Glutamine Supplementation in Cystic Fibrosis: A Randomized Placebo-Controlled Trial

Doug L. Forrester, PhD,[1] Alan J. Knox, FRCP,[1] Alan R. Smyth, FRCP,[2] Helen L. Barr, PhD,[1] Rebecca Simms, BSc,[1] Sarah J. Pacey, MPhil,[3] Ian D. Pavord, FRCP,[4] David Honeybourne, FRCP,[5] Jane Dewar, PhD,[6] Andy Clayton, PhD,[6] and Andrew W. Fogarty, FRCP[7]*

Summary. Rationale: Pulmonary infection and malnutrition in cystic fibrosis are associated with decreased survival. Glutamine has a possible anti-microbial effect, with a specific impact against *Pseudomonas aeruginosa*. We aimed to test the hypothesis that oral glutamine supplementation (21 g/day) for 8 weeks in adults with cystic fibrosis would decrease pulmonary inflammation and improve clinical status. Methods: The study design was a randomized double-blind placebo-controlled study design with an iso-nitrogenous placebo. The primary analysis was intention to treat, and the primary outcome was change in induced sputum neutrophils. Results: Thirty-nine individuals were recruited and thirty-six completed the study. Glutamine supplementation had no impact on any of the outcome measures in the intention-to-treat analysis. In the per protocol analysis, glutamine supplementation was associated with an increase in induced sputum neutrophils ($P = 0.046$), total cells ($P = 0.03$), and in *Pseudomonas* isolation agar colony forming units ($P = 0.04$) compared to placebo. Conclusions: There was no effect of glutamine supplementation on markers of pulmonary inflammation in the intention-to-treat analysis. **Pediatr Pulmonol. 2016;51:253–257.**　© 2015 Wiley Periodicals, Inc.

Key words: glutamine; cystic fibrosis; infection; nutrition.

INTRODUCTION

Cystic fibrosis (CF) is a disease characterized by chronic pulmonary infections and impaired nutrition.[1] In recent decades, new interventions have been developed and these collectively have resulted in large improvements in survival over this period.[2] However, the median age at death in 2008 was approximately 27 years,[3] and further interventions are required to build on the current knowledge and hence enable individuals with CF to live longer. These interventions should ideally be easily deliverable in the home, reducing the need for recurrent hospital visits.

Glutamine is an amino acid that may be clinically active against infection. In an early randomized controlled trial of acute trauma patients,[4] those allocated to parenteral glutamine had a large reduction in secondary infections, including pneumonia and sepsis. Clinical benefits have also been reported after glutamine supplementation in a recent meta-analysis of the studies in critically ill and surgical patients, with a significant decrease in infections.[5] The biological mechanism of any anti-microbial effect is unclear, although a model of skin burns in animals demonstrated a large reduction in infection with *Pseudomonas aeruginosa*,[6] suggesting a

[1]Division of Respiratory Medicine, University of Nottingham, Nottingham, United Kingdom.

[2]Division of Child Health, Obstetrics and Gynaecology, University of Nottingham, Nottingham, United Kingdom.

[3]Department of Pharmacy, NUH NHS Trust, Nottingham, United Kingdom.

[4]Division of Respiratory Medicine, Oxford University, United Kingdom.

[5]Department of Respiratory Medicine, Heartlands Hospital, Birmingham, United Kingdom.

[6]Department of Respiratory Medicine, Nottingham City Hospital, Nottingham, United Kingdom.

[7]Division of Public Health and Epidemiology, University of Nottingham, Nottingham, United Kingdom.

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*Correspondence to: Andrew Fogarty, Division of Public Health and Epidemiology, University of Nottingham, Nottingham, United Kingdom. E-mail: andrew.fogarty@nottingham.ac.uk

possible specific activity against this bacterium that may also explain some of the clinical benefits seen in patients with severe burns.[7,8] More generally, phagocytosis is impaired in the context of protein–energy malnutrition, possibly as a consequence of decreased complement levels.[9]

Individuals with CF often have a low lean body mass and this is associated with increased subsequent mortality.[10] The disease is also characterized by chronic infection with *Pseudomonas aeruginosa*, which is an independent indicator of accelerated decline in lung function[11] and decreased survival.[12] Children with CF have lower intracellular glutamine levels in their neutrophils than controls, which may modify their ability to mount an effective response to infection,[13] as neutrophils are an important component in protecting the healthy lungs from infection.[14] We hypothesized that supplementation with glutamine may thus have clinical benefits to individuals with CF and completed a randomized double-blind placebo-controlled trial to test this using sputum biomarkers of inflammation as the main outcome measure.[15]

MATERIALS AND METHODS

The study used a randomized double-blind parallel group trial design. The study was registered as a clinical trial (EudraCT2007-006204; ISRCTN22534872) and approved by the Nottingham Research Ethics Committee. Informed consent was obtained from all participants.

Study Population

We recruited participants from Nottingham University Hospitals NHS Trust and Birmingham Heartlands Hospital Heart of England NHS Trust. The inclusion criteria were diagnosis of CF based on genotype and/or positive sweat test, forced expiratory volume in one second (FEV_1) of 25–80% predicted, and chronic pulmonary *Pseudomonas aeruginosa* infection with a positive isolation of *Pseudomonas aeruginosa* from the airways in the previous 12 months. Individuals were excluded if they were pregnant or breastfeeding, had a pulmonary exacerbation or change in pulmonary therapy in the preceding 4 weeks, had received a lung transplant, had poorly controlled diabetes mellitus, liver failure, had a liver function assay greater than three times the upper limit of normal, or were involved in another interventional clinical trial.

Randomization and Intervention

The randomization schedule was generated using STATA (TX) stratified for center and used variable block sizes. The randomization schedule was held in pharmacy in Nottingham and both researchers and participants were blinded to allocation throughout the study. Participants were randomized to receive either 21 g L-glutamine daily (one sachet of 7 g glutamine powder to be dissolved in water and taken three times a day, Essential Nutrition, Brough, UK) or a matched iso-nitrogenous placebo (a mixture of 82% asparagine and 18% glycine) in identical sachets for an 8-week period. The dose of glutamine was pragmatically chosen as being consistent with the dose that appeared both biologically active based on the literature available at the time of study and a tolerable size to facilitate adherence by the patients.

Data Collection

Prospective participants were seen at a screening visit and for three further visits. First, before starting the intervention/placebo and then at week 4 (+/−7 days) and 8 (+/−7days). Participants were then reviewed by telephone call 1 month after completing the study to ensure there were no deferred consequences of participation in the study.

We collected baseline demographic and clinical data before randomization: weight, height, spirometry (FEV_1, forced vital capacity [FVC], peak expiratory flow [PEF]). Based upon examination findings and symptoms, a composite clinical score as described by Jensen et al.[16] was calculated. Participants also completed the Cystic Fibrosis Questionnaire-Revised (CFQ-R), a validated quality of life questionnaire.[17] This has 11 domain scores from 0 to 100, with higher scores representing a perception of greater well being.

Laboratory Measurements

Induced sputum sample collection and processing were performed as previously described.[18] Briefly, sputum plugs were selected and mixed with 4× weight/volume 0.1% dithiothreitol (DTT) and an equal volume of Dulbecco's phosphate-buffered saline (PBS). Samples were filtered and assessed for cell viability and differential cell counts were performed using Rappi Diff II. Colony forming unit (CFU) counts were performed on blood and Pseudomonas Isolation Agar using serial dilution with 0.9% saline, counted at 24 and 48 hr. Multiplex cytokine analyses for TNF-a, IL-8, IL-6, and IL-1b were performed using a Bio-plex® (BioRad) using an eight-point standard curve and the average of samples run in duplicate.

Statistical Analysis

Analysis of changes in measurements used Student's *t*-tests and Wilcoxon rank sum tests as appropriate. The intention to treat analysis replaced any missing values with baseline values as part of the statistical analysis plan. Secondary analysis used per protocol analysis of all

available paired data. The primary outcome measure was the change in induced sputum neutrophil count over 8 weeks and the other measures were assessed as secondary outcomes.

With 36 individuals providing data, the study would have over 80% power to detect an absolute 75% reduction in sputum neutrophil count using data from Ordonez et al.[19] All statistical analyses were performed using STATA SE 13.0 (Stata Corporation, College Station, TX) software.

RESULTS

Four hundred and eighty-nine patients were screened, and of these 180 patients were approached with 39 individuals being randomized into the study and 36 attending for the final visit (Fig. 1). Twenty-four (62%) were male, the mean age was 30 years and the mean percent predicted FEV_1 was 47% (Table 1), with a value of 41% in the glutamine supplementation group and 53% in the placebo group.

In the intention-to-treat analysis, there was no difference between those who received glutamine supplementation compared to placebo for either the clinical measurements or the markers of pulmonary inflammation ($P > 0.05$ for all values). In the per protocol analysis, supplementation with oral glutamine was associated with an increase in sputum neutrophils ($P = 0.046$), total cells ($P = 0.03$) and *Pseudomonas* Isolation Agar CFUs ($P = 0.04$) compared to those who received placebo (Table 2). There was no change in any of the quality of life measurements in these paired data (data not presented).

Questioning of 30 participants at the end of the study assessed effective blinding of the study. Thirteen were unsure, twelve thought they were receiving glutamine (eight correct), five thought they were not (four correct). There were no adverse events or side effects of treatment or placebo detected in the study.
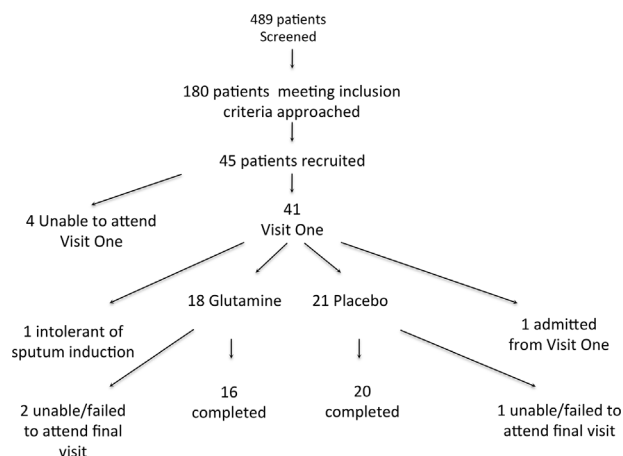

489 patients
Screened

180 patients meeting inclusion
criteria approached

45 patients recruited

4 Unable to attend
Visit One

41
Visit One

18 Glutamine   21 Placebo

1 intolerant of
sputum induction

1 admitted
from Visit One

2 unable/failed
to attend final
visit

16
completed

20
completed

1 unable/failed to
attend final visit

Fig. 1. Flow diagram of patient screening and participation on the study.

DISCUSSION

This is the first randomized double-blind placebo-controlled trial to test the hypothesis that glutamine supplementation may have clinical benefit in individuals with CF. Although there was no change in the main outcome measures in the intention-to-treat analysis, the per protocol demonstrated that the group who received glutamine supplementation had an increase in sputum neutrophils, total cells, and *Pseudomonas* Isolation Agar colony forming units after 8 weeks.

The strengths of these data are the randomized controlled study design that permits evaluation of single interventions avoiding the risk of confounding. The main outcome measures were measured by an individual who was not in contact with the study participant, and this can be regarded as another level of blinding to ensure objective endpoints. Compliance with the intervention appeared good, with almost all participants attending subsequent study visits to obtain further supplies of their allocated intervention, although there was no way of being sure that every participant completed every sachet of amino acid that they were given. The blinding was successful as demonstrated by the fact that 60% of participants were unaware of their randomization status. The patients were recruited from busy cystic fibrosis centers and covered a range from mild to severe clinical disease, making these data generalizable to other comparable centers. Ninety-two of patients completed the study suggesting that the intervention was generally acceptable to these individuals.

There are a number of limitations that require consideration. The per protocol analysis involved a number of outcome measures, so the significant changes could be a consequence of multiple hypothesis testing, and not a true biological effect and the results should be considered hypothesis generating rather than definitive. The baseline *Pseudomonas* Isolation Agar colony forming units in the patients allocated to placebo were higher than those allocated to glutamine supplementation, and the increase in this outcome measure may represent regression to the mean. The choice of pulmonary inflammatory markers as the main outcome measures for this clinical trial was a necessary compromise to permit an evaluation of the potential of glutamine supplementation with CF as they are considered to be biomarkers of surrogate clinical response to anti-microbial interventions.[15] Other outcome measures such as time to next pulmonary exacerbation, a longer intervention, or an intervention in a different population with CF such as children may provide better indications of the potential clinical benefits of glutamine in cystic fibrosis. Another possibility is that a biochemical measure of systemic inflammation such as C-reactive protein may have provided a more sensitive outcome measure. We did not have the ability to measure objective measures of adherence, such as

TABLE 1—Baseline Characteristics of Study Population

	All (N = 39)	Glutamine (N = 18)	Placebo (N = 21)
	Clinical measurements		
Mean age, years (sd)	30 (10)	32 (9)	28 (9)
Number of males (%)	24 (62)	10 (56)	14 (67)
Δ508 genotype			
Homozygote	26 (67)	12 (67)	14 (67)
Heterozygote	10 (25)	4 (22)	6 (29)
Other	3 (8)	2 (11)	1 (5)
Mean weight, Kg (sd)	61.9 (10.8)	60.7 (7.8)	62.9 (13.0)
Mean height, m (sd)	1.69 (0.07)	1.69 (0.07)	1.68 (0.08)
Mean BMI, kg/m^2 (sd)	21.6 (3.1)	21.1 (2.0)	22.1 (3.8)
Mean FEV_1, L (sd)	1.71 (0.64)	1.46 (0.33)	1.92 (0.76)
Mean percent predicted FEV_1 (sd)	47 (16)	41 (9)	53 (20)
Mean FVC, L (sd)	3.08 (0.84)	2.83 (0.72)	3.30 (0.89)
Mean FEV_1/FVC (sd)	66.6 (12.4)	64.1 (11.3)	68.8 (13.1)
Mean PEF, L/min (sd)	339 (102)	316 (70)	358 (122)
Median Clinical Score (IQR)	4 (2–5) (N = 37)	4.5 (3–6) (N = 18)	3 (2–5) (N = 19)
	Median induced sputum measurements (IQR)		
Neutrophils (per ml × 10^6)	2.3 (1.3–3.7) (N = 34)	2.8 (1.8–4.5) (N = 16)	2.0 (0.9–2.8) (N = 18)
Total cells (per ml × 10^6)	2.5 (1.3–3.9) (N = 35)	2.9 (1.9–4.6) (N = 16)	2.3 (0.9–3.8) (N = 19)
Blood agar CFU (×10^7)	3.0 (1.5–15.6) (N = 34)	3.0 (1.1–1.7) (N = 15)	3.6 (2.0–1.6) (N = 19)
PIA CFU (×10^6)	6.7 (2.1–34.8) (N = 35)	4.1 (1.2–8.8) (N = 16)	23 (3–54) (N = 19)
TNFα (pg/ml)	5.6 (1.8–31.3) (N = 36)	6.3 (1.8–17.0) (N = 16)	5.3 (1.9–39.4) (N = 20)
IL1-β (pg/ml)	126 (56–211) (N = 35)	126 (57–211) (N = 17)	130 (29–203) (N = 18)
IL-6 (pg/ml)	2.7 (0.8–12.3) (N = 19)	1.1 (0.4–2.4) (N = 8)	9.0 (1.7–40.0) (N = 11)
IL-8 (pg/ml)	345 (266–489) (N = 36)	349 (286–461) (N = 17)	285 (210–500) (N = 19)

BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow; IQR, interquartile range; CFU, colony forming units; PIA, *Pseudomonas* isolation agar; TNFα, tumor necrosis factor alpha; IL1-β, interleukin 1 beta; IL6, interleukin 6; IL8, interleukin 8.

intra-neutrophil glutamine levels, or in the sputum generally and hence have no confirmation that all the glutamine supplements were taken as prescribed, or taken but not absorbed. As the main outcome measure was sputum inflammatory markers, we were unable to also measure the impact of glutamine on lean muscle mass, or protein or energy intake. The study was designed in response to the observation that other patient groups appeared to benefit from glutamine supplementation, particularly those who were in the critical care setting[4,5] and patients with burns.[7,8] However, patients with cystic fibrosis have a chronic disease that is associated with malnutrition, and hence may not respond to glutamine supplementation in the same manner, as populations with acute disease and no diagnosis of CF.

TABLE 2—Per Protocol Analysis of Change in Outcome Measures for all Participants Who Completed the Study

	Glutamine group (N = 16)	Placebo (N = 20)	Mean difference of glutamine compared to placebo
	Mean change in clinical measurements		
Weight, Kg	+0.71	+0.45	+0.26 (−1.04 to +1.56)
FEV_1, ml	+81	+26	+55 (−94 to +205)
FVC, ml	+174	+76	+97 (−175 to +370)
Clinical score	+0.6 (N = 16)	+0.5 (N = 17)	+0.1 (−2.2 to +2.4)
	Median change in induced sputum measurements		
			P-value
Neutrophils (per ml × 10^6)	+3.63 (N = 10)	+0.07 (N = 15)	0.046
Total cells (per ml × 10^6)	+3.8 (N = 10)	−0.02 (N = 16)	0.03
Blood agar CFU (×10^7)	0.00 (N = 11)	+1.0 (N = 15)	0.74
PIA CFU (×10^7)	+12.3 (N = 9)	−5.2 (N = 14)	0.04
TNFα (pg/ml)	−0.96 (N = 12)	+0.08 (N = 14)	0.96
IL1-β (pg/ml)	−33 (N = 15)	−18 (N = 15)	0.76
IL-6 (pg/ml)	−1.6 (N = 3)	−2.5 (N = 5)	0.88
IL-8 (pg/ml)	+132 (N = 14)	+17 (N = 15)	0.08

Statistical comparisons used unpaired *t*-test for clinical measurements and Mann–Whitney test for induced sputum measurements.
LFEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow; CFU, colony forming units; PIA, *Pseudomonas* isolation agar; TNFα, tumor necrosis factor alpha; IL1-β, interleukin 1 beta; IL6, interleukin 6; IL8, interleukin 8.

Finally, only 25% of those individuals who were eligible entered the study, with many citing time limitations as the reason for their not participating.

The main outcome of our study is that there was no change in markers of pulmonary inflammation in the intention-to-treat analysis, although we did observe that pulmonary neutrophils and total cell counts increased significantly in the glutamine supplementation group compared to those who received placebo in the per protocol analysis. The clinical significance of this is unclear, as an increased inflammatory response may be beneficial in some acute situations but potentially detrimental if this results in chronic inflammatory response in the lungs. It is unclear if this is a true biological effect or a chance finding as a consequence of the many statistical analyses on these data. Glutamine has been demonstrated in a recent meta-analysis of studies[5] to have a possible clinical benefit with no clear biological mechanism, and one possibility is that glutamine results in stimulation of the cellular immune response, mobilizing a more effective challenge to the presence of pathogens. However, a recent large multicenter randomized controlled trial of glutamine in critical care patients demonstrated no clinical benefit or reduction in secondary infections,[20] and an association with increased mortality at 6 months. This study was in a very different patient group to CF, and gave much higher doses of glutamine than we used in our study, so direct comparisons with our data are impossible. The only prior study of glutamine in the context of CF was alongside human growth hormone in nine children and did not report any protein gain over 4 weeks.[21]

In summary, there was no change in the primary outcome measures after glutamine supplementation in the intention-to-treat analysis, and as a consequence, these data suggest that glutamine has no clear clinical benefits in patients with cystic fibrosis.

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