

Substance P and Hemokinin I in Nasal Lavage Fluid of Patients with Chronic Sinusitis and Nasal Polyposis

Ashley Lonergan^{1,2}, Theoharis Theoharides, MD, PhD³,
Eirini Tsilioni, PhD³, and Elie Rebeiz, MD²

No sponsorships or competing interests have been disclosed for this article.

Abstract

This pilot study was undertaken to isolate and quantify substance P (SP) and hemokinin I (HK-I) in the nasal lavage fluid of patients with chronic rhinosinusitis with nasal polyps to better elucidate the pathophysiology underlying this inflammatory process, which remains poorly understood. Mucus samples were collected from this introductory cohort of 10 patients diagnosed with chronic rhinosinusitis with nasal polyps at Tufts Medical Center (Boston, Massachusetts). Relative levels of SP and HK-I were measured with enzyme-linked immunosorbent assay methods. Both inflammatory neuropeptides were found in detectable and comparable amounts in patient samples and in concentrations up to 100-fold those established in past literature. The presence of SP and HK-I necessitates further investigation into their role in nasal polyposis and the potentiation of the chronic inflammation inherent to chronic rhinosinusitis. Downregulating these peptides could therefore provide novel treatment targets to manage this disease process.

Keywords

nasal polyposis, chronic rhino sinusitis, rhinology, substance P, hemokinin I, nasal lavage

Received April 22, 2019; accepted August 20, 2019.

Nasal polyps are edematous outgrowths of degenerated paranasal sinus mucosa, which often coexist with chronic inflammatory processes, most notably allergy and chronic rhinosinusitis (CRS).^{1,2} Polyps primarily consist of mast cells known to be involved in allergic and inflammatory processes.³ Common symptoms of nasal polyposis include nasal obstruction, anosmia, rhinorrhea, and subsequent reduced quality of life.⁴ Polyps can also obstruct the sinus drainage pathways and potentiate CRS, for which

there is an established association. Chronic sinusitis predisposes patients to recurrent infections, headaches, and antibiotic resistance due to repetitive antibiotic use, making this disease process not only limiting to individuals suffering from it but also part of a larger public health concern.

The pathogenesis of nasal polyposis is thought to be multifactorial, though it remains poorly understood and an active area of research. Previously proposed mechanisms include compromised integrity of the airway epithelial barrier, altered expression of cytokines and chemokines, activation of innate and adaptive immunity, and tissue remodeling.⁵

Treatment modalities for polyposis are limited to intranasal and systemic corticosteroids as well as surgical resection for cases refractory to medical management, but neither has been successful in preventing recurrence—in fact, it is estimated that up to 60% to 70% of patients will have a recurrence of nasal polyps after definitive functional endoscopic sinus surgery by 18 months.^{6–8} Thus, further knowledge of this disease process is necessary to create more lasting treatment strategies.⁹

Previous studies have affirmed the role of inflammatory cytokines, such as IL-6 and IL-33, in nasal polyposis.^{10,11} It was our hypothesis that 2 particular neuropeptides—substance P (SP) and hemokinin 1 (HK-1)—could also contribute to the inflammation involved in CRS and nasal polyposis. SP and HK-1 act at the neurokinin 1 receptor on mast

¹School of Medicine, Tufts University, Boston, Massachusetts, USA

²Department of Otolaryngology, Tufts Medical Center, Boston, Massachusetts, USA

³Department of Integrative Physiology and Pathobiology, School of Medicine, Tufts University, Boston, Massachusetts, USA

This article was presented at the AAO-HNSF 2018 Annual Meeting & OTO Experience; October 7–10, 2018; Atlanta, Georgia.

Corresponding Author:

Ashley Lonergan, School of Medicine, Tufts University, 145 Harrison Avenue, Boston, MA 02111, USA.

Email: Ashley.Lonergan@tufts.edu



Table 1. Mean Concentrations of SP and HK-1 in Nasal Lavage Fluid Samples of 10 Patients with CRSwNP as Measured by ELISA.

| Patient | Age, y | Sex | Mean Concentration, ng/mL | |
|---------|--------|-----|---------------------------|-------|
| | | | SP | HK-1 |
| 1 | 58 | F | 2.153 | 2.655 |
| 2 | 40 | M | 2.113 | 3.791 |
| 3 | 29 | F | 6.715 | 3.146 |
| 4 | 86 | M | 2.881 | 3.584 |
| 5 | 34 | M | 3.700 | 3.411 |
| 6 | 67 | M | 4.362 | 3.101 |
| 7 | 67 | F | 4.835 | 3.189 |
| 8 | 53 | M | 3.851 | 3.342 |
| 9 | 34 | M | 2.511 | 2.950 |
| 10 | 69 | M | 3.364 | 2.347 |

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; ELISA, enzyme-linked immunosorbent assay; F, female; HK-1, hemokinin 1; M, male; SP, substance P.

cells and lymphocytes, and both have been implicated in chronic inflammation as well as nasal polyp formation and regrowth after surgical and steroid treatment. SP and HK-1 have also been shown to potentiate inflammatory states, such as asthma and arthritic disease.¹²⁻¹⁴ SP was previously isolated in the nasal polyp tissue of patients with chronic sinusitis, but relevant literature has lain dormant for >2 decades^{15,16}; the presence of HK-1 in nasal polyp tissue has not yet been recorded, though its shared receptor and function with SP makes this feasible.¹⁷ The following pilot study embodies a preliminary investigation into the hypothesized role of both these neuropeptides in CRS with nasal polyps (CRSwNP) as a means to inform future studies, which would thereby bolster the expanding knowledge base of this disease process.

Methods

In this pilot study, mucus samples were collected at Tufts Medical Center from patients diagnosed with CRSwNP. The study was approved by the Tufts Medical Center Institutional Review Board. The introductory cohort consisted of 3 women and 7 men with a mean \pm SD age of 53.7 ± 18.92 years (range, 29-86 years) who were known to have nasal polyps and CRS. Samples were suctioned from the nasal cavity, collected in a Lukens trap collection device, and then stored in 1 mL of normal saline in a -80°C freezer until analysis. After thawing on ice, samples were centrifuged at 3000 rpm for 5 minutes. Supernatants were analyzed for the presence of SP and HK-1 with the ELISA protocol.

Results

SP and HK-1 were measured in all 10 mucus samples in comparable concentrations (**Table 1**). The range of concentrations of SP was larger than that for HK-1, with the former present at a mean concentration of 3.649 ± 1.411

ng/mL and the latter found at a mean concentration of 3.152 ± 0.4266 ng/mL. Notably, previously established levels of SP in mucus samples of control patients were recorded on the order of mcg/mL; comparing these data with the mean concentration of the neuropeptide in our cohort, we found a 100-fold increase in concentration relative to baseline.^{18,19}

Conclusion

Both neuropeptides SP and HK-1 were found in detectable amounts in the samples of patients with nasal polyps and CRS, suggesting their production and secretion by either the nasal polyp tissue itself or peripheral sensory nerve endings, lending credence to their role in potentiating CRS refractory to standard-of-care treatments. Combining knowledge of SP and HK-1 in systemic inflammation with past research efforts to categorize and typify the molecular basis of CRSwNP suggests that these endogenous neuropeptides stimulate nasal polyps to release inflammatory cytokines into the surrounding mucus and nasal mucosal tissue.

The results of our study, as compared with the relatively low amounts in the controls of previous studies, suggest an association among SP, HK-1, and the inflammatory processes involved in CRSwNP. Preliminarily isolating these neuropeptides in the nasal lavage fluid of patients with CRSwNP in this pilot study informs future studies investigating their role in the pathogenesis of nasal polyposis and the chronic inflammation inherent to CRS, while providing novel targets for treatment. The study is limited in that the results were not compared with a control population of patients with nasal polyps who did not have CRS. Further studies will include patients with allergic rhinitis and compare the levels of SP and HK-1 in the nasal lavage fluid of those patients with levels in patients with CRS but without nasal polyps as well as control subjects who have nasal polyps but do not have CRS.

Author Contributions

Ashley Lonergan, primary author, specimen collection, data collection and analysis, drafting, final approval, accountability for work; **Theoharis Theoharides**, coauthor, data collection and analysis, drafting, final approval, accountability for work; **Eirini Tsilioni**, coauthor, data collection and analysis, drafting, final approval, accountability for work; **Elie Rebeiz**, senior author, data collection and analysis, drafting, final approval and revisions, accountability for work.

Disclosures

Competing interests: None.

Sponsorships: None.

Funding source: None.

References

1. Kucuksezer UC, Ozdemir C, Akdis M, Akdis CA. Chronic rhinosinusitis: pathogenesis, therapy options, and more. *Expert Opin Pharmacother*. 2018;19:1805-1815.

2. Georgy MS, Peters AT. Nasal polyps. *Allergy Asthma Proc.* 2012;33(suppl 1):S22-S23.
3. Zhai GT, Wang H, Li JX, et al. IgD-activated mast cells induce IgE synthesis in B cells in nasal polyps. *J Allergy Clin Immunol.* 2018;142:1489-1499.
4. Dietz de Loos DA, Hopkins C, Fokkens WJ. Symptoms in chronic rhinosinusitis with and without nasal polyps. *Laryngoscope.* 2013;123:57-63.
5. Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. *Clin Exp Allergy.* 2015;45:328-346.
6. Chong LY, Head K, Hopkins C, et al. Saline irrigation for chronic rhinosinusitis. *Cochrane Database Syst Rev.* 2016;4: CD011995.
7. Gudis D, Zhao KQ, Cohen NA. Acquired cilia dysfunction in chronic rhinosinusitis. *Am J Rhinol Allergy.* 2012;26:1-6.
8. DeConde AS, Mace JC, Levy JM, Rudmik L, Alt JA, Smith TL. Prevalence of polyp recurrence after endoscopic sinus surgery for chronic rhinosinusitis with nasal polyposis. *Laryngoscope.* 2016;126:550-555.
9. Georgy MS, Peters AT. Nasal polyps. *Allergy Asthma Proc.* 2012;33(suppl 1):S22-S23.
10. Bolta M, Bucca C, Riva G, Heffler E, Rolla G. Release of type 2 cytokines by epithelial cells of nasal polyps. *J Immunol Res.* 2016;2016:2643297.
11. Kato A. Immunopathology of chronic rhinosinusitis. *Allergol Int.* 2015;64:121-130.
12. Harrison S, Geppetti P. Substance P. *Int J Biochem Cell Biol.* 2001;33:555-576.
13. Metwali A, Blum AM, Elliott DE, Setiawan T, Weinstock JV. Cutting edge: hemokinin has substance P-like function and expression in inflammation. *J Immunol.* 2004;172:6528-6532.
14. Taracanova A, Alevizos M, Karagkouni A, et al. SP and IL-33 together markedly enhance TNF synthesis and secretion from human mast cells mediated by the interaction of their receptors. *Proc Natl Acad Sci.* 2017;114:E4002-E4009.
15. Beatrice F, Aluffi P, Bottomicca F, Perlasco L, Sartoris A. Nasal polyps and substance P: a preliminary report. *Acta Otorhinolaryngol Ital.* 1994;14(suppl 41):35-39.
16. Borbély, É, Helyes Z. Role of hemokinin-1 in health and disease. *Neuropeptides.* 2017;64:9-17.
17. Mortuaire G, Leroy X, Gengler I, Chevalier D, Prin L, Picry A. Histopathological classification of refractory chronic rhinosinusitis with nasal polyps. *Histol Histopathol.* 2015;30:1447-1454.
18. Drake-Lee A, Jones V, Lewin I, Nayyar S, Wells A, Stanworth D. Levels of substance P and IgE decapeptide in nasal polyp fluid and matching sera. *J Laryngol Otol.* 1996; 110:225-227.
19. Schäper C, Noga O, Koch B, et al. Anti-inflammatory properties of montelukast, a leukotriene receptor antagonist in patients with asthma and nasal polyposis. *J Investig Allergol Clin Immunol.* 2011;21:51-58.