

Denatonium benzoate bitter taste perception in chronic rhinosinusitis subgroups

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Background: Chronic rhinosinusitis (CRS) with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP), and aspirin-exacerbated respiratory disease (AERD) have varying levels of inflammation and disease severity. Solitary chemosensory cells (SCCs) are enriched in nasal polyps, are the primary source of interleukin 25 (IL-25) in upper airways, leading to type 2 inflammation, and are activated by bitter-tasting denatonium benzoate (DB). Thus, we sought to evaluate DB taste perception at a range of concentrations in order to identify 1 that most differentiates CRS subgroups from controls.

Methods: CRSsNP (n = 25), CRSwNP (n = 26), and AERD (n = 27) patients as well as controls (n = 25) tasted 6 DB concentrations in a fixed, random order, rating on a category scale of 0 (no intensity) to 12 (extremely intense). Sinonasal epithelial cultures were treated with and without denatonium and analyzed for IL-25 via flow cytometry.

Results: CRSsNP patients rated DB as significantly less intense than did controls at all concentrations: 5.62×10^{-9} M, 1.00×10^{-8} M, 1.78×10^{-8} M, 3.16×10^{-8} M, 5.62×10^{-8} M, and 1.00×10^{-7} M (all $p < 0.0083$). CRSwNP patients did not show significant differences from controls. AERD

patients rated DB as significantly more intense than did controls at concentrations of 1.00×10^{-8} M and 3.16×10^{-8} M ($p < 0.0083$). In vitro data demonstrated significant increase in IL-25-positive cells after denatonium stimulation (n = 5), compared to control (n = 5) ($p = 0.012$).

Conclusion: Our findings link in vitro DB stimulation of sinonasal tissue with increased IL-25 and show differential DB taste perception in CRS subgroups relative to the control group, with CRSsNP being hyposensitive and AERD being hypersensitive. We propose a concentration of 3.16×10^{-8} M for future study of clinical utility. © 2020 ARS-AAOA, LLC.

Key Words:

bitter taste receptors; sensory perception; bitterness; AERD; solitary chemosensory cells; type 2 inflammation; mucosal immunity

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Chronic rhinosinusitis (CRS) is 1 of the most common chronic conditions in the United States, affecting up to 16% of the population and invoking a direct treatment cost of \$10 billion to \$13 billion per year.^{1–3} Despite the tremendous socioeconomic burden and diminished quality of life, treatment for CRS continues to be both a medical and surgical challenge. These patients are classically categorized into 2 subtypes: without (sans) nasal polyps (CRSsNP) and with nasal polyps (CRSwNP).⁴ Aspirin-exacerbated respiratory disease (AERD) is a distinct chronic pro-inflammatory

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condition characterized by CRSwNP, asthma, and sensitivity to medications that inhibit cyclooxygenase-1 (COX-1) enzymes. These 3 subgroups share a number of clinical similarities but are characterized by different inflammatory pathways and different levels of disease severity. Comparing CRSsNP and CRSwNP, CRSsNP has an increased incidence of neutrophilic inflammation, whereas CRSwNP has increased incidence of type 2 (T helper 2 [Th2]) eosinophilic inflammation.^{5–7} CRSwNP patients prove to be more difficult to treat compared to CRSsNP patients.^{8,9} Interleukin-25 (IL-25), an epithelium-derived cytokine, leads to type 2 inflammation and has been implicated in the pathogenesis of CRSwNP, with the amount of IL-25 correlating with disease severity.^{5,6,10,11} Similar to CRSwNP, a hallmark of AERD is Th2 inflammation with increased IL-25 levels; however, levels of eosinophil and mast cell degranulation and disease severity are greater in AERD.^{12–15} In addition, AERD, unlike CRSwNP, also involves baseline prostaglandin E₂ (PGE₂) deficiency and increased 5-lipoxygenase activity that is amplified by COX-1 inhibition (eg, by aspirin).^{14,15} Overall, the underlying inflammatory mediators have important clinical implications.

Bitter taste receptors (T2Rs) have been studied for their role in sinonasal immunity and contributions to CRS.^{16–23} They are capable of modulating the immune system through at least 2 different cell types in the upper airway: ciliated cells and solitary chemosensory cells (SCCs). Stimulation of bitter taste receptors, such as T2R38, on sinonasal ciliated cells has a well-described role in fighting bacteria.^{17,19,24–26} CRSsNP patients with specific non-functional variants of T2R38 have been found to be more susceptible to gram-negative sinusitis¹⁷ and more likely to form biofilms,²⁷ as well as to have suboptimal outcomes from sinus surgery.^{28,29} The other cell type, SCCs, are a niche epithelial cell population that also expresses bitter taste receptors. When stimulated by bacterial byproducts³⁰ or other bitter-tasting compounds such as denatonium benzoate (DB),^{29,31} SCCs induce release of antimicrobial peptides from adjacent epithelial cells.^{32,33} More recently, SCCs have been found to be enriched in nasal polyps and to be the exclusive source of epithelial-derived IL-25 in sinonasal tissue.^{5,6} Cellular hyperresponsiveness to DB predicts worse surgical outcomes in CRSwNP.³⁴ It is not yet known if DB stimulation also elicits IL-25 release, but other compounds that stimulate SCCs are known to release IL-25, such as leukotrienes and extracts from the fungus *Alternaria*.^{10,35}

Given the immune response stimulated by bitter compounds in the upper airway, previous studies by our group have evaluated the use of phenotypic taste testing in differentiating CRS from controls. This past work comprised 652 CRSsNP and CRSwNP patients tested for responses to 3 different bitter compounds, including DB. A subset of those data published by our group revealed that at a concentration of 1.8×10^{-6} M there was a decrease in DB taste sensitivity scores, relative to controls, in the CRSsNP and CRSwNP groups, with a statistically significant difference in CRSsNP.²⁸ This may reflect a decrease in CRS patients'

T2R-mediated release of antimicrobial peptides by SCCs. Importantly, this concentration yielded average DB taste intensity scores ranging from 7 to 9, which is on the upper end of our scale of 0 to 12. This concentration is presumably much higher than the physiologic one at which receptors in the upper airway can be stimulated.

With the newer understanding that bitter-taste receptor-expressing SCCs release IL-25 and are expanded in polyp tissue,⁶ DB taste testing may have further utility in measuring disease severity and in elucidating the mechanisms by which some patients are predisposed to upper airway inflammation. The objective of this study was to identify a more accurate and relevant concentration of DB than used previously, capturing larger differences in taste perception between controls and CRS subgroups, for use in future studies. This was accomplished by testing DB taste perception across a full spectrum of concentrations from near-threshold to clearly suprathreshold in control subjects compared to CRSsNP, CRSwNP, and AERD patients. Furthermore, we aimed to evaluate in vitro IL-25 expression in response to DB stimulation of sinonasal cultures.

Patients and methods

Patient recruitment

With University of Pennsylvania Institutional Review Board (IRB) approval, patients were enrolled from the Rhinology and Allergy clinics at the Perelman Center for Advanced Medicine. Control subjects were enrolled from individuals accompanying patients to clinic visits. Control subjects had no current symptoms or history of treatment for CRS, no prior surgery for recurrent sinus infections, and ≤ 3 diagnosed acute sinus infections in the past 12 months. Test subjects were patients who had been diagnosed with CRS and met the objective and subjective guidelines for CRS outlined by the clinical practice guideline for adult sinusitis set forth by the Academy of Otolaryngology–Head and Neck Surgery.^{4,36–38} Inclusion criteria for patients included immune competence, age ≥ 18 years, and endoscopic evidence of sinonasal inflammation. Exclusion criteria included rhinologic granulomatous disease, sarcoidosis, immune deficiencies, genetic disorders affecting mucociliary clearance (eg, cystic fibrosis, primary ciliary dyskinesia), history of chemotherapy, and history of facial trauma.

Patients with CRS were subcategorized based on the endoscopic visualization and/or computed tomography (CT) review of sinonasal polyposis by a fellowship-trained rhinologist. AERD required challenge-proven aspirin intolerance characterized by worsening of upper and/or lower airway symptoms with ingestion of aspirin, a diagnosis of asthma, and endoscopic or CT evidence of CRS with nasal polyposis.³⁹ CRSwNP required CRS with sinonasal polyposis without AERD. CRSsNP required no visualization of nasal polyposis.

Subjects completed questionnaires collecting demographic and background information (including history

of smoking, allergies, asthma, nasal therapeutics, and sinus surgery), and the 22-item Sino-Nasal Outcome Test (SNOT-22), a validated, symptom-based rhinosinusitis outcome measure.⁴⁰ Following this data collection, they provided taste intensity ratings for 6 concentrations of DB, a common bitter psychophysical tastant used in our previous studies of CRS susceptibility.²⁸

Taste testing method

DB solutions were prepared at the Monell Chemical Senses Center, an internationally recognized research institution with expertise in the genetic, sensory, and cellular aspects of human taste perception. The 6 concentrations, 5.62×10^{-9} M, 1.00×10^{-8} M, 1.78×10^{-8} M, 3.16×10^{-8} M, 5.62×10^{-8} M, and 1.00×10^{-7} M, were dissolved in Millipore-filtered water. Taste kits were taken to the outpatient clinic, where subjects were presented with the 6 concentrations in a fixed, random order in order to ensure that all subjects' ratings were given in the same immediate sensory context and reduce the potential impact of subject expectations (eg, with an ascending or descending series): (1) 1.00×10^{-8} M, (2) 5.62×10^{-9} M, (3) 3.16×10^{-8} M, (4) 5.62×10^{-8} M, (5) 1.78×10^{-8} M, (6) 1.00×10^{-7} M. Subjects were instructed to refrain from eating, drinking tea/coffee, smoking, and brushing their teeth for at least 1 hour prior to taste testing. Subjects were provided with a bottle of spring water (UPC 099482418885) and a plastic cup for expectoration. All subjects rinsed their mouths once with water before and after tasting each solution, and then rated the taste intensity on a 13-point, validated category scale with equidistant verbal descriptors: "Extremely intense" (#12), "Very intense" (#9), "Moderately intense" (#6), "Slightly intense" (#3), and "No intensity at all" (#0). This scale has been used in multiple clinical studies at the Monell Chemical Senses Center.^{28,41}

Sinonasal cell culture

Tissue was obtained from patients recruited from the Department of Otorhinolaryngology–Head and Neck Surgery, Division of Rhinology at the University of Pennsylvania with IRB approval. Tissue for establishing cultures was obtained from nasal polyps or inferior turbinate of patients undergoing sinonasal surgery. Diagnosis of CRSwNP was made per established diagnostic standards and the exclusion criteria included history of systemic diseases (eg, granulomatosis polyangiitis, sarcoidosis, cystic fibrosis, and immunodeficiency). Human sinonasal epithelial cells were enzymatically dissociated from sinonasal mucosal specimens obtained from polyp and turbinate tissue as described in prior studies.^{5,42} Cells were plated onto 75-cm² (75-mL) flasks (Corning, Corning, NY) and grown in proliferation media (Lonza, Basel, Switzerland). Upon reaching 80% confluency, cells were trypsinized and plated onto 25-cm² (25-mL) flasks, and treated with 500µM denatonium or Hank's Balanced Salt Solution without glucose (HBSS) every other day for 1 week.

Cell staining and flow cytometry

Cells were incubated with 5 µg/mL brefeldin A (Sigma-Aldrich, St Louis, MO) for 4 hours to stop Golgi-mediated release of IL-25. Cells were then trypsinized and single-cell suspensions were stained with viability dye Aqua at 1:600 dilution (Thermo Fisher Scientific, Waltham, MA) for dead cell exclusion. Fc receptors were blocked with TruStain FcXTM (BioLegend, San Diego, CA). Fluorescence-minus-one (FMO) controls were prepared for each marker. Isotype control antibodies were used in FMOs for IL-25. Cell surface markers were stained using anti-EpCAM–PECy7 (eBioscience, Santa Clara, CA) to mark epithelial cells and anti-CD45–eFlour450 (eBioscience) to exclude lymphocytes. After fixation and permeabilization, IL-25 was stained overnight at 4°C using anti-IL-25 fluorescein isothiocyanate (Invitrogen, Carlsbad, CA). Antibody-labeled cell suspensions were analyzed on an LSR FortessaTM using FACSDiva version 8 (BD Biosciences, San Jose, CA). At least 500,000 events were acquired per sample. Data were analyzed using FlowJo10 (FlowJo, LLC, Ashland, OR). Gating on forward scatter (FSC) and side scatter (SSC) was used to exclude doublets and debris. Positive gates were determined using <1% of events on FMO samples. Singlets, Aqua Live, CD45[−], EpCAM⁺, and IL-25⁺ events were recorded as IL-25-positive cells.

Statistical analysis

Subjects were grouped and compared by their case-control status for demographic and clinical variables using the Fisher's exact test for categorical variables and *t* tests for continuous variables. The taste intensity scores were adjusted in a general linear model by including covariates of sex, age, race, smoking status, comorbid asthma, and comorbid allergies within each study group (CRSsNP, CRSwNP, AERD, and control). The means and standard deviations (SDs) of the adjusted values were calculated and dose-response curves were constructed for each group by performing a logit regression method for DB concentrations using the "drc" R package (R Foundation for Statistical Computing, Vienna, Austria; <https://www.r-project.org/>).⁴³ Receiver-operating characteristic (ROC) curves⁴⁴ were performed with vectors (group, adjusted taste intensity scores) as an ROC function⁴⁵ to assess the difference in the adjusted taste intensity scores for each concentration between cases and controls. For each comparison, a cutoff value was derived by maximizing the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$). The *J* measure of sensitivity was used to summarize the measure of the ROC curve that enables selection of an optimal threshold value (cutoff point),⁴⁶ and the significance of the area under the ROC curve (AUC) compared to 0.50 was calculated,⁴⁷ with $p < 0.0083$ (Bonferroni correction for 6 tests = $0.05/6 = 0.0083$) as the significance level. Statistical analysis was performed using R version 3.5.3 and RStudio v1.1.456 (R Studio, Boston, MA). For the in vitro data, the Mann-Whitney *U* test was

TABLE 1. Baseline patient characteristics

Characteristic	Control (n = 25)	CRSsNP (n = 25)	<i>p</i>	CRSwNP (n = 26)	<i>p</i>	AERD (n = 27)	<i>p</i>
Age (years), mean $\pm$ SD	46.72 $\pm$ 15.79	50.60 $\pm$ 13.75	0.359	53.62 $\pm$ 12.33	0.090	47.15 $\pm$ 15.98	0.923
Gender (male), n (%)	10 (40.0)	10 (40.0)	1.000	15 (57.7)	0.267	12 (44.4)	0.785
Ethnicity (Non-Hispanic), n (%)	25 (100.0)	25 (100.0)	1.000	22 (84.6)	0.110	27 (100.0)	1.000
Race (White), n (%)	16 (64.0)	22 (88.0)	0.095	22 (84.6)	0.116	22 (81.5)	0.215
Ever smoker, n (%)	7 (28.0)	6 (24.0)	1.000	13 (50.0)	0.153	11 (40.7)	0.392
Ever asthma, n (%)	3 (12.0)	8 (62.8)	0.171	14 (53.8)	0.003	27 (100.0)	<0.001
Ever allergies, n (%)	7 (28.0)	14 (56.0)	0.085	18 (69.2)	0.005	23 (85.2)	<0.001
SNOT-22, mean $\pm$ SD	6.68 $\pm$ 7.19	32.28 $\pm$ 20.78	<0.001	21.31 $\pm$ 22.15	0.003	18.04 $\pm$ 14.96	0.001

AERD = aspirin-exacerbated respiratory disease; CRSsNP = chronic rhinosinusitis without (sans) nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; SD = standard deviation; SNOT-22 = 22-item Sino-Nasal Outcome Test.

used to evaluate the differences in IL-25 between the control and DB-treated groups.

Results

In total, 78 CRS (25 CRSsNP, 26 CRSwNP, and 27 AERD) patients and 25 controls were included in the analyses. Our patients were predominantly middle-aged and white. The control group was similar with respect to age, gender, race, and ethnicity. As expected, the CRS subgroups had significantly higher SNOT-22 scores indicating higher disease burden. CRSwNP and AERD were significantly more likely to have comorbid asthma and/or allergies (Table 1).

Our results show differing DB taste perception among the different patient groups compared to controls. The CRSsNP group was found to have lower mean taste scores, with the ROC analysis finding significant differentiation at concentrations 5.62×10^{-9} M ($p < 0.001$), 1.00×10^{-8} M ($p < 0.001$), 1.78×10^{-8} M ($p < 0.001$), 3.16×10^{-8} M ($p < 0.001$), 5.62×10^{-8} M ($p = 0.003$), and 1.00×10^{-7} M ($p < 0.001$). The CRSwNP group rated DB solutions lower than controls for the majority of the concentrations, excluding the lowest 2. The ROC analysis did not yield significant results at any concentration in this subgroup. The AERD group was found to have overall higher mean taste scores, with the ROC analysis finding significant differentiation at concentrations 1.00×10^{-8} M ($p < 0.001$) and 3.16×10^{-8} M ($p < 0.001$). The average scores, AUCs, *p* values, and cutoff values at each concentration are presented in Table 2 and Figure 1. The concentrations with both $p < 0.0083$ and AUC > 70 are demonstrated in more detail in Figure 1 (C shows the AERD group, D shows the CRSsNP group), including appropriate cutoff scores to differentiate the patient group from controls while maximizing sensitivity and specificity.

The concentration with the largest difference from controls in mean taste score in the CRSsNP group was found at 1.00×10^{-7} M (CRSsNP mean: 5.32; compared to con-

trol: -2.60), with decreases in the separation at lower concentrations. The largest difference from controls in the CRSwNP group was found at 5.62×10^{-8} M (CRSwNP mean: 5.00; compared to control: -0.80). Last, the largest difference from controls in the AERD group was found at 3.16×10^{-8} M (AERD mean: 6.30; compared to control: $+2.62$), with decreases in the separation at the lower and higher concentrations.

In vitro analysis of cell cultures demonstrated increased frequency of IL-25-positive cells following “chronic” denatonium exposure compared to HBSS exposure (Fig. 2). Distinct cell samples from the same 5 cultures were used in both the control and treatment groups. The mean $\pm$ SD frequency of IL-25-positive epithelial cells (singlet, aqua live, CD45⁺, EpCAM⁺, IL-25⁺) was 17.76 ± 9.91 in the control group and 44.34 ± 15.30 in the DB-treatment group. Mann-Whitney *U* test generated a *p* value of 0.012.

Discussion

In this study, we identified DB concentrations that yield differing taste responses in CRS subgroups compared to controls. Specifically, CRSsNP patients had significantly decreased scores compared to controls at numerous concentrations, and AERD patients had significantly increased scores compared to controls at some concentrations. Both groups had significant findings at concentrations of 1.00×10^{-8} M and 3.16×10^{-8} M. Furthermore, in vitro DB incubation was found to yield a significant increase in IL-25-positive cells compared to control incubation. To our knowledge, this is the only study to date to test psychophysical taste testing in a wide variety of DB concentrations, the only one to test taste perception in AERD, and the only one to measure IL-25 expression in response to DB.

The differences in mean taste ratings across subgroups of patients suggest an association between the different underlying disease processes and the patients’ subjective taste perception. ROC analysis is a widely-accepted method

TABLE 2. Comparison of mean DB taste scores between controls and cases*

Concentration	Control (mean $\pm$ SD)	Case (mean $\pm$ SD)	p^a	AUC % (95% CI)	p^b	Cutoff
Control (n = 25) vs CRSsNP (n = 25)						
$5.62 \times 10^{-9}M$	1.32 $\pm$ 0.93	0.40 $\pm$ 0.29	0.001	84.96 (72.19–97.74)	<0.001	0.743
$1.00 \times 10^{-8}M$	1.04 $\pm$ 0.59	0.56 $\pm$ 0.23	0.001	81.92 (69.06–94.78)	<0.001	0.708
$1.78 \times 10^{-8}M$	3.08 $\pm$ 1.17	1.24 $\pm$ 0.99	0.001	87.20 (77.18–97.22)	<0.001	2.426
$3.16 \times 10^{-8}M$	3.68 $\pm$ 1.38	2.04 $\pm$ 0.71	0.001	84.80 (73.46–96.14)	<0.001	2.853
$5.62 \times 10^{-8}M$	5.80 $\pm$ 1.74	4.00 $\pm$ 2.45	0.004	74.72 (60.74–88.70)	0.003	4.133
$1.00 \times 10^{-7}M$	7.92 $\pm$ 1.46	5.32 $\pm$ 2.42	0.001	82.72 (70.27–95.17)	<0.001	5.972
Control (n = 25) vs CRSwNP (n = 26)						
$5.62 \times 10^{-9}M$	1.32 $\pm$ 0.93	1.62 $\pm$ 0.78	0.223	62.62 (46.72–78.51)	0.122	1.550
$1.00 \times 10^{-8}M$	1.04 $\pm$ 0.59	1.54 $\pm$ 1.05	0.043	66.00 (50.22–81.78)	0.050	1.193
$1.78 \times 10^{-8}M$	3.08 $\pm$ 1.17	2.88 $\pm$ 1.64	0.629	62.62 (46.29–78.94)	0.122	2.518
$3.16 \times 10^{-8}M$	3.68 $\pm$ 1.38	3.58 $\pm$ 1.60	0.806	53.85 (37.57–70.12)	0.638	4.031
$5.62 \times 10^{-8}M$	5.80 $\pm$ 1.74	5.00 $\pm$ 1.36	0.073	63.08 (47.39–78.77)	0.109	5.699
$1.00 \times 10^{-7}M$	7.92 $\pm$ 1.46	7.23 $\pm$ 1.71	0.129	59.31 (43.38–75.23)	0.254	5.825
Control (n = 25) vs AERD (n = 27)						
$5.62 \times 10^{-9}M$	1.32 $\pm$ 0.93	1.48 $\pm$ 0.66	0.470	55.41 (39.33–71.49)	0.504	1.793
$1.00 \times 10^{-8}M$	1.04 $\pm$ 0.59	2.19 $\pm$ 0.57	0.001	90.67 (82.46–98.88)	<0.001	1.326
$1.78 \times 10^{-8}M$	3.08 $\pm$ 1.17	2.56 $\pm$ 1.07	0.098	66.67 (51.16–82.17)	0.039	2.544
$3.16 \times 10^{-8}M$	3.68 $\pm$ 1.38	6.30 $\pm$ 2.34	0.001	82.07 (70.40–93.75)	<0.001	5.061
$5.62 \times 10^{-8}M$	5.80 $\pm$ 1.74	6.89 $\pm$ 1.84	0.033	66.82 (51.88–81.75)	0.038	6.782
$1.00 \times 10^{-7}M$	7.92 $\pm$ 1.46	8.11 $\pm$ 1.84	0.682	52.00 (35.74–68.26)	0.805	10.128

*Bold values are significant.

^a p values comparing the mean DB test scores between controls and cases.^b p values of tests with the null hypothesis that the AUC = 0.50, with $p < 0.0083$ as the significance level.

AERD = aspirin-exacerbated respiratory disease; AUC = area under the ROC curve; DB = denatonium benzoate; CI = confidence interval; CRSsNP = chronic rhinosinusitis without (sans) nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; ROC = receiver-operating characteristic; SD = standard deviation.

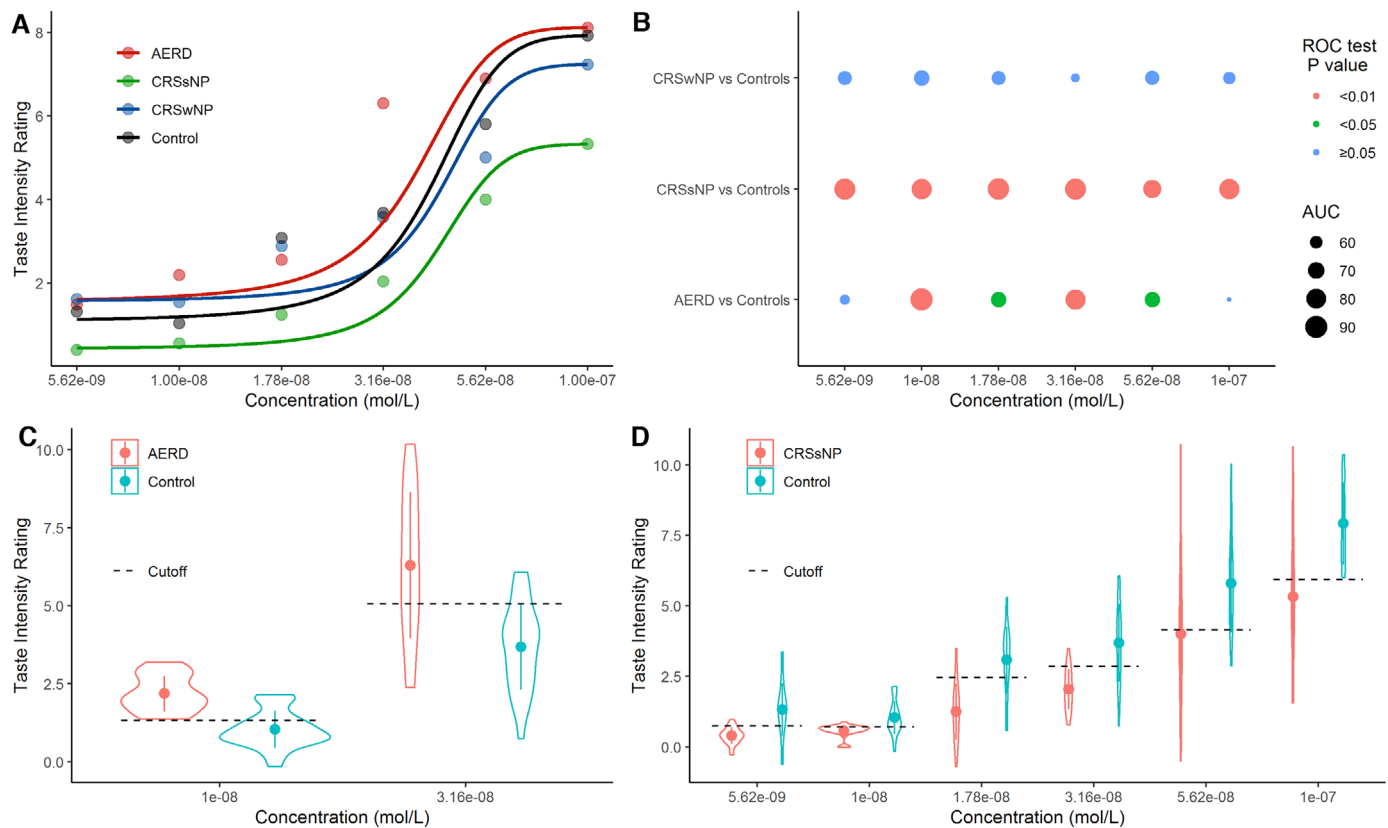


FIGURE 1. Patients with AERD or CRSsNP differed in DB bitter taste perception compared to the control group. (A) A log dose-response curve of mean taste score for each of the 6 DB concentrations per subgroup, starting at 5.62×10^{-9} mol/L and going up to 1.00×10^{-7} mol/L with predicted 10,000 points in between. (B) Summary of ROC curve statistical tests at each concentration per patient group compared to controls. The p value of the ROC test and the AUC size are demonstrated using dot sizes and color. (C,D) Violin plots with appropriate cutoff values for the concentrations with a significant difference ($p < 0.0083$, $AUC > 70$) in DB bitter taste intensity scores. (C) AERD patient group vs control group. (D) CRSsNP patient group vs control group. AERD = aspirin-exacerbated respiratory disease; AUC = area under the ROC curve; CRSsNP = chronic rhinosinusitis without (sans) nasal polyps; DB = denatonium benzoate; ROC = receiver-operating-characteristic.

that allows assessment of the strength of a test (DB taste intensity scores in our case) in distinguishing between cases and controls. In CRSsNP patients, we found significantly decreased mean DB taste scores, which is consistent with our prior study.²⁸ In CRSwNP, the scores were decreased at the 4 higher concentrations and increased at the 2 lower concentrations compared to controls, but not at statistically significant levels. In AERD, we observed significantly increased scores, which is a novel finding. Of note, the previous study by Workman et al.²⁸ also tested salt, which patients reported to be of comparable intensity to that reported by controls. Thus, the DB effects did not appear to be due to a nonspecific taste loss in CRS.

These results suggest a spectrum of bitter taste perception in CRS subgroups, which could potentially reflect underlying pathophysiology. DB is known to activate SCCs, and SCCs are known to be the primary source of epithelial IL-25.^{5,6,29,31} In the data presented here, we demonstrate an increase in IL-25-positive epithelial cells following DB incubation compared to control incubation. Though we cannot know for certain based on the current data, it is possible that low DB perception may reflect low SCC receptor activity, leading to an inability to defend against pathogens.

On the other hand, high DB perception may reflect high SCC receptor activity, leading to hyper-inflammation. At this point, the present analysis cannot make a determination. Regardless, the diversion of taste sensitivities of CRS subgroups may reflect differences in the cellular biology associated with CRS (1) in the absence of polyps, (2) with generic polyps, and (3) with AERD polyps.

As we hypothesized, by lowering the DB concentration to one that yields an average taste intensity score of 5 to 7 in patient groups (on our scale of 0 to 12), we achieved larger separations from the control group than we had in our previous testing. The largest separations for each subgroup occurred at 1.00×10^{-7} M for CRSsNP with a mean intensity score of 5.32 (−2.60 from control mean), at 5.62×10^{-8} M for CRSwNP with a mean intensity score of 5.00 (−0.80 from control mean), and at 3.16×10^{-8} M for AERD with a mean intensity score of 6.30 (+2.62 from control mean). Previously, the DB mean scores were around 8 for both CRSsNP and CRSwNP groups (AERD was not specifically evaluated), with differences from controls being <1.²⁸ Our previous test's concentration of 1.8×10^{-6} M may have been too intense for our scale, leading to a ceiling effect. Taste testing works when the ligand is at

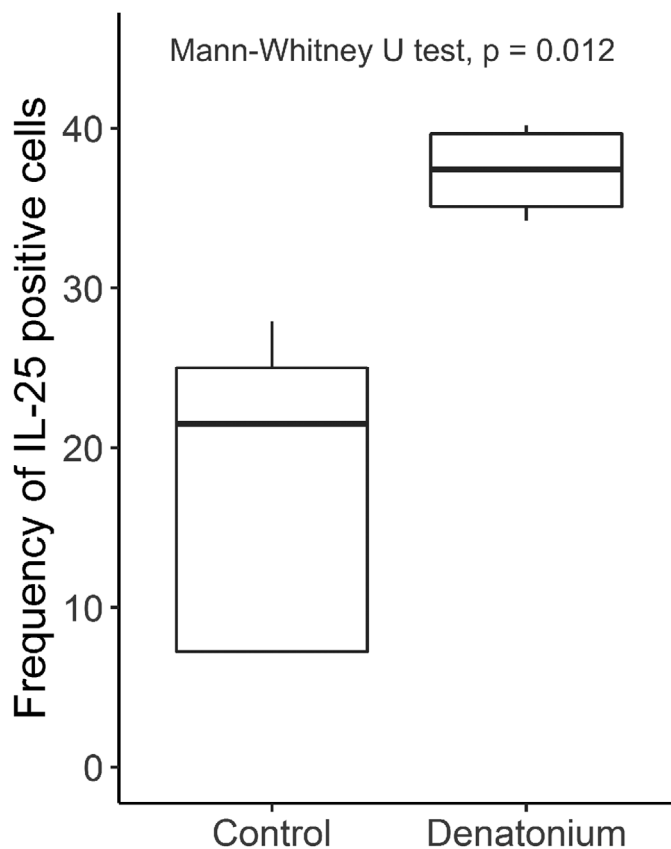


FIGURE 2. Flow cytometry was used to analyze DB-induced IL-25 expression. Sinonasal epithelial cultures derived from polyp and turbinate tissue were stimulated with denatonium (0.5mM) ($n = 5$) or HBSS (control solution) ($n = 5$) every other day for 7 days and then analyzed for intracellular IL-25 via flow cytometry. Distinct cell samples from the same 5 cultures were used in each group. Using the Mann-Whitney U test, there was a significant increase in IL-25-positive cells following DB incubation compared to control incubation ($p = 0.012$). DB = denatonium benzoate; HBSS = Hank's Balanced Salt Solution without glucose; IL-25 = interleukin-25.

micromolar to nanomolar concentrations and is therefore very sensitive. Identifying a concentration that optimizes the separation seems to increase the potential for clinical utility. However, given the spectrum we observed, each subgroup reaches their moderate mean score, and thus widest separation from controls, at a different concentration. We suggest $3.16 \times 10^{-8}\text{M}$ as a reasonable concentration for further testing as it is the mutually significant concentration that yields wider separations from controls.

The complexity of CRS is evidenced by the numerous research efforts aimed at uncovering the pathophysiology and discovering the optimal treatment modalities for each subset of patients.^{48–51} However, as studies are uncovering the cellular mechanisms underlying the different subsets of CRS and the need for individualized treatment plans is becoming apparent,⁴⁹ there is still no readily accessible diagnostic tool to guide treatment. Furthermore, there is no established method to determine which patients will benefit from certain interventions and which will not. This leads to widespread use of steroids, antibiotics, and eventually sur-

gical intervention in an effort to quell the inflammatory burden. Therefore, an inexpensive taste test would have broad implications if differences in taste perception do in fact reflect aberrant inflammatory responses and predisposition to inflammatory or infectious sinonasal disorders. If that is the case, with the appropriate parameters, a taste test could help distinguish patients likely to improve with conservative medical management from those likely to require, and respond to, more aggressive treatment such as functional endoscopic sinus surgery. This taste test could lead to more effective treatment paradigms including refined patient selection for the varied therapeutic modalities. The potential utility of a low-cost taste test is high, with implications for healthcare expenditures, antibiotic stewardship, and quality of life.

This study examines a possible taste test for clinical use in sinonasal disorders, proposing DB as the potential tool. DB is known to activate SCCs, and SCCs play a role in sinonasal disease. DB is particularly well-suited as a clinically useful taste test because it is a broad bitter agonist, stimulating many different bitter taste receptors.^{52,53} Thus, responses to it are not expected to vary significantly as a result of genetic differences in any single receptor. Other bitter compounds that target 1 or 2 receptors may be less generalizable in a patient population. Moreover, DB is commonly used in human taste studies^{28,54–56} and is known to be nontoxic.⁵⁵ After evaluating DB taste responses across a wide spectrum of concentrations, we found differential taste scores in CRS subgroups relative to the control group, with CRSsNP being hyposensitive and AERD being hypersensitive to DB. Through this dose-response testing, we can have more confidence in concentration selection for future study.

Our study was limited by small sample sizes for taste testing of CRS subgroups as well as for flow cytometry. Given the logistical and time challenges of administering a taste test that includes many concentrations during a busy outpatient clinic, the goal was to screen these concentrations in a small group to optimize the concentration(s) to be used on a larger scale. It is possible results could shift with increased sample size and power. There was also a large amount of variance in our results, with overlapping SDs that may decrease diagnostic potential. Future studies with larger samples sizes are needed to evaluate our conclusions further. Lastly, although our analysis controlled for multiple variables that we incorporated into the model, there may have been unknown confounders we did not identify.

Conclusion

Bitter taste receptors are expressed in human upper respiratory epithelial cells where they are activated by bitter compounds such as DB. Prior studies have revealed multiple roles for bitter taste receptors in the immune response to pathogens, including both an antimicrobial response and an inflammatory cytokine release by SCCs. In this work, we

explored the relationship between DB bitter taste perception and upper airway disorders, and analyzed in vitro IL-25 expression following DB stimulation. We demonstrated increase in IL-25 positivity in response to DB stimulation of sinonasal cultures. After taste testing a range of DB concentrations, our findings show differential taste scores in

CRS subgroups relative to the control group, with CRSsNP being hyposensitive and AERD being hypersensitive to DB. We have also identified concentrations and score cutoffs to be used for further study, namely 3.16×10^{-8} M. Through future work, we hope to unlock diagnostic, prognostic, and therapeutic possibilities. 🌐

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