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Forrester, Doug L. and Knox, Alan J. and Smyth, Alan R. and Barr, Helen L. and Simms, Rebecca and Pacey, Sarah J. and Pavord, Ian D. and Honeybourne, David and Dewar, Jane and Clayton, Andy and Fogarty, Andrew W. (2015) Glutamine supplementation in cystic fibrosis: a randomized placebo-controlled trial. *Pediatric Pulmonology*, 51 (3). pp. 253-257. ISSN 1099-0496

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Glutamine Supplementation in Cystic Fibrosis: A randomised placebo-controlled trial

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Running title: Oral glutamine supplementation in cystic fibrosis

Word count: 2049

Key words: glutamine, cystic fibrosis, infection, nutrition

Abstract

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 (21g/day) for eight weeks in adults with cystic fibrosis would decrease pulmonary inflammation and improve clinical status. Methods: The study design was a randomised 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: 39 individuals were recruited and 36 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.

Introduction

Cystic fibrosis (CF) is a disease characterised 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 randomised 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 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 characterised 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 hypothesised that supplementation with glutamine may thus have clinical benefits to individuals with CF and completed a randomised 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 randomised 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 (FEV₁) 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.

Randomisation and intervention

The randomisation schedule was generated using STATA (Texas, USA) stratified for centre and used variable block sizes. The randomisation schedule was held in pharmacy in Nottingham and both researchers and participants were blinded to allocation throughout the study. Participants were randomised to receive either 21g L-glutamine daily (one sachet of 7g 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. Firstly, before starting the intervention/placebo and then at week 4 (+/-7 days) and week 8 (+/-7days). Participants were then reviewed by telephone call one 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 randomisation: weight, height, spirometry (FEV₁, 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 questionnaire¹⁷. This has 11 domain scores from 0-100, with higher scores representing a perception of greater well-being.

Laboratory measurements

Induced sputum sample collection and processing were performed as previously described¹⁸. Briefly, sputum plugs were selected and mixed with 4x 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 24hrs and 48hrs. Multiplex cytokine analyses for TNF- α , IL-8, IL-6 and IL-1 β were performed using a Bio-plex® (BioRad) using an 8-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 eight 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, Texas) software.

Results

489 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 (Figure 1). 24 (62%) were male, the mean age was 30 years and the mean percent predicted FEV₁ 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. 13 were unsure, 12 thought they were receiving glutamine (8 correct), 5 thought they were not (4 correct). There were no adverse events or side effects of treatment or placebo detected in the study.

Discussion

This is the first randomised 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 eight weeks.

The strengths of these data are the randomised 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 randomisation status. The patients were recruited from busy cystic fibrosis centres and covered a range from mild to severe clinical disease, making these data generalisable to other comparable centres. 92% 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 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. 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, mobilising a more effective challenge to the presence of pathogens. However, a recent large multicenter randomised 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 six 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 four 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.

Acknowledgements

The study was funded by the Cystic Fibrosis Foundation (CFF RK0811) and the Nottingham Respiratory Biomedical Research Unit. Doug Forrester is funded by Wellcome Trust Fellowship WT088614MA. We would like to thank all the study participants for their involvement in the study.

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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)
Δ 508 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 to 5) (N=37)	4.5 (3 to 6) (N=18)	3 (2 to 5) (N=19)
Median induced sputum measurements (IQR)			
Neutrophils (per ml x 10 ⁶)	2.3 (1.3 to 3.7) (N=34)	2.8 (1.8 to 4.5) (N=16)	2.0 (0.9 to 2.8) (N=18)
Total cells (per ml x 10 ⁶)	2.5 (1.3 to 3.9) (N=35)	2.9 (1.9 to 4.6) (N=16)	2.3 (0.9 to 3.8) (N=19)
Blood agar CFU (x10 ⁷)	3.0 (1.5 to 15.6) (N=34)	3.0 (1.1 to 1.7) (N=15)	3.6 (2.0 to 1.6) (N=19)
PIA CFU (x10 ⁶)	6.7 (2.1 to 34.8) (N=35)	4.1 (1.2 to 8.8) (N=16)	23 (3 to 54) (N=19)
TNF α (pg/ml)	5.6 (1.8 to 31.3) (N=36)	6.3 (1.8 to 17.0) (N=16)	5.3 (1.9 to 39.4) (N=20)
IL1- β (pg/ml)	126 (56 to 211) (N=35)	126 (57 to 211) (N=17)	130 (29 to 203) (N=18)
IL-6 (pg/ml)	2.7 (0.8 to 12.3) (N=19)	1.1 (0.4 to 2.4) (N=8)	9.0 (1.7 to 40.0) (N=11)
IL-8 (pg/ml)	345 (266 to 489) (N=36)	349 (286 to 461) (N=17)	285 (210 to 500) (N=19)

BMI = body mass index

FEV₁ = 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

TNF α = Tumour necrosis factor alpha

IL1- β = Interleukin 1 beta

IL6 = Interleukin 6

IL8 = Interleukin 8

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 x 10 ⁶)	+3.63 (N=10)	+0.07 (N=15)	0.046
Total cells (per ml x 10 ⁶)	+3.8 (N=10)	-0.02 (N=16)	0.03
Blood agar CFU (x10 ⁷)	0.00 (N=11)	+1.0 (N=15)	0.74
PIA CFU (x10 ⁷)	+12.3 (N=9)	-5.2 (N=14)	0.04
TNF α (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

FEV₁ = Forced expiratory volume in one second

FVC = Forced vital capacity

PEF = Peak expiratory flow

CFU = colony forming units

PIA = *Pseudomonas* isolation agar

TNF α = Tumour necrosis factor alpha

IL1- β = Interleukin 1 beta

IL6 = Interleukin 6

IL8 = Interleukin 8

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