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Original Article

Randomized controlled study of aerosolized hypertonic xylitol versus hypertonic saline in hospitalized patients with pulmonary exacerbation of cystic fibrosis

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ABSTRACT

Background: Cystic fibrosis (CF) lung disease is characterized by chronic bacterial infection and recurrent pulmonary exacerbations. Xylitol is a 5-carbon sugar that can lower the airway surface salt concentration and augment innate immunity. We examined the safety and efficacy of aerosolized xylitol use for 2 weeks in subjects hospitalized with a pulmonary exacerbation of CF.

Methods: In a 2-week study, 60 subjects with cystic fibrosis and $FEV_1 > 30\%$ predicted were enrolled to receive aerosolized 7% hypertonic saline (4 ml) or 15% xylitol (5 ml) twice a day for 14 days. Outcomes assessed included change from baseline in $FEV_1\%$ predicted, change in sputum microbial density, revised CF quality of life questionnaire including the respiratory symptom score, time to next hospitalization for a pulmonary exacerbation, and frequency of adverse events.

Results: 59 subjects completed the study (one subject in the saline group withdrew before any study product administration). No significant differences were noted between the 2 arms in mean changes in lung function, sputum microbial density for *Pseudomonas aeruginosa* and *Staphylococcus aureus*, body weight, quality of life, and frequency of adverse events.

Conclusions: Aerosolized hypertonic xylitol was well-tolerated among subjects hospitalized for CF pulmonary exacerbation. Future studies examining efficacy for long term use in patients with CF lung disease would be worthwhile.

The clinical trial registration number for this study is NCT00928135.

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1. Introduction

Cystic fibrosis (CF) lung disease is characterized by chronic bacterial infection, recurrent pulmonary exacerbations and relentless decline in forced expiratory volume in the first second (FEV_1) [1–3]. These infections eventually damage the lungs and contribute to significant morbidity and mortality in patients with CF. It is well known that antibacterial activity of innate immune mediators, such as lysozyme and beta defensins, in human airway surface liquid (ASL) is salt-sensitive. An increase in salt concentration inhibits activity of these immune mediators [4], whereas their activity is increased by low ionic strength [5–7]. Lowering the ASL salt concentration might potentiate innate immunity and decrease or prevent airway infections in subjects with CF.

In previous in vitro studies, we found that salt concentration in CF and non-CF epithelia could be significantly reduced by administration of artificial sweetener, xylitol [8]. Xylitol is a five-carbon sugar that has been successfully used in chewing gums to prevent dental caries [9,10] and acute otitis media [11] without significant adverse effects [12]. In addition to augmenting airway immunity by lowering ASL salt concentration, there is the added potential advantage of enhancing mucociliary clearance with hypertonic doses.

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In our phase 1 safety studies, single doses of inhaled isoosmolar and hypertonic xylitol (15% weight/volume) were well tolerated by healthy volunteers and stable subjects with CF [13,14]. Two-week toxicology studies in rats and dogs confirmed that xylitol was well-tolerated in concentrations up to 21 mg/kg body weight [15]. In a study of pharmacokinetics of aerosolized xylitol in normal volunteers, the terminal half-life of a single dose of isoosmotic xylitol in the airways was found to be 45 min [16].

In this Phase II study, we tested the hypothesis that aerosolized hypertonic xylitol given twice daily for 2 weeks will be safe and well tolerated compared with 7% saline, a commonly used mucolytic therapy in patients hospitalized for pulmonary exacerbation.

2. Methods

2.1. Participants

Subjects with CF (medical record evidence of cystic fibrosis transmembrane conductance regulator mutation or sweat chloride test or nasal voltage difference, and 1 or more clinical findings of CF), age 12 or greater, were included in the study if they were admitted for a pulmonary exacerbation as determined by the CF physician, had a recent FEV_1 >30% predicted and oxygen saturation ≥ 90% on FiO2 ≤ 50%. Subjects were excluded for the following reasons: pregnancy; hemoptysis >60 ml; use of any investigational study drug, or initiation of hypertonic saline within the last 30 days; known intolerance to hypertonic saline; serum creatinine 2 mg/dl or more; active malignancy in the last year; on waiting list for lung transplantation or history of a solid organ transplant; lack of FEV_1 data from the last 14 days; and previous participation in this study.

Written informed consent was obtained from all participants (or their parent or guardian). The study was approved by the University of Iowa Institutional Review Board as well as the Food and Drug Administration.

2.2. Study design and treatment

This Phase II trial was a prospective controlled study that randomized CF subjects in a 1:1 ratio to aerosolized 15% xylitol (5 ml twice a day for up to 14 consecutive days) vs. hypertonic 7% saline (4 ml twice a day for up to 14 consecutive days). Xylitol or hypertonic saline (Hypersal, Pari Respiratory Equipment Inc., Midlothian, VA) were administered using Pari LC nebulizer with Vios compressor (Pari Respiratory Equipment Inc., Midlothian, VA). Xylitol crystals are made as food additives by Danisco Cultor, USA. These crystals were used by UI Pharmaceuticals (Iowa City, IA) to make a sterile, non-pyrogenic, preservative free solution for inhalation. Blinding to subjects was not assured because of the distinct sweet or salty tastes of the study treatments.

Subjects were randomized in a 1:1 ratio to either xylitol or saline, in five blocks of 12 each using a random number generator. Within 48 h of admission, after obtaining informed consent, eligible subjects were assigned to either xylitol or saline arm by a research pharmacist from the investigational drug services at the University of Iowa Hospitals and Clinics based on a sequence generated by the study statistician. Subjects were asked to stop inhaled hypertonic saline if they were using it at home. Subjects were screened for airway hyperresponsiveness (AHR) using a trial dose of their assigned study drug as follows. After baseline spirometry was done, subjects were premedicated with albuterol inhalation. After 20–30 min, the study drug was administered, followed by repeat spirometry in 20–30 min. Subjects were discontinued from the study if oxygen saturation was <89% or FEV_1 declined by 20% or greater from baseline. In subjects who tolerated trial inhalation, demographics, current medication use and, the revised CF quality of life questionnaire (CFQ-R) [17] were collected. Spontaneously expectorated sputum was collected and processed for cell count and cytokines, and quantitative cultures. Blood was collected for chemistry and liver function tests (LFT) if not done for clinical care. All subjects received intravenous antibiotics, twice daily dornase alpha and four times daily airway clearance sessions (i.e., manual chest physiotherapy plus postural drainage, vest, pneumatic percussion), daily aerobic exercise and multidisciplinary team input. The study drug was administered before inhaled antibiotics or steroids and airway clearance sessions and was monitored by a study team member.

Data from spirometry that was routinely done for clinical care were recorded. All spirometry testing followed the 2005 American Thoracic Society guidelines [18]. At discharge (days 10–14), subjects provided sputum samples and completed CFQ-R questionnaires. If subjects were discharged prior to 10 days of hospitalization to complete antibiotic infusions at home, they were asked to continue study drug at home and return for follow-up any day between days 10–15. As part of study followup, subjects were contacted by phone at week 1, day 90 and day 180 after discharge to enquire about pulmonary exacerbations, hospitalizations, and other adverse events.

2.3. Outcome assessments

2.3.1. Outcomes

Safety endpoints inlcuded change in FEV_1 from baseline, withdrawal from the study, rescue bronchodilator use, incidence of treatment-related adverse events, and laboratory tests (chemistry and LFTs). Exploratory efficacy outcomes included difference in density of bacteria per gram of sputum from baseline to day 14, time to next hospitalization for a pulmonary exacerbation, difference in CFQ-R, difference in sputum cytokine levels and cell counts.

2.3.2. Adverse events

Any untoward medical occurrence (abnormal symptom, sign or laboratory value) in a subject during and through 7 days after study drug inhalation was reported as an adverse event.

2.4. Statistical analysis

This pilot safety study was powered to detect common adverse events. Assuming that the likelihood of a subject experiencing an adverse event is no <5%, a sample size of 30 subjects per group ensures 80% power towards event detection [19]. More specifically, with a sample size of 30, the probability of observing at least one adverse event is 80%.

Data from all randomized subjects who received at least one dose of xylitol or saline (excluding trial dose of study drug) were analyzed. Baseline characteristics for continuous variables were summarized using mean and standard deviation (SD) while frequency and percentage were used to summarize categorical variables. Differences in mean change from baseline for outcomes in the two treatment groups were analyzed using either a two sample *t*-test or a Wilcoxon rank-sum test. To analyze group differences in time to next exacerbation, a time-to-event analysis was conducted. Kaplan-Meier estimates were obtained for each survival curve (where failure was defined by exacerbation). To compare the survival distributions, a log rank test was employed. The proportion of subjects in each group who experienced adverse events was compared using a Fisher's exact test. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina). All tests were two-sided, and conducted using a significance level of 0.05.

Table 1
Demographics and baseline characteristics of study subjects.

Characteristic*	Xylitol ($N = 30$)	Hypertonic Saline ($N = 29$)
Age in years, Mean (SD)	29.7 (12.3)	30.4 (12.8)
Male, N (%)	15 (50)	17 (59)
Caucasian race, N (%)	30 (100)	28 (97)
Baseline BMI, Mean (SD)	22.3 (3.4)	22.0 (3.8)
Baseline serum white blood cell count, K/mm3, Mean (SD)	11.6 (3.8)	11.0 (3.1)
FEV_1% predicted, Mean (SD)	58.8 (20.1)	53.3 (18.8)
CFTR Genotype: Homozygous F508*del*, N (%)	18 (60)	17 (59)
Pseudomonas aeruginosa, Median log CFU (Min - Max)[a]	2.0 (0–7.6)	6.2 (0–7.3)
Staphylococcus aureus, Median log CFU (Min - Max)[a]	3.5 (0–7.0)	4.7 (0–7.1)
Azithromycin use, N (%)	28 (93)	28 (97)
Dornase alfa use, N (%)	13 (43)	14 (48)

Abbreviations: BMI = body mass index; FEV_1 = Forced expiratory volume in first second; CFTR = cystic fibrosis transmembrane conductance regulator; CFU = colony forming units; SD = standard deviation.

* There were no significant differences ($P > 0.05$) between the groups. The p value for continuous variables was based on Student's t-test, except the comparisons for *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which were based on a Wilcoxon rank-sum test. The p value for nominal variables was derived from a chi-square test, except the comparisons for race and Azithromycin use, which was based on Fisher's exact test.

[a] Data were not available on *Pseudomonas aeruginosa* and *Staphylococcus aureus* for 2 patients in the xylitol group and 3 patients in the HS group.

2.5. Results

Seventy subjects were screened and 62 randomized to receive trial dose (e-Figure 1; online supplement). Two subjects failed trial inhalation of xylitol since their FEV_1 dropped >20% from baseline. One subject in the saline group withdrew from the study after randomization but before any study drug exposure and was not included in the final analysis.

Demographics and baseline distributions were similar across both cohorts (Table 1). Forty eight percent of the cohort used inhaled hypertonic saline regularly prior to admission.

One subject in the xylitol group stopped using study drug after three doses due to excessive cough (no change in spirometry) but remained in the study and completed the day 14 assessments. One subject in each group did not return for 14 day follow-up (e-Table 1; online supplement), but completed the telephone follow-ups. In the saline group, subjects took a mean 23 doses, compared to 22 doses in the xylitol group. The mean duration of study participation was 12 days for both groups.

2.5.1. Outcomes

The change in lung function from baseline was not significantly different for either outcome between the groups (Table 2).

Subjects in both the groups showed improvement in lung function, body mass index (BMI) and white blood cell (WBC) count at treatment completion compared to baseline (Fig. 1). As with lung function, no significant differences were observed in BMI and WBC count between the hypertonic saline and xylitol groups (data not shown).

Table 2
Lung function outcomes.

Lung Function	Xylitol ($N = 30$)	Hypertonic Saline ($N = 29$)	Difference
	Mean difference from baseline (95% CI)		Mean difference between groups (95% CI)
FEV_1, % predicted	8.8 (4.7, 12.9)	11.8 (8.1, 15.5)	3.0 (−2.4, 8.5)
FVC % predicted	8.6 (3.7, 13.6)	11.3 (7.1, 15.6)	2.7 (−3.7, 9.1)

Abbreviations: FEV_1 = forced expiratory volume in first second; FVC = Forced Vital Capacity; CI = confidence interval.

2.6. Microbiology outcomes

Among subjects who were able to produce sputum for cultures at baseline and treatment completion there was a reduction in bacterial densities in both groups (Table 3). Analyses of mean change between groups yielded mixed results. Less than five subjects in each group grew *Stenotrophomonas* or non-typeable *Hemophilus influenzae* and therefore those organisms were not analyzed.

2.7. Time to next exacerbation

Of the 30 subjects in the xylitol arm, 11 subjects experienced another pulmonary exacerbation by the 6 month follow-up. Among 29 subjects in the saline arm 15 subjects were hospitalized for another pulmonary exacerbation at 6 month follow-up. There was no difference in the median time to next exacerbation between the groups ($p = 0.36$, Fig. 2).

3. Adverse events

There was no significant difference in the incidence of adverse events between the 2 groups. Eight subjects in the xylitol group experienced an adverse event compared to 11 in the saline group ($p = 0.58$). For serious adverse events, two subjects in the saline group were diagnosed with distal intestinal obstruction syndrome during the study, which were determined to be unrelated to study drug. Adverse events are described in detail in e-Table 2 (online supplement).

3.1. CFQR

There were no significant differences in any of the domain scores between the two treatment groups. (e-Table 3; online supplement).

3.2. Sputum cytokines

Final study day sputum was available only in 10 subjects in the saline group and 8 subjects in the xylitol group. The other subjects were unable to expectorate due to overall improvement in respiratory status after hospitalization. There was no significant difference in sputum interleukin (IL)-8 levels between xylitol and saline groups (data not shown). IL-6, IL-10, IL-13 and tumor necrosis factor alpha were below detectable range in all of these samples.

4. Discussion

In this pilot safety study, we observed that aerosolized hypertonic xylitol when given twice daily for up to 14 days was well tolerated in subjects admitted for a CF pulmonary exacerbation. We opted to study a hypertonic concentration of xylitol in order to potentially augment mucociliary clearance similar to hypertonic saline. In addition, hypertonic xylitol because of the ASL salt lowering property would have the added advantage of augmenting innate immunity., Another osmolyte, dry powdered mannitol has been shown to improve some measures of lung function in CF [20].

The most important finding observed in this study was the acceptance and tolerance to inhaled xylitol. According to the 2016 Cystic Fibrosis Foundation Patient Registry Annual Data Report, approximately 71% of all CF patients, 6 years and older, use inhaled hypertonic saline [21]. However, side effects associated with hypertonic saline use such as chest tightness, sore throat and hemoptysis [22] often lead to permanent discontinuation of therapy. In

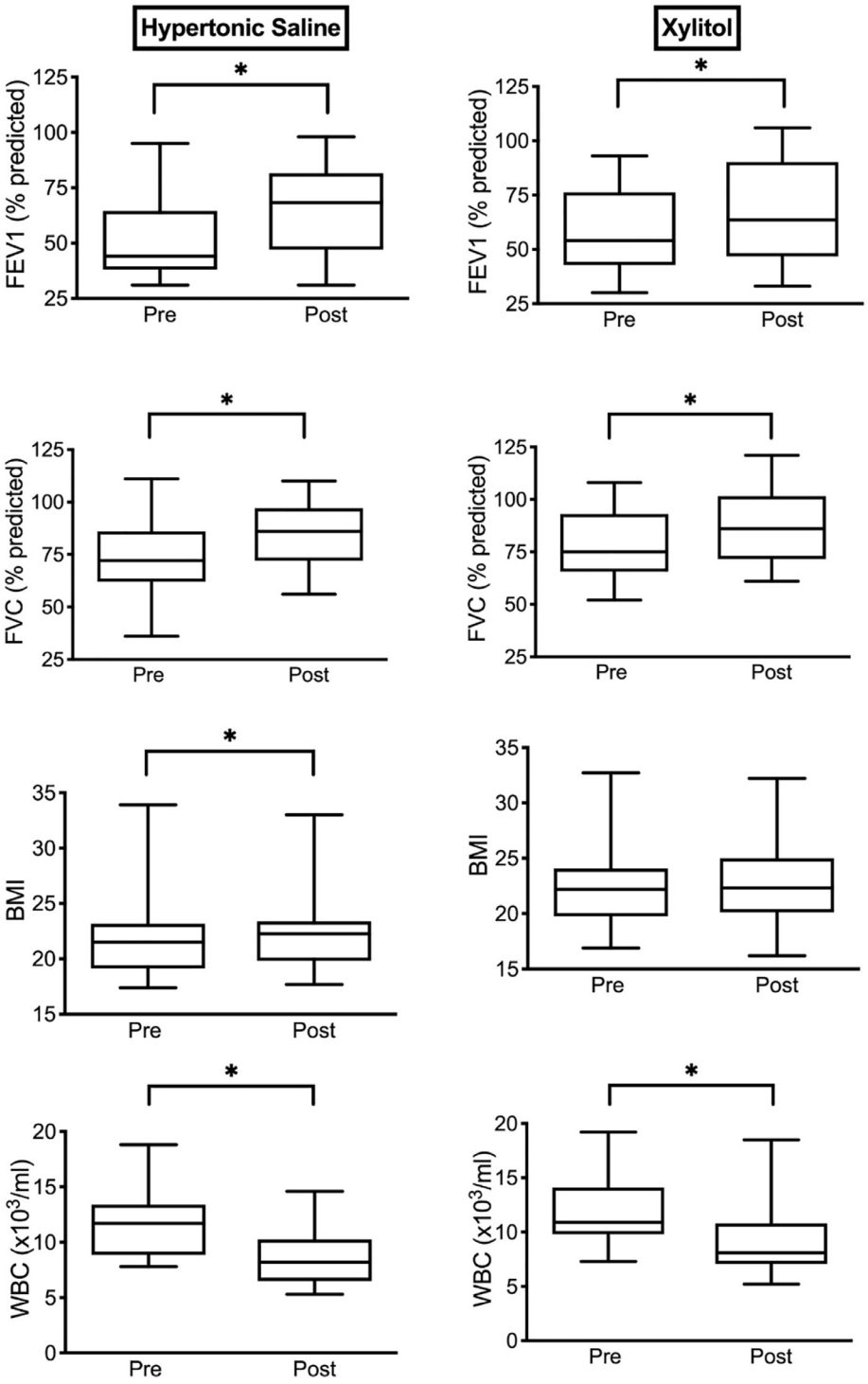

Fig. 1. Distribution of lung function, body mass index (BMI) and white blood cell (WBC) count in the pre- and post-intervention periods in the two treatment groups.
*Statistically significant mean difference from baseline within group at a significance level of 0.05.

Table 3
Microbiology Outcomes in the Two Treatment Groups.

Microorganism	Xylitol ($N = 22$)[a]	Hypertonic Saline ($N = 18$)[a]	Difference
	Mean difference from baseline (95% CI)		Mean difference between groups (95% CI)
Pseudomonas aeruginosa, log CFU	−0.9 (−2.3, 0.4)	−1.4 (−2.8, −0.1)	−0.5 (−2.3, 1.3)
Pseudomonas aeruginosa mucoid, log CFU	−1.0 (−2.0, 0.1)	−2.8 (−4.3, −1.3)	−1.9 (−3.6, −0.2)
Staphylococcus aureus, log CFU	−1.7 (−2.7, −0.7)	−1.2 (−2.5, 0.2)	0.5 (−1.1, 2.1)

Abbreviations: CFU = colony forming units; CI = confidence interval.
[a] Analysis included only subjects that provided pre- and post-intervention sputum samples.

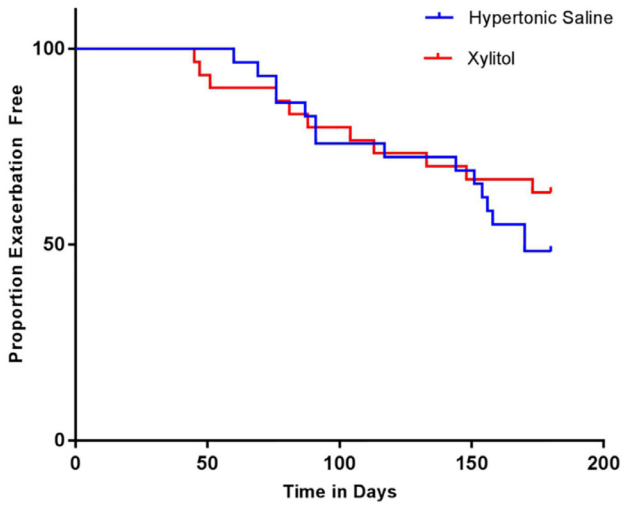

Fig. 2. A Kaplan-Meier plot of the proportion of subjects that remained free of exacerbation in the inhaled hypertonic saline (blue) and xylitol (red) treatment groups. Time to next exacerbation was not statistically different between the two groups (log rank, p value = 0.36). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

our study, 29 out of 32 subjects who were randomized to xylitol group, tolerated the trial inhalation well and had excellent compliance once enrolled. Subjects reported less gagging sensation due to the sweet taste of xylitol, therefore better tolerance. Our study excluded subjects who could not tolerate hypertonic saline and therefore study drug tolerance could not be compared between groups.

At study completion, both groups showed improvement in lung function, body weight, WBC count as well as bacterial density. However, we did not find a significant mean difference in lung function, body weight, WBC count and quality of life measures, between treatment groups at study completion. Bacterial density analyses showed mixed results; there was a significant reduction in mean density of *Pseudomonas aeruginosa* in the saline group and a significant reduction in density of *Staphylococcus aureus* in the xylitol group at study completion. Possible sampling errors (inability to provide good quality sputum), and wide standard deviations make microbiological endpoints particularly challenging to interpret in small studies.

An important study limitation includes lack of blinding which may have affected the quality of life assessments; however it is unlikely to change the direction of the study results. This study was not powered to study efficacy endpoints. However, a few possible explanations for lack of trend in efficacy include, lower osmolarity of xylitol solution used in this study (approximately half that of inhaled hypertonic saline, 1120 ± 30 mOsm compared to ~2400 mOsm for 7% sodium chloride), or a ceiling effect given co-administration of intensive treatments such as intravenous antibiotics, rigorous bronchial hygiene, aerobic exercise, supervised nutrition, etc. In addition, we used twice daily dosing which may have not been enough given the short half- life in the ASL. We chose to study twice daily dosing for compliance reasons given the already enormous burden of multiple therapies in these patients. It is possible that more frequent dosing may have improved efficacy [23]. Finally, these patients exhibited advanced disease and high density of airway infection which are unlikely to be amenable to any therapeutic interventions targeting innate immunity. Ultimately hypertonic xylitol may lack efficacy in the setting of CF exacerbation. It is possible that this intervention, based on augmented innate immunity, may be less likely to show efficacy in lung function or microbiology in CF patients with advanced bronchiectasis, acute illness, and short duration of therapy (2 weeks in our study).

We opted to study this group of hospitalized subjects for 2 reasons: a confined study population which ensures good compliance and follow-up once enrolled; and a sicker group of subjects to examine safety. In a study of hospitalized CF patients, inhaled hypertonic saline was well tolerated and was associated with greater reduction in pulmonary exacerbation symptoms [24]. In our study, both the study treatments were well tolerated and were associated with improvement in lung function. A group likely to benefit more from inhaled xylitol is the group of subjects with early disease with mild to no airway infection where manipulation of ASL salt concentration is likely to make an impact in airway immunity. Thus hypertonic xylitol may fit better among chronic treatments for early CF lung disease.

Recently, CFTR modulator treatments have shown promise in decelerating CF lung decline [25]; however, many CF patients with established bronchiectasis continue to require novel therapies that reduce airway infection and pulmonary exacerbations.

In summary, the addition of inhaled hypertonic xylitol to treatment of an inpatient CF exacerbation is well tolerated and produces similar outcomes as hypertonic saline. Future larger studies of longer treatment duration to assess the efficacy in chronic management of CF pulmonary disease are warranted.

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Declarations of competing interests

The authors except JZ do not have any conflicts of interest to report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2019.06.016.

References

[1] Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005;352(19):1992–2001.
[2] Zabner J, et al. Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia in vitro. Mol Cell 1998;2:397–403.
[3] Welsh MJ, et al. Cystic fibrosis The metabolic and molecular basis of inherited disease. Scriver CR, et al., editors. New York: McGraw-Hill; 2001.
[4] Goldman MJ, et al. Human beta-defensin-1 is a salt-sensitive antibiotic inlung that is inactivated in cystic fibrosis. Cell 1997;88(4):553–60.
[5] Bals R, et al. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J Clin Invest 1998;102(5):874–80.
[6] Travis SM, et al. Activity of abundant antimicrobials of the human airway. Am J Respir Cell Mol Biol 1999;20(5):872–9.
[7] Singh PK, et al. Production of beta-defensins by human airway epithelia. Proc Natl Acad Sci U S A 1999;95(5):14961–6.
[8] Zabner J, et al. The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing. Proc Natl Acad Sci U S A 2000;97:11614–19.
[9] Makinen KK, et al. Xylitol chewing gums and caries rates: a 40-month cohort study. J Dent Res 1995;74(12):1904–13.
[10] Soderling E, et al. Effect of sorbitol, xylitol, and xylitol/sorbitol chewing gums on dental plaque. Caries Res 1989;23(5):378–84.
[11] Uhari M, et al. Xylitol chewing gum in prevention of actue otitus media: double blind randomised trial. BMJ 1996;313(7066):1180–4.

[12] Makinen KK. Long-term tolerance of healthy human subjects to high amounts of xylitol and fructose: general and biochemical findings. Int Z Vitam Ernahrungsforsch Beih 1976;15:92–104.
[13] Durairaj L, et al. Safety assessment of inhaled xylitol in mice and healthy volunteers. Respir Res 2004;5(1):13.
[14] Durairaj L, et al. Safety assessment of inhaled xylitol in subjects with cystic fibrosis. J Cyst Fibros 2007;6(1):31–4.
[15] Reed MD, et al. Safety assessment of nebulized xylitol in beagle dogs. Inhal Toxicol 2012;24(6):365–72.
[16] Durairaj L, et al. Bronchoscopic assessment of airway retention time of aerosolized xylitol. Respir Res 2006;7:27.
[17] Quittner AL, et al. Development and validation of the cystic fibrosis questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. Chest 2005;128(4):2347–54.
[18] Miller MR, et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319–38.
[19] O'Neill RT. The assessment of safety: biopharmaceutical statistics for drug development, Vol Chapter 13. New York: Marcel Dekker; 1988.
[20] Nevitt SJ, et al. Inhaled mannitol for cystic fibrosis. Cochrane Database Syst Rev 2018(2):CD008649.
[21] CysticFibrosisFoundation. Patient registry 2016 annual data report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2017.
[22] Elkins MR, et al. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. N Engl J Med 2006;354(3):229–40.
[23] Donaldson SH, et al. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. N Engl J Med 2006;354(3):241–50.
[24] Dentice RL, et al. A randomised trial of hypertonic saline during hospitalisation for exacerbation of cystic fibrosis. Thorax 2016;71(2):141–7.
[25] Southern KW, et al. Correctors (specific therapies for class II CFTR mutations) for cystic fibrosis. Cochrane Database Syst Rev 2018(8):CD010966.