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Original Article

A randomized controlled trial of inhaled L-Arginine in patients with cystic fibrosis

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Abstract

Background: Cystic fibrosis (CF) airways are nitric oxide (NO) deficient. We studied safety and efficacy of repeated inhalations of nebulized L-arginine, the substrate for NO synthase (NOS), in patients with CF.
Methods: Double-blind, randomized, placebo-controlled crossover treatment trial of twice daily inhalation of 500 mg L-arginine for two weeks compared to inhalation of saline in 19 CF patients (ClinicalTrials.gov Identifier: NCT00405665).
Results: L-Arginine inhalation was well tolerated and resulted in a significant increase in exhaled NO. FEV_1 increased by an average of 56 ml compared to −8 ml after saline solution; but this difference did not reach statistical significance. Sputum concentrations of L-ornithine, the product of arginase activity, increased significantly while the L-ornithine derived polyamines did not. There was no change in inflammatory markers in sputum.
Conclusion: Repeated inhalation of L-arginine in CF patients was safe and well tolerated. Inhaled L-arginine increased NO production without evidence for changes in airway inflammation.

Keywords: Nitric oxide; L-Arginine; Polyamines; Clinical trial; Airway inflammation

1. Introduction

Nitric oxide (NO), which is important in the regulation of airway smooth muscle tone, is formed from L-arginine by nitric oxide synthases (NOSs). The availability of L-arginine for NOS and the production of NO are reduced in cystic fibrosis (CF) airways [1,2] Recent evidence also suggests that the competitive NOS inhibitor asymmetric dimethylarginine (ADMA), which is found in CF airways in high concentrations, contributes to the known NO deficiency and increases the availability of L-arginine for arginase, an enzyme that also uses L-arginine as substrate [3]. The activity of arginase is increased in CF airway secretions, and L-ornithine, the product of arginase activity, further reduces the availability of L-arginine for intracellular NOS by competing for its cellular uptake [2]. L-Ornithine is the precursor for polyamine biosynthesis. The polyamines putrescine, spermidine and spermine are present in CF airways in high concentrations and, while it remains unclear what role polyamines play in CF lung disease, there is evidence that spermine may contribute to airway smooth muscle constriction [4,5]. While supplementation of L-arginine has the potential to improve airway NO production and pulmonary function in CF patients, an increase in L-arginine availability may augment the production of L-arginine metabolites such as ADMA or spermine with untoward side effects. In addition, the effect of increased NO production on airway inflammation in CF has not been defined.

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Pulmonary function in patients with CF is closely linked to airway NO production, as patients with better function were found to have higher exhaled NO and sputum NOx levels compared to those with poor lung function [6–8]. Antibiotic treatment for a CF pulmonary exacerbation improves pulmonary function and increases airway NO formation [2,3,9,10]. A single inhalation of nebulized L-arginine transiently increased both exhaled NO and FEV_1 in one previous clinical study in CF patients [11]. We therefore conducted a double-blind, randomized, placebo-controlled treatment trial to assess the safety and efficacy of twice daily inhalation of L-arginine in CF patients. In this study we tested the hypothesis that repeated doses of inhaled L-arginine would: 1. improve airway NO production and pulmonary function in CF patients, and 2. be well tolerated and have a negligible effect on airway inflammation.

2. Methods

This is a double-blind, randomized, placebo-controlled crossover treatment trial to assess the safety and efficacy of twice daily inhalation of a 500 mg L-arginine solution for 2 weeks compared to inhalation of saline solution matched for tonicity in 20 patients with CF at the Hospital for Sick Children (ClinicalTrials.gov identifier: NCT00405665). The study was approved by the Institutional Research Ethics Board; written informed consent was obtained in all cases.

Patients were eligible for this study if they had a confirmed diagnosis of CF as defined by two or more clinical features of CF and a documented sweat chloride concentration greater than 60 mEq/L or two well characterized disease causing CFTR gene mutations, were 14 years of age and older, clinically stable at enrollment and had a FEV_1 between 40% and 80% of predicted values [12]. Exclusion criteria include a respiratory culture positive for *Burkholderia cepacia* complex within the past year or at screening, use of systemic corticosteroids (1 mg/kg or $\geq$20 mg of prednisone per day) within 30 days of screening, use of intravenous antibiotics or oral quinolones within 14 days of screening, history of biliary cirrhosis, portal hypertension, or splenomegaly on physical exam at screening or enrollment, other major organ dysfunction, investigational drug use within 30 days of screening, history of alcohol, illicit drug or medication abuse within 1 year of screening, history of lung transplantation or currently on lung transplant list, positive pregnancy test at screening (performed on all post-menarche females), acute respiratory symptoms including wheezing at the time of study, inability to take any form of bronchodilator, peripheral oxygen saturation below 95% on room air or currently receiving supplemental oxygen therapy.

2.1. Intervention

A 10% L-arginine solution (5 ml) was administered by inhalation twice daily for 14 days with crossover to 5 ml of saline inhaled twice daily for 14 days in randomized sequence using sequentially numbered containers assigned by the Sick Kids Research pharmacy. The active treatment consisted of L-arginine 250 mg/ml dispensed in 2.2 ml vials, from which the patient took 2 ml (500 mg) and diluted it with 3 ml of sterile water to give 5 ml of a 100 mg/ml solution. A placebo of similar osmolarity and appearance was formulated consisting of 2.2 ml vials of 1110 mmol/L (2.6%) hypertonic saline. A PARI eFlow prototype (PARI, Starnberg, Germany) was used for nebulization. Participants and caregivers were blinded to treatment allocation.

The justification for this regimen was based on the tolerance and safety of a previous trial on inhaled L-arginine in CF patients, where a single dose of 1.3 g L-arginine was administered with the same inhalation device [11]. The total volume of this solution was 18 ml and we reasoned that an additional daily inhalation was only feasible if the volume does not exceed 5 ml per inhalation for a twice daily regimen. We therefore opted to use a twice daily inhalation of 5 ml of a 100 mg/ml solution resulting in a cumulative daily dose of 1 g L-arginine.

The primary efficacy endpoint was the absolute change in lung function, measured by the change in FEV_1 from baseline to completion of the 14 days treatment periods. Secondary efficacy endpoints included absolute changes in FVC and in FEV_{25-75} from baseline to completion of the 2 week treatment periods, relative changes in these lung function parameters from baseline to completion of the 2 week treatment period, changes in exhaled NO as well as changes in L-arginine metabolite concentrations and in inflammatory markers in sputum including absolute neutrophil counts, neutrophil elastase activity and interleukin (IL)-8.

Patients underwent a screening assessment that included a history and physical examination, review of current medications, spirometry and respiratory tract cultures for microbiology. The enrolment visit occurred within 14 days of the screening visit. If all eligibility criteria were still met, patient was randomized to a treatment arm. A repeat visit was performed at day 15, the day after the last inhalation of study medication. Subsequently, patients entered a 4 week washout period before crossover into the second treatment phase that was identical in scheduled visits to the first phase of the study.

Participants performed spirometry according to ATS guidelines using a mass flow sensor (Vmax series, SensorMedics Corporation, Yorba Linda, California, USA) at screening Day-14, enrollment Day 1 and Day 15. The fraction of exhaled NO (F_{ENO}) was measured using a chemiluminescence analyzer (Eco Physics CLD 88 sp® NO analyzer, Dürnten, Switzerland) at Day 1, and 15. Single breath on-line measurements were performed at a constant expiratory flow of 50 $ml \times min^{-1}$, ($F_{ENO\ 50}$) in accordance with published ERS/ATS standards [13]. The mean of three measurements within 15% variation are used for analysis.

Participants supplied spontaneously expectorated sputum specimens for the measurements of L-arginine metabolite concentrations and markers of inflammation at screening Day 14, enrollment Day 1 and Day 15. The amino acids L-arginine, L-citrulline, and L-ornithine and ADMA were measured in sputum supernatant using liquid chromatography tandem mass spectrometry (LC/MS/MS), as recently described [3]. Quantification of the putrescine, spermidine and spermine was performed using LCMS, according to the method described by Byun et al. [14]. Sputum Il-8 concentrations were measured by ELISA