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Original Article

Antibiotic exposure and interpersonal variance mask the effect of ivacaftor on respiratory microbiota composition

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Abstract

Background: G551D is a class III mutation of the cystic fibrosis transmembrane regulator (CFTR) that results in impaired chloride channel function in cystic fibrosis (CF). Ivacaftor, a CFTR-potentiating agent improves sweat chloride, weight, lung function, and pulmonary exacerbation rate in CF patients with G551D mutations, but its effect on the airway microbiome remains poorly characterised.
Methods: Twenty CF patients with at least one G551D mutation from a single centre were recruited to a 4 month double-blind, placebo-controlled, crossover study of ivacaftor with 28 days of active treatment. Sputum microbiota composition was assessed by 16S rRNA gene amplicon sequencing and quantitative PCR at five key time points, along with regular clinical review, respiratory function assessment, and peripheral blood testing.
Results: No significant difference in microbiota composition was observed in subjects following ivacaftor treatment or placebo (PERMANOVA $P = 0.95$, square root ECV = −4.94, 9479 permutations). Microbiota composition variance was significantly greater between subjects, than within subjects over time ($P < 0.0001$, Mann Whitney U test), and an additional within-patient paired assessment of microbiota similarity was therefore performed. Again, change in microbiota composition was not significantly greater during treatment with ivacaftor compared to placebo (Wilcoxon test, $P = 0.51$). A significant change in microbiota composition was however associated with any change in antibiotic exposure, regardless of whether ivacaftor or placebo was administered ($P = 0.006$). In a small, subgroup analysis of subjects whose antibiotic exposure did not change within the study period, a significant reduction in total bacterial load was observed during treatment with ivacaftor ($P = 0.004$, two-tailed paired Student's t-test).
Conclusions: The short-term impact of ivacaftor therapy on sputum microbiota composition in patients with G551D mutations are modest compared to those resulting from antibiotic exposure, and may be masked by changes in antibiotic treatment regimen.

Keywords: Airway microbiome; Ivacaftor; Antibiotics

1. Introduction

Cystic fibrosis (CF) is a recessively-inherited disease that results from mutation of the gene which encodes the cystic fibrosis transmembrane regulator (CFTR), a chloride ion channel [1]. CF affects multiple organs due to widespread CFTR protein channel distribution. The G551D mutation (Class III) affects the

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ion-gating characteristics of CFTR function. Ivacaftor, a CFTR-potentiating agent is registered for treatment of patients with this mutation at many CF centres [2].

One of the major complications of CF is chronic infection of the airways with *Pseudomonas aeruginosa* and other bacterial infections resulting in generalised bronchiectasis, progressive deterioration in lung function and frequent requirement for appropriately-targeted antibacterial agents to manage both acute, infective exacerbations and chronic lung infection.

Pivotal ivacaftor studies have shown reduced sweat chloride (SCT) and increased FEV_1 and weight with treatment up to 48 weeks [2]. Improved lung function is partially correlated with sweat chloride decrease [3], suggesting ivacaftor response is multifactorial. The microbiome of CF airways is likely to change as a result of ivacaftor treatment and the associated change in airway physiology and micro-environment, but the quality and magnitude of this change remains unclear. Our aim in this study was to assess the impact of ivacaftor on the airway microbiome of CF patients with the G551D mutation who were previously naïve to this medication as part of a single-centre, placebo-controlled crossover study.

2. Materials and methods

2.1. Study design and outcome variables

Study approval was obtained from the Alfred Health Human Ethics Committee. We performed a double-blind, randomized, placebo-controlled crossover study of ivacaftor 150 mg twice daily in all 20 eligible adult patients with at least one copy of the G551D gene mutation in our centre over 4 months. Eligible patients underwent tests at screening (day −28), at baseline randomisation (day 0) and then at the end of each 28-day cycle of either active or placebo treatment with a washout period in between (Fig. 1). All patients had their CF diagnosis and G551D status confirmed by sweat test, genotype and phenotype assessment. Clinical assessment, spirometry, cardio-pulmonary exercise testing and blood tests, sputum sampling and microbiology testing were performed at each of the five study visits (Fig. 1). Routine CF care, including airway clearance and exercise routines remained unchanged. All hospital admissions and antibiotic use (inpatient and outpatient) during the period of the study were recorded. Exclusion criteria included a positive pregnancy test, strong CYP3A inducers or liver dysfunction (ALT or AST 5× upper limit of normal). Adverse events were documented and reported to the IEC and Vertex Pharmaceuticals Inc.

This component of the study focused on the impact of ivacaftor on the patients' microbiome. The primary outcome variable of interest was total bacterial load, with *P. aeruginosa* quantitation and microbiota composition being key secondary variables. Other clinical, physiological and biochemical end-points for the study are reported elsewhere [4].

2.2. Sputum processing

Spontaneously expectorated sputa were collected at all study time points. They were immediately refrigerated after collection and processed within 2 h. An equal volume of diluted (10%) dithiothreitol in phosphate buffer (Sputalysin®; Calbiochem Corp., San Diego, USA) was added to the sputa and suspended by vortexing for 30 s. An equal volume of a glycerol broth solution (23% *v/v* glycerol, 0.76% *w/v* BD Bacto tryptone, 0.38% *w/v* yeast extract, 0.76% *w/v* sodium chloride and deionized water; Monash University, Melbourne, Australia) was then added. This solution was briefly vortexed. The processed sputa were aliquoted and frozen at −80 °C.

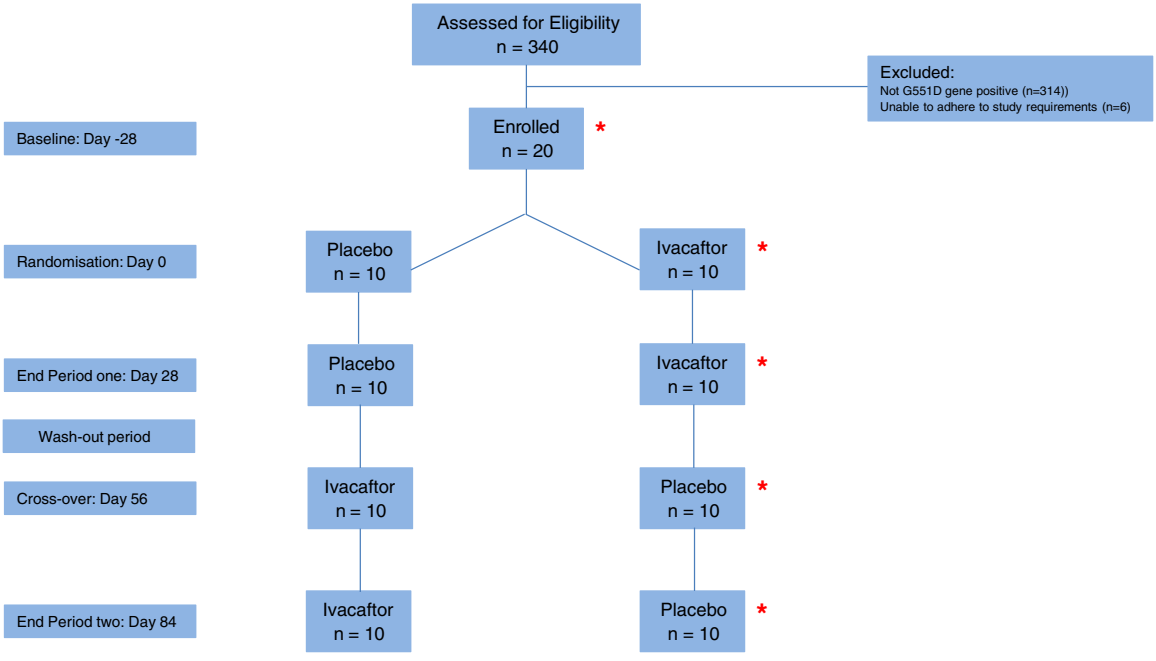

Fig. 1. Consort diagram for study (* *specified sputum collection time points*).

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