Comparison of Nasal Sprays and Irrigations in the Delivery of Topical Agents to the Olfactory Mucosa

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Objectives/Hypothesis: Sinonasal diseases are often treated with topical agents administered through various application techniques, but few prior studies have examined their distribution to the olfactory mucosa. The purpose of this study was to compare the distribution of nasal irrigations to sprays within the olfactory cleft.

Study Design: Human cadaveric study.

Methods: Eight cadaveric heads, providing a total of 15 nasal sides, underwent treatment with methylene blue solution. Application utilized a pressurized spray device followed by an irrigation squeeze bottle, both used according to manufacturer instructions. Videos and images from six standardized anatomical positions were recorded by rigid nasal endoscopy prior to and following each method of agent delivery. Using the acquired images, three reviewers blinded to the means of application scored the approximate surface area stained. An image-analysis program was additionally calibrated and used to measure pixel intensity in order to quantify surface delivery of methylene blue.

Results: Based on both blinded reviewer ratings and image pixel intensity measurements, irrigations demonstrated a greater extent and intensity of staining than sprays within the sphenethmoid recess, superior turbinate, and superior olfactory cleft (all $P < 0.05$). Sprays and irrigations, however, were comparable in the extent of staining at the nasal vestibule ($P > 0.05$), inferior turbinate ($P = 0.04$), and middle turbinate ($P > 0.05$).

Conclusions: Compared to sprays, irrigations provide a more effective method of delivering topical agents to the posterior and superior aspects of the nasal cavity. The thorough distribution of irrigations has important clinical implications for improving the delivery of therapeutic agents to the olfactory mucosa.

Key Words: Nasal sprays, nasal irrigation, olfactory mucosa, intranasal topical therapy.

Level of Evidence: N/A.

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INTRODUCTION

Medical management of sinonasal diseases increasingly involves the use of topical agents administered through techniques ranging from sprays and nebulizers to irrigations. Topical therapies offer an improved ability to deliver high concentrations of pharmacologic agents to the mucosa of the nasal cavity for localized, rather than systemic, effects. Given the significant role that topical therapies play in conditions such as chronic rhinosinusitis (C.R.S.) and allergic rhinitis (A.R.), immense interest in the efficacy of specific application techniques has become evident.1,2

Among intranasal topical therapies, saline irrigations have demonstrated improvements in the severity and duration of symptoms related to sinonasal diseases.3 The mechanism by which saline irrigations reduce sinonasal symptoms and improve quality of life may be partly attributed to the diffuse delivery of topical agents to the intranasal mucosa. Despite the uses of irrigations for a variety of sinonasal diseases, the distribution pattern of irrigations to the olfactory epithelium has not been fully characterized.

The goal of this study was to evaluate the effectiveness of irrigations in delivering topical agents to the human olfactory epithelium. It was hypothesized that irrigations provide a more extensive and intense delivery of solutions to the olfactory mucosa than do sprays.

MATERIALS AND METHODS

Cadaveric Specimen Selection

This study was conducted within the Department of Otolaryngology–Head and Neck Surgery at Northwestern University. Eight formaldehyde-preserved cadaveric heads were obtained from the Northwestern University Department of Physical Therapy and Human Movement Sciences. Because no identifying information was included with the specimens, this study was deemed by the institutional review board (IRB) at Northwestern University to be exempt from official IRB review.
Each cadaveric head was initially evaluated by nasal endoscopy using a 30-degree, 3.0-mm rigid endoscope (Karl Storz, Tuttingen, Germany) to assess for anatomical anomalies and gross pathology. Of the 16 nasal sides, 15 were included for treatment purposes. One side was excluded because of difficult endoscopic access secondary to a deviated septum.

Administration of Treatment Solutions
Sprays were simulated with a 4-mL pressurized spray device (delivered in an emptied azelastine spray bottle; Meda Pharmaceuticals Inc., Somerset, NJ). Application of irrigations utilized a 240-mL squeeze bottle (Sinus Rinse; NeilMed Pharmaceuticals, Santa Rosa, CA). Topical solutions prepared for sprays consisted of 0.75 mL of 10% sterile methylene blue (Akorn, Inc., Lake Forest, IL) in 20 mL of water, while the irrigation solutions consisted of 0.8 mL of 19% methylene blue in 240 mL of water.

Each cadaveric specimen underwent trials with methylene blue first applied with the spray device and then the irrigation squeeze bottle. For spray application, two sprays (0.137 mL/spray) were administered into each nare while the specimen was held in a forward-tilted position with sprays directed away from the septum, as is commonly recommended by nasal spray manufacturers. Following documentation of spray deposition with nasal endoscopy, irrigation solution was administered using the squeeze bottle with technique, as directed by the manufacturer. A total of 240 mL of solution was flushed through both nares of a single cadaveric head while it was held in a head-over-sink position. Runoff methylene blue solution was allowed to exit via the oropharynx and contralateral nasal cavity.

Endoscopic Data Collection
Endoscopic videos and still images were recorded at six standardized anatomical positions prior to and following each treatment application. These positions were the nasal vestibule, inferior turbinate, middle turbinate, sphenoid recess, superior turbinate, and superior olfactory cleft.

Semi-Quantitative Scoring of the Extent of Distribution
In order to facilitate data analysis, images of the nasal sides before and after methylene blue treatment were collated together for review. These series of images were sorted according to anatomical subsites, but were otherwise randomly ordered. Three reviewers blinded to the method of agent delivery scored these images, using an ordinal grading scale to rate the amount of surface area staining. The values for the grading scale were as follows: “1” for 0% to 20% of subsite surface area with staining, “2” for 21% to 40% of subsite surface area with staining, “3” for 41% to 60% of subsite surface area with staining, “4” for 61% to 80% of subsite surface area with staining, and “5” for 81% to 100% of subsite surface area with staining. The average scores from the three reviewers were taken to compare the extent of staining produced by sprays and irrigations. Examples of the observed gradation of staining in the middle turbinate specimens with their corresponding average scores are included (Fig. 1).

In addition to comparing the reviewer scores at individual subsites, data collected at the olfactory subsites, which specifically included the sphenoid recess, superior turbinate, and superior olfactory cleft, were also tallied and analyzed as a single group in order to determine results as a composite. Scores from the nasal vestibule, inferior turbinate, and middle turbinate were likewise tallied into one group to calculate the composite score for the anterior/middle subsites.

Quantitative Image Measurements of Staining Intensity
To quantify staining intensities following spray and irrigation deliveries, the endoscopic images were evaluated using a publicly available image-analysis program (ImageJ; National Institutes of Health, Washington, DC). All still images were normalized to a standard size (640 × 480 pixels) and separated into three 8-bit grayscale images grouped according to red, green, and blue channels. A representative circular and standardized (716 square pixels) region of interest was selected for each specimen subsite. For each region of interest, the mean gray value—a measure of the average pixel intensity within the indicated area—was determined in each of the separated color (red, green, blue) matrices.

Since the spectroscopic absorption profile of methylene blue selectively absorbs red wavelengths compared to blue wavelengths, the staining intensity was calculated as the ratio of the average pixel intensity in blue channels to red channels. A positive correlation between the blue-to-red ratio and the concentration of methylene blue was established using a calibration model utilizing white filter papers stained with five different volume concentrations of methylene blue (370 µL/mL, 37 µL/mL, 3.7 µL/mL, 0.37 µL/mL, 0.037 µL/mL). Collection and analysis of filter paper images utilized the same methodology as specimen images.

After calculating the intensity ratios of nasal subsites before and after methylene blue treatments, the ratios were

Fig. 1. Representative endoscopic images of various middle turbinate specimens following methylene blue treatment and their corresponding average reviewer scores. Images were taken with a 30-degree, 3.0-mm rigid endoscope. A score of “1” reflected 0% to 20% of surface area coverage as determined by three blinded reviewers. A score of “3” reflected 41% to 60% of surface area coverage. A score of “5” reflected 81% to 100% of surface area coverage. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
compared against the calibration model to determine the relative amount of methylene blue applied by each delivery method. Composite ratios and corresponding methylene blue concentrations were also calculated for the three anterior/middle subsites and the three olfactory subsites by tallying the data of the respective positions into combined groups.

**Statistical Analysis**

For individual and composite anatomical subsites, average reviewer scores and quantitative measurements of staining intensity were separately compared between sprays and irrigations using a 2-tailed $t$ test for continuous variables. A Bonferroni correction was applied to each of the calculated $P$ values to account for multiple comparisons in six subsites. Statistical significance was determined as $P \leq 0.05$. Calculations were performed with Microsoft Excel version 14.0 (Microsoft Corporation, Redmond, WA). A Krippendorff’s alpha coefficient was also determined with an online program (http://dfreelon.org/utils/recalfront/recal-oir/) to measure the interrater reliability among the three reviewers.

**RESULTS**

**Extent of Distribution: Ordinal Scale Rating Scores**

Representative images displaying the various degrees of intranasal deposition of methylene blue in the anterior/middle subsites (Fig. 2) and olfactory subsites (Fig. 3) of one specimen are included.

Based on average reviewer ratings, irrigations demonstrated a greater extent of distribution within all six anatomical subsites than sprays (Fig. 4), but a more widespread distribution of irrigations was particularly noted in the sphenethmoid recess (sprays: $1.9 \pm 0.8$, irrigations: $4.3 \pm 0.93$, $P < 0.05$), superior turbinate...
sprays: 1.8 ± 1.3, irrigations: 3.9 ± 1.3, \( P < 0.05 \), and superior olfactory cleft (sprays: 1.8 ± 1.3, irrigations: 4.3 ± 1.0, \( P < 0.05 \)). For the olfactory subsites as a composite, the average score for the spray group was 2.9 ± 1.2, while the irrigation group received a significantly higher average score of 4.3 ± 1.1 (\( P < 0.05 \)). Among the three reviewers, the alpha coefficient for interrater reliability was 0.76 for a total of 180 image sets.

The spray group was more comparable to the irrigation group in methylene blue distribution within the anterior/middle subsites. Specifically, no significant difference between sprays and irrigations existed at the nasal vestibule (spray: 4.5 ± 0.7, irrigation: 4.9 ± 0.2, \( P > 0.05 \)) and middle turbinate (spray: 3.5 ± 1.6, irrigation: 4.3 ± 0.9, \( P > 0.05 \)). In terms of the composite of anterior/middle subsites, the spray group received an average score of 4.0 ± 1.2, while the irrigation group scored an average of 4.6 ± 0.8 (\( P < 0.05 \)).

### Intensity of Staining: Image Quantification Analysis

Analysis of the calibration model revealed that increased methylene blue concentrations were strongly correlated with an intensification of the blue-to-red intensity ratio. The resulting calibration curve established a semilogarithmic relationship between the blue-to-red ratio and methylene blue concentration (Fig. 5).

Compared to sprays, irrigations had significantly higher average intensity ratios at all six individual subsites (\( P < 0.05 \)) (Fig. 6). There were also statistically significant differences between the methods in the composite intensity of anterior/middle and olfactory subsites.

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**Fig. 3.** Representative endoscopic images of a left-sided cadaveric nasal side at the olfactory subsites and during various stages of methylene blue treatment. At the sphenoethmoid recess, images were taken prior to treatment (A), following spray administration (B), and following irrigation application (C). At the superior turbinate, images were again taken prior to treatment (D), following spray administration (E), and following irrigation application (F). At the superior olfactory cleft, images were taken prior to treatment (G), following spray administration (H), and following irrigation application (I). The images of this cadaveric nasal side came from the same specimen as depicted in the images of Fig. 2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
The calibration curve was further used to convert staining intensity ratios at the different subsites into estimates of surface concentrations, as demonstrated in Figure 5. For the irrigation group, an average blue-to-red ratio of 1.2 at the olfactory composite correlated with a surface methylene blue concentration of 1.7 μL/L.

**Fig. 4.** Average reviewer scores (utilizing an ordinal scale ranging from 1 to 5) in cadaveric nasal specimens (n = 15) organized by individual and composite subsites, as well as by topical delivery techniques. Average reviewer scores and standard deviations were taken from a total of three reviewers blinded to delivery methods. Average reviewer scores were compared between the spray and irrigation groups using a 2-tailed t test, with statistical significance (P < 0.05) indicated.

The calibration curve was further used to convert staining intensity ratios at the different subsites into estimates of surface concentrations, as demonstrated in Figure 5. For the irrigation group, an average blue-to-red ratio of 1.2 at the olfactory composite correlated with a surface methylene blue concentration of 1.7 μL/L.

**Fig. 5.** Relationship of volume concentration of methylene blue used for staining (log μL/mL) and the resultant average staining intensity ratio (average pixel intensity in the blue channels-to-average pixel intensity in red channels) for the calibration model. The substrate for the calibration model was white filter paper. A total of five different volume concentrations was used for calibration purposes, with average intensity ratios taken for each stained filter paper a total of three times. The best-fit line has been included. Examples of two nasal specimens at the sphenoid recess and their corresponding average intensity ratios have been included on the best-fit line to show the relationship of image pixel measurements and methylene blue staining. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
mL. For the spray group, the average intensity ratio of 0.9 at the anterior/middle composite correlated with a surface concentration of 0.2 µL/mL. Extrapolating these values meant that irrigations delivered a surface concentration 7.1 times higher than that delivered by sprays.

**DISCUSSION**

Topical therapies are commonly used in the treatment of various sinonasal diseases. To date, the most popularly prescribed preparation of topical therapies is the nasal spray. Recent published studies, however, have highlighted the therapeutic benefits of nasal irrigations.6,7 Direct comparisons between sprays and irrigations have evaluated different outcome measures associated with their use, including symptomatic improvement and safety profiles.8 While there is strong evidence that irrigations are more effective than sprays in reaching the mucosa of the paranasal sinuses,9–11 no prior studies have specifically compared the relative distribution of sprays and irrigations within the olfactory subsites.

This study is thus the first in the literature to show the ability of irrigations to thoroughly deposit topical agents to the olfactory mucosa. In contrast, as demonstrated in prior studies12–14 and confirmed in this investigation, sprays are minimally successful in delivering topical solutions to the olfactory subsites. Irrigations and sprays, however, are comparably effective in delivering agents to the anterior and middle nasal cavity. The greater distributive efficacy of irrigations over sprays within the olfactory subsites may be explained by the administration of a large solution volume with a high positive pressure.

While prior investigations on the intranasal deposition patterns of topical agents have routinely utilized semiquantitative means to characterize agent staining,9,10,15 this study’s methodology is unique in its use of quantitative measurements with image pixel intensity to correlate with staining intensity. Recent studies by Bleier et al.16,17 have detailed a novel method of quantifying intranasal fluorescein concentrations by calculating image luminosity with a light filtration system and image graphics program. In this study, measurements of image pixel intensity were modeled after this previously described method, but the decision was made to use methylene blue as the staining agent instead of fluorescein in order to obviate the need for specialized, noncommercially available filters. With pixel intensity measurements in the split grayscale images, larger values of the blue-to-red ratio were expected to represent enhanced surface staining. Furthermore, the present methodology controlled for differences in illumination resulting from subtle differences in endoscope positioning during image acquisition.

Based on this investigation’s results, the staining intensity ratio provided consistent measurements of effective agent accumulation. The intensity ratio for untreated mucosal surfaces at all six subsites, for example, fell reliably within a tight range (0.79–0.83). Presence of visible agent accumulation in images also consistently resulted in intensity ratios that increased from the baseline values for untreated substrates. While the subjective measurements utilizing reviewer-based observations assessed...
surface area staining with good interrater reliability; objective measurements involving pixel intensity ratios also correlated well, validating both methods of intranasal staining assessment.

Calculations of staining intensity ratios additionally allowed for the correction of starting differences in methylene blue concentrations of the spray and irrigation groups. In the study, the larger volume of methylene blue was necessary for irrigations to provide visible staining. Each irrigation application started with 40 times as much methylene blue as a spray application (irrigation: 0.4 mL of 10% methylene blue, spray: 0.010 mL of 10% methylene blue). Irrigations, however, delivered surface concentrations 7.1 times higher than sprays to the olfactory subites. Given the initial concentration differences of spray and irrigation groups, this study establishes a relative concentration factor of 5.6 in irrigations in order to achieve surface isodose. The large applied volume of irrigation serves as a vehicle for topical agent delivery to the olfactory mucosa, but in return requires a larger concentration of agent than that needed for sprays.

While this study is the first to assess the success of irrigations in reaching the olfactory cleft, its findings supplement the few investigations that have used semiquantitative methods to evaluate access of various delivery techniques to the olfactory subsites (Table I). Scheibe et al.\textsuperscript{12} compared the distribution of a blue-dyed solution to the olfactory cleft in healthy adults, as applied by a syringe-based squirting system, pressurized sprayer, and pipette-based dropper. The squirting system was found to be more effective than the other two techniques in reaching the olfactory cleft. This study identified the squirting system as an innovative delivery method, but the method has yet to supplant more traditional application techniques, perhaps due to its difficulty of self-administration.

Rudman et al.\textsuperscript{13} used computed tomography in healthy participants to assess the deposition of contrast solution delivered by spray bottles and eye dropper bottles. This investigation showed that neither sprays nor drops reliably reached regions superior to the middle turbinate. In another study evaluating the sinonasal distribution of a powered nasal nebulizer, Manes et al.\textsuperscript{18} demonstrated in fresh-frozen cadavers that penetration into the middle meatus was significantly greater than into the olfactory cleft. The limited role of nebulizers in accessing the olfactory cleft was consistent in specimens before and after endoscopic sinus surgery.

The importance of effective drug delivery to the olfactory subsites is reflected in the limitations of medical management for sinonasal olfactory disorders. The various mechanisms of sinonasal pathology, including mucosal edema, epithelial inflammation, and mechanical obstruction from CRS, AR, and nasal polyposis are often attributed to instigating and exacerbating olfactory dysfunction. Corticosteroid therapy improves olfactory function by reducing the inflammatory reactions within the olfactory mucosa,\textsuperscript{19} but the most appropriate method of administration of therapy is debatable. While prolonged use of oral corticosteroid preparations is limited by systemic side effects, evidence suggests the anatomical constraints of the olfactory cleft hinder the use of spray applications.\textsuperscript{12,13,18} The olfactory cleft’s narrow configuration and small surface area make delivery of traditional aerosolized corticosteroid sprays technically difficult.

The extensive sinonasal access achieved by irrigations highlights the current and potential utility of irrigations in medical therapeutics. Recent in vitro investigations, for example, have found that irrigations containing topical mupirocin\textsuperscript{20} and diluted baby shampoo\textsuperscript{21} may have a role in treating recalcitrant CRS. While topical mupirocin at high concentrations eradicates biofilms produced by \textit{Staphylococcus aureus}, baby shampoo—a widely available surfactant—inhibits the formation of \textit{Pseudomonas aeruginosa} biofilms. Subsequent use of these products in CRS patients has furthermore improved subjective symptomatology scores.\textsuperscript{21,22}

The irrigation method may provide a future treatment modality for sinonasal olfactory dysfunction. Studies have already found that irrigations containing a mixture of saline and budesonide may be safe and effective topical alternatives to systemic corticosteroids and traditional steroid sprays for treatment of recurrent
nasal polyposis.23 No prior studies, however, have evaluated the effects of intranasal corticosteroid irrigations on olfactory function. Further investigation in this direction is warranted.

Despite the revealing findings in this original study, clinical generalizations are limited by the use of cadaveric specimens. In the cadaveric model, simulation of topical agent administration is affected by the inability to reproduce the mucociliary clearance and epithelial barriers characteristic of the physiologic state. Cadaveric studies also neglect important patient-related variables, which in the case of topical agent administration includes patient comfort and proper usage of delivery devices. Because these factors influence the eventual distribution of irrigations, future investigations involving participants with and without sinonasal diseases should better elucidate the clinical relevance of nasal irrigations.

An additional limitation inherent in this study's methodology resulted from the irreversible manner of methylene blue staining. Following administration of sprays, residual staining could not be entirely removed from the specimens with rinsing of water. Such a limitation is expected to overestimate the extent and intensity of the irrigation method as compared to the spray technique, but as noted in the included endoscopic images (Fig. 3), reviewer assessments, and quantitated pixel intensity, sprays did not effectively reach the olfactory subsites—the primary areas of interest for this investigation. The data obtained for the olfactory subsites thus reflect the distributive effects of squeeze bottle, as opposed to spray, delivery techniques.

CONCLUSION

This study demonstrated that irrigations, performed with large solution volumes and positive pressure, effectively deliver topical solutions to all intranasal regions, including the olfactory epithelium within the sphenethmoid recess, superior turbinate, and superior olfactory cleft. Irrigations provide a more extensive and intense distribution pattern along the olfactory mucosa when compared to sprays. However, since irrigations require a higher volume, a relative concentration factor of 5.6 is necessary to achieve a similar surface isodose to sprays. These findings suggest irrigations can potentially be utilized in the physician's armamentarium to improve clinical outcomes in olfactory disorders by enhancing the delivery of topical therapies to intended olfactory sites.

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BIBLIOGRAPHY