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SAFETY OF 2-WEEK USE OF AEROSOLIZED XYLITOL IN SUBJECTS WITH CYSTIC FIBROSIS

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ABSTRACT

Cystic fibrosis (CF) lung disease is characterized by chronic bacterial colonization and recurrent infection of the airways. Disruption of the cystic fibrosis transmembrane conductance regulator chloride channels in subjects with CF results in altered fluid and electrolyte transport across the airway epithelium thereby initiating infections. Lowering the ASL salt concentration and increasing the ASL volume might therefore potentiate innate immunity and therefore decrease or prevent airway infections in subjects with CF. Xylitol is a 5-carbon sugar that can lower the airway surface salt concentration, thus enhancing innate immunity. We recently tested the safety and tolerability of inhaling increasing concentrations of single doses of hypertonic xylitol (10% followed by 15%) in stable subjects with CF. All six subjects tolerated both the doses well. There was no significant change in FEV_1 or respiratory symptom score. In this protocol, we propose to test the safety and tolerability of aerosolized xylitol used daily for 2 weeks in subjects with cystic fibrosis. In a pilot, 2-week study, 60 subjects with cystic fibrosis with an FEV_1 >30% predicted will be randomized to receive aerosolized 7% hypertonic saline (4 ml) or 15% xylitol, (5 ml) twice a day for 14 days. The primary outcomes will be safety as assessed by FEV_1 change from baseline, adverse events and respiratory symptom score. Outcomes for trend in efficacy include density of colonization per gram of sputum, time to next exacerbation, sputum cytokines and revised CF quality of life questionnaire.

BACKGROUND

Cystic fibrosis (CF) lung disease is characterized by chronic bacterial colonization and recurrent infection of the airways. Disruption of the cystic fibrosis transmembrane conductance regulator chloride channels in subjects with CF results in altered fluid and electrolyte transport across the airway epithelium thereby initiating infections [1, 2]. These infections eventually destroy 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 their activity [3]. Conversely, their activity is increased by low ionic strength [4-6]. Lowering the ASL salt concentration and increasing the ASL volume might therefore potentiate innate immunity and therefore decrease or prevent airway infections in subjects with CF.

Xylitol, a five-carbon sugar with low transepithelial permeability, which is poorly metabolized by bacteria can lower the salt concentration of both cystic fibrosis (CF) and non-CF epithelia *in vitro* [7]. Xylitol is an artificial sweetener that has been successfully used in chewing gums to prevent dental caries [8, 9]; it has been used as an oral sugar substitute without significant adverse effects [10]. It has also been shown to decrease the incidence of acute otitis media by 20-40% [11]; nasal application to normal human subjects was found to decrease colonization with coagulase negative staphylococcus [7].

We found that aerosolized iso-osmolar xylitol was safe in mice and healthy volunteers when administered over a single day [12]. We then tested the safety of a single dose aerosolized iso-osmotic xylitol in subjects with stable CF and found that it was well tolerated [13] In a study of pharmacokinetics of aerosolized xylitol in normal volunteers, the terminal half-life of a single dose of iso-osmotic xylitol (10 mL) was 45 minutes [14]. In a recent study, we observed that single doses of 10% followed by 15% xylitol was well tolerated by subjects with cystic fibrosis who were stable (safety reported submitted to the FDA).

We conducted a 2 week toxicology study under good laboratory practices done in rats and dogs in collaboration with Lovelace Respiratory Research Institute (manuscript in preparation). Aerosolized xylitol was generated from a 450 mg/mL nebulizer solution through two Aerotech II nebulizers. Exposure levels were defined by time of exposure (high, 3 hours; mid, 1.5 hours, and low, 0.5 hours). Mean xylitol aerosol concentrations were 3.14 ± 0.19 mg/L for the 3-hour (high) exposure group, 3.03 ± 0.12 for the 1.5-hour (mid) exposure group, and 2.91 ± 0.18 for the 0.5-hour (low) exposure group. Mean particle sizes of xylitol were determined to be in the inhalable size range for rodents [(range 1–3) μm mass median aerodynamic diameter]. Mean total inhaled doses were estimated as 354.2 (target of 394.8), 170.9 (target of 197.4), and 54.7 (target of 65.8) mg/kg, for the high, mid, and low exposure groups, respectively. Mean total deposited doses were estimated as 37.55 (target of 55.27), 18.12 (target of 27.64), and 5.80 (target of 9.21) mg/kg, for the high, mid, and low exposure groups, respectively.

All rats survived to the scheduled necropsy. Xylitol exposures were generally well-tolerated. No specific xylitol-related findings were observed in clinical signs, body

weights, organ weights, or histopathology. Single and 14 consecutive-day xylitol exposures were associated with a resolvable discoloration of lungs in rats sacrificed prior to 24 h after exposure. Non-specific inflammatory and remodeling responses of the upper airway were observed after 14 days of exposure. The latter is commonly observed in rodent inhalation studies and was not determined to be related to any specific chemical toxicity of xylitol. A No Observed Effect Level (NOEL) was not determined based on the acute pulmonary findings. The No Observed Adverse Effect Level (NOAEL) was determined to be the low exposure level due to the resolvable nature of the acute gross pulmonary findings and the lack of any corroborating pulmonary histopathology in main study and recovery animals at all exposure levels.

For the dog study, beagle dogs were exposed to aerosolized xylitol for varying durations (high exposure group at 1 hour, mid exposure group at 0.5 hours, and low exposure group at 0.25 hours). A control group was exposed for 1 hour to a nebulized normal saline solution. Mean xylitol aerosol concentrations were 3.49 ± 0.15 mg/L for the high exposure group, 3.55 ± 0.18 for the mid exposure group, and 3.54 ± 0.18 for the low exposure group. Mean particle sizes of xylitol were determined to be in the inhalable size range for dogs [(actual range 2–3) μm mass median aerodynamic diameter]. Mean total inhaled doses were estimated as 105, 53, and 27 mg/kg, for the high, mid, and low exposure groups, respectively. Mean total deposited doses to the pulmonary region were estimated as 21, 11, and 5 mg/kg, for the high, mid, and low exposure groups, respectively.

All dogs survived to the scheduled necropsy and xylitol exposures were generally well-tolerated. No treatment-related findings were observed in clinical signs, body

weights, physical (with ECGs) and ophthalmic examinations, food consumption, clinical pathology, gross pathology, organ weights, or histopathology. The No Observed Effect Level (NOEL) was determined to be the high exposure level.

In this pilot study we propose to test the hypothesis that aerosolized hypertonic xylitol given twice daily for 2 weeks, will be safe and well tolerated and potentially lower the density of colonization in subjects with CF compared to hypertonic saline. We chose hypertonic concentration of xylitol to be comparable in part to hypertonic saline which is being offered as a routine treatment in hospitalized patients with CF exacerbation. In addition, hypertonic concentration allows us to deliver adequate amounts of xylitol with twice a day dosing which will help with compliance.

Protocol

Randomized Controlled Study of Aerosolized hypertonic xylitol versus hypertonic saline in hospitalized patients with exacerbation of cystic fibrosis

The study has been approved by the University of Iowa Institutional Review Board as well as the Food and Drug Administration. This will be a randomized, controlled study. Blinding is not assured because of ability to taste the sweetness of xylitol.

Inclusion Criteria: 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 with an FEV_1 >30% predicted (within the last 14 days) and oxygen saturation ≥90% on FiO2 ≤ 50%, admitted for an exacerbation, able to provide written informed consent or assent.

Exclusion Criteria: Pregnancy, hemoptysis more than 60 mL within the last 30 days, use of any investigational study drug within the last 30 days, 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, received a solid organ transplant, lack of FEV_1 data from the last 14 days and previous participation in this study.

Experimental Treatment: Aerosolized 15% xylitol, 5 ml twice a day for 2 weeks using Pari LC neubulizer with Vios compressor, (Pari Inc, Monterey, CA) . Xylitol crystals are

made as food additives by Danisco Cultor, USA. Aerosol solution is sterile, non-pyrogenic, preservative-free solution for inhalation made by UI Pharmaceuticals (Iowa City, IA).

Control Treatment: Hypertonic 7% saline (UIHC pharmacy) 4 ml twice a day for 2 weeks using the same kind of nebulizer.

Screening and Baseline Visit: On the day of admission for exacerbation, informed consent obtained in subjects whose admission spirometry (or spirometry within the last 14 days prior to admit date) shows an FEV_1 >30% predicted. Once consented, subjects will be randomized to either xylitol or saline group as per the randomization code previously generated by the statistician. Demographics, current medications use and the revised CF quality of life questionnaire (CFQ-R) will be completed. Spontaneously expectorated sputum will be collected and processed for cell count and cytokines and quantitative cultures. Blood will be collected for chemistry and liver function tests (LFT). Pregnancy test will be done in women. All medication use during hospitalization will be recorded. Treatment will begin the day after admission for an exacerbation. Results from spirometry that is routinely done twice a week for inpatients will be recorded. All spirometry testing will follow the 2005 American Thoracic Society guidelines.

First dose pre and post spirometry: All subjects will undergo spirometry before and 20 minutes after the first study drug dose as follows.

Perform baseline spirometry and measure SpO2. Multiply the patient's best FEV_1 by 0.80 to obtain 20% fall in FEV_1 value.

Premedicate subject with 4 puffs albuterol and wait 15-30 min.

Begin study drug nebulization.

Wait for 15-30 minutes and repeat spirometry and SpO2.

Subject is excluded from the study of SpO2 is <89% or FEV_1 declines by 20% or greater from baseline.

Administration: Subjects will take their routine inhaled medicines followed by chest physical therapy. All subjects will then receive 2-4 puffs of albuterol metered dose inhaler 15-30 minutes before the study drug. Finally the study drug will be inhaled. All empty, unused and partially used study drug containers will be tracked by the investigators. Subjects will be required not to use any new treatments if not medically indicated during the study period.

Day 14 follow-up: Subjects will undergo spirometry and sputum collection and answer CFQ-R questionnaire. Blood draw for LFTS and electrolytes will be done if not done for clinical reasons. If subjects are discharged prior to 14 days of xylitol use, nebulizer solutions will be sent home. They will be asked to keep a log of nebulizer use and bring back empty and unused vials on follow-up visit (Day 10-15, preferably day 14). They will undergo spirometry and sputum collection and questionnaire administration on that day. If subjects don't return on this day, they will have a phone follow-up for adverse events and compliance.

Phone follow-up: Week 1, Day 90 and Day 180 after discharge: Subjects will be contacted by phone at these time points to enquire about further pulmonary exacerbations, admissions, and other AEs.

In the event of bronchospasm: Subjects will alert the nurses or study team members. Nebulized bronchodilators will be immediately administered. Once symptoms resolve, they will undergo spirometry using a portable handheld spirometer by a trained user. If the subjects dropped FEV_1 by 20% or greater from their baseline, they will not receive further study drug. However, they will remain in the study for completion of data collection.

Endpoints: Primary outcome will be FEV_1 change from baseline. Other safety assessments include vital signs within 30 minutes after nebulization, withdrawal from the study, rescue bronchodilator use, incidence of treatment related adverse events, and laboratory tests (chemistry and liver function tests). Efficacy outcomes include difference in density of colonization per gram of sputum from baseline to day 14, time to next hospitalization, difference in CFQ-R, sputum cytokine levels and cell counts.

Adverse events such as cough, chest tightness, and headache will be scored on a visual analog scale. Other variables collected include antibiotic and other medication use during the hospitalization, demographics, organ involvement with CF, genotype data from medical records. Follow-up phone calls will be made 1 week, 3 months and 6 months

after study completion for any complications and to assess the time to next hospitalization.

Adverse events: Any untoward medical occurrence (abnormal symptom, sign or laboratory value) in a study subject that is temporally associated with the study drug will be reported as an adverse event. The event does not have to have a causal relationship with the treatment. The event will be classified by the investigator as mild, moderate or severe. The relationship of an adverse event to the study drug will be determined by the investigator to be 'unlikely to be related, possibly related, probably related or definitely related'.

Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining unlinked sputum and blood specimens for possible use in future research studies. Samples may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Each sample will be labeled only with a study ID to protect subject's confidentiality.

There are no benefits to subjects in the future research use of their specimens. Reports about future research done with these samples will not be kept in their health records. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject's decision can be changed at any time prior to the end of the study by notifying the study investigators in writing.

However, if a subject consents to future use and some of their specimens have already been used for research purposes, the information from that research may still be used.

Statistical analysis: We plan to enroll a total of 60 subjects for this study. These subjects will be randomly assigned to the treatment (xylitol) and control (saline) groups, with a goal of 30 subjects in each group. Since this is a pilot safety study, it has been primarily powered to detect common adverse events. Assuming that the likelihood of a subject experiencing an adverse event is no less than 5%, a sample size of 30 subjects ensures 80% power towards event detection [16]. (In other words, with a group sample size of 30, the probability of observing at least one adverse event in the group is at least 80%.)

Study Performance

Accrual will be assessed be on a monthly basis. We will examine the number of screened subjects, those meeting eligibility criteria, the number of persons consenting to participate and the number of randomized. The rate of accrual will be tracked versus the expected number to achieve the target of 60 participants randomized.

We will also track the completion number and proportion by time. The number of study terminations and reason for termination will be measured. The subject will undergo a spirometry and if the FEV_1 has declined by 20%, the subject will be terminated from the study.

Baseline

The composition of the study sample will be described in aggregate. Comparison of randomized participants of baseline characteristics will be analyzed using 2 sample t-test,

Wilcoxon rank sum test, and analysis of variance (ANOVA) as indicated by the type and characteristics of the variable.

Outcomes

To assess the adequacy of these group sample sizes for testing a trend in efficacy, we consider the outcome of log CFU. We assume an average density of colonization of 6.5 log CFU with a SD of 1.5 log CFU among CF subjects. To detect a reduction of 1.5 log CFU in the xylitol group with 80% power, the required sample size is 17 subjects per group. (This computation is based on a two-sided, two-sample t-test conducted at the 0.05 level of significance.) Data from all patients who have received at least one dose of the drug or placebo will be analyzed. Thus, the analysis will be based on intention to treat. All tests will be two-sided, and conducted using a significance level of 0.05.

The primary outcome is FEV_1 change from baseline. To test for a difference in the mean change between the treatment group and the control group, a two-sample t-test will be conducted. To estimate the difference in the mean change, the equivalent confidence interval procedure will be used. Prior to conducting the test, normality will be assessed for each group based on normal probability plots. If gross departures from normality are observed, a Wilcoxon rank sum test will be performed instead of the t-test. (With group sample sizes of 30, the t-test is fairly robust to departures from normality.)

The same analytical approach will be used to assess differences in the group means for the following variables: the primary outcome of change in respiratory symptom score, and the efficacy outcomes of change in density of colonization (per gram of sputum),

change in sputum cytokines and cell counts, and responses to all Likert-scale questions on the CFQ-R.

To analyze group differences in the efficacy outcome of time to next exacerbation, a time-to-event analysis will be conducted. Kaplan-Meier estimates will be obtained for each survival curve (where failure is defined by exacerbation). To compare the survival distributions, a log rank test will be employed.

To compare the proportion of subjects in each group who have experienced any adverse events, Fisher's exact test will be used. Since certain subjects may experience multiple adverse events, we will also compare the incidence of adverse events in each group. For this comparison, a test will be conducted to contrast the mean number of events in each group, where the two samples of event counts are treated as arising from independent Poisson distributions. To estimate the difference in group proportions and the difference in group means, equivalent confidence interval procedures will be employed.

The preceding inferential procedures will be conducted for individual types of adverse events (e.g., cough, chest tightness, headache), as well as the aggregate of all adverse events. Fisher's exact test will also be used to compare the compliance proportion and the proportion of rescue bronchodilator use in each group.

Since this study is a randomized, controlled clinical trial, we expect the treatment group and the control group to be balanced with respect to factors that could affect the primary

and efficacy outcomes, such as age and gender. For such variables, descriptive statistics will be compiled for each group to ensure that the randomization has been effective. If a marked difference is observed in the distribution of any variables, an analytical approach will be utilized that will allow us to control for these variables. Specifically, the t-test can be replaced with an analysis of covariance model, the log-rank test can be replaced with a Cox proportional hazards model, Fisher's exact test can be replaced with a logistic regression model, and the two-sample Poisson test can be replaced with a Poisson regression model.

All analyses will be performed by the study statistician using SAS version 9.2 (SAS Institute, NC).

Risks: Exposure to xylitol and hypertonic saline is expected to not elicit any significant symptoms other than some cough; however we recognize that there may be a possibility of bronchospasm with the hyperosmolar xylitol in a subgroup of subjects with CF who have bronchial hyperresponsiveness that was not known previously.

Blood draw and spirometry are a part of standard care and will not pose added risks. With the admission labs that are routinely done, we will collect another red top tube for liver function tests if they were not planned. Answering questions may involve some concentration and may be boring.

Risks are minimized as follows: In the event of bronchospasm with the study drug, immediate treatment with bronchodilators is available. The subject will undergo a spirometry and if the FEV_1 has declined by 20%, the subject will be terminated from the

study. Blood draw will be performed by trained nurses or the hospital IV team with minimal discomfort. Spirometry will be done by experienced respiratory therapists. Symptom questionnaire will be administered by well-trained interviewers and will be as brief as possible.

Confidentiality: All the test results and questionnaire will be stored in the research office under lock and key with access only to the investigators. Once all testing is complete and data is entered for analysis, there will be no identifiers connecting the subject to the data. All results will be reported in summary without identifying the subjects. Blood and urine specimens will not be stored for future use.

Stopping criteria: If any subject drops his/her FEV_1 % predicted by 20%, we will stop further exposures. If 3 subjects drop their FEV_1 by 20% the treatment assignment will be unblinded and they were all in the xylitol group, the study will be closed for further recruitment.

Safety Monitoring: Any unexpected fatal or life-threatening adverse event will be reported to the Food and Drug Administration (FDA) by phone, fax or email no later than 7 days of occurrence. Any other serious and unexpected adverse event associated with the use of xylitol will be reported to the FDA within 15 days of occurrence. Please see DSMP for a detailed safety plan.

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