen) was calculated to provide a measure of both the size and density of growth within the traditional "zone of clearance." This value was adjusted for the blank well space, and represented as a percentage of the antibiotic control to normalize between plates using the formula: (IntDen SNOT − IntDen WELL)/(IntDen ANTIBIOTIC − IntDen Antibiotic WELL) × 100%. Therefore, a higher percentage correlates with more microbial killing by the secretions in the sample.

Concentration of lysozyme and lactoferrin in secretions

Enzyme-linked immunosorbent assay (ELISA) was used to quantify the concentrations of key antimicrobial peptides within nasal secretion samples (human lysozyme = EL3010-1; human lactoferrin = EL2011-1; AssayMax ELISA kit; Assaypro, St. Charles, MO).

Statistical analysis

To provide an estimation of antimicrobial peptide concentration in nasal secretions the levels of lysozyme and lactoferrin were combined for each time point. Antimicrobial activity and lysozyme+lactoferrin levels were calculated as a percentage of baseline levels using the formula: $T_i/T_0 \times 100\%$ where $i = 1, 6,$ and 24 hours.

A Wilcoxon-Mann-Whitney test was used to detect a significant difference between CRS and healthy volunteer
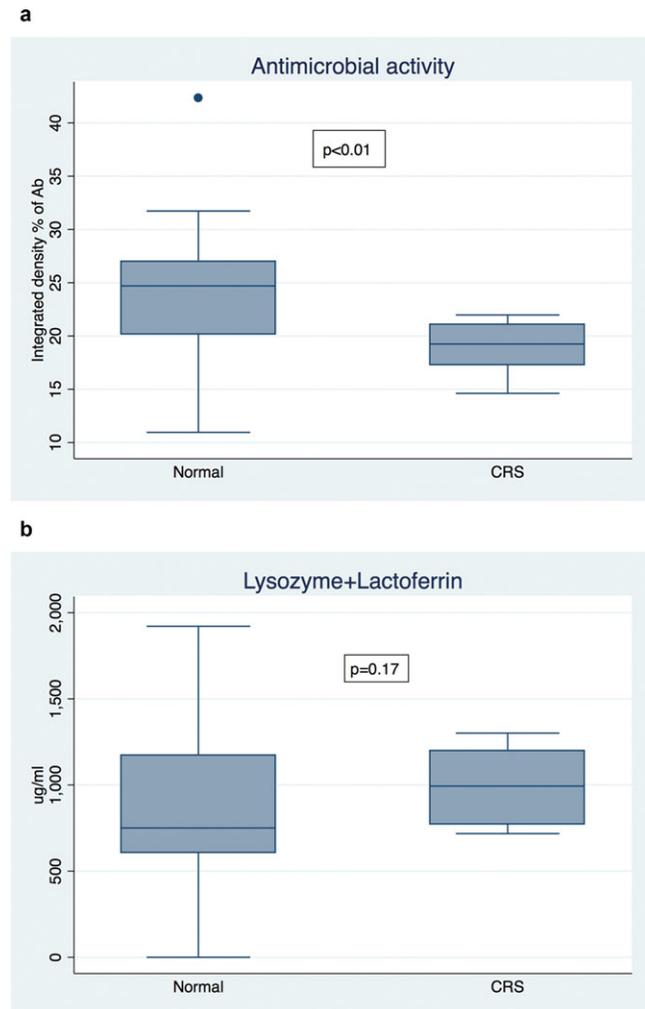

FIGURE 1. Box plot of antimicrobial activity (A) and lysozyme+lactoferrin (B) levels in nasal secretions between normal (n = 20) and CRS groups (n = 10). Ab = antibiotic; CRS = chronic rhinosinusitis.

groups for antimicrobial activity and lysozyme+lactoferrin levels. A mixed-effect linear regression model was applied in STATA version 13.0 (StataCorp., College Station, TX) to determine the antimicrobial activity of nasal secretions prior to, and 1, 6, and 24 hours following nasal irrigation with 4 commercial irrigation solutions. A maximum-likelihood estimation procedure was used to compare the significant differences over time and between solutions. The model was used to determine the irrigation effects (adjusted mean change of 4 solutions at each time point) and interaction effects (overall effects on the 4 solutions after 1 hour, 6 hours, and 24 hours). All analysis was performed with 2-tailed tests and the level of significance was set at $p < 0.05$. Where appropriate, 95% confidence intervals (95% CIs) were reported along with p values.

Results

Antimicrobial activity was significantly lower in secretions from CRS patients compared to normal controls ($p < 0.01$;
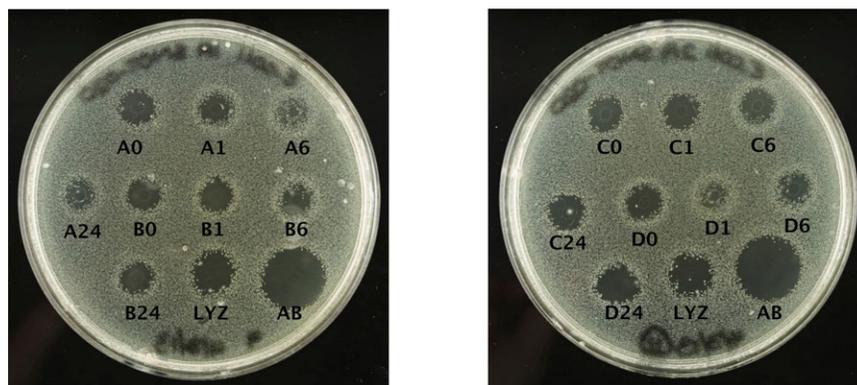
FIGURE 2. Radial diffusion assay demonstrating effects of irrigation solution on the antimicrobial activity of nasal secretions in 1 "normal" individual. Sinonasal secretions were collected before (0 hours) and after (1 hour, 6 hours, 24 hours) irrigation with 4 different formulations (A = isotonic saline formulation; B = hypertonic saline formulation; C = lactated Ringer's formulation; D = low salt formulation) from 1 individual and the antimicrobial activity was determined. Zones of inhibition are apparent. A "halo" of increased growth can be observed associated with some samples. The antibiotic (Ab) was utilized for normalization between plates and repeated assays, and lysozyme (LYZ) was utilized as an internal positive control. Ab = antibiotic; LYZ = lysozyme.
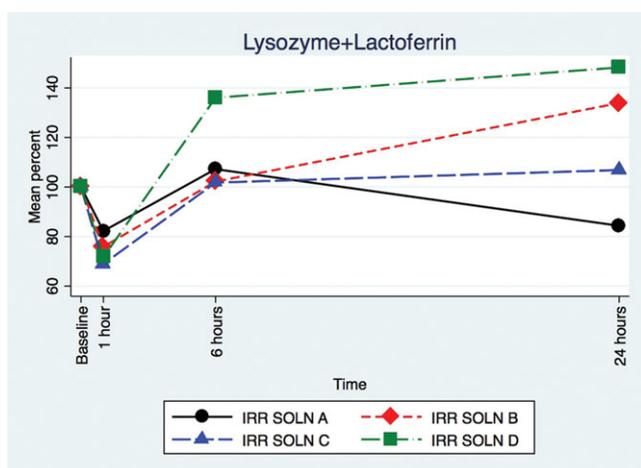

FIGURE 3. Change in antimicrobial peptide concentration in nasal secretions following nasal irrigation with 4 different irrigation formulations. Nasal secretions were collected prior to and at 1 hour, 6 hours, and 24 hours after nasal irrigation in normal participants (n = 10 for each solution). Data was normalized to percentage of baseline level for each participant. IRR = irrigation; SOLN = solution; SOLN A = isotonic saline; SOLN B = hypertonic saline; SOLN C = lactated Ringer's; SOLN D = low salt.

FIGURE 4. Change in antimicrobial activity of nasal secretions following nasal irrigation with 4 different irrigation formulations. Nasal secretions were collected prior to and at 1 hour, 6 hours, and 24 hours after nasal irrigation in normal participants (n = 10 for each solution). Data was normalized to percentage of baseline level for each participant. IRR = irrigation; SOLN = solution; SOLN A = isotonic saline; SOLN B = hypertonic saline; SOLN C = lactated Ringer's; SOLN D = low salt.
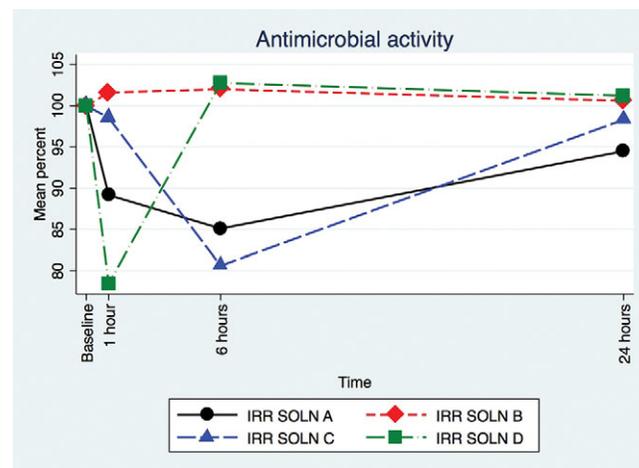
Fig. 1A). Higher median levels of lysozyme+lactoferrin were detected in the CRS group compared to the normal group, but this did not reach statistical significance ($p = 0.17$; Fig. 1B).

The effect of nasal irrigation formulation on cationic antimicrobial peptide concentration and antimicrobial activity was investigated in normal volunteers. The effect of irrigation over a 24-hour time period on these parameters is depicted in Figures 2, 3, and 4. All irrigation solutions caused an initial reduction in lysozyme+lactoferrin concentrations. For the isotonic solutions (saline and lactated Ringer's) this returned to baseline levels by 6 hours. The low-salt solution stimulated peptide secretion by approximately 40% at 6 hours and 24 hours with the hypertonic solution stimulating peptide secretion by approximately 30% at 24 hours.

Very different profiles were observed for antimicrobial activity. Low-salt solution produced a transient decrease in antimicrobial activity, returning to baseline levels by 6 hours. Isotonic solutions resulted in a sustained decrease in antimicrobial activity approaching baseline levels by 24 hours, despite lysozyme+lactoferrin levels normalizing at 6 hours. The hypertonic formulation maintained baseline levels of antimicrobial activity, despite the initial decrease in lysozyme+lactoferrin levels.

Statistical modeling and predictions of antimicrobial activity and peptide concentrations following nasal irrigation

The observed antimicrobial activity and peptide concentrations were entered into a mixed effects linear regression

TABLE 1. A mixed effects linear regression model predicting time effects for antimicrobial activity and cationic peptide concentration across the 4 irrigation solutions*

Test	Time (hours)	Solution A	Solution B	Solution C	Solution D
Antimicrobial activity	1	−10.8 (−28.9 to 7.3)	1.6 (−17.6 to 20.8)	−1.1 (−18.8 to 16.6)	−21.6 (−42.1 to 1.0)†
	6	−14.9 (−33.0 to 3.2)	3.1 (−16.8 to 23.1)	−20.4 (−38.6 to −2.0)†	2.8 (−17.8 to 23.3)
	24	−5.5 (−23.6 to 12.6)	0.6 (−18.6 to 19.8)	−1.7 (−18.9 to 15.5)	1.2 (−19.3 to 21.7)
Lysozyme+lactoferrin	1	−15.8 (−68.5 to 36.9)	−24.7 (−74.3 to 24.9)	−31.1 (−73.9 to 11.8)	−28.3 (−79.5 to 22.9)
	6	8.2 (−41.4 to 57.9)	2.8 (−46.8 to 52.4)	0.9 (−44.6 to 46.4)	36.0 (−15.2 to 87.3)
	24	−14.6 (−64.3 to 35.0)	33.9 (−14.0 to 81.8)	6.8 (−36.0 to 49.7)	48.3 (0.01 to 99.5)†

*Data are presented as percent change from baseline activity. Marginal mean change (95% CI); changes at 1, 6, and 24 hours from baseline for each group.
†$p < 0.05$.
CI = confidence interval; Solution A = isotonic saline; Solution B = hypertonic saline; Solution C = lactated Ringer's; Solution D = low salt.

model to predict differences between irrigation formulations over time (Table 1). This model predicts an approximate 25% decrease in antimicrobial peptide levels immediately following irrigation, which return to near baseline levels by 6 hours. A sustained significant increase in peptide concentration of approximately 40% is predicted 6 hours and 24 hours after irrigation with the low-salt solution. With the exception of the hypertonic solution, antimicrobial activity is predicted to decrease by 15% to 20% following irrigation. Antimicrobial activity is predicted to return to baseline levels within 6 hours after irrigation with low-salt solution, and by 24 hours with isotonic saline or lactated Ringer's solutions. The hypertonic formulation is predicted to maintain antimicrobial activity following irrigation, despite the predicted decrease in antimicrobial peptide concentration at 1 hour.

Discussion

This study evaluated the combined concentration of antimicrobial peptides lysozyme and lactoferrin, as well as the functional antimicrobial activity present in sinonasal secretions of CRS patients when compared to normal healthy individuals. Antimicrobial activity was significantly diminished in CRS patients compared to normal controls; however, comparable levels of the antimicrobial peptides were still present. Our findings suggest that in CRS, it is the functionality of these innate peptides that is reduced. Measuring the tonicity of nasal secretions is very complex and was not attempted in this study; however, we hypothesize that a small increase in the tonicity of ASL (perhaps increased NaCl concentration as a result of "plasma" transfer through a diseased, damaged, and ineffective epithelial barrier) may be inhibiting the functional activity of cationic antimicrobial peptides, thereby leading to impaired innate defense of the sinonasal epithelium and contributing to the development of CRS. Further work will be required to advance this hypothesis.

In rhinological research, nasal secretions are most commonly collected via nasal lavage[33]; however, this process would have altered the fluid composition and was therefore not appropriate for this type of research. Other commonly used methods of obtaining minimally manipulated nasal secretions include filter paper, sinus pack, microsuction, nose blow, or polyurethane foam sponge.[33] For this study it was essential to identify a method by which enough nasal fluid could be obtained for the assays without causing trauma to the mucosa or activation of nasal reflexes that stimulate secretion.

The results of this study also demonstrate that both the use and the formulation of irrigation solutions impacts on the normal function of the innate immune sinonasal defenses. The use of large-volume nasal irrigation solutions is evidence-based and recommended for the removal of thickened nasal secretions in patients with CRS.[34,35] There is also evidence for their efficacy in allergic rhinitis, the common cold, and other upper respiratory tract infections.[36] In the postsurgical scenario, douching provides symptomatic relief through the physical action of removing thick eosinophilic mucin, crusts, and postoperative blood clots.[34]

However, a potential negative effect of sinonasal irrigation is the reduction/removal of normal nasal secretions from the nasal cavity (and sinonasal cavity in the postsurgical patient).[28] It has been previously estimated that the cationic antimicrobial peptides start to reconstitute themselves within 10 to 20 minutes, but that it may require 4 to 24 hours before they return to normal preirrigation ASL concentrations.[37] This study has clearly demonstrated that, following irrigation, cationic antimicrobial peptides are reconstituted more quickly than previously thought, with a return to baseline levels at 1 to 6 hours. Nevertheless, during this postirrigation period, the innate immune defenses of the nasal epithelium remain potentially compromised. Patients are often instructed to irrigate repeatedly during the day, potentially leaving the epithelium with decreased levels of antimicrobial peptides for a prolonged period, providing favorable conditions for microbial colonization of the sinonasal epithelium, especially in patients with already impaired mucociliary clearance pathways (ie, CRS).

Of particular interest, the low-salt formulation (which was well tolerated by the patients in this study) stimulated a 40% increase in cationic peptide secretion between 1 and 6 hours postirrigation. This was maintained at 24 hours, suggesting a potential benefit to innate nasal defenses with this irrigation solution.

In addition to the concentration of cationic antimicrobial peptides in sinonasal secretions, it is important to consider the impact of irrigation formulation on the functional activity of these peptides. It has been well established that cationic antimicrobial peptides are salt-sensitive[23–27] and there is potential that the sodium in any residual irrigation solution could affect functionality of these peptides. The radial diffusion assay utilized in this study was chosen because it uses minimal amounts of sample and is highly sensitive to determine the antimicrobial activity of cationic antimicrobial peptides.[31,32] Nevertheless, the assay only measures activity against 1 microorganism and it is entirely possible that different results would be obtained for other bacteria or fungi.

Despite the levels of lysozyme and lactoferrin returning to baseline levels by 1 to 6 hours, antimicrobial activity remained impaired by 15% to 20% at 6 hours after irrigation with isotonic saline or lactated Ringer's solution, demonstrating an ongoing negative effect of irrigation on the functionality of antimicrobial peptides secreted into the sinonasal secretions. In contrast, the low-salt formulation resulted in a transient decrease in antimicrobial activity that returned to baseline levels within 1 to 6 hours. An additional advantage of the low-salt irrigation formulation may be an increase in mucociliary function, given that it has been demonstrated that extracellular sodium blocks ciliary activity in the airway.[38]

Of particular note, no decrease in antimicrobial activity was observed following irrigation with hypertonic saline, despite a 25% decrease in peptide concentration. Many patients report tingling or pain after irrigation with hypertonic solutions, believed to be a result of activation of nociceptive nerves stimulating substance P release into secretions.[39] Serosal substance P has also been reported to stimulate submucosal gland secretion, which would theoretically increase secretion volume (ie, water and bicarbonate) as well as antimicrobial peptides.[40] We observed a 25% decrease in lysozyme and lactoferrin concentrations during the immediate postirrigation period; this could have been simply due to an excess of water and bicarbonate over peptide stimulation. It should also be considered that substance P and other neuropeptides are also known to have both direct and indirect antimicrobial properties themselves,[41,42] which could explain the sustained antimicrobial activity observed following hypertonic saline irrigation. The antimicrobial action of these neuropeptides would be separate from the lysozyme/lactoferrin pathways and their concentrations were not measured in this study. Other possible explanations of the observed hypertonic effect could include: (1) The high concentration of NaCl itself inhibits microbial growth (NaCl is a known preservative in the food industry). Pilot assay work for this study used increased NaCl concentrations in the media and demonstrated inhibition of *E. coli* growth, such that no bacterial lawn could be established at a concentration of NaCl similar to commercially-available hypertonic saline (unpublished data). (2) Residual hypertonic saline may liberate bound cathelicidin LL-37 from glycosaminoglycan, providing an additional source of active antimicrobial peptide in secretions (previously demonstrated in patients with cystic fibrosis[43]).

The strengths of this study are as follows: (1) the use of an established assay designed to measure the effect of antimicrobial peptides; and (2) the in vivo collection method after irrigation with commercially available solutions. The weaknesses include: (1) activity was only assayed against 1 bacterial strain; (2) the in vivo irrigation trial was only performed on normal controls and not patients with CRS; (3) only the 2 most prevalent innate antimicrobial peptides were measured; and (4) there may be additional unidentified variables in our ex vivo assay.

Conclusion

This study identified impaired antimicrobial activity of sinonasal secretions in patients with CRS, and that formulation of irrigation solutions affects the antimicrobial activity of sinonasal secretions in healthy individuals. Low-salt and hypertonic formulations would appear to provide benefits for antimicrobial activity following irrigation, perhaps via different salt-dependant and salt-independent physiological mechanisms, respectively. 🕸

References

1. Anand VK. Epidemiology and economic impact of rhinosinusitis. *Ann Otol Rhinol Laryngol Suppl.* 2004;193:3–5.

2. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl.* 2012;(23):3 p preceding table of contents, 1-298.

3. Tan BK, Schleimer RP, Kern RC. Perspectives on the etiology of chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg.* 2010;18:21–26.

4. Timperley D, Schlosser RJ, Harvey RJ. Chronic rhinosinusitis: an education and treatment model. *Otolaryngol Head Neck Surg.* 2010;143:S3–S8.

5. Hsu J, Peters AT. Pathophysiology of chronic rhinosinusitis with nasal polyp. *Am J Rhinol Allergy.* 2011;25:285–290.

6. Reh DD, Wang Y, Ramanathan M Jr, et al. Treatment-recalcitrant chronic rhinosinusitis with polyps is associated with altered epithelial cell expression of interleukin-33. *Am J Rhinol Allergy.* 2010;24:105–109.

7. Ramanathan M Jr, Lane AP. Innate immunity of the sinonasal cavity and its role in chronic rhinosinusitis. *Otolaryngol Head Neck Surg.* 2007;136:348–356.

8. Ooi E, Psaltis A, Witterick I, et al. Innate immunity. *Otolaryngol Clin North Am.* 2010;43:473–487,vii.

9. Ooi EH, Wormald P-J, Tan LW. Innate immunity in the paranasal sinuses: a review of nasal host defenses. *Am J Rhinol.* 2008;22:13–19.

10. Cole AM, Liao HI, Stuchlik O, et al. Cationic polypeptides are required for antibacterial activity of human airway fluid. *J Immunol.* 2002;169:6985–6991.

11. Kirby AJ. The lysozyme mechanism sorted—after 50 years. *Nat Struct Biol.* 2001;8:737–739.

12. Ibrahim HR, Matsuzaki T, Aoki T. Genetic evidence that antibacterial activity of lysozyme is independent of its catalytic function. *FEBS Lett.* 2001;506:27–32.

13. Laible NJ, Germaine GR. Bactericidal activity of human lysozyme, muramidase-inactive lysozyme, and cationic polypeptides against *Streptococcus sanguis* and *Streptococcus faecalis*: inhibition by chitin oligosaccharides. *Infect Immun.* 1985;48:720–728.

14. Masschalck B, Deckers D, Michiels CW. Lytic and nonlytic mechanism of inactivation of Gram-positive bacteria by lysozyme under atmospheric and high hydrostatic pressure. *J Food Prot.* 2002;65:1916–1923.

15. Woods CM, Hooper DN, Ooi EH, et al. Human lysozyme has fungicidal activity against nasal fungi. *Am J Rhinol Allergy.* 2011;25:236–240.

16. Woods CM, Lee VS, Hussey DJ, et al. Lysozyme expression is increased in the sinus mucosa of patients with chronic rhinosinusitis. *Rhinology*. 2012;50:147–156.

17. Verkman AS, Song Y, Thiagarajah JR. Role of airway surface liquid and submucosal glands in cystic fibrosis lung disease. *Am J Physiol Cell Physiol*. 2003;284:C2–C15.

18. Joris L, Dab I, Quinton PM. Elemental composition of human airway surface fluid in healthy and diseased airways. *Am Rev Respir Dis*. 1993;148:1633–1637.

19. Govindaraju K, Cowley EA, Eidelman DH, et al. Microanalysis of lung airway surface fluid by capillary electrophoresis with conductivity detection. *Anal Chem*. 1997;69:2793–2797.

20. Washington N, Steele RJ, Jackson SJ, et al. Determination of baseline human nasal pH and the effect of intranasally administered buffers. *Int J Pharm*. 2000;198:139–146.

21. England RJ, Homer JJ, Knight LC, et al. Nasal pH measurement: a reliable and repeatable parameter. *Clin Otolaryngol Allied Sci*. 1999;24:67–68.

22. Fabricant ND. Significance of the pH of nasal secretions in situ. *Arch Otolaryngol*. 1941;33:13.

23. Smith JJ, Travis SM, Greenberg EP, et al. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell*. 1996;85:229–236.

24. Singh PK, Tack BF, McCray PB Jr, et al. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:L799–L805.

25. Travis SM, Conway BA, Zabner J, et al. Activity of abundant antimicrobials of the human airway. *Am J Respir Cell Mol Biol*. 1999;20:872–879.

26. Tomee JF, Hiemstra PS, Heinzel-Wieland R, et al. Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. *J Infect Dis*. 1997;176:740–747.

27. Woods CM, Hooper DN, Ooi EH, et al. Fungicidal activity of lysozyme is inhibited in vitro by commercial sinus irrigation solutions. *Am J Rhinol Allergy*. 2012;26:298–301.

28. Low TH, Woods CM, Ullah S, et al. A double-blind randomized controlled trial of normal saline, lactated Ringer's, and hypertonic saline nasal irrigation solution after endoscopic sinus surgery. *Am J Rhinol Allergy*. 2014;28:225–231.

29. Lu FX, Esch RE. Novel nasal secretion collection method for the analysis of allergen specific antibodies and inflammatory biomarkers. *J Immunol Methods*. 2010;356:6–17.

30. Cole AM, Dewan P, Ganz T. Innate antimicrobial activity of nasal secretions. *Infect Immun*. 1999;67:3267–3275.

31. Lehrer RI, Rosenman M, Harwig SS, et al. Ultrasensitive assays for endogenous antimicrobial polypeptides. *J Immunol Methods*. 1991;137:167–173.

32. Steinberg DA, Lehrer RI. Designer assays for antimicrobial peptides. Disputing the "one-size-fits-all" theory. *Methods Mol Biol*. 1997;78:169–186.

33. Scadding G, Hellings P, Alobid I, et al. Diagnostic tools in Rhinology EAACI position paper. *Clin Transl Allergy*. 2011;1:2.

34. Harvey R, Hannan SA, Badia L, et al. Nasal saline irrigations for the symptoms of chronic rhinosinusitis. *Cochrane Database Syst Rev*. 2007;(3):CD006394.

35. Achilles N, Mosges R. Nasal saline irrigations for the symptoms of acute and chronic rhinosinusitis. *Curr Allergy Asthma Rep*. 2013;13:229–235.

36. Kassel JC, King D, Spurling GK. Saline nasal irrigation for acute upper respiratory tract infections. *Cochrane Database Syst Rev*. 2010;(3):CD006821.

37. Kaliner MA. Human nasal host defense and sinusitis. *J Allergy Clin Immunol*. 1992;90:424–430.

38. Ma W, Korngreen A, Uzlaner N, et al. Extracellular sodium regulates airway ciliary motility by inhibiting a P2X receptor. *Nature*. 1999;400:894–897.

39. Baraniuk JN, Ali M, Yuta A, et al. Hypertonic saline nasal provocation stimulates nociceptive nerves, substance P release, and glandular mucous exocytosis in normal humans. *Am J Respir Crit Care Med*. 1999;160:655–662.

40. Choi JY, Khansaheb M, Joo NS, et al. Substance P stimulates human airway submucosal gland secretion mainly via a CFTR-dependent process. *J Clin Invest*. 2009;119:1189–1200.

41. Kowalska K, Carr DB, Lipkowski AW. Direct antimicrobial properties of substance P. *Life Sci*. 2002;71:747–750.

42. Augustyniak D, Nowak J, Lundy FT. Direct and indirect antimicrobial activities of neuropeptides and their therapeutic potential. *Curr Protein Pept Sci*. 2012;13:723–738.

43. Bergsson G, Reeves EP, McNally P, et al. LL-37 complexation with glycosaminoglycans in cystic fibrosis lungs inhibits antimicrobial activity, which can be restored by hypertonic saline. *J Immunol*. 2009;183:543–551.