Received: 30 August 2023 | Revised: 20 November 2023 | Accepted: 11 January 2024

DOI: 10.1002/lio2.1219

ORIGINAL RESEARCH

Laryngoscope Investigative Otolaryngology

Inhaled fosamprenavir for laryngopharyngeal reflux: Toxicology and fluid dynamics modeling

Alexandra Lesnick BS[1] | Tina L. Samuels MS[1] | Donna Seabloom BS[2] | Beverly Wuertz BS[2] | Abhilash Ojha MS[3] | Davis Seelig DVM, PhD, DACVP[4] | Frank Ondrey MD[2] | Timothy S. Wiedmann PhD[5] | Chris Hogan PhD[3] | Emma Torii BVSc, MANZCVS, DACVP[4] | Hui Ouyang PhD[6] | Ke Yan PhD[7] | Guilherme J. M. Garcia PhD[1,8] | Jonathan M. Bock MD, FACS[1] | Nikki Johnston PhD[1,9]

[1]Otolaryngology and Communication Sciences, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

[2]Otolaryngology Head and Neck Surgery, University of Minnesota, Minneapolis, Minnesota, USA

[3]Mechanical Engineering, University of Minnesota, Minneapolis, Minnesota, USA

[4]Comparative Pathology Shared Resource, Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA

[5]Pharmaceutics, University of Minnesota, Minneapolis, Minnesota, USA

[6]Mechanical Engineering, University of Texas-Dallas, Dallas, Texas, USA

[7]Pediatrics Quantitative Health Sciences, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

[8]Biomedical Engineering, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

[9]Microbiology and Immunology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Correspondence
Nikki Johnston, Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, 9200 W Wisconsin Ave, Milwaukee, WI 53226, USA.
Email: njohnston@mcw.edu

Abstract

Objectives: Approximately 25% of Americans suffer from laryngopharyngeal reflux (LPR), a disease for which no effective medical therapy exists. Pepsin is a predominant source of damage during LPR and a key therapeutic target. Fosamprenavir (FOS) inhibits pepsin and prevents damage in an LPR mouse model. Inhaled FOS protects at a lower dose than oral; however, the safety of inhaled FOS is unknown and there are no inhalers for laryngopharyngeal delivery. A pre-Good Lab Practice (GLP) study of inhaled FOS was performed to assess safety and computational fluid dynamics (CFD) modeling used to predict the optimal particle size for a laryngopharyngeal dry powder inhaler (DPI).

Methods: Aerosolized FOS, amprenavir (APR), or air (control) were provided 5 days/week for 4 weeks ($n = 6$) in an LPR mouse model. Organs (nasal cavity, larynx, esophagus, trachea, lung, liver, heart, and kidney) were assessed by a pathologist and bronchoalveolar lavage cytokines and plasma cardiotoxicity markers were assessed by Luminex assay. CFD simulations were conducted in a model of a healthy 49-year-old female.

Results: No significant increase was observed in histologic lesions, cytokines, or cardiotoxicity markers in FOS or APR groups relative to the control. CFD predicted that laryngopharyngeal deposition was maximized with aerodynamic diameters of 8.1–11.5 μm for inhalation rates of 30–60 L/min.

Conclusions: A 4-week pre-GLP study supports the safety of inhaled FOS. A formal GLP assessment is underway to support a phase I clinical trial of an FOS DPI for LPR.

Level of Evidence: NA.

Portions of data herein were presented on July 7, 2016 at the 14th Biennial Phonosurgery Symposium and Modeling Laryngeal Biology Conference, Madison, WI, USA; on January 28, 2023 at the Triological Society Combined Sections Meeting, Coronado, CA USA; and on April 28, 2023 at the 30th Congress of Union of the European Phoniatricians, Antalya, Turkey.

Funding information
Medical College of Wisconsin, Department of Otolaryngology and Communication Sciences; Bank of America, N.A.: Dr. Ralphand Marian Falk Medical Research Trust; N-Zyme Biomedical Incorporated

KEYWORDS
computational fluid dynamics, dry powder inhaler, laryngopharyngeal delivery, laryngopharyngeal reflux, pepsin

1 | INTRODUCTION

Laryngopharyngeal reflux (LPR) is an inflammatory condition of upper aerodigestive tract tissues related to the backflow of gastroduodenal contents. The most prevalent symptoms of LPR include dysphonia, globus sensation, throat pain, odynophagia, accumulation of viscous mucus, throat clearing, and cough.[1] LPR can dramatically impact the quality of life and lead to serious health consequences such as airway stenosis, reactive airway disease, and laryngeal cancer.[2–6]

LPR is estimated to affect 10%–30% of the US population,[1,7–9] yet there is no gold-standard medical therapy. Although acid-suppressing proton pump inhibitors (PPIs) are the mainstay therapy for GERD, their efficacy for LPR is poor.[10] Multichannel intraluminal impedance pH-monitoring (MII-pH) has demonstrated that many episodes of LPR are nonacidic and gaseous, and that weakly or nonacidic LPR is often associated with persistent symptoms in acid-suppressed patients.[11–14] These symptoms are alleviated by anti-reflux surgery[15–21] and may be ameliorated by less invasive strategies that limit reflux occurrence or neutralize reflux constituents beyond acid (e.g., dietary and lifestyle modification and over-the-counter alginate products).[22–25] It is therefore reasonable to assume that one or more nonacid constituents of refluxate are responsible for LPR symptoms. Among nonacid components of refluxate, the digestive enzyme pepsin is considered a predominant damaging agent, biomarker, and therapeutic target for reflux-attributed diseases.[5,26–35] At the pH of the laryngopharynx (pH $\sim$ 6.8), pepsin is enzymatically inactive, whereas studies in experimental models demonstrate that it is endocytosed and retained in acidic endosomes in which its enzymatic activity would be restored. Exposure to pepsin leads to inflammatory and carcinogenic alterations irrespective of pH in vitro and in vivo including altered transcriptomic profiles; promotion of apoptotic resistance, cell migration, anchorage-independent growth, and glycolysis; and development of tumors in a hamster cheek model.[31,33–40]

We recently identified the protease inhibitor fosamprenavir (FOS; prodrug of amprenavir, APR), an FDA-approved therapy for HIV/AIDS, as a candidate therapy for LPR. FOS binds and inhibits pepsin in the low micromolar range and oral gavage of FOS at the manufacturer-recommended dose for HIV prevented inflammatory damage in an LPR mouse model.[41] Inhaled FOS provided similar protection at 1/20th of the oral dose.[41] Although oral FOS has a good safety profile, topical administration at a lower dose should reduce the potential for side effects. Such topical administration may be achieved through a dry powder inhaler (DPI). DPIs were originally developed to treat asthma and chronic obstructive pulmonary disease (COPD), delivering active drugs in powdered form through the oral cavity into the lungs. Currently, no commercial DPI has been developed specifically for drug delivery to the laryngopharynx. Aerodynamic particle size distribution (APSD), inhalation rate, and airway diameter are key determinants of the regional doses of inhaled drugs along the airway.[42,43] Current DPIs have drug particles with diameters smaller than 5 μm, which minimize drug deposition in the upper airway and maximize drug delivery to the lungs. The optimal particle size for drug delivery to the laryngopharynx has not been established yet. To develop aerosolized FOS for LPR, the toxicology of inhaled FOS requires evaluation and a device optimized for drug delivery to the laryngopharynx will need to be developed. Herein we evaluated inhaled FOS with a pre-Good Lab Practice (GLP) toxicity study and investigated the optimal particle size of FOS as a dry powder for laryngopharyngeal delivery using computational fluid dynamics (CFD) simulations in a model of the adult respiratory tract.

2 | METHODS

2.1 | In vivo toxicology

Experiments were approved by the University of Minnesota (UMN) Institutional Animal Care and Use Committee (protocol 2102-38881A) and utilized a previously validated LPR mouse model wherein mechanical wounding and instillation are used to directly injure the larynx.[41] The model reliably replicates epithelial alterations observed in patients with LPR and confers advantages over existing surgical animal models of reflux in which refluxate inconsistently reaches the laryngopharynx and the horizontally positioned upper digestive tract promotes liquid, as opposed to the gaseous refluxate typical of LPR.[7,41,44–48] The sham group of the LPR mouse model, which employs mechanical laryngeal wounding and instillation with saline, was utilized to examine the safety of inhaled FOS and APR herein (see treatment schema in Figure 1). In 6-week-old female Jackson A/J mice (Jackson Laboratory, Bar Harbor, ME) acclimated 1 week post-arrival, a laryngeal scratch was performed on days 2 and 9 by the same personnel to the anterior cricoid/tracheal cartilage and saline instilled 3 days/week for 4 weeks. FOS (Mylan Laboratories Limited, Hyderabad, Telangana, India) and APR (MedChemExpress LLC, Monmouth Junction, NJ) dry powder, and supermicrometer aerosols were generated and administered 5 days/week using a six-port exposure chamber (Intox); air alone was administered as control.[41] Details are provided in the Supporting information, including a description of the dry powder aerosolization, particle size distribution measurements, and mass exposure calculations. The average aerosol concentrations were 0.020 mg/L FOS and 0.016 mg/L APR; given the respiratory minute volume of mice (0.020 L/min), the inhaled mass of FOS and APR were 1.1 and 0.96 mg/kg/day, respectively.

FIGURE 1 In vivo treatment schema.

Mice were weighed, euthanized by CO_2, placed on their back, skin cut down the midline starting at the lower lip, and trachea exposed by separating fatty tissue containing salivary glands and submandibular lymph nodes. A small slit (~1 mm) was made on the exposed surface of the trachea. To collect bronchoalveolar lavage (BAL), a 1 mL syringe containing 0.5 mL DPBS (no calcium or magnesium, ThermoFisher Scientific, Waltham, MA) was connected to a dispensing tip (blunted syringe needle with a 45° bend), the tip inserted into the slit in the trachea, and a curved tip forceps used to grasp nearby fatty tissue, compressing trachea and extra tissue around the syringe tip to create a seal. The DPBS was slowly injected into the lungs, and then the syringe plunger was drawn back slowly to aspirate DPBS from the lungs. BAL was placed on ice. Whole blood was collected by severing major vessels in the subclavian space and pipetting pooled blood from the pleural cavity. Plasma was obtained by placing blood in EDTA tubes and incubating on ice. BAL and plasma were centrifuged to clear cells. Supernatants were collected. Plasma was diluted 1:10 in DPBS. Both were stored at −80 °C until analysis.

Organs were collected in 10% neutral buffered formalin for pathology including the tongue, larynx, trachea, esophagus, mediastinal tissue (containing thymus and tracheal lymph nodes), heart, lungs, liver, both kidneys, and head for nasal turbinates. Tissues in fixative were transported to UMN Comparative Pathology Shared Resource for analysis. Tissues were embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin. Slides were reviewed by two board-certified veterinary pathologists who were not blinded to treatment groups.

Toxicity markers were assessed via Luminex assay by Eve Technologies (Calgary, Alberta, Canada). BAL was analyzed using Mouse High Sensitivity T cell 18-Plex Discovery Assay and plasma-EDTA was analyzed using Mouse Cardiovascular Disease Panel 2 9-Plex Assay. Samples were run in duplicate in each assay and means were compared across groups by Student's *t*-test. Owing to the skewness of the data, the Mann–Whitney test was used to compare the groups. Results were considered statistically significant for $p < .05$.

2.2 | CFD simulations

A model of a healthy adult was built from a magnetic resonance imaging scan of a 49-year-old female with normal airway anatomy and no pathology as verified by an ear-nose-throat surgeon (J.B.; Figure 2A). The anatomical sites (oral cavity, pharynx, larynx, trachea, and main bronchi) were mapped on the walls to quantify the regional doses. CFD simulations of airflow and particle transport were performed for constant inhalation rates of 30, 45, and 60 L/min in ANSYS Fluent™ 14.0 (ANSYS Inc., Lebanon, New Hampshire), representing typical inhalation rates of adult patients using DPIs.

The laryngopharyngeal deposition fraction (i.e., the fraction of inhaled particles that deposit at the oropharynx and larynx) was plotted against the impaction parameter (IP, units of µm^2 L/min), a parameter that accounts for the effects of both particle size and inhalation rate; it is defined as $IP = d_a{}^2 Q$, where d_a is the particle aerodynamic diameter in µm and Q is the inhalation rate in L/min. The CFD simulations were validated by comparing the predicted extrathoracic dose (i.e., oral cavity, oropharynx, larynx, and trachea) with in vitro experiments reported by Stahlhofen et al., showing good agreement (Figure 2B).[42] The laryngopharyngeal deposition fraction was estimated assuming that DPIs generate aerosol clouds with a log-normal particle size distribution characterized by its mass median aerodynamic diameter d_{50} (i.e., half of the aerosol mass is contained in particles with $d_a \le d_{50}$) and geometric standard deviation σ_g, a measure of the width of the particle size distribution. Further details regarding CFD simulations and analysis methods may be found in Supporting information.

3 | RESULTS

3.1 | In vivo toxicology

One mouse from each control and FOS groups died; death in the control group suggests the cause was stress of procedures rather than the FOS drug, consistent with the absence of other signs of drug-specific toxicity. All mice, regardless of the treatment group, had a variable amount of predominantly perivascular to less frequently peribronchiolar and subpleural inflammatory foci. This varied from predominantly lymphoplasmacytic, to a mixture of lymphocytes, plasma cells, granulocytes, and histiocytes. Granulocytes were present in mice from each treatment group. One mouse from the control group had rare, mild, and predominantly mononuclear myocarditis. Other findings in other organs were thought to be background and/or incidental lesions. Cytokines (granulocyte-macrophage colony-stimulating factor, GM-CSF; interferon gamma, IFN-γ; monocyte chemoattractant protein-1, MCP-1; tumor necrosis factor-alpha, TNFα;

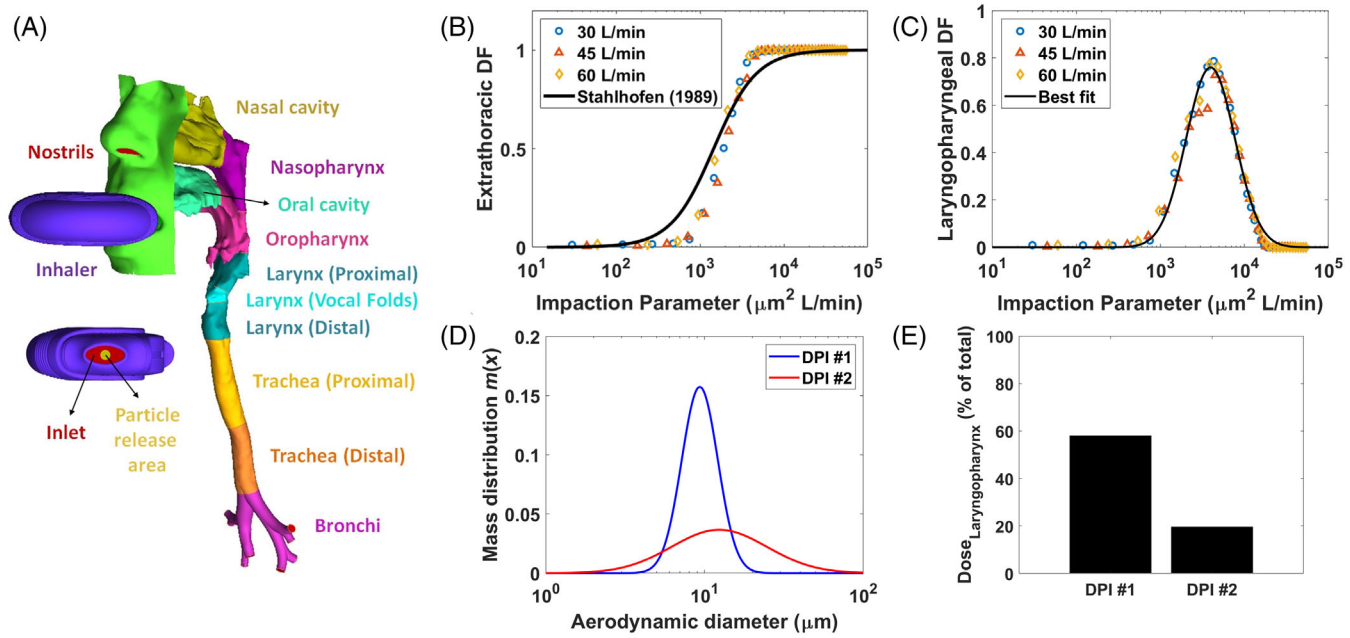

FIGURE 2 (A) 3D model of a healthy adult airway built from MRI scan of a 49-year-old female. (B) Comparison of extrathoracic deposition fraction (DF) predicted by CFD for inhalation rates of 30, 45, and 60 L/min with experimental data from Stahlhofen et al. (C) Laryngopharyngeal deposition fraction predicted by the CFD simulations and fitted with Equation (S3) in the Supporting information. (D) Log-normal mass distribution of two hypothetical DPIs (DPI #1: $d_{50} = 10$ μm, $\sigma_g = 1.3$; DPI #2: $d_{50} = 20$ μm, $\sigma_g = 2$). (E) Estimated dose delivered to the laryngopharynx by the two hypothetical DPIs at an inhalation rate of 30 L/min.

TABLE 1 Cytokine expression in bronchoalveolar lavage.

	Mean (IQR) pg/mL			*p*-value	
	Control	APR	FOS	APR vs. control	FOS vs. control
GM-CSF	4.05 (4.05–6.15)	2.40 (0.00–5.25)	5.72 (0.00–6.89)	.36	>.99
IFN-γ	2.09 (1.79–4.23)	2.21 (0.91–19.74)	1.68 (1.47–2.96)	.93	.83
IL-1α	38.46 (31.15–56.09)	29.50 (3.25–57.24)	42.52 (0.00–48.92)	.85	>.99
IL-1β	2.21 (1.82–3.44)	2.40 (1.13–2.96)	2.59 (2.46–3.20)	.52	.83
IL-2	8.95 (8.00–11.88)	6.76 (5.72–9.98)	9.47 (5.89–15.29)	.65	.83
IL-4	0.58 (0.50–0.87)	0.23 (0.08–0.28)	0.56 (0.16–0.64)	.0081	.53
IL-5	3.77 (3.49–5.06)	2.80 (2.35–3.53)	5.54 (3.41–6.22)	.083	>.99
IL-6	4.79 (2.90–6.55)	4.98 (1.95–8.14)	5.76 (5.07–6.83)	.78	.40
IL-7	1.33 (1.13–2.04)	1.33 (1.13–1.74)	2.56 (1.03–2.56)	.85	.83
IL-10	2.25 (2.01–3.09)	1.59 (1.41–2.25)	2.37 (2.01–2.61)	.12	.92
IL-12p70	3.40 (2.89–4.64)	1.69 (1.27–3.40)	2.10 (0.00–2.63)	.46	.094
KC	111.29 (79.34–157.69)	71.37 (66.25–102.87)	171.74 (93.37–186.73)	.083	.68
LIX	0.00 (0.00–13.58)	2.07 (0.00–7.12)	9.15 (6.77–13.58)	>.99	.34
MCP-1	52.45 (33.44–65.21)	20.28 (18.51–22.04)	40.45 (26.71–50.59)	.053	.68
MIP-2	145.72 (132.56–159.20)	139.18 (109.02–163.28)	154.93 (135.37–169.09)	.71	.83
TNF-α	1.93 (1.47–2.65)	0.50 (0.00–2.70)	2.18 (0.32–2.32)	.31	.83

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL, interleukin; KC, keratinocyte-derived cytokine (IL-8 homolog); LIX, lipopolysaccharide-induced CXC chemokine (IL-8 homolog); MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein 2-alpha (IL-8 homolog); TNF-α, tumor necrosis factor-alpha.

IL-8 homologs keratinocyte-derived cytokine (KC), lipopolysaccharide-induced CXC chemokine (LIX), and macrophage inflammatory protein 2-alpha (MIP-2); and IL-1α, 1β, 2, 4, 5, 6, 7, 10, 12p70, 13, and 17A) were not significantly elevated in BAL of FOS or APR groups relative to control (Table 1). LIGHT (TNF superfamily member 14), Oncostatin M, phosphatidylinositol-glycan biosynthesis class F

TABLE 2 Cardiotoxicity marker expression in plasma.

	Median (IQR) pg/mL			*p*-value	
	Control	APR	FOS	APR vs. control	FOS vs. control
CXCL16	16.39 (0.00–26.45)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	.14	.072
Endocan	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	.36	.42
LIGHT	ND	ND	ND		
Follistatin	0.00 (0.00–129.25)	0.00 (0.00–39.88)	0.00 (0.00–261.45)	.75	>.99
Oncostatin M	ND	ND	ND		
sCD40L	ND	ND	ND		
PIGF-2	ND	ND	ND		
Troponin I	67,639.82 (128.20–111,665.77)	76,276.77 (59,003.83–275,983.99)	108,149.33 (42,504.33–249,926.09)	.52	.40
Troponin T	3927.90 (0.00–4403.37)	5568.25 (3327.45–20,113.41)	9584.82 (2484.50–15,301.53)	.23	.21

Abbreviation: ND, not detected.

protein-2 (PIGF-2), and soluble cluster of differentiation 40 ligand (sCD40L) were undetectable in plasma-EDTA; remaining cardiotoxicity markers (chemokine ligand 16 (CXCL16), Endocan, Follistatin, Troponin I, and Troponin T) demonstrated no elevation in FOS or APR groups relative to control (Table 2).

3.2 | CFD modeling

The CFD simulations predicted that a monodisperse aerosol with aerodynamic diameter $d_a = 11.5$ μm would provide a laryngopharyngeal deposition fraction of 0.76 (i.e., 76% of the DPI dose would deposit in the laryngopharynx) for an inhalation rate of 30 L/min (corresponding to IP = 3964 μm^2 L/min) (Figure 2C). Similarly, for inhalation rates of 45 and 60 L/min, the optimal aerodynamic diameter to maximize laryngopharyngeal deposition was predicted to be 9.4 and 8.1 μm, respectively. Assuming that FOS particles in a DPI formulation have a hypothetical density of $\rho_p = 1400$ kg/m^3, the optimal geometric diameter to maximize laryngopharyngeal deposition computed from the aerodynamic diameter (see Equation S2 in the Supporting information) would be 6.9–9.7 μm for inhalation rates of 30–60 L/min based on the relationship between aerodynamic diameter and geometric diameter (see Supporting information).[49]

However, DPIs generate polydisperse aerosols. To illustrate how the APSD affects the DPI dose delivered to the laryngopharynx, we considered two hypothetical DPIs with different mass median diameters (d_{50}) and geometric standard deviations (σ_g). DPI #1 ($d_{50} = 10$ μm, $\sigma_g = 1.3$) has a narrower APSD with d_{50} near the aerodynamic diameter that maximizes laryngopharyngeal deposition, while DPI #2 ($d_{50} = 20$ μm, $\sigma_g = 2$) has a wider APSD with d_{50} shifted from the optimal value (Figure 2D). For an inhalation rate of 30 L/min, we estimated that 58.1% of the dose from DPI #1 would deposit in the laryngopharynx, while only 19.6% of the dose from DPI #2 would deposit in the laryngopharynx (Figure 2E). After accounting for the log-normal distribution of pharmaceutical aerosols, we estimated that the optimal mass median aerodynamic diameter for laryngopharyngeal deposition would be $d_a = 9.1$ μm at an inhalation rate of 30 L/min and assuming a geometric standard deviation of $\sigma_g = 1.3$. These results illustrate that the particle size distribution generated by the DPI has a major impact on the dose delivered to the laryngopharynx.

4 | DISCUSSION

The hypothesis that inhibitors of peptic activity and/or receptor antagonists hold potential for treatment of LPR is gaining acceptance, with over a decade of supportive research, lending toward the production of novel therapeutic options.[26,28,34,50] Proof-of-concept that peptic inhibition reduces aerodigestive tract damage has been established in animal models of LPR.[41,51] Local delivery by inhalation confers the benefit of reduced dosing as verified in our LPR mouse model.[41] Although oral FOS has a reasonably good safety profile, that of inhaled FOS has not yet been characterized. Further, commercial inhalers for laryngopharyngeal delivery do not currently exist. Therefore, the development of aerosolized FOS for LPR will require its toxicologic assessment and design, development, and testing of an inhaler optimized for laryngopharyngeal delivery.

Standard GLP assessment required for registration of an investigative new drug with the Food and Drug Administration involves a 28-day toxicologic trial in rodent and non-rodent models. Herein, treatment of equal duration produced no toxicity in a rodent model as indicated by organ pathology, BAL inflammatory cytokines, and plasma cardiotoxicity markers. This preliminary assessment bodes well for formal GLP assessment which is currently underway to support an FDA-regulated phase I, randomized clinical trial to assess the safety and toxicity of inhaled FOS as a dry powder for LPR.

Current commercial DPIs maximize pulmonary delivery by generating plumes of fine particles of 1–4 μm to bypass the upper airways.[52] A prior CFD study estimated that in a monodisperse aerosol (i.e., consisting of particles of a single size), 8–10 μm particles would provide maximal laryngeal deposition,[53] which is in good agreement with the optimal aerodynamic diameter of 8.1–11.5 μm predicted in

this study. However, although monodisperse aerosols may be generated under controlled laboratory conditions, DPIs are polydisperse. To our knowledge, this is the first study to examine how the APSD influences laryngopharyngeal drug delivery. After accounting for the log-normal distribution of pharmaceutical aerosols, we estimated that the optimal mass median aerodynamic diameter for laryngopharyngeal deposition would be $d_a = 9.1$ μm at an inhalation rate of 30 L/min which is slightly smaller than the optimal aerodynamic diameter of 11.5 μm for a monodisperse aerosol. Our analysis of two hypothetical DPIs with moderately different APSD revealed a threefold difference in the laryngopharyngeal dose. These results illustrate that DPIs for laryngopharyngeal drug delivery may need to be redesigned as compared to DPIs for pulmonary drug delivery to generate plume properties that maximize drug delivery to the target site.

Limitations of the study include unmasked evaluation of organ pathology. While this increases risk of bias, negative organ pathology was corroborated by absence of drug-related effects on inflammatory and cardiotoxicity markers in BAL and plasma. In addition, as with any experimental observation, caution should be exercised when translating in vivo findings from a limited number of animals to the clinical situation. Animal toxicology studies will continue to be critical for demonstrating the safety of investigational new drugs until superior models are substantiated as acceptable alternatives; however, differences between mouse and human physiology should be kept in mind when evaluating the implications of these data. Limitations of CFD analysis include the small sample size ($n = 1$), use of a simplified turbulence model in the steady-state CFD simulations, lack of consideration of mucociliary clearance, and drug physicochemical properties (e.g., solubility). Future research to address these limitations could include CFD simulations in a larger cohort to investigate interindividual variability and the development of a physiologically based pharmacokinetic model of drug dissolution, transport, and absorption to account for mucociliary clearance and dissolution rates. Such future work may determine the optimal APSD and inhalation rate (which is associated with the airflow resistance of the device) to maximize drug delivery to the laryngopharynx. These data would inform the design of the FOS DPI and patient instructions for use regarding optimal breathing technique. Finally, whereas the most prevalent symptoms of LPR (globus sensation, throat clearing, and hoarseness) primarily involve the laryngopharynx, LPR produces symptoms affecting more proximal regions of the aerodigestive tract. A randomized placebo-controlled trial using a validated symptom questionnaire as an outcome measure will ultimately yield the best assessment of the efficacy of a laryngopharyngeal FOS DPI for the resolution of the various symptoms associated with LPR.

5 | CONCLUSION

FOS is a candidate LPR therapeutic with a lower effective dosage required by inhalation versus ingestion. To develop inhaled FOS for LPR, the safety of inhaled FOS was verified over 4 weeks in a rodent model, and the optimal particle size for DPI delivery to the laryngopharynx was estimated via CFD. The results indicate a good safety profile and feasibility of a laryngopharyngeal FOS DPI and support a formal preclinical GLP assessment and phase I clinical trial in LPR patients.

FUNDING INFORMATION

This work was funded by the Dr. Ralph and Marian Falk Medical Research Trust of the Bank of America, N-Zyme Biomedical Inc., and the Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, Milwaukee, WI, USA.

CONFLICT OF INTEREST STATEMENT

N.J. is a co-founder, Chief Scientific Officer, and an investor in N-Zyme Biomedical. N.J. is an inventor on International Patent Application PCT/US2021/027758, Aerosolized formulations of HIV protease inhibitors for the treatment of airway reflux, filed on April 16, 2021, and International Patent Application: PCT/US2023/071204, Sustained-release oral fosamprenavir for the treatment of reflux, filed on July 28, 2023. T.S. is an investor in N-Zyme Biomedical. Other authors have no financial relationships or conflicts of interest to disclose.

ORCID

Alexandra Lesnick https://orcid.org/0009-0000-7052-7762
Tina L. Samuels https://orcid.org/0000-0002-7045-357X
Guilherme J. M. Garcia https://orcid.org/0000-0003-2995-9226
Jonathan M. Bock https://orcid.org/0000-0002-4367-7527

REFERENCES

1. Lechien JR, Akst LM, Hamdan AL, et al. Evaluation and management of laryngopharyngeal reflux disease: state of the art review. *Otolaryngol Head Neck Surg*. 2019;160:762-782.
2. Bianchi ET, Guerreiro Cardoso PF, Minamoto H, Bibas BJ, Salati M, Pego-Fernandes PM. Impact of fundoplication for gastroesophageal reflux in the outcome of benign tracheal stenosis. *J Thorac Cardiovasc Surg*. 2019;158:1698-1706.
3. Esposito C, Saxena A, Irtan S, Till H, Escolino M. Laparoscopic Nissen fundoplication: an excellent treatment of GERD-related respiratory symptoms in children – results of a multicentric study. *J Laparoendosc Adv Surg Tech A*. 2018;28:1023-1028.
4. Kim SY, Park B, Lim H, Kim M, Kong IG, Choi HG. Increased risk of larynx cancer in patients with gastroesophageal reflux disease from a national sample cohort. *Clin Otolaryngol*. 2019;44:534-540.
5. Parsel SM, Wu EL, Riley CA, McCoul ED. Gastroesophageal and laryngopharyngeal reflux associated with laryngeal malignancy: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2019;17:1253-1264.e1255.
6. Riley CA, Marino MJ, Hsieh MC, Wu EL, Wu XC, McCoul ED. Detection of laryngeal carcinoma in the U.S. elderly population with gastroesophageal reflux disease. *Head Neck*. 2019;41:1434-1440.
7. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope*. 1991;101:1-78.
8. Connor NP, Palazzi-Churas KL, Cohen SB, Leverson GE, Bless DM. Symptoms of extraesophageal reflux in a community-dwelling sample. *J Voice*. 2007;21:189-202.

9. Kamani T, Penney S, Mitra I, Pothula V. The prevalence of laryngopharyngeal reflux in the English population. *Eur Arch Otorhinolaryngol*. 2012;269:2219-2225.
10. Spantideas N, Drosou E, Bougea A, AlAbdulwahed R. Proton pump inhibitors for the treatment of laryngopharyngeal reflux. A systematic review. *J Voice*. 2020;34:918-929.
11. Sharma N, Castell DO. Further comment on proton pump inhibitor failures. *Clin Gastroenterol Hepatol*. 2009;7:363.
12. Tamhankar AP, Peters JH, Portale G, et al. Omeprazole does not reduce gastroesophageal reflux: new insights using multichannel intraluminal impedance technology. *J Gastrointest Surg*. 2004;8:890-897. (discussion 897–898).
13. Tutuian R, Mainie I, Agrawal A, Adams D, Castell DO. Nonacid reflux in patients with chronic cough on acid-suppressive therapy. *Chest*. 2006;130:386-391.
14. Lee JS, Jung AR, Park JM, Park MJ, Lee YC, Eun YG. Comparison of characteristics according to reflux type in patients with laryngopharyngeal reflux. *Clin Exp Otorhinolaryngol*. 2018;11:141-145.
15. Falk GL, Van der Wall H, Burton L, Falk MG, O'Donnell H, Vivian SJ. Fundoplication for laryngopharyngeal reflux despite preoperative dysphagia. *Ann R Coll Surg Engl*. 2017;99:224-227.
16. Iqbal M, Batch AJ, Spychal RT, Cooper BT. Outcome of surgical fundoplication for extraesophageal (atypical) manifestations of gastroesophageal reflux disease in adults: a systematic review. *J Laparoendosc Adv Surg Tech A*. 2008;18:789-796.
17. Klimara MJ, Randall DR, Allen J, Figueredo E, Johnston N. Proximal reflux: biochemical mediators, markers, therapeutic targets, and clinical correlations. *Ann N Y Acad Sci*. 2020;1481:127-138.
18. Lechien JR, Dapri G, Dequanter D, et al. Surgical treatment for laryngopharyngeal reflux disease: a systematic review. *JAMA Otolaryngol Head Neck Surg*. 2019;145:655-666.
19. Mainie I, Tutuian R, Shay S, et al. Acid and non-acid reflux in patients with persistent symptoms despite acid suppressive therapy: a multicentre study using combined ambulatory impedance-pH monitoring. *Gut*. 2006;55:1398-1402.
20. Sidwa F, Moore AL, Alligood E, Fisichella PM. Surgical treatment of extraesophageal manifestations of gastroesophageal reflux disease. *World J Surg*. 2017;41:2566-2571.
21. Zhang C, Hu ZW, Yan C, et al. Nissen fundoplication vs proton pump inhibitors for laryngopharyngeal reflux based on pH-monitoring and symptom-scale. *World J Gastroenterol*. 2017;23:3546-3555.
22. Giacchi RJ, Sullivan D, Rothstein SG. Compliance with anti-reflux therapy in patients with otolaryngologic manifestations of gastroesophageal reflux disease. *Laryngoscope*. 2000;110:19-22.
23. McGlashan JA, Johnstone LM, Sykes J, Strugala V, Dettmar PW. The value of a liquid alginate suspension (Gaviscon advance) in the management of laryngopharyngeal reflux. *Eur Arch Otorhinolaryngol*. 2009;266:243-251.
24. Zalvan CH, Hu S, Greenberg B, Geliebter J. A comparison of alkaline water and Mediterranean diet vs proton pump inhibition for treatment of laryngopharyngeal reflux. *JAMA Otolaryngol Head Neck Surg*. 2017;143:1023-1029.
25. Koufman JA. Low-acid diet for recalcitrant laryngopharyngeal reflux: therapeutic benefits and their implications. *Ann Otol Rhinol Laryngol*. 2011;120:281-287.
26. Bardhan KD, Strugala V, Dettmar PW. Reflux revisited: advancing the role of pepsin. *Int J Otolaryngol*. 2012;2012:646901.
27. Calvo-Henriquez C, Ruano-Ravina A, Vaamonde P, Martinez-Capoccioni G, Martin-Martin C. Is pepsin a reliable marker of laryngopharyngeal reflux? A systematic review. *Otolaryngol Head Neck Surg*. 2017;157:385-391.
28. Johnston N, Dettmar PW, Ondrey FG, Nanchal R, Lee SH, Bock JM. Pepsin: biomarker, mediator, and therapeutic target for reflux and aspiration. *Ann N Y Acad Sci*. 2018;1434:282-289.
29. Weitzendorfer M, Antoniou SA, Schredl P, et al. Pepsin and oropharyngeal pH monitoring to diagnose patients with laryngopharyngeal reflux. *Laryngoscope*. 2019;130:1780-1786.
30. Sereg-Bahar M, Jerin A, Hocevar-Boltezar I. Higher levels of total pepsin and bile acids in the saliva as a possible risk factor for early laryngeal cancer. *Radiol Oncol*. 2015;49:59-64.
31. Dai YF, Tan JJ, Deng CQ, Liu X, Lv ZH, Li XP. Association of pepsin and DNA damage in laryngopharyngeal reflux-related vocal fold polyps. *Am J Otolaryngol*. 2020;41:102681.
32. Johnston N, Yan JC, Hoekzema CR, et al. Pepsin promotes proliferation of laryngeal and pharyngeal epithelial cells. *Laryngoscope*. 2012;122:1317-1325.
33. Li H, Zhang S, Zhou S, et al. Pepsin enhances glycolysis to promote malignant transformation of vocal fold leukoplakia epithelial cells with dysplasia. *Eur Arch Otorhinolaryngol*. 2023;280:1841-1854.
34. Niu K, Guo C, Teng S, et al. Pepsin promotes laryngopharyngeal neoplasia by modulating signaling pathways to induce cell proliferation. *PLoS One*. 2020;15:e0227408.
35. Tan JJ, Wang L, Mo TT, Wang J, Wang MG, Li XP. Pepsin promotes IL-8 signaling-induced epithelial–mesenchymal transition in laryngeal carcinoma. *Cancer Cell Int*. 2019;19:64.
36. Samuels TL, Johnston N. Pepsin in gastroesophageal and extraesophageal reflux: molecular pathophysiology and diagnostic utility. *Curr Opin Otolaryngol Head Neck Surg*. 2020;28:401-409.
37. Doukas PG, Vageli DP, Sasaki CT, Judson BL. Pepsin promotes activation of epidermal growth factor receptor and downstream oncogenic pathways, at slightly acidic and neutral pH, in exposed hypopharyngeal cells. *Int J Mol Sci*. 2021;22:4275.
38. Adams J, Heintz P, Gross N, et al. Acid/pepsin promotion of carcinogenesis in the hamster cheek pouch. *Arch Otolaryngol Head Neck Surg*. 2000;126:405-409.
39. Kelly EA, Samuels TL, Johnston N. Chronic pepsin exposure promotes anchorage-independent growth and migration of a hypopharyngeal squamous cell line. *Otolaryngol Head Neck Surg*. 2014;150:618-624.
40. Samuels TL, Zimmermann MT, Zeighami A, et al. RNA sequencing reveals cancer-associated changes in laryngeal cells exposed to non-acid pepsin. *Laryngoscope*. 2021;131:121-129.
41. Johnston N, Samuels TL, Goetz CJ, et al. Oral and inhaled fosamprenavir reverses pepsin-induced damage in a laryngopharyngeal reflux mouse model. *Laryngoscope*. 2023;133(Suppl 1):S1-s11.
42. Stahlhofen W, Rudolf G, James AC. Intercomparison of experimental regional aerosol deposition data. *J Aerosol Med*. 1989;2:285-308.
43. Food and Drug Administration. Metered dose inhaler (MDI) and dry powder inhaler (DPI) products – quality considerations – guidance for industry. Accessed June 20, 2023. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/metered-dose-inhaler-mdi-and-dry-powder-inhaler-dpi-drug-products-quality-considerations
44. Adhami T, Goldblum JR, Richter JE, Vaezi MF. The role of gastric and duodenal agents in laryngeal injury: an experimental canine model. *Am J Gastroenterol*. 2004;99:2098-2106.
45. Little FB, Koufman JA, Kohut RI, Marshall RB. Effect of gastric acid on the pathogenesis of subglottic stenosis. *Ann Otol Rhinol Laryngol*. 1985;94:516-519.
46. Roh JL, Yoon YH. Effect of acid and pepsin on glottic wound healing: a simulated reflux model. *Arch Otolaryngol Head Neck Surg*. 2006;132:995-1000.
47. Yellon RF, Szeremeta W, Grandis JR, Diguisseppe P, Dickman PS. Subglottic injury, gastric juice, corticosteroids, and peptide growth factors in a porcine model. *Laryngoscope*. 1998;108:854-862.
48. Caicedo-Granados E, Galbraith AR, Schachern MG, et al. N-methylnitrosourea-induced carcinoma as a model for laryngeal carcinogenesis. *Head Neck*. 2014;36:1802-1806.
49. ChemSRC. Fosamprenavir. Accessed June 20, 2023. https://www.chemsrc.com/en/cas/226700-79-4_399132.html

50. Johnston N, Wells CW, Samuels TL, Blumin JH. Rationale for targeting pepsin in the treatment of reflux disease. *Ann Otol Rhinol Laryngol*. 2010;119:547-558.
51. Nagahama K, Yamato M, Nishio H, Takeuchi K. Essential role of pepsin in pathogenesis of acid reflux esophagitis in rats. *Dig Dis Sci*. 2006; 51:303-309.
52. Newman SP. Fine particle fraction: the good and the bad. *J Aerosol Med Pulm Drug Deliv*. 2022;35:2-10.
53. Perkins EL, Basu S, Garcia GJM, Buckmire RA, Shah RN, Kimbell JS. Ideal particle sizes for inhaled steroids targeting vocal granulomas: preliminary study using computational fluid dynamics. *Otolaryngol Head Neck Surg*. 2018;158:511-519.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Lesnick A, Samuels TL, Seabloom D, et al. Inhaled fosamprenavir for laryngopharyngeal reflux: Toxicology and fluid dynamics modeling. *Laryngoscope Investigative Otolaryngology*. 2024;9(1):e1219. doi:10.1002/lio2.1219