P-glycoprotein inhibition with verapamil overcomes mometasone resistance in Chronic Sinusitis with Nasal Polyps*

Maie S. Taha[1,2,3], Angela Nocera[1,2], Alan Workman[1], Mansoor M. Amiji[2], Benjamin S. Bleier[1]

[1] Department of Otolaryngology, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, United States of America
[2] The Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, MA, United States of America
[3] The Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Rhinology 59: 2, 205 - 211, 2021
https://doi.org/10.4193/Rhin20.551

***Received for publication:**
October 26, 2020
Accepted: December 6, 2020

Abstract

Background: P-glycoprotein (P-gp) is a membrane efflux pump which is overexpressed in Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) and promotes Type 2 inflammation. Glucocorticoids (GC) are substrates of P-gp suggesting that overexpression may additionally contribute to GC resistance in CRSwNP. This study aims to determine whether P-gp inhibition using verapamil enhances mometasone retention and efficacy in nasal polyp explants.

Methodology: IRB approved study in which organotypic polyp explants were exposed to mometasone (4.15 µg/mL) and verapamil (125 µg/mL) as mono and combination therapy. The effect of verapamil on mometasone tissue retention over time was determined using HPLC. The effect of verapamil on mometasone anti-inflammatory function was determined using ELISA for secreted IL-5. Groups were compared using Kruskal-Wallis test.

Results: P-gp expression strongly and significantly inversely correlated with mometasone retention 1hr after exposure, with a nearly 6-fold reduction in tissue retention between the lowest and highest P-gp expressing polyp explants. P-gp inhibition reversed this effect and significantly improved mometasone retention at 1hr relative to mometasone alone. The combination of mometasone and verapamil significantly reduced IL-5 secretion relative to vehicle control and outperformed either treatment alone.

Conclusions: Our study confirms that P-gp contributes to mometasone resistance. This P-gp mediated resistance was successfully reversed by addition of the P-gp inhibitor verapamil. Verapamil further significantly enhanced the anti-inflammatory effect of mometasone when given as a combination therapy.

Key words: Chronic Rhinosinusitis with Nasal Polyps, mometasone resistance, P-glycoprotein, verapamil

Introduction

Chronic Rhinosinusitis (CRS) represents a spectrum of diseases unified by the presence of chronic inflammation of the sinonasal mucosa. Glucocorticoids (GC) have long been a mainstay in the treatment of CRS [1-4] with the goal of broad, non-targeted, suppression of the various inflammatory pathways leading to the clinical disease state. Mometasone furoate, in particular, has emerged as one of most favorable topical GCs due to its high potency and low bioavailability [5]. While generally effective [6-9], it has been suggested that up to 50% of patients may demonstrate resistance to GCs [10] for reasons which have yet to be fully elucidated.

P-glycoprotein (P-gp) is transmembrane efflux pump which utilizes ATP hydrolysis to transport a wide range of substrates across the plasma membrane. Prior studies by our group have demonstrated that P-gp is locally overexpressed in the epithelium of CRS patients with Type 2 inflammation [11, 12] and that it is capable of regulating epithelial secretion of multiple pro-inflammatory cytokines [13-15]. Our lab has also shown that inhibition of P-gp improves prednisone retention in organotypic nasal polyp explants [16] raising the possibility that P-gp may participate in GC resistance through the active efflux of GC substrates. This

phenomenon has been previously reported among steroid resistant patients with Crohn's disease who overexpress P-gp within their intestinal epithelium [17].

Verapamil hydrochloride is a calcium channel blocker which binds to the alpha subunit of L-type voltage dependent calcium (Cav1) channels thereby blocking the influx of calcium ions into the host cell [18]. In addition to this function, verapamil was also one of the first inhibitors of P-gp to be identified in the 1980s [19]. Several studies, including by our group [14], have reported that verapamil is capable of modulating inflammatory responses in human T-cells, animal models of asthma, and nasal polyps [18, 20-23] through its P-gp inhibitory function.

Based on the confluence of these findings, we hypothesized that 1) mometasone furoate may be a substrate for P-gp and 2) overexpression of P-gp would result in reduced mometasone tissue retention and efficacy. The purpose of this study was to therefore test these hypotheses and to further determine whether P-gp inhibition using verapamil could reverse resistance to mometasone.

Methods

Materials

Mometasone furoate, verapamil hydrochloride, dexamethasone-21-acetate and CelLytic™ MT cell lysis reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Bronchial epithelial growth medium (BEGM) was purchased from Lonza (Basel, Switzerland). Pierce™ BCA Protein Assay Kit was purchased from ThermoFisher Scientific (Waltham, MA, USA). Human P-gp ELISA kit was purchased from Cedarlane (Burlington, NC, USA). Custom Human Cytokine Q-Plex Array was purchased from Quansys Biosciences (Logan, UT, USA). CytoTox 96® Non-Radioactive Cytotoxicity Assay was purchased from Promega (Madison, WI, USA). All solvents used were purchased from Fisher scientific and were HPLC grade.

Primary human mucosal sampling

Tissue sampling was approved by the Massachusetts Eye and Ear Institutional Review Board. All samples were taken from patients who had not been exposed to antibiotics or steroids for at least 4 weeks. Inclusion criteria included patients diagnosed with CRSwNP and healthy patients (i.e. controls) undergoing turbinate reduction surgery for non-inflammatory disease. Exclusion criteria included patients with ciliary dysfunction, autoimmune disease, cystic fibrosis, immunodeficiency and smoking. Among controls, additional exclusion criteria included the presence of allergy or asthma. Diagnostic criteria for asthma, aspirin-exacerbated respiratory disease (AERD), and allergic rhinitis were based on both clinical history and allergy testing. Not all patients were used in all experiments but rather within each results subsection the patients used were the same between control and experimental groups to maintain consistency.

Explant incubation for mometasone retention evaluation

Harvested polyps; from patients washed out of oral or topical steroid for 4 weeks, were immediately sectioned into 5-mm explants using standard biopsy punch (Integra™ Meltex™), taking care to maintain an intact epithelial layer in each explant as previously described [13]. Explants were individually placed in tubes containing 350 µL of hydrocortisone-free BEGM containing 0.5 µg/mL of *Staphylococcus aureus* enterotoxin B (SEB) and either mometasone alone or a combination of mometasone and the P-gp inhibitor, verapamil (2.08, 4.15 µg/mL and 125 µg/mL for mometasone and verapamil concentrations, respectively). Tubes were incubated at 37°C, 5% CO_2 for 30 minutes, media was then removed, and explants washed with BEGM to remove any surface-bound drug. Explants were then incubated in mometasone-free BEGM for 30, 60 or 120 minutes as washout periods. For the combination group, explants were washed out in BEGM containing 125 µg/mL verapamil to maintain the P-gp blockade. At the end of the washout period, explants were rinsed with BEGM and stored at -80°C for later analysis of mometasone concentration. Turbinate (control) tissues were sectioned and treated similar to polyps, with exception that the BEGM used for incubation did not contain SEB.

Quantification of mometasone retention and P-gp levels in explants

Explants were homogenized in 400 µL of cell lytic buffer and the homogenates (200 µL) were spiked with 1 µL of dexamethasone-21-acetate ethanolic stock, used as an internal standard for extraction. The spiked homogenates were extracted with 800 µL of ethyl acetate by vortexing for 15 min. The organic layer was separated by centrifugation at 4000 x g for 10 min at 4ºC, and 600 µL of it was transferred to new tubes and dried in air. Dried films were reconstituted in 75 µL of acetonitrile and analyzed with high performance liquid chromatography (HPLC) for mometasone concentration. Standards for HPLC analysis were prepared by spiking tissue homogenate (125 mg/mL in cell lytic buffer, from same patient's tissue) with mometasone (from standard stocks) to final concentration of 5, 2.5, 1.25, 0.63, 0.13, 0.08 and 0.04 µg/mL. Standards were treated similarly for extraction and analysis. The remainder of the tissue homogenates were centrifuged at 13000 x g for 20 min at 4°C and supernatants were collected for protein quantification using a BCA assay and for analysis of P-gp expression using an ELISA kit following the manufacturer's protocol.

Explant incubation for anti-IL-5 effect evaluation

Polyps (sectioned as above) were placed in tubes containing 350 µL of hydrocortisone-free BEGM containing 0.5 µg/mL of SEB and were incubated at 37°C, 5% CO_2. After 24 h, media was collected and stored at -80°C (Day 1) and replaced with 350 µL of BEGM containing 0.5 µg/mL of SEB and either mometasone

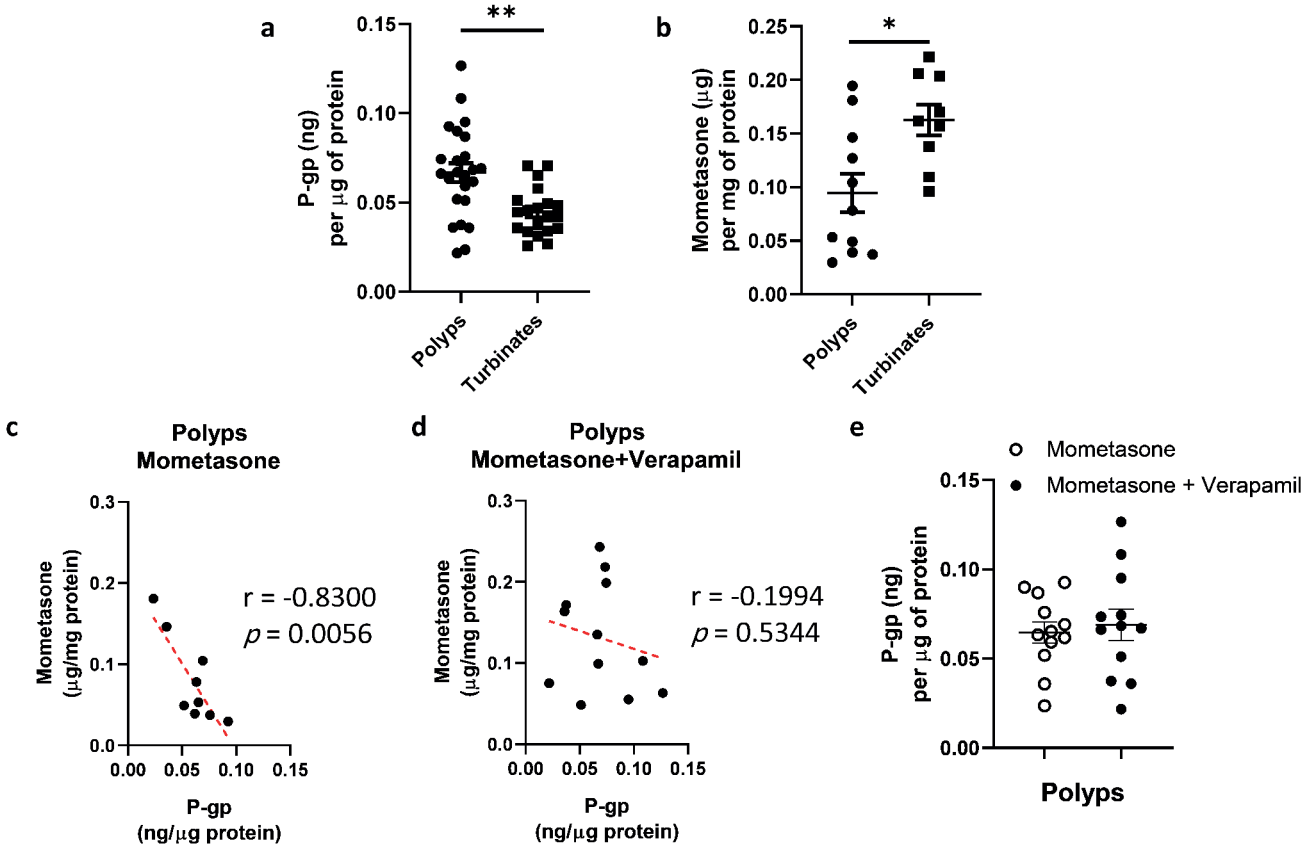

Figure 1. P-gp expression inversely correlates with mometasone retention. (a) P-gp expression in nasal polyp explants (n=24, 4 explants from each of 6 patients) versus control turbinate explants (n=20, 3-4 explants from each of 3 patients). (b) Mometasone tissue concentration after 60 min washout period. (c-d) Pearson correlation between P-gp expression and mometasone retention in nasal polyps following treatment with (c) mometasone alone (4.15 µg/mL), or (d) mometasone in combination verapamil (4.15 µg/mL and 125 µg/mL, respectively) after 60 min washout period. (e) P-gp levels within polyps with respect to the treatment condition demonstrating no change in treatment related expression. Data is presented as mean ± SEM. *, p <0.05 and **, p <0.01, unpaired two-tailed t-test.

or a combination of mometasone and verapamil (4.15 µg/mL and 125 µg/mL for mometasone and verapamil concentrations, respectively). After 24 h in the treatment condition, media was collected and stored at -80°C (Day 2) for later cytokine and cytotoxicity analysis. Explants incubated in media (BEGM or BEGM containing 0.5 µg/mL of SEB) with no treatment were included as controls. Secreted IL-5 was quantified in day 1 and 2 samples, using the Quansys Q-Plex array. Cytokine secretion in response to the different treatments was normalized to Day 1 secretion level and expressed as Day2/Day1 ratio to make direct comparisons between polyp samples as previously described [14]. The released LDH in the samples was assayed using CytoTox 96® cytotoxicity assay, as an indicator for cytotoxicity caused by the treatment groups.

Statistical analysis

All data is presented as means ± standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism 8 (La Jolla, CA, USA). Values falling outside 1.5 times the interquartile range of their respective data set were considered outliers and indiscriminately excluded from analysis as previously described [13]. Data were analyzed by Shapiro-Wilk test for normality, two-tailed student t-test, two-way ANOVA, Mann-Whitney test, Kruskal-Wallis test or Pearson correlation, as indicated. A p-value of <0.05 was considered statistically significant.

Results

P-gp Expression Inversely Correlates with Mometasone Retention

P-gp concentrations in the polyp explants were significantly higher than in control turbinate tissues (p<0.01, unpaired two-tailed t-test) (Figure 1a). This P-gp overexpression in polyps resulted in significantly lower tissue mometasone retention than in the lower P-gp expressing turbinates (p <0.05, unpaired two-tailed t-test) (Figure 1b). P-gp expression in polyp explants correlated significantly and inversely with mometasone reten-

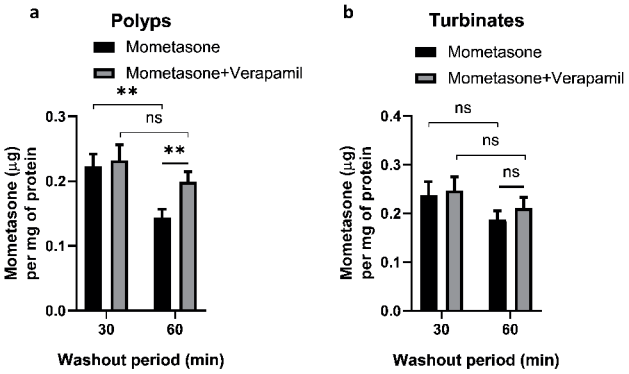

Figure 2. Verapamil enhances mometasone retention in organotypic polyp explants. Mometasone tissue concentrations in (a) polyp explants (minimum n=32, 4-6 explants from each of 6 patients) and (b) turbinate explants (minimum n=10, 5-6 explants from each of 2 patients), after 30 min exposure to either mometasone alone (4.15 µg/mL) or mometasone in combination with verapamil (4.15 µg/mL and 125 µg/mL, respectively), followed by washout. All data is presented as mean ± SEM. ns, non-significant and **, p <0.01, unpaired two-tailed t-test.

tion (Pearson's r= -0.8300, p = 0.0056). Polyps with high P-gp expression levels retained the least mometasone after a 60 min washout period (Figure 1c), with a 6-fold reduction in retention between the lowest and highest P-gp expressing polyp explants. This strong and significant inverse correlation was abrogated (Pearson's r= -0.1994, p = 0.5344) when mometasone was treated in combination with the P-gp inhibitor, verapamil (Figure 1d). There was no significant difference between P-gp levels in polyps exposed to either mometasone alone or in combination with verapamil (Figure 1e).

Verapamil enhances mometasone retention in organotypic polyp explants in a dose dependent manner

P-gp inhibition with verapamil did not influence the initial mometasone uptake in polyp explants, as indicated by mometasone tissue concentration after 30 min of washout. However, it resulted in significantly improved retention of mometasone in polyps over time (Figure 2a). Polyps exposed to mometasone only demonstrated mometasone efflux over 1 hour (30 min versus 60 min washout, p <0.01, unpaired two-tailed t-test). In contrast, co-treatment with verapamil maintained mometasone tissue concentration over the entire 60 min washout period and resulted in significantly higher tissue mometasone concentration as compared to the mometasone-only group (154% ± 52%, p <0.01, unpaired two-tailed t-test). Of note, verapamil did not influence mometasone retention in the control tissue (Figure 2b) at any of the washout time points. It is noteworthy that the significant enhancement in mometasone retention upon verapamil co-treatment was also observed at half the mometasone dose (156% ± 32%, p <0.05,Mann-Whitney test) (Figure 3a), resulting in a similar fold increase in mometasone level from the single treatment (Figure 3b). Increasing verapamil concentrations did not result in a concentration-dependent response (Figure 3c).

Verapamil significantly enhances the anti-IL-5 effect of mome-

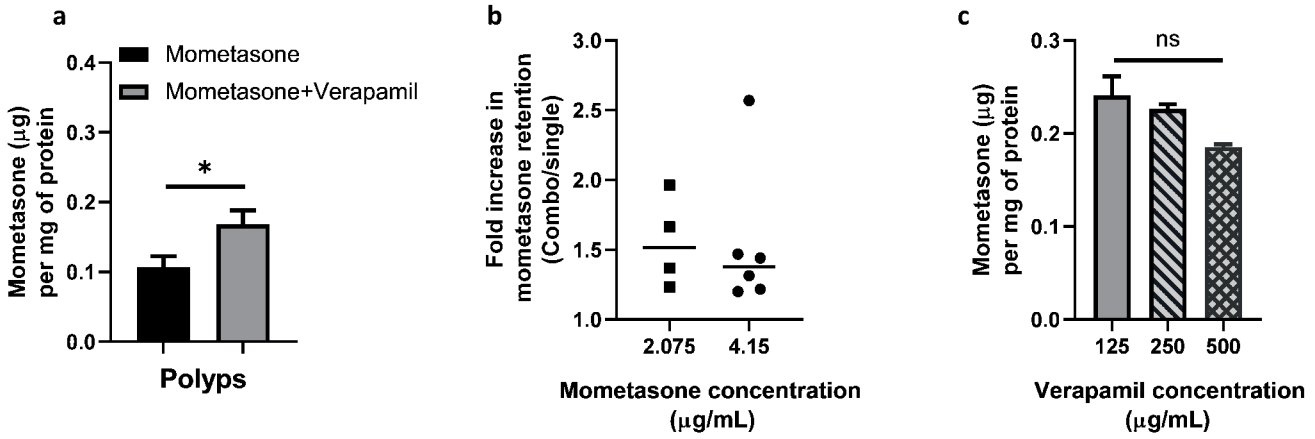

Figure 3. Dose response of mometasone retention in organotypic polyp explants. (a) Mometasone tissue concentration in polyp explants after 30 min exposure to either mometasone alone (2.075 µg/mL) or mometasone in combination with verapamil (2.075 µg/mL and 125 µg/mL, respectively), followed by 60min washout (minimum n= 20/group, 4-6 explants from each of 4 patients), (b) Relative fold change in verapamil (125 µg/mL) mediated mometasone retention in polyp explants by mometasone dose. (c) Mometasone retention in polyp explants (minimum n=4/group, 4-6 explants from 1 patient) upon co-treatment with 125, 250 or 500 µg/mL of verapamil for 30 min followed by 60 min washout period. All data is presented as mean ± SEM. ns, non-significant and *, p <0.05, Mann-Whitney test.

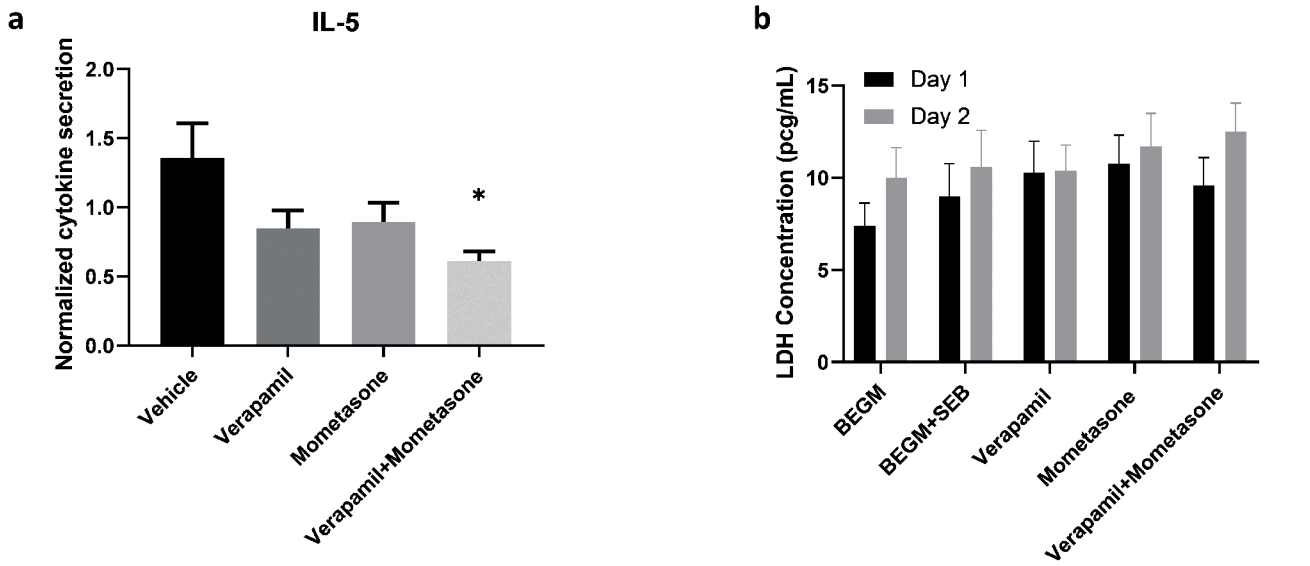

Figure 4. Verapamil significantly enhances the anti-inflammatory effect of mometasone. (a) Histogram demonstrating normalized IL-5 secretion from organotypic nasal polyp explants in response to mometasone (4.15 µg/mL) or verapamil (125 µg/mL) treatments both in isolation and in combination. Only the combination of verapamil and mometasone significantly decreased IL-5 secretion relative to control (* $p < 0.05$, Kruskal-Wallis test). (b) LDH assay demonstrating lack of cytotoxicity within all conditions relative to vehicle control (BEGM). Day 1 represents 24h incubation in BEGM + 0.5 µg/mL of SEB and Day 2 BEGM + 0.5 µg/mL of SEB + treatment condition.

tasone

We next tested the influence of P-gp inhibition on the anti-inflammatory effect of mometasone. Co-treatment of verapamil with mometasone for 24 h significantly decreased the secretion of IL-5 as compared to both the untreated explants and those treated with either monotherapy alone ($p < 0.05$, Kruskal-Wallis test) (Figure 4a), without inducing cytotoxicity (Figure 4b).

Discussion

Within the past decade, there has been an increased focus on topical GC therapeutic strategies for the management of CRS, both with and without Nasal Polyps [24-26]. Mometasone furoate has garnered particular interest due to its unique pharmacochemistry. The addition of halogen and chloride at positions 9 and 21 increases the compound's affinity for the corticosteroid receptor and decreases its susceptibility degradation while promoting hepatic metabolism [27]. Simultaneously, it has been recognized that a subset of patients with CRS exhibit resistance to GCs [10] thereby limiting the efficacy of even the most potent molecules.

P-glycoprotein has long been recognized as a mechanism for clearing GCs from the cytoplasm however this effect is highly dependent on the specific amino acid moieties within the molecule [28]. For example, dexamethasone, prednisolone and budesonide were found to have a high affinity for P-gp whereas triamcinolone acetonide did not [29]. Despite these findings, no studies specifically examined whether mometasone furoate acted as a substrate for P-gp. Given the established presence of P-gp overexpression in Type 2 endotypes of CRS [11, 12], we chose to study this phenomenon within a previously described organotypic nasal polyp explant model [13].

Using this approach, we first validated that within our sample set P-gp was overexpressed within nasal polyps as compared to healthy inferior turbinate controls. Inferior turbinate tissue was chosen on the basis of prior studies demonstrating minimal P-gp expression relative to sinus tissue; however, it is possible that use of a different control tissue could have impacted the results. We then demonstrated that the mean mometasone retention at 1 hour following exposure was statistically significantly reduced within nasal polyps as compared to low P-gp expressing inferior turbinates. While these results suggested mometasone was acting as a substrate for P-gp, we then confirmed this by correlating mometasone retention and P-gp expression within each individual explant. Using this approach, we found a 6-fold decrease in mometasone retention between the highest and lowest P-gp expressing explants, a value which would likely have significant implications for clinical efficacy.

We next sought to determine whether we could prevent this mometasone efflux by blocking P-gp activity. We elected to utilize verapamil given its established P-gp inhibitory activity [30] and its successful use in prior CRS clinical trials [23]. We first demonstrated that the co-administration of verapamil abrogated the inverse relationship between P-gp expression and mometasone retention within nasal polyps. This effect translated into a significant increase in tissue mometasone concentration within polyps co-treated with verapamil relative to those treated with

mometasone alone. Finally, our inflammatory assays confirmed that co-administration of mometasone with verapamil was superior with respect to reducing the canonical Type 2 cytokine IL-5 relative to either drug alone. One of the limitations of our study is the non-specificity of verapamil as a P-gp inhibitor. Some of our observed results could be attributable to off target effects related to verapamil's calcium channel blocking activity in addition to its role in inhibiting P-gp.

Conclusion

The results of these studies have important clinical implications with regards to the topical treatment of CRS using mometasone. Based on prior reports, we can infer that the patients with more severe Type 2 CRS endotypes will tend to have the highest levels of P-gp expression [12, 31]. This study therefore suggests that these patients will also tend to be the most resistant to topical mometasone therapy. While verapamil has previously been shown to be effective in reducing both subjective and objective indices of CRSwNP as a monotherapy, this data indicates that verapamil may also play in an important role in potentiating topical mometasone efficacy when given together.

Acknowledgement

We acknowledge funding from the Boston Biomedical Innovation Center and Massachusetts Eye and Ear Summit Grant.

Authorship contribution

MST: design of the work, acquisition, analysis, and interpretation of the data and drafting the work. AN: acquisition and analysis of the cytokine data. AW: analysis and interpretation of the cytokine data. MMA: conception of the work and revising it critically for important intellectual content. BSB: conception of the work and revising it critically for important intellectual content.

Conflict of interest

Conflict of Interest Statement: Dr. Benjamin S. Bleier has consultant relationships with Inquis Medical, Olympus, Medtronic, Karl Storz, Baxter, and 3D Matrix, receives royalties from Theime, on holds patents regarding treatments for CRS and drug delivery to the CNS.

References

1. Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, Brook I, Ashok Kumar K, Kramper M, et al. Clinical practice guideline (update): adult sinusitis. Otolaryngol Head Neck Surg. 2015;152(2 Suppl):S1-S39.
2. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. Rhinol. 2012;23:1-298.
3. Scadding GK, Durham SR, Mirakian R, Jones NS, Drake-Lee AB, Ryan D, et al. BSACI guidelines for the management of rhinosinusitis and nasal polyposis. Clin Exp Allergy. 2008;38(2):260-75.
4. Desrosiers M, Evans GA, Keith PK, Wright ED, Kaplan A, Bouchard J, et al. Canadian clinical practice guidelines for acute and chronic rhinosinusitis. J Otolaryngol Head Neck Surg. 2011;40 Suppl 2:S99-193.
5. Derendorf H, Meltzer EO. Molecular and clinical pharmacology of intranasal corticosteroids: clinical and therapeutic implications. Allergy. 2008;63(10):1292-300.
6. Vaidyanathan S, Barnes M, Williamson P, Hopkinson P, Donnan PT, Lipworth B. Treatment of chronic rhinosinusitis with nasal polyposis with oral steroids followed by topical steroids: a randomized trial. Ann Intern Med. 2011;154(5):293-302.
7. Rupa V, Jacob M, Mathews MS, Seshadri MS. A prospective, randomised, placebo-controlled trial of postoperative oral steroid in allergic fungal sinusitis. Eur Arch Otorhinolaryngol. 2010;267(2):233-8.
8. Van Zele T, Gevaert P, Holtappels G, Beule A, Wormald PJ, Mayr S, et al. Oral steroids and doxycycline: two different approaches to treat nasal polyps. J Allergy Clin Immunol. 2010;125(5):1069-76 e4.
9. Hissaria P, Smith W, Wormald PJ, Taylor J, Vadas M, Gillis D, et al. Short course of systemic corticosteroids in sinonasal polyposis: a double-blind, randomized, placebo-controlled trial with evaluation of outcome measures. J Allergy Clin Immunol. 2006;118(1):128-33.
10. Gurrola J, 2nd, Borish L. Chronic rhinosinusitis: Endotypes, biomarkers, and treatment response. J Allergy Clin Immunol. 2017;140(6):1499-508.
11. Bleier BS. Regional expression of epithelial MDR1/P-glycoprotein in chronic rhinosinusitis with and without nasal polyposis. Int Forum Allergy Rhinol. 2012;2(2):122-5.
12. Feldman RE, Lam AC, Sadow PM, Bleier BS. P-glycoprotein is a marker of tissue eosinophilia and radiographic inflammation in chronic rhinosinusitis without nasal polyps. Int Forum Allergy Rhinol. 2013;3(8):684-7.
13. Bleier BS, Singleton A, Nocera AL, Kocharyan A, Petkova V, Han X. P-glycoprotein regulates Staphylococcus aureus enterotoxin B-stimulated interleukin-5 and thymic stromal lymphopoietin secretion in organotypic mucosal explants. Int Forum Allergy Rhinol. 2016;6(2):169-77.
14. Bleier BS, Kocharyan A, Singleton A, Han X. Verapamil modulates interleukin-5 and interleukin-6 secretion in organotypic human sinonasal polyp explants. Int Forum Allergy Rhinol. 2015;5(1):10-3.
15. Bleier BS, Nocera AL, Iqbal H, Hoang JD, Alvarez U, Feldman RE, et al. P-glycoprotein promotes epithelial T helper 2-associated cytokine secretion in chronic sinusitis with nasal polyps. Int Forum Allergy Rhinol. 2014;4(6):488-94.
16. Kocharyan A, Feldman R, Singleton A, Han X, Bleier BS. P-glycoprotein inhibition promotes prednisone retention in human sinonasal polyp explants. Int Forum Allergy Rhinol. 2014;4(9):734-8.
17. Farrell RJ, Murphy A, Long A, Donnelly S, Cherikuri A, O'Toole D, et al. High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. Gastroenterology. 2000;118(2):279-88.
18. Khakzad MR, Mirsadraee M, Mohammadpour A, Ghafarzadegan K, Hadi R, Saghari M, et al. Effect of verapamil on bronchial goblet cells of asthma: an experimental study on sensitized animals. Pulm Pharmacol Ther. 2012;25(2):163-8.
19. Tsuruo T, Iida H, Yamashiro M, Tsukagoshi S, Sakurai Y. Enhancement of vincristine- and adriamycin-induced cytotoxicity by verapamil in P388 leukemia and its sublines resistant to vincristine and adriamycin. Biochem Pharmacol. 1982;31(19):3138-40.
20. Hashioka S, Klegeris A, McGeer PL. Inhibition of human astrocyte and microglia neurotoxicity by calcium channel blockers. Neuropharmacology. 2012;63(4):685-91.
21. Li G, Qi XP, Wu XY, Liu FK, Xu Z, Chen C, et al. Verapamil modulates LPS-induced cytokine production via inhibition of NF-kappa B activation in the liver. Inflamm Res. 2006;55(3):108-13.
22. Matsumori A, Nishio R, Nose Y. Calcium channel blockers differentially modulate cytokine production by peripheral blood mononuclear cells. Circ J. 2010;74(3):567-71.
23. Miyake MM, Nocera A, Levesque P, Guo R, Finn CA, Goldfarb J, et al. Double-blind placebo-controlled randomized clinical

trial of verapamil for chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol. 2017;140(1):271-3.
24. Forwith KD, Chandra RK, Yun PT, Miller SK, Jampel HD. ADVANCE: a multisite trial of bioabsorbable steroid-eluting sinus implants. Laryngoscope. 2011;121(11):2473-80.
25. Douglas RG, Psaltis AJ, Rimmer J, Kuruvilla T, Cervin A, Kuang Y. Phase 1 clinical study to assess the safety of a novel drug delivery system providing long-term topical steroid therapy for chronic rhinosinusitis. Int Forum Allergy Rhinol. 2019;9(4):378-87.
26. Sindwani R, Han JK, Soteres DF, Messina JC, Carothers JL, Mahmoud RA, et al. NAVIGATE I: Randomized, Placebo-Controlled, Double-Blind Trial of the Exhalation Delivery System With Fluticasone for Chronic Rhinosinusitis With Nasal Polyps. Am J Rhinol Allergy. 2019;33(1):69-82.
27. Szefler SJ. Pharmacokinetics of intranasal corticosteroids. J Allergy Clin Immunol. 2001;108(1 Suppl):S26-31.
28. Mares-Samano S, Badhan R, Penny J. Identification of putative steroid-binding sites in human ABCB1 and ABCG2. Eur J Med Chem. 2009;44(9):3601-11.
29. Webster JI, Carlstedt-Duke J. Involvement of multidrug resistance proteins (MDR) in the modulation of glucocorticoid response. J Steroid Biochem Mol Biol. 2002;82(4-5):277-88.
30. Bleier BS, Nocera AL, Iqbal H, Hoang JD, Feldman RE, Han X. P-glycoprotein functions as an immunomodulator in healthy human primary nasal epithelial cells. Int Forum Allergy Rhinol. 2013;3(6):433-8.
31. Nocera AL, Meurer AT, Miyake MM, Sadow PM, Han X, Bleier BS. Secreted P-glycoprotein is a noninvasive biomarker of chronic rhinosinusitis. Laryngoscope. 2017;127(1):E1-E4.

Maie S. Taha, PhD
Kasr El-Aini St.
Faculty of Pharmacy
Cairo University
Cairo, 11562
Egypt

E-mail:
maie.shaaban@pharma.cu.edu.eg