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Original Article

Impact of azithromycin on the clinical and antimicrobial effectiveness of tobramycin in the treatment of cystic fibrosis

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Abstract

Background: Concomitant use of oral azithromycin and inhaled tobramycin occurs in approximately half of US cystic fibrosis (CF) patients. Recent data suggest that this combination may be antagonistic.
Methods: Test the hypothesis that azithromycin reduces the clinical benefits of tobramycin by analyses of clinical trial data, *in vitro* modeling of *P. aeruginosa* antibiotic killing, and regulation of the MexXY efflux pump.
Results: Ongoing administration of azithromycin associates with reduced ability of inhaled tobramycin, as compared with aztreonam, to improve lung function and quality of life in a completed clinical trial. In users of azithromycin FEV_1 (L) increased 0.8% during a 4-week period of inhaled tobramycin and an additional 6.4% during a subsequent 4-week period of inhaled aztreonam ($P < 0.005$). CFQ-R respiratory symptom score decreased 1.8 points during inhaled tobramycin and increased 8.3 points during subsequent inhaled aztreonam ($P < 0.001$). A smaller number of trial participants not using azithromycin had similar improvement in lung function and quality of life scores during inhaled tobramycin and inhaled aztreonam. *In vitro*, azithromycin selectively reduced the bactericidal effects tobramycin in cultures of clinical strains of *P. aeruginosa*, while up regulating antibiotic resistance through MexXY efflux.
Conclusions: Azithromycin appears capable of reducing the antimicrobial benefits of tobramycin by inducing adaptive bacterial stress responses in *P. aeruginosa*, suggesting that these medications together may not be optimal chronic therapy for at least some patients.

Keywords: Inhaled antibiotics; Drug interaction; *Pseudomonas aeruginosa*; Clinical trial; Azithromycin; Tobramycin; MexXY; Cystic fibrosis

1. Introduction

In clinical trials testing suppressive treatment of chronic *P. aeruginosa* infections in people with cystic fibrosis (CF), both inhaled tobramycin and oral azithromycin demonstrate clear benefit to lung function and quality of life [1–3]. Concomitant use of the two agents is now common, but has not been tested prospectively. Several independent in vitro studies have reported antagonism between azithromycin and tobramycin against *P. aeruginosa* [4–6]. We have hypothesized that combined therapy with these two medications may unexpectedly provide less rather than more clinical improvement.

Herein we report the post-hoc analyses of a second CF clinical trial in which subjects received 4 weeks of inhaled tobramycin immediately preceding four weeks of inhaled aztreonam [7]. Focusing on outcomes within subjects reporting use of

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azithromycin, we observe a pattern of interaction in which concomitant azithromycin treatment was associated with poorer response to inhaled tobramycin compared with inhaled aztreonam. This differential pattern of effect between classes of inhaled antibiotics was not observed in a smaller group of subjects not using azithromycin, which is consistent with our recent post-hoc analyses of parallel subject groups exposed to inhaled tobramycin or aztreonam in a separate clinical trial [8]. To test directly if azithromycin may impair the response to tobramycin, as opposed to enhancing the response to aztreonam, we tested bacterial killing with these and other clinically relevant antibiotics using 30 CF clinical isolates of *P. aeruginosa* collected from unique individuals. Antimicrobial activity was selectively reduced when azithromycin was added to tobramycin and had either no effect or improved killing when added to several other anti-pseudomonal antibiotics used in CF clinical care. Importantly, we found that azithromycin, particularly when combined with tobramycin, greatly increased gene expression of a *Pseudomonas* efflux pump, MexXY, which is a central mechanism of inducible aminoglycoside resistance [9–12]. Both aminoglycoside and macrolide antibiotics target the bacterial ribosome. Overlap in the bacterial response to ribosomal perturbation from each antibiotic may help explain how chronic azithromycin can lessen the antimicrobial effectiveness of tobramycin [12]. Genetic removal of *mexX* in *P. aeruginosa* resulted in an additive rather than antagonistic antimicrobial pattern between azithromycin and tobramycin.

Our prior and current post-hoc analyses of CF clinical trials, combined with the complementary in vitro findings, provide additional evidence and biological plausibility indicating that in the setting of chronic *P. aeruginosa* infection, the common clinical practice of combining oral azithromycin with inhaled tobramycin as a maintenance therapy may, in some patients, be less effective than tobramycin alone. This contrasts with some earlier reports of synergy when combining azithromycin and tobramycin in vitro [13,14]. The methods used for multiple combined antibiotic testing vary and have produced, at times, inconsistent findings. Our approach to this question of drug interaction has begun with observations in clinical data sets, which inspired both microbiological and mechanistic research based on published work of others. Our bacterial culture methods are distinct from those used in prior testing and it is not known which in vitro model may best predict clinical response. The discrepant findings and potential clinical importance of such an adverse drug interaction indicates that confirmatory, prospective testing and additional mechanistic research are necessary; therefore, we are now conducting a dedicated, prospective clinical trial and continue to investigate how *P. aeruginosa* responds to this antibiotic combination. Some of these data have previously been reported in abstracts or oral presentation [15].

2. Methods

2.1. Clinical trial dataset

De-identified data from the completed AIR-CF2 clinical trial (Clinicaltrials.gov NCT00104520) were requested through an investigator initiated research process and provided by the sponsor, Gilead Sciences [7]. In this trial, subjects with CF and chronic *P. aeruginosa* all received 4 weeks of inhaled tobramycin immediately followed by 4 weeks of inhaled aztreonam or placebo. We obtained the following from all subjects who consented to share data: azithromycin use, gender, FEV_1 (Liters and % predicted), scores on cystic fibrosis quality of life-revised respiratory symptom scale (CFQ-R RSS) [16], and sputum density of *P. aeruginosa*. At enrollment, 128 of 176 subjects (73%) reported concomitant azithromycin use. Baseline mean FEV_1% predicted and gender did not significantly differ based on azithromycin use (FEV_1 58% vs. 54%) or randomization to aztreonam vs. placebo (see Table S1). All subjects without missing data, including those randomized to placebo, were included in our analysis when applicable. This results in larger groups during the tobramycin study phase compared with aztreonam study phase. Subjects randomized to twice vs. thrice daily inhaled aztreonam demonstrated similar outcomes and were pooled for our analyses, as previously done by others in the original publication of this trial [7].

2.2. Bacterial non-surface attached aggregate cultures

30 CF clinical isolates of *P. aeruginosa* were collected from 3 separate sources: 10 from Gilead Sciences banked during a clinical trial of CF subjects [17], 10 from National Jewish Health Microbiology Laboratory collected from adult CF patients in Denver, CO, and 10 randomly selected from the CF Isolate Core at Seattle Children's Research Institute. Additional details on this culture method are available in the on-line supplement and comprehensive characterization and comparison with planktonic cultures have previously been published [4]. In brief, bacteria were cultured with gentle, constant movement in low-nutrient media with human plasma and lysed human neutrophils added to form *Pseudomonas aeruginosa* aggregates over 48 h. Antibiotics were then added to cultures for an additional 16 h, at which point bacterial density was measured.

Gene expression was measured by qPCR at 5 time points between 5 and 240 min following addition of antibiotics to biofilm aggregates in a subset of the same clinical isolates of *P. aeruginosa* (N = 13). The following genes were tested for maximum expression in the 4 h following antibiotic challenge: *PA5471, mexX, mexY, mexA, mexB*. RNAprotect Bacteria Reagent (QIAGEN) was added to samples, which were stored at −80 °C. RNA was extracted with the QIAGEN QIACube and RNeasy kit (QIAGEN) using previously calculated amount of RNA, Random Hexamer (IDT 51–01–18-15), M-MLV Reverse Transcriptase (ThermoFisher Scientific 28,025–031) and 2.5 mM dNTPs (ThermoFisher Scientific AM8200) per manufacturer protocol. SYBR Green 2× (Maxima SYBR Green qPCR Master Mix ThermoFisher Scientific K0253) and 7.4ul nuclease-free water were used in a 20ul qPCR reaction, along with the primers provided in the online supplement (*PA5471, mexX, mexY, mexA, mexB*, IDT DNA). Relative gene expression was calculated using ΔCT values and internal control *rpoD* gene expression. Control cultures of each bacterial strain grown under identical conditions were used to determine