In vitro effects of anthocyanidins on sinonasal epithelial nitric oxide production and bacterial physiology

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ABSTRACT

Background: T2R bitter taste receptors play a crucial role in sinonasal innate immunity by upregulating mucociliary clearance and nitric oxide (NO) production in response to bitter gram-negative quorum-sensing molecules in the airway surface liquid. Previous studies showed that phytochemical flavonoid metabolites, known as anthocyanidins, taste bitter and have antibacterial effects. Our objectives were to examine the effects of anthocyanidins on NO production by human sinonasal epithelial cells and ciliary beat frequency, and their impact on common sinonasal pathogens Pseudomonas aeruginosa and Staphylococcus aureus.

Methods: Ciliary beat frequency and NO production were measured by using digital imaging of differentiated air-liquid interface cultures prepared from primary human cells isolated from residual surgical material. Plate-based assays were used to determine the effects of anthocyanidins on bacterial swimming and swarming motility. Biofilm formation and planktonic growth were also assessed.

Results: Anthocyanidin compounds triggered epithelial cells to produce NO but not through T2R receptors. However, anthocyanidins did not impact ciliary beat frequency. Furthermore, they did not reduce biofilm formation or planktonic growth of P. aeruginosa. In S. aureus, they did not reduce planktonic growth, and only one compound had minimal antibiofilm effects. The anthocyanidin delphinidin and anthocyanin keracyanin were found to promote bacterial swimming, whereas anthocyanidin cyanidin and flavonoid myricetin did not. No compounds that were tested inhibited bacterial swarming.

Conclusion: Results of this study indicated that, although anthocyanidins may elicit an innate immune NO response from human cells, they do not cause an increase in ciliary beating and they may also cause a pathogenicity-enhancing effect in P. aeruginosa. Additional studies are necessary to understand how this would affect the use of anthocyanidins as therapeutics. This study emphasized the usefulness of in vitro screening of candidate compounds against multiple parameters of both epithelial and bacterial physiologies to prioritize candidates for in vivo therapeutic testing.

Chronic rhinosinusitis (CRS) affects more than one in eight adults in the United States and accounts for >$8 billion in direct health care costs and >$20 billion in total economic burden annually. CRS is a syndrome of complex etiologies that involve chronic infection and/or persistent inflammation of the paranasal sinuses, often linked to ineffective sinonasal mucociliary clearance and/or chronic bacterial infection and biofilm formation. Patients with CRS who require surgery report worse quality-of-life scores for physical pain and social functioning than those patients with chronic obstructive pulmonary disease, congestive heart failure, or angina.

CRS accounts for one in five adult antibiotic prescriptions in the United States, a modality that is becoming less effective with the emergence of antibiotic-resistant microorganisms. Nonantibiotic topical CRS therapeutics are needed. Bitter taste receptors (T2Rs) are emerging potential therapeutic targets. Originally identified on the tongue, T2Rs are also located throughout the sinonasal cavity, where they stimulate nitric oxide (NO) production or antimicrobial peptide secretion. Moreover, a nonfunctional (“nontaster”) polymorphism in the TAS2R38 gene encoding T2R38 is an independent risk factor for CRS and predicts surgical outcome in non-polypoid CRS. Because T2Rs regulate important host defense responses in the sinonasal cavity, bitter compounds may be useful as topical therapeutics to stimulate endogenous immune responses.

Anthocyanidins are a class of naturally occurring plant flavonoids found in red berries and other fruits, with antioxidant activity in vitro. Moreover, anthocyanidins can activate some T2R receptors and have been linked to endothelial NO production. Because NO is an important component of sinonasal innate immunity, we hypothesized that anthocyanidins may activate sinonasal T2Rs to generate NO. In addition, anthocyanidin-rich plant extracts have antibacterial properties against Staphylococcus aureus, Escherichia coli, Corynebacterium diphtheriae, Moraxella catarrhalis, Bacillus cereus, and Pseudomonas aeruginosa. However, other studies have indicated that anthocyanidins do not have antibacterial effects against B. cereus, E. coli, or P. aeruginosa, and that anthocyanidins only reduce inflammation. The interactions between anthocyanidins and bacteria are complex but interest in anthocyanidins has increased with the correlation between consumption of grapes and grape products (e.g., wine) with lower incidences of cancers and inflammatory diseases.

We hypothesized that anthocyanidins may be potential natural topical therapeutics for CRS, either as T2R agonists or direct antimicrobials. Because plant compounds can have multiple effects on airway epithelial cells, we sought to investigate the effects of various representative anthocyanidins on primary human epithelial cells and common sinonasal pathogens, P. aeruginosa and S. aureus. In addition to microbiologic assays, we used optical imaging of air-liquid interface (ALI) cultures of primary human sinonasal cells isolated from residual surgical material, a state-of-the-art in vitro model for testing epithelial cell responses to novel compounds. Analysis of the results of this study emphasizes the
importance of testing multiple parameters of epithelial cell responses when screening candidate compounds in vitro, which may help to identify and prioritize the best compounds for subsequent in vivo testing.

METHODS

Solutions and Reagents

Anthocyanidins (Fig. 1) were obtained from Cayman Chemical (Ann Arbor, MI). Stock solutions of chemicals were dissolved in dimethyl sulfoxide before dissolving in Dulbecco’s phosphate-buff- ered saline (PBS), with the highest working concentrations re- flecting no more than 0.1% dimethyl sulfoxide, a level that does not affect sinonasal ALIs.19,20,42,43 In the experiments, PBS was primarily used on the apical side and Hank’s Balanced Salt Solution (which contained 10 mM HEPES and Minimal Essential Medium amino acids) basolateral- ly, as described elsewhere.19,20,42–44 4-Amino-5-methylamino-2,7’-difluorescein (DAF-FM) was from Life Technologies, Inc. (Carlsbad, CA). All other reagents were from Sigma-Aldrich (St. Louis, MO).

Sinonasal ALI Cultures

ALIs were prepared as described elsewhere19,20,43–46. Patients were recruited from the Department of Otorhinolaryngology—Head and Neck Surgery, University of Pennsylvania, with full institutional re- view board approval (protocol 800614, “Pathogenesis of Chronic Rhinosinusitis”). Informed consent was obtained during the preoperative clinic visit or in the preoperative waiting room. The criterion for patient selection was those patients undergoing sinonasal surgery. Criteria for exclusion were a history of systemic diseases (Wegener’s granulomatosis, cystic fibrosis, sarcoid, immunodeficiencies) and the use of antibiotics, oral corticosteroids, or antibiologics (e.g., omalizumab) within 1 month of surgery.

ALIs were established from sinonasal epithelial cells enzymically dissociated from residual surgical material and grown in proliferation medium that consisted of Dulbecco’s Modified Eagle’s Medium (Invitrogen/Life Technologies, Inc.)/Nutrient Mixture F-12 Ham and bronchial epithelial basal medium (Lonza Group, Basel, Switzerland) for 7 days. Cells were then trypsinized and seeded on Transwell cell culture inserts (6–7 × 10^4 cells per membrane; Corning Inc., Corning, NY) coated with bovine serum albumin, bovine collagen, and fibronectin. Five days later, the culture medium was removed from the upper compartment and the epithelium was allowed to differentiate in medium that consisted of 1:1 Dulbecco’s Modified Eagle’s Medium and bronchial epithelial basal medium (Lonza Group) with human epidermal growth factor, epinephrine, bovine pituitary extract, hydrocortisone, insulin, triiodothyronine, and transferrin, with penicil- lin, streptomycin, retinoic acid, and fetal bovine serum in the basal compartment.

Low-Light–Level Live-Cell Fluorescence Imaging of Intracellular NO Production

Cells were loaded with DAF-FM as described elsewhere19,20,43,44,47 by incubation in 10 μM DAF-FM diacetate and 5 μM 2-(4-carboxy- phenyl)-4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, a cell-per- meant NO scavenger to prevent NO production during loading. After 30 minutes, cultures were washed with PBS to remove 2-(4-carboxy- phenyl)-4,5,5-tetramethylimidazoline-1-oxyl-3-oxide and unloaded DAF-FM, followed by 15 minutes of incubation to allow for dye retention. Images were acquired on an IX-81 microscope (×10, 0.3 NA, UPlanFLN objective; Olympus, Tokyo, Japan) at 5-second intervals by using a 488 laser and Fluoview confocal system (Olympus). Because magnitudes of fluorescence changes were used to approximate NO production, care was taken to follow the loading protocol strictly to normalize dye loading, and microscope and/or software settings were identical for each experiment. No gain, offset, or gamma alterations were used.

High-Speed Digital Imaging of Ciliary Beat Frequency

Ciliary beat frequency (CBF) was performed as described elsewhere.49–51 Briefly, 100 μL of 0.5 McFarland P. aeruginosa (PAO1 or ATCC 27853) or S. aureus (methicillin-resistant strain M252) cultures in 50% lysogeny broth (LB) were added to a 96-well plate and grown for 48 hours statically at 37°C. Media were changed after 24 hours. The plates were washed two to three times with water, then stained with 1% crystal violet for 30–60 minutes, followed by a second wash. Stained biofilms were solubilized in crystal violet with 30% acetic acid and read in a spectrophotometer plate reader at 550 nm.

Biofilm Formation Assays

Crystal violet biofilm assays were carried out as described elsewhere.49–51 Briefly, 100 μL of 0.5 McFarland P. aeruginosa (PAO1 or ATCC 27853) or S. aureus (methicillin-resistant strain M252) cultures in 50% lysogeny broth (LB) were added to a 96-well plate and grown for 48 hours statically at 37°C. Media were changed after 24 hours. The plates were washed two to three times with water, then stained with 1% crystal violet for 30–60 minutes, followed by a second wash. Stained biofilms were solubilized in crystal violet with 30% acetic acid and read in a spectrophotometer plate reader at 550 nm.

Figure 1. Compounds tested in this study. Two anthocyanidins, delphinidin and cyanidin, were tested. Keracyanin, the glycosylated anthocyanin derivative of cyanidin, also was examined. The non–anthocyanidin flavonoid myricetin was used as a control.
Figure 2. Anthocyanins and anthocyanidins can stimulate sinonasal epithelial nitric oxide (NO) production. (A) Average traces of 4-amino-5-methylamino-2',7'-difluorescein (DAF-FM) fluorescence (n = 6 ALI cultures from three patients), showing increases in reactive nitrogen species (RNS) production in response to 9 and 90 μM delphinidin, an anthocyanidin. (B) Pharmacology of the delphinidin-induced response. DAF-FM fluorescence increases in response to 90 μM delphinidin (103 ± 23 units; n = 3 cultures from three patients) were inhibited in the presence of L-N^6^-nitroarginine methyl ester (L-NAME) (11 ± 7); n = 5 cultures from three patients; p < 0.01) but not D-N^6^-nitroarginine methyl ester (91 ± 13; n = 4 cultures from three patients; p = not significant [n.s.]), U73122 (90 ± 14; n = 3 cultures from three patients; p = n.s.), or U73343 (88 ± 17; n = 4 cultures from three patients; p = n.s.). (C) DAF-FM fluorescence increases in response to another anthocyanidin, cyanidin (90 μM; 112 ± 17; n = 5 cultures from three patients), were likewise inhibited by L-NAME (8 ± 1; n = 3 cultures from three patients; p < 0.01) but not U73122 (93 ± 9; n = 3 cultures from three patients; p = n.s.). (D) The anthocyanin keracyanin similarly caused an increase in RNS production at 3 μM (58 ± 10; n = 5 cultures from four patients) and 30 μM (107 ± 8; n = 7 cultures from four patients). Response to 30 μM was inhibited by L-NAME (8 ± 6; n = 3 cultures from three patients; p < 0.01) but not U73122 (104 ± 12; n = 7 cultures from four patients; p = n.s.). For all bar graphs, **p < 0.01 compared with control (first bar) by one-way analysis of variance with the Dunnett posttest.
Anthocyanidins Trigger Sinonasal Epithelial Cells to Produce NO

NO generation can be activated by receptor-driven changes in intracellular calcium concentrations that stimulate NO synthase activation.19,42,56 Cellular NO production was imaged in real time by using DAF-FM, which reacts with NO-derived reactive nitrogen species to form a fluorescent benzotriazole.19 Treating the apical side of ALIs with delphinidin, a representative anthocyanidin found in Cabernet Sauvignon grapes,57,58 elicited DAF-FM fluorescence increases over ~5 minutes, which signaled production of NO (Fig. 2 A). This was confirmed by using the NO synthase inhibitor L-N^ω-nitroarginine methyl ester (L-NAME) and inactive stereoisomer D-N^ω-nitroarginine methyl ester. L-NAME, but not D-N^ω-nitroarginine methyl ester, blocked DAF-FM fluorescence increases in response to delphinidin (Fig. 2 B), which demonstrated that this indeed reflected NO production. However, the DAF-FM fluorescence responses were not blocked by phospholipase C inhibitor U73122 (Fig. 2 F). The inactive analog of U73122, U73343, also showed no effect (Fig. 2 B). The structurally similar cyanidin activated similar NO responses that were inhibited by L-NAME but not U73122 (Fig. 2 C).

Anthocyanidins are often found as glycosylated anthocyanins (Fig. 1). Keracyanin is the anthocyanin derivative of cyanidin, found in black currants and raspberries.59 To determine if anthocyanins have similar effects, we tested keracyanin on sinonasal ALIs. Keracyanin similarly stimulated NO production that was inhibited by L-NAME but not by U73122 (Fig. 2 D). In addition, delphinidin- and keracyanin-induced DAF-FM fluorescence responses were not dependent on the TAS2R38 gene, which encodes T2R38.60,61 There was no significant difference between cultures homozygous for the functional TAS2R38 PAV “taster” allele and those homozygous for the nonfunctional AVI “nontaster” allele (Fig. 3), which confirmed the lack of involvement of this taste receptor in these responses.

Anthocyanidins and Anthocyanins Do Not Impact Ciliary Beat Frequency

To determine if anthocyanin- and/or anthocyanidin-stimulated NO production results in increased ciliary beating, we imaged CBF

![Figure 3. T2R38 is not involved in anthocyanin- and/or anthocyanidin-stimulated nitric oxide (NO) production. 4-Amino-5-methylamino-2',7'-difluorescein (DAF-FM) measured NO production in human sinonasal epithelial cells in response to delphinidin or keracyanin stimulation did not differ between patient cultures homozygous for the functional “taster” TAS2R38 haplotype (PAV) or the nonfunctional “nontaster” haplotype (AVI). DAF-FM fluorescence increases were 104 ± 11 units (90 µM delphinidin, PAV/PAV; n = 6 cultures from three patients), 99 ± 13 units (90 µM delphinidin, AVI/AVI; n = 6 cultures from three patients), 111 ± 10 units (30 µM keracyanin; PAV/PAV; n = 6 cultures from three patients), and 109 ± 6 units (30 µM keracyanin; AVI/AVI; n = 6 cultures from three patients). Significance determined by one-way analysis of variance with the Bonferroni posttest comparing bracket-indicated conditions.](image-url)

![Figure 4. Delphinidin and keracyanin did not stimulate significant increases in ciliary beat frequency (CBF). Mean traces of normalized CBF are shown for delphinidin (A) (n = 6 cultures from three patients) and keracyanin (B) (n = 6 cultures from three patients). Responses to ATP, added at the end, confirmed the responsiveness and viability of cultures. ATP = adenosine 5'-trisphosphate.](image-url)
in vitro by using high-speed digital imaging. Changes in CBF were reported as a ratio of stimulated basal frequencies. No significant differences were observed when ALIs were stimulated with delphinidin (Fig. 4 A) or keracyanin (Fig. 4 B). Application of adenosine 5′-trisphosphate (ATP), a purinergic agonist and a positive control to assess responsiveness, induced a robust 40–50% CBF increase.

Anthocyanidins and Anthocyanins Have Minimal Effects on P. aeruginosa and S. aureus Growth

We sought to determine if anthocyanidins or anthocyanins affect biofilm or planktonic growth of P. aeruginosa or S. aureus, common sinonasal pathogens sensitive to sinonasal NO production.14 We tested two wild-type P. aeruginosa strains, ATCC 27853 and PAO1. Although biofilm mass appeared to be slightly reduced in the presence of both keracyanin and delphinidin, the results were not statistically significant (Fig. 5 A), which indicated that these compounds had minimal or no antibiofilm effects. We also tested these compounds on P. aeruginosa planktonic growth. No significant reduction of CFUs was observed with either delphinidin or keracyanin (Fig. 5 B). As a control, the potent gram-negative antibiotic colistin sulfate reduced CFUs by ~1000-fold (Fig. 5 B). Similarly, we tested biofilm and planktonic growth in methicillin-resistant S. aureus strain M2. There was no significant difference in biofilm mass in the presence of cyanidin or delphinidin, although there seemed to be a slight reduction in the latter. A small but statistically significant reduction was observed in the presence of keracyanin (Fig. 5 C). There was no significant difference in the percentage of S. aureus CFUs that remained in the presence of cyanidin, delphinidin, or keracyanin relative to an LB-only control (Fig. 5 D).

Figure 5. Anthocyanidins had minimal effects on Pseudomonas aeruginosa and Staphylococcus aureus planktonic and biofilm growth. (A) Bar graph showing P. aeruginosa normalized biofilm mass (PAO1) grown in the presence of LB-only (control) or LB that contained delphinidin or keracyanin at indicated concentrations. Although biofilm mass seemed to be slightly reduced, the values were not significant when compared with LB-only by one-way analysis of variance (ANOVA) with the Dunnett posttest. Each biofilm mass measurement represents the average of five experiments, with each experiment being the average of at least eight wells grown in a single 96-well plate. Normalized biofilm masses were 100 ± 6 (LB-only), 72 ± 7 (0.9 μM delphinidin), 71 ± 5 (9 μM delphinidin), 75 ± 5 (90 μM delphinidin), 88 ± 14 (0.3 μM keracyanin), 70 ± 6 (3 μM keracyanin), and 66 ± 4 (30 μM keracyanin). (B) Bar graph, showing planktonic growth, measured by a colony-forming unit (CFU) spotting and counting assay of two P. aeruginosa wild-type strains, PAO1 and ATCC 27853. CFUs were not significantly reduced in the presence of delphinidin or keracyanin at indicated concentrations (ANOVA with the Dunnett posttest). The potent gram-negative antibiotic colistin sulfate was included as a control; *p < 0.01. CFUs recovered for PAO1 were 15,571 ± 2119 (LB), 10,417 ± 1031 (0.9 μM delphinidin), 15,333 ± 1312 (9 μM delphinidin), 8722 ± 1370 (90 μM delphinidin), 5667 ± 623 (0.3 μM keracyanin), 9000 ± 816 (3 μM keracyanin), 11,000 ± 3391 (30 μM keracyanin), and 5 ± 3 (10 μg/mL colistin). CFUs recovered for ATCC 27853 were 22,000 ± 3536 (LB), 17,667 ± 4327 (0.9 μM delphinidin), 27,667 ± 4249 (9 μM delphinidin), 12,667 ± 4784 (90 μM delphinidin), 35,000 ± 3342 (0.3 μM keracyanin), 16,333 ± 1546 (3 μM keracyanin), 27,667 ± 5573 (30 μM keracyanin), and 6 ± 2 (10 μg/mL colistin). (C) Bar graph, showing S. aureus normalized biofilm mass grown in the presence of LB-only (control) or LB that contained cyanidin, delphinidin, or keracyanin at indicated concentrations. Normalized S. aureus biofilm masses were 100 ± 4% (LB), 97 ± 6% (9 μM cyanidin), 106 ± 5% (90 μM cyanidin), 94 ± 5% (90 μM delphinidin), 92 ± 2% (90 μM keracyanin), and 68 ± 9% (90 μM keracyanin). (D) Normalized S. aureus CFUs remaining were 100 ± 7% (LB), 108 ± 11% (0.9 μM cyanidin), 75 ± 22% (9 μM cyanidin), 105 ± 12% (90 μM cyanidin), 88 ± 11% (9.9 μM delphinidin), 728 ± 6% (9 μM delphinidin), 93 ± 4% (90 μM delphinidin), 116 ± 8% (0.9 μM keracyanin), 95 ± 4% (9 μM keracyanin), 111 ± 10% (90 μM keracyanin); *p < 0.05 by ANOVA with Dunnett post-test. LB = lysogeny broth.
Swimming requires intact flagellar function and the production of rhamnolipids,\(^5\) linked to airway infection,\(^6\) as well as 3-hydroxy-alkanoic acids.\(^5\) However, a plate-based swarming assay\(^6\) revealed that neither delphinidin, cyanidin, nor keracyanin caused reductions in bacterial swimming (Fig. 6A).

**Delphinidin and Keracyanin Promote Bacterial Swimming Mobility**

Swimming is a method of *Pseudomonas* motility that occurs near surfaces,\(^5,6,6\) is involved in chemotaxis,\(^6\) requires twitching\(^5\) and flagellar motility, and is implicated in pathogenesis\(^6,9,26\) and biofilm formation.\(^71,72\) Swimming is evidenced by an even colony spread, characteristic of uncooperative motion, on lower-agar plates.\(^5\) Delphinidin and keracyanin caused a statistically significant increase in swimming of both *P. aeruginosa* strains (*p < 0.01*) (Fig. 6, B and C). However, this was not statistically significant with cyanidin and the non–anthocyanidin flavonoid myricetin, which indicated that variation exists in terms of flavonoid stimulation of bacterial swimming.

**DISCUSSION**

Our results indicated that anthocyanidins stimulated sinonasal NO production, previously shown to have antibacterial effects,\(^19,21,22\) but not through bitter taste receptor stimulation. Although cyanidin can activate both T2R39 (effective concentration [EC], 32 \(\mu\)M; EC\(_{50}\) 187 \(\mu\)M\(^9\)) and T2R14 (EC, 250 \(\mu\)M\(^*)\), the lower EC observed in this study (90 \(\mu\)M) combined with the lack of inhibition by U73122 indicated that these effects of anthocyanidins are not mediated through T2Rs. Moreover, cells from patients who were homozygous for both functional (PAV) T2R38 and nonfunctional (AVI) T2R38 responded equally to delphinidin and keracyanin, which indicated that T2R38, a major T2R isoform in sinonasal cilia, was not involved. Phospholipase C\(_{\beta}\) is an essential downstream component of taste signal transduction,\(^1,2,3,5,16,19,20\) and the lack of an effect of U73122 here indicated that the anthocyanin-stimulated production of NO observed is likely not due to a taste receptor response. We previously demonstrated that U73122 inhibits NO downstream of the T2R38 taste receptor expressed in sinonasal cilia.\(^19,42\) The mechanism of this NO production remains unknown. We recently reported on a secreted *S. aureus* compound that likewise stimulated acute taste receptor–independent NO production.\(^42\) It remains to be determined if anthocyanidins activate this same pathway.

No mucociliary clearance, driven by ciliary beating, removes foreign debris, including bacteria, from the respiratory tract.\(^7\) NO can elevate CBF through activation of protein kinase G. It remains unclear why anthocyanidin-driven NO increases do not increase CBF. Possibilities include off-target effects, including changes in intracellular pH or protein kinase C activity\(^29\) to “cancel out” stimulatory effects of protein kinase G, or, perhaps the NO response is somehow uncoupled from downstream protein kinase G signaling. The lack of CBF increases, lack of inhibition of biofilm or planktonic growth or swelling, and promotion of bacterial swimming indicated that anthocyanidins may have less therapeutic benefit than compounds that stimulate both NO and CBF together (e.g., agonists that activate sinonasal T2R isoforms\(^9\)). Analysis of these data also indicated that anthocyanidins alone had minimal antibacterial effects against *Pseudomonas* and *Staphylococcus* biofilm or planktonic growth. Although a statistically significant reduction in *S. aureus* biofilm growth was seen with keracyanin, it is unclear whether this small decrease (<1 log) in vitro would result in clinically significant biofilm reduction in vivo. Cranberry proanthocyanidins, another flavonoid class, were previously shown to have antibiofilm properties\(^56\) and block *P. aeruginosa* swimming,\(^77\) which indicates that they may be more useful as therapeutics than anthocyanidins.

Analysis of these results indicates that in vitro screening of compounds under investigation for topical application in CRS, including
naturally occurring compounds, e.g., anthocyanins, against both human sinonasal cells in vitro and sinonasal bacteria can be useful for identification and prioritization of which compounds may have the most therapeutic benefit. Although a lack of effect in vitro (e.g., as with the lack of effects on ciliary beating observed in this study) does not necessarily mean a lack of benefit in vivo, the in vitro testing of two of the most common natural CRS therapeutics, Sinupret (Bionorica SE, Neumarkt in der Oberpfalz, Germany)78,79 and GeloMyrtol (G. Pohl-Boskamp GmbH & Co. KG, Hohenlockstedt, Germany),80 demonstrated significant in vitro enhancement of components of sinonasal innate immunity. In vitro screening may also be useful to identify compounds with off-target effects that may increase bacterial virulence.

CONCLUSION

Although analysis of our data indicates that some anthocyanin or anthocyanidin compounds activate a potentially beneficial NO response from sinonasal cells, the same compounds can stimulate activation of a possibly pathogenicity-enhancing response in P. aeruginosa. Further study is required to determine (1) whether this bacterial response would offset the potential beneficial responses of anthocyanins and/or anthocyanidins in vitro and (2) which specific anthocyanidin isoforms might stimulate the most efficacious host NO production without modulating bacterial physiology. However, therapeutic effects of anthocyanins and/or anthocyanidins nonetheless may be reduced by their lack of a net effect on cilia. Analysis of these data indicated that in vitro screening of compounds against multiple parameters of epithelial and bacterial physiology may allow prioritization of classes of compounds for in vitro testing by identifying which compound isoforms activate the most beneficial responses.

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