Mometasone Absorption in Cultured Airway Epithelium

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Abstract

Topical mometasone is frequently used as an intranasal spray, on drug eluting stents, and compounded by specialty pharmacies as a sinus rinse. A typical sinus rinse contains 1.2 mg of mometasone dissolved in 240 mL of buffered saline and is flushed through the sinonasal cavity. The mometasone irrigation rapidly flows to the contralateral sinonasal cavity or the nasopharynx with a contact time on the order of 5–10 seconds. However, no information is available on the absorption rate of topical mometasone on the sinonasal surface. To determine the absorption characteristics of mometasone, we harvested nasal epithelium from 2 healthy donors and differentiated them into a mature ciliated epithelium on Millicell membranes. We applied mometasone to the apical surface at various time points and then rinsed off non-absorbed mometasone with phosphate buffered saline. Millicell membranes with the adherent epithelial cells were then harvested and stored in guanidine hydrochloride for quantification using a high-performance liquid chromatography-mass spectrometry method. Fifty percent of the maximal absorption occurred 37.8 minutes after application, and maximal absorption occurred after 2 hours. Our data provide an estimate for rates of absorption of mometasone applied to the sinonasal cavity and suggest that the absorption rates poorly match contact time during saline lavage.

Keywords

topical steroids; chronic rhinosinusitis; mometasone

INTRODUCTION:

Chronic rhinosinusitis (CRS) is defined as symptomatic inflammation of the sinonasal cavity for more than 12 weeks and is a clinical diagnosis based on the presence of 2 of the following symptoms: mucopurulent drainage, nasal obstruction, facial pain/pressure/fullness or decreased sense of smell, in addition to purulent mucus, nasal polyps, or radiographic evidence of mucosal inflammation.[1,2] Recent evaluations of the medical and socioeconomic impacts of CRS estimate that the disease affects 2–16% of the US population and has an economic burden of greater than $60 billion annually.[3] Sinusitis accounts for nearly 5% of overall US health care expenditures.[3]

In addition to the financial implications, CRS substantially decreases quality of life, in a magnitude similar to or exceeding that experienced by patients with Parkinson's disease, congestive heart failure, or chronic obstructive pulmonary disease.[4] Despite the substantial burden of CRS, sufficient treatment options have yet to be identified.

Modern molecular-based therapies for CRS are lagging behind therapies for other airway diseases such as cystic fibrosis (CF) and asthma. For people with asthma, multiple immune modulators have come to market and more are in the drug discovery pipeline.[5] In CF, targeting apical ion channels has been shown to decrease disease burden and is hoped to increase life expectancy.[6] Despite these advances in lower airway diseases, little progress has been made in the medical management of CRS, which still relies on antibiotics and corticosteroids.

Corticosteroids are used to decrease inflammation and suppress the adaptive immune response.[7] Clinical trials have demonstrated symptomatic improvement in patients with CRS with nasal polyps while on oral steroids.[8] However, oral administration of steroids can result in severe adverse effects, including hyperglycemia, mania, glaucoma, cataracts, osteoporosis, and avascular necrosis of the femoral head.[9]

Topical administration of corticosteroids via spray or lavage theoretically provides the benefits of steroids with fewer adverse effects. Of the currently approved intranasal corticosteroid sprays, only mometasone furoate (Nasonex; Merck, Kenilworth, NJ) is specifically approved for chronic sinusitis. Despite the indication for CRS, topical sprays have poor penetration in the sinuses, and deposition of spray-based intranasal steroids occurs primarily on the inferior turbinate and internal valve. [10] Because of the poor penetration of nasal sprays into the sinuses, many clinicians use high-volume steroid rinses formulated by compounding pharmacies. Although this treatment is not approved by the Food and Drug Administration, numerous studies have shown benefit of corticosteroid-containing rinses, [11–13] and a recent double-blind, randomized control trial demonstrated improved symptoms from mometasone rinses compared with mometasone nasal spray in the postoperative setting.[14]

Mometasone furoate has high affinity for the glucocorticoid receptor and has minimal systemic absorption.[15] It is highly lipophilic, which helps facilitate absorption into the epithelium and has a low systemic bioavailability, which is desired to reduce systemic side effects.[16] Mometasone furoate has an estimated systemic bioavailability of <1% while

fluticasone propionate has a systemic bioavailability of ~17%.[17] These properties make mometasone furoate ideal for topical sinonasal application, and it is one of the most commonly used steroids for saline lavage.

While it is understood that a significant portion of the medication will flow through the sinonasal cavity and be lost to the drain, the rate of absorption of mometasone on the sinonasal epithelium is unknown. We developed a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) method to quantitatively determine the amount of mometasone absorbed in the cultured airway epithelium.

METHODS:

Human Nasal Epithelial Air-Liquid Interface (ALI) Cultures:

A middle turbinate (donor 1) that was excised as part of a transsphenoidal approach was obtained from a patient with no evidence of inflammatory sinus disease using IRB protocols #03–1396. Curettage (donor 2) of the inferior turbinate was performed to obtain second nasal epithelia cell line from a healthy donor using IRB protocols #98–1015.

The middle turbinate was processed overnight in 0.1% protease XIV and DNAse solution. Protease was neutralized with fetal bovine serum (Sigma Aldrich; St. Louis, MO), and the turbinate was then scraped using a scalpel to isolate human nasal epithelial cells (HNECs). Cells were pelleted, counted, and expanded using the conditionally reprogrammed cell (CRC) method as previously described.[18,19] After expanding for one passage, cells were plated onto 12-mm diameter Millicell CM inserts (Millipore; Burlington, MA) at a density of 250,000 cells per insert.[20]

The inferior turbinate underwent curettage, and cells were pelleted, counted, and expanded using the CRC method as previously described. [18,19] After expansion, cells were passed and plated onto 12-mm diameter Millicell CM inserts at a density of 250,000 cells per insert.[20]

HNECs were washed with 1X phosphate buffered saline (PBS) and fed with ALI media[19] apically and basal-laterally for approximately 3 days. Once cells reached 90% confluence, they were only fed basal laterally to create an ALI culture, therefore facilitating differentiation into a mature ciliated epithelium (Figure 1). HNEC cultures were maintained until day 28 and then treated with mometasone or utilized for histology.

Mometasone Treatment:

Mometasone was diluted to 1.2 mg/240 mL in buffered saline (Standard Neil-Med packet in distilled H2O). HNECs were treated with 200 μL of mometasone solution for the indicated times. The mometasone exposure was stopped by washing the apical and basal surface of the membrane 3 times with PBS (200 μl apically and 1 mL on the basal surface). Membrane inserts were excised and placed in 200 μL of guanidine hydrochloride (6M). To ensure lysis, 3 freeze-thaw cycles were performed.

Membrane inserts without HNECs were treated with mometasone and incubated for 24 hours prior to washing exactly as in the experimental group.

Non-Specific Absorption Controls:

Mature HNEC ALI cultures were either washed three times with PBS then fixed in 4% paraformaldehyde or fixed without washing (unwashed; Figure 4). Cells were then permeabilized and blocked. Slides were made from these cell blocks. The slides were then incubated in primary antibodies against MUC5AC (45M1, ThermoFisher; Waltham, MC) and MUC5B (in-house rabbit anti-human polyclonal antibody). After washing, slides were incubated with appropriate fluorescent secondary antibodies (Jackson ImmunoResearch; Westgrove, PA) and chemical stains for filamentous actin (phalloidin) and nuclei (Hoechst).

Four single-layer x-y images of the apical surfaces of each condition were acquired in triplicate with an Olympus Fluoview 1000 microscope with a 60X oil objective. ImageJ[20] software was used to process images and quantify mucin.[21] MUC5AC and MUC5B were quantified using ImageJ and normalized to the unwashed Millicells (Figure 4).

HPLC-MS/MS Sample Preparation:

Lysed cells were treated with 25 μL of 200 ng/mL mometasone furoate-d_3 internal standard (IS) and vortexed for 15 s. One mL of methyl tert-butyl ether (MTBE) was added to each sample, and all samples were shaken for 30 min. Samples were centrifuged for 5 min at 5,000 rpm. The supernatant MTBE layer was collected and evaporated under vacuum. The extract was reconstituted in 200 μL of methanol, vortexed, and transferred to an auto-sampler vial.

HPLC-MS/MS Parameters:

Gradient-programmed mobile phase delivery was performed with Shimadzu LC-20AD HPLC pumps, and chromatographic separations were carried out on an Atlantis T3 C18 column (3 μm, 2.1 × 50 mm; Waters Corporation, Milford, MA) maintained at 40°C. A mobile phase flow rate of 0.3 mL/min was used. Mobile phase A was water (Optima LC-MS grade; Fisher Scientific, Fair Lawn, NJ) with 0.1% formic acid, and mobile phase B was acetonitrile (Optima LC-MS grade; Fisher Scientific, Fair Lawn, NJ) with 0.1% formic acid. The gradient program was mobile phase B, 40% (0 min), 80% (5 min), 95% (5.1–6 min), 40% (6.1–10 min). An injection volume of 10 μL was used for all samples, calibrators, and quality control (QC) samples. HPLC was coupled to the electrospray ionization (ESI) source of a PESciex 3000 triple quadrupole mass spectrometer. ESI voltage was set to +4 kV, nitrogen was used as the nebulizing gas (10 arb units), and the source was held constant at 400 °C. The mass spectrometer was operated in multiple-reaction monitoring (MRM) mode with nitrogen collision gas (10 arb units). The de-clustering potential was 62 V for all MRM transitions. One quantifying and 2 qualifying MRM transitions were monitored for both analyte and IS, each with a dwell time of 50 ms equating to a cycle time of 0.33 s. For mometasone furoate, the precursor ion to product ion transitions were $m/z\,521 \rightarrow m/z\,503$ (Quantifying, Collision Energy = 15 V), $m/z\,355$ (Qualifying, CE = 21), and $m/z\,279$ (Qualifying, CE = 26). For mometasone furoate-d_3, the precursor ion to product ion transitions were $m/z\,524 \rightarrow m/z\,506$ (Quantifying, Collision Energy = 15 V), $m/z\,355$ (Qualifying, CE = 30), and $m/z\,279$ (Qualifying, CE = 26). Stock solutions of mometasone furoate and mometasone furoate-d_3 were prepared in methanol (Optima LC-MS grade; Fisher Scientific, Fair Lawn, NJ). Six calibrators ranging from 1.5 to 100 ng/mL and QCs

were prepared in methanol, and 25 ng/mL IS was added to match the final IS concentration in samples (Figure 1). Any samples outside of the dilution range were diluted, and a dilution factor was applied to determine the concentration.

Data Analysis:

Time of 50% maximal absorption was calculated by non-linear regression assuming one site-specific binding (Prism 7; GraphPad Software, Inc)

RESULTS:

Using the method we developed, we were able to detect mometasone to quantities below 1 ng (Figure 2) with a high signal to noise ratio. Upon application of mometasone, 50% maximal binding occurred in donor 1 and donor 2 at 35.6 min (95% CI 20.3 – 63.1 min) and 40.0 min (95% CI 22.4 – 72.5 min) respectively. Maximal mometasone absorption in cells derived from donor 1 was 193.4 ng/mL (95% CI 154.7–258.1 ng/mL) and 221 ng/mL (95% CI 174 304.5 ng/mL) in donor 2 (Figure 3). After 107 minutes in donor 1 and 120 minutes in donor 2, 75% of the maximal mometasone was absorbed. In the first 30 seconds of contact only, 2.6% and 2.7% of the maximal absorption of mometasone occurred in donor 1 and donor 2, respectively. After 300 minutes of incubation with the mometasone solution, membrane controls had undetectable mometasone (Figure 3).

To determine whether mometasone binds non-specifically to the mucus layer of the cells, we quantified MUC5AC and MUC5B before washing the apical and basal surfaces 3 times with PBS (200 μL apically and 1 mL on the basal surface; Figure 4).Mucus was significantly decreased (p = 0.031) from 100% (SEM 14.96%) in unwashed HNECs to 1.804% (SEM 1.028%) in washed HNECs.

DISCUSSION:

Using a robust HPLC-MS/MS assay with an *in vitro* airway epithelial model, we were able to determine the quantity of mometasone within the airway epithelium. Our data show that mometasone is not instantly absorbed into the epithelium. In the 2 HNEC cell lines derived from different healthy donors, half of the maximal absorption occurred at an average of 37.8 minutes of contact, and 75% maximal absorption is not reached until after approximately 2 hours of contact. , Only 2.7% of maximal mometasone absorption is achieved during the initial 30 seconds of contact. By contrast, The primary bolus of a nasal rinse contacts the sinonasal surface for 5–10 seconds, a time-frame that does not allow the majority of mometasone to be absorbed into the epithelium

Our findings do not dispute the clinical benefits of mometasone rinses; however, they highlight the different time scales of mometasone rinse versus absorption. It is possible that a thin coating of mometasone and pooling of the rinse in surgically opened sinuses allows for substantial absorption over a much longer time period. It is also possible that a severely inflamed mucosa absorbs mometasone at a faster rate than our *in vitro* model.

Clinical benefits are noted with mometasone rinses, even with the kinetic discrepancy we have described. These findings suggest that if corticosteroid absorption during a sinonasal rinse were optimized, the clinical effects could be significantly enhanced with less drug. Furthermore, our study characterized a new HPLC-MS/MS method to quantify mometasone in cells that could be applied to nasal tissue to determine the physiological deposition of mometasone after topical sprays or irrigations in patients.

CONCLUSION:

This study demonstrates that the rate of mometasone absorption in epithelial tissue is dramatically longer than the contact time in a typical saline lavage. While we do not dispute the clinical results of mometasone irrigation and routinely utilize it in our patients, the methods developed here could be used to better understand the patterns of mometasone absorption in the sinonasal cavity and suggest that a corticosteroid with a better absorption profile may enhance the benefits for patients.

Acknowledgments

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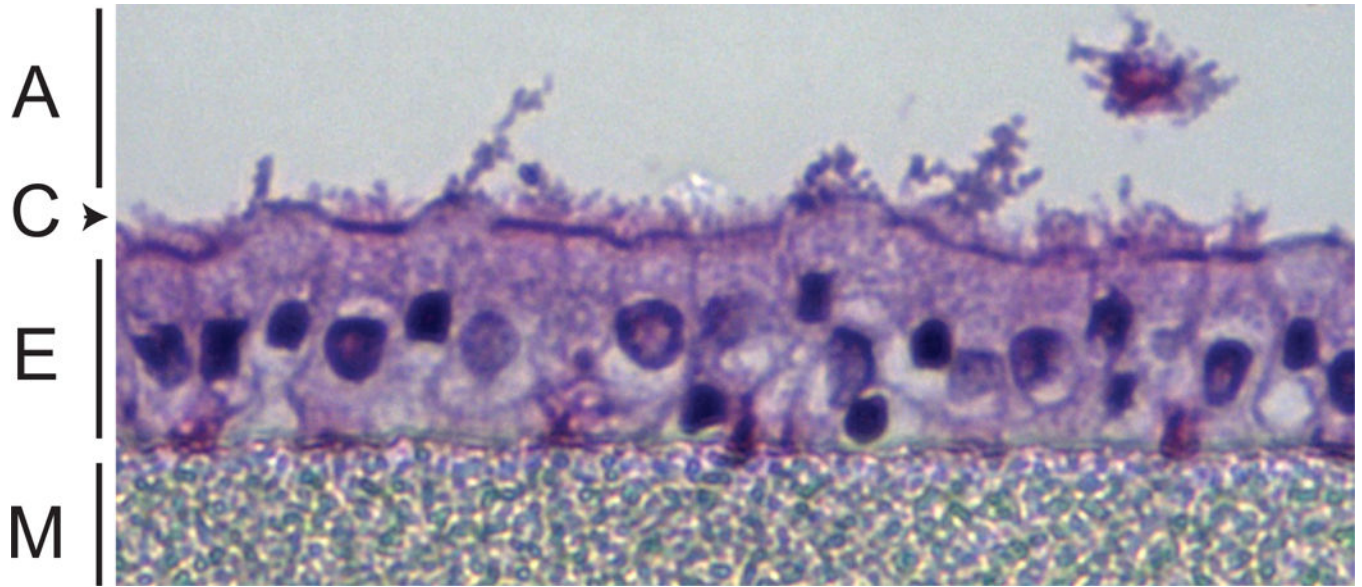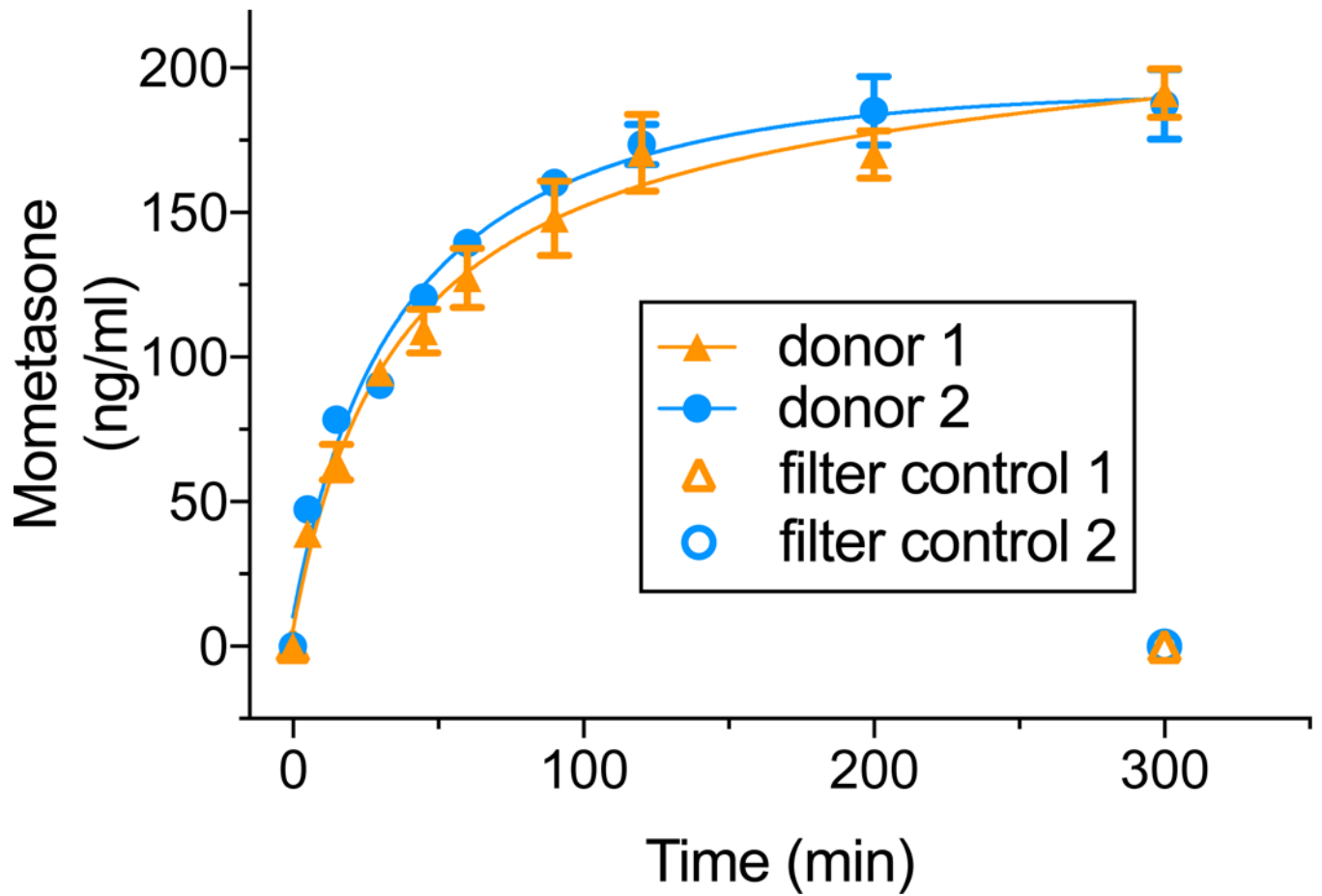
Figure 1.
Airway epithelial cells were grown on a semi-permeable membrane and differentiated into a ciliated epithelium. The apical surface of the epithelial layer is exposed to air overlying mucus and cilia. The cells are differentiated on a semi-permeable membrane. Mometasone is applied to the apical surface to mimic lavage by sinus rinse.
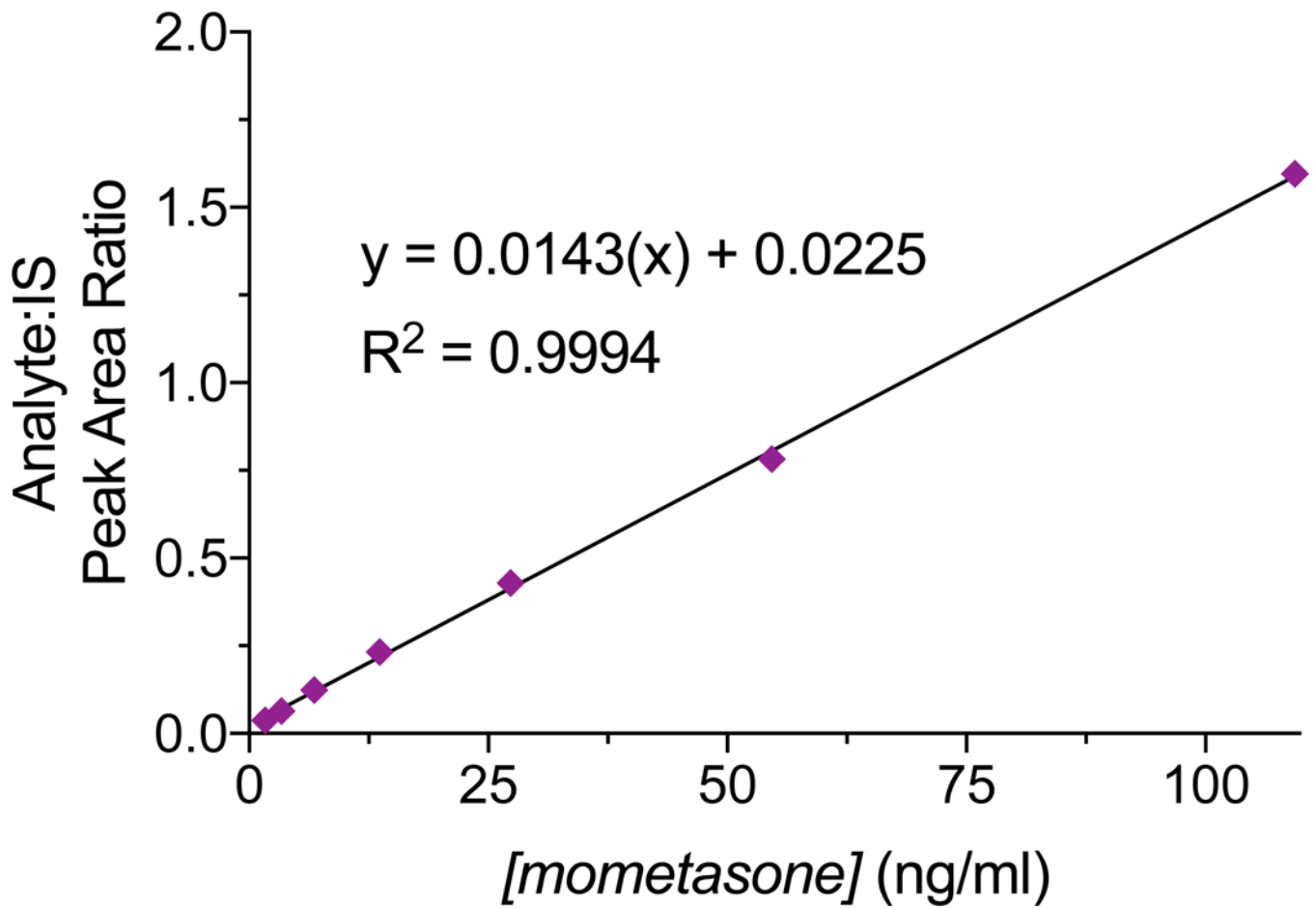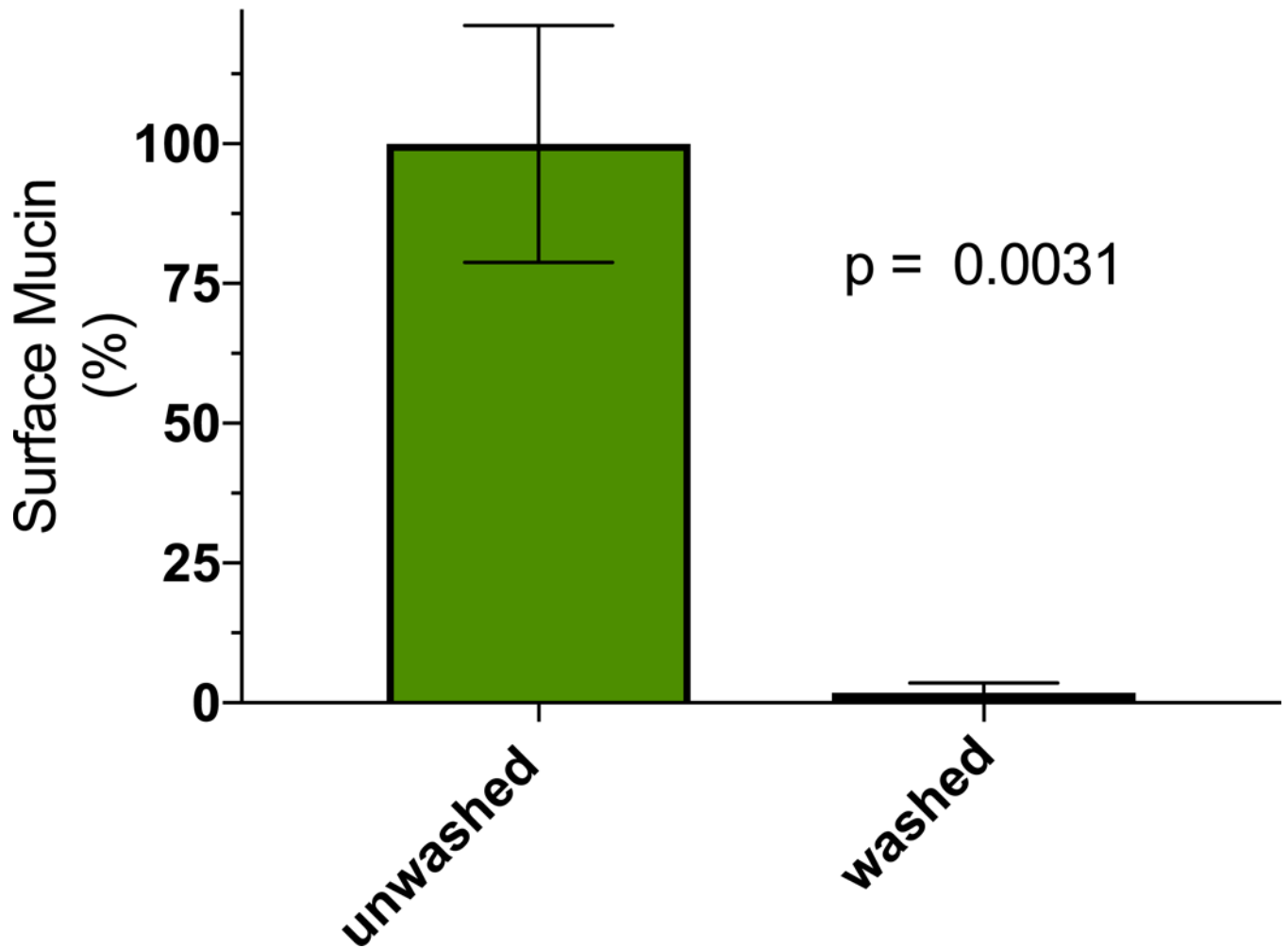
Figure 2.
Calibration curve for mometasone furoate as measured by analyte to internal standard peak area ratios from HPLC-MS/MS analysis.

Figure 3.
Mometasone is absorbed slowly on airway epithelium. Mometasone was applied to the apical surface of ciliated epithelium for the indicated time on 2 separate human nasal epithelial cell lines derived from healthy donors. The amount of absorbed mometasone was then quantified using LC-MS/MS. Non-specific binding to a Millicell insert without overlying cells was also determined.

Figure 4.

Washing human nasal epithelial cells removes the majority of mucus. Overall mucus was estimated by staining washed and unwashed HNECs for MUC5AC and MUC5B. There was significantly less mucus (p = 0.0031) after washing, with approximately 98% of mucus removed.