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Original Article

Iron supplementation does not worsen respiratory health or alter the sputum microbiome in cystic fibrosis

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Abstract

Background: Iron supplementation for hypoferremic anemia could potentiate bacterial growth in the cystic fibrosis (CF) lung, but clinical trials testing this hypothesis are lacking.
Methods: Twenty-two adults with CF and hypoferremic anemia participated in a randomized, double-blind, placebo-controlled, crossover trial of ferrous sulfate 325 mg daily for 6 weeks. Iron-related hematologic parameters, anthropometric data, sputum iron, Akron Pulmonary Exacerbation Score (PES), and the sputum microbiome were serially assessed. Fixed-effect models were used to describe how ferrous sulfate affected these variables.
Results: Ferrous sulfate increased serum iron by 22.3% and transferrin saturation (TSAT) by 26.8% from baseline ($p < 0.05$) but did not affect hemoglobin, sputum iron, Akron PES, and the sputum microbiome.
Conclusions: Low-dose ferrous sulfate improved hypoferremia without correcting anemia after 6 weeks. We did not observe significant effects on sputum iron, Akron PES, and the sputum microbiome. Although we did not identify untoward health effects of iron supplementation, a larger blinded randomized controlled trial would be needed to fully demonstrate safety.

Keywords: Hypoferremia; Anemia; Cystic fibrosis; Iron; Hepcidin-25; Microbiome

Abbreviations: CBC, complete blood count; CF, cystic fibrosis; CFPE, cystic fibrosis pulmonary exacerbation; CFRD, cystic fibrosis-related diabetes; CFTR, cystic fibrosis transmembrane conductance regulator; ELISA, enzyme-linked immunosorbent assay; FEV_1%, percent-predicted forced expiratory volume in one second; IL-6, interleukin-6; IRB, institutional review board; OTU, operational taxonomic unit; *P.a.*, *Pseudomonas aeruginosa*; PEG, polyethylene glycol; PES, Pulmonary Exacerbation Score; SDI, Simpson Diversity Index; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

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1. Introduction

Anemia affects an estimated 10–29% of adult cystic fibrosis (CF) patients [1–3]. We [4] and others [2,3] have observed low circulatory iron stores (hypoferremia) in 23–100% of anemic CF patients, suggesting that iron deficiency may restrict erythropoiesis [5]. Nonetheless, accurate assessment of iron status is challenging in CF because serum ferritin [6] and transferrin saturation (TSAT) [7] are often increased and decreased, respectively, by inflammation, leading to overestimation or underestimation of total body iron reserves. In CF, an elevated serum soluble transferrin receptor (sTfR) level reflects hypoferremic anemia [7] and is not influenced by the acute phase response of infective exacerbation [8], but it cannot distinguish between iron-limited erythropoiesis and anemia of chronic disease [9] wherein iron is not mobilized for erythropoiesis [10]. Therefore, no single blood test explains the finding of hypoferremia in CF.

However, iron supplementation is warranted for selected patients [11], but this practice is associated with several theoretical concerns. Bacteria in the CF lung require iron for growth and possess mechanisms to obtain this micronutrient from human tissues [12,13]. Iron enhances the formation of *Pseudomonas aeruginosa* (*P.a.*) biofilm communities [14] which can be visualized in the sputum of patients [15]. In an epithelial co-culture model, ΔF508-CFTR increases iron in airway surface liquid and augments *P.a.* biofilm growth and antibiotic resistance [16,17]. Neovascular changes in bronchial arteries may lead to hemoptysis [18], also introducing iron into the airways. These observations prompted us to ask three related questions about oral iron supplementation: 1) does it increase sputum iron?; 2) does it alter bacterial communities (i.e., the microbiome of sputum from the CF lung)?; and 3) compared to placebo, does it increase the frequency of CF pulmonary exacerbation (CFPE)?

That iron supplementation might be harmful in CF is a concern of clinicians who reported the onset of CFPE symptoms in patients following infusion of intravenous iron [19]. Ambiguity in the literature about the definition of CF-related anemia and its underlying mechanisms arguably contribute to the use of iron supplements in patients for whom additional iron is unlikely to be beneficial. We conducted this study to more fully understand the clinical ramifications of iron supplementation in CF.

2. Methods

2.1. Subjects

Adults who were ≥18 years old with CF confirmed by genotype analysis were recruited from the programs at Dartmouth-Hitchcock Medical Center (DHMC) and Maine Medical Center (MMC). They provided written informed consent as part of identical protocols approved by the institutional review boards (IRBs) at both sites. Participants were required to have serum transferrin saturation (TSAT) ≤21% and hemoglobin concentration <15.5 g/dl (men) or <13.6 g/dl (women) at screening. TSAT ≤21% is below the mean for 20–39 year old Caucasian women in the third National Health and Nutrition Examination Survey (NHANES III) [20]. Cutoffs for hemoglobin are below the gender-specific means for 20–29 year old Caucasians in NHANES III [21]. All subjects had a history of ≥1 *P.a.*-positive sputum culture. Exclusion criteria included use of iron-containing vitamins, history of an iron-overload condition or cirrhosis, pregnancy or breastfeeding, and recent visible hemoptysis.

2.2. Study design

This investigation was a randomized, double-blind, placebo-controlled, crossover trial of ferrous sulfate 325 mg taken orally once a day for 6 weeks. Subjects were randomized in a 1:1 allocation. A 30-day washout period occurred between arms. Subjects attended follow-up visits at weeks 0, 3, and 6 of each arm. The CONSORT flow chart (Fig. 1) further describes subject participation. Treatment adherence was determined by pill counts and asking subjects about remaining pills at each visit. Because constipation is a common problem in CF [22] that could be worsened by iron, study personnel tracked this symptom. Subjects were advised to avoid taking the study drug at the same time as fluoroquinolones, as iron limits their absorption [23]. Systemic antibiotic use was documented at each visit because of its effect on iron homeostasis [24]. CFPE was defined by an Akron Pulmonary Exacerbation Score (PES) ≥5 [25].

2.3. Sample size and statistical analyses

The primary endpoint of this study was the absolute change from baseline in hemoglobin concentration attributed to ferrous sulfate. Ater et al. [26] found that 8 out of 22 CF patients (36%) treated with ferrous sulfate (6 mg/kg/day) experienced a ≥1.0 g/dl increase in hemoglobin after 4–5 weeks. We calculated that 28 subjects would be needed to observe this endpoint with power of 80% and $\alpha = 0.05$. Secondary endpoints were absolute changes from baseline attributed to ferrous sulfate and antibiotic use for the following parameters: serum iron, hepcidin-25, TSAT, sputum iron, and incremental and dichotomized PES (< or ≥5 points). Paired Student's t-tests were used to compare sputum microbiome parameters.

All data were checked for normality (Kolmogorov–Smirnov test) and were otherwise log-transformed. Heterogeneity of the carry-over effects between the two treatment sequences was refuted by permutation test. Fixed-effect models accounted for repeated measurements within subjects and treatment sequence [27]. For log-transformed predictor variables, the estimated effect is expressed as percent change relative to baseline. Otherwise, the estimated effect signifies the absolute change from baseline and (standard error) that is explained by each variable. Multicollinearity disallowed simultaneous inclusion of serum iron and TSAT in the models. Fisher's exact test was used to compare side effect frequency and adherence between arms. SAS 9.3® (SAS Institute, Inc., Cary, NC) and GraphPad Prism® 5.04 (GraphPad Software, Inc., La Jolla, CA) were used for all analyses. A two-tailed p-value <0.05 was significant.