Azithromycin May Antagonize Inhaled Tobramycin When Targeting *Pseudomonas aeruginosa* in Cystic Fibrosis

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Abstract

Rationale: Recent studies of inhaled tobramycin in subjects with cystic fibrosis (CF) find less clinical improvement than previously observed. Nonhuman data suggest that in some strains of *Pseudomonas aeruginosa*, azithromycin can antagonize tobramycin.

Objectives: We tested the hypothesis that concomitant azithromycin use correlates with less improvement in key outcome measures in subjects receiving inhaled tobramycin while not affecting those receiving a comparative, nonaminoglycoside inhaled antibiotic.

Methods: We studied a cohort of 263 subjects with CF enrolled in a recent clinical trial comparing inhaled tobramycin with aztreonam lysine. We performed a secondary analysis to examine key clinical and microbiologic outcomes based on concomitant, chronic azithromycin use at enrollment.

Measurements and Main Results: The cohort randomized to inhaled tobramycin and reporting azithromycin use showed

a significant decrease in the percent predicted FEV₁ after one and three courses of inhaled tobramycin when compared with those not reporting azithromycin use (28 d: −0.51 vs. 3.43%, $P < 0.01$; 140 d: −1.87 vs. 6.07%, $P < 0.01$). Combined azithromycin and inhaled tobramycin use was also associated with earlier need for additional antibiotics, lesser improvement in disease-related quality of life, and a trend toward less reduction in sputum *P. aeruginosa* density. Subjects randomized to inhaled aztreonam lysine had significantly greater improvement in these outcome measures, which were unaffected by concomitant azithromycin use. Outcomes in those not using azithromycin who received inhaled tobramycin were not significantly different from subjects receiving aztreonam lysine. Azithromycin also antagonized tobramycin but not aztreonam lysine in 40% of *P. aeruginosa* clinical isolates tested *in vitro*.

Conclusions: Oral azithromycin may antagonize the therapeutic benefits of inhaled tobramycin in subjects with CF with *P. aeruginosa* airway infection.

Keywords: cystic fibrosis; *Pseudomonas aeruginosa*; inhaled antibiotic; azithromycin; drug-drug interaction

(Received in original form October 9, 2013; accepted in final form December 29, 2013)

Clinical data and cystic fibrosis clinical specimens of *Pseudomonas aeruginosa* provided by Gilead Sciences. Supported by National Institutes of Health/ National Center for Advancing Translational Sciences Colorado grant CTSI KL2 TR000156 (D.P.N., J.A.N.), Gilead Sciences as part of an investigator-initiated study, and a kind gift from Susan and Jim Goodwin. Additional financial support from the Rebecca Runyon Bryan Chair for Cystic Fibrosis (J.A.N.).

Contents are the authors' sole responsibility and do not necessarily represent official views of the National Institutes of Health, Gilead Sciences, or other sponsors.

Author Contributions: J.A.N.: involvement in conception, hypotheses delineation, design, data interpretation, and manuscript preparation. S.M.M.: involvement in conception, data acquisition, data interpretation, and manuscript revision. J.F.C.: involvement in conception, data interpretation, and manuscript revision. A.V.F.: involvement in data analysis and manuscript preparation. S.H.K.: involvement in data acquisition, data interpretation, and manuscript revision. M.T.S.: involvement in conception, data interpretation, and manuscript revision. L.S.: involvement in conception, data interpretation, and manuscript revision. J.L.T.-C.: involvement in conception, data interpretation, and manuscript revision. D.P.N.: involvement in conception; hypotheses delineation; design; data acquisition, analysis, and interpretation; and manuscript preparation and revision.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Ann Am Thorac Soc Vol 11, No 3, pp 342–350, Mar 2014

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DOI: 10.1513/AnnalsATS.201310-352OC

Internet address: www.atsjournals.org

Projected median survival of those with cystic fibrosis (CF) has dramatically improved in recent decades, largely as a result of new medications and therapies. *Pseudomonas aeruginosa* is the most common pathogen in the airways of people with CF. By early adolescence, half of all patients with CF are chronically infected with *P. aeruginosa* (1). This prevalence increases to nearly 80% by early adulthood, and persistent *P. aeruginosa* airway infection is linked to more rapid loss of lung function and earlier mortality (2, 3). Strategies to eradicate initial *P. aeruginosa* airway infection or treat persistent infection have proven beneficial (4–7). For those chronically infected, inhaled antibiotics achieve high airway concentrations, and cycled, chronic use of these drugs improves lung function and reduces the frequency of pulmonary exacerbations while avoiding many safety concerns associated with prolonged systemic drug exposure (8, 9).

The aminoglycoside tobramycin was the first inhaled antipseudomonal antibiotic commercially developed for CF and remains the most prescribed inhaled antibiotic used in the United States (1). Roughly two-thirds of all U.S. patients with CF and chronic *P. aeruginosa* are prescribed inhaled tobramycin—typically as 28 days of on–off cycles. Long-term use of oral azithromycin has also been shown to improve lung function and reduce exacerbations in patients with CF with chronic *P. aeruginosa* airway infection

(10, 11). Although the mechanism of action is still unclear, azithromycin may function as an antiinflammatory agent in CF (12–15). Azithromycin is widely prescribed and is now the most common chronic antibiotic used in patients with CF with persistent *P. aeruginosa* (1). In a query of the U.S. CF Foundation National Patient Registry in April 2013, we found that 71% of all patients with *P. aeruginosa*, and 75% of those prescribed inhaled tobramycin, are now also prescribed oral azithromycin on a chronic basis.

Potential interactions between concomitant medications and mechanisms for antibiotic resistance in CF are poorly understood. These issues have been cited in current CF consensus treatment guidelines as two key unanswered questions in need of further study (16). Based on recent preclinical research demonstrating antagonism between azithromycin and tobramycin, but not the fluoroquinolone ciprofloxacin (17), we hypothesized that azithromycin may selectively inhibit tobramycin when targeting *P. aeruginosa* airway infection in CF lung disease. Our primary objective was to describe the differences in key clinical and microbiological outcomes between subjects participating in a trial of inhaled antipseudomonal antibiotics, with and without concomitant azithromycin use. The primary outcome in our secondary analysis was change in lung function based on the FEV₁% predicted after one and three

courses of inhaled antibiotics (28 d and 140 d). Secondary outcomes include: time to need of additional antibiotic treatment, self-reported disease-related quality of life, and change in sputum *P. aeruginosa* density. We further tested the impact of azithromycin in addition to tobramycin or aztreonam lysine *in vitro* using a collection of *P. aeruginosa* clinical isolates from this trial under biofilm culture conditions. Some of the results of these studies have been previously reported in the form of an abstract (18).

Methods

Dataset

Through an investigator-initiated project mechanism, we received unfiltered, deidentified data from Gilead Sciences, the sponsor of a randomized comparator trial in 268 subjects with CF, which compared the clinical effectiveness of inhaled aztreonam lysine versus inhaled tobramycin (5). Subjects were randomized to receive three 28-day courses of inhaled aztreonam lysine (75 mg three times/d) or tobramycin inhalation solution (300 mg twice/d), separated by 28-day intervals without inhaled antibiotics in an open-label, parallel-group design. After completing three courses, 50% of subjects crossed over to a continuation phase of open-label aztreonam lysine. Nearly all clinical trial sites agreed to share data, and we received unfiltered datasets for 263 of 268 (98%)

Table 1. Baseline demographic and clinical data

	Tobramycin	Tobramycin + Azithromycin	Aztreonam	Aztreonam + Azithromycin	P Value*
Subjects, n	37	91	45	90	
Age, yr [†]	22.3 (7.4)	26.3 (9.6)	27.6 (9.3)	24.9 (8.9)	0.04
Sex, % female	54.1	48.4	53.3	48.9	0.96
Baseline FEV ₁ %	54.9 (13.7)	51.3 (15.0)	53.6 (14.8)	51.6 (16.0)	0.58
Baseline <i>Pseudomonas aeruginosa</i> log ₁₀ cfu/g sputum	7.2 (1.4)	6.7 (1.4)	6.5 (1.6)	6.8 (1.4)	0.41
TBRA MIC > 4, [‡] %	24	36	25	42	0.10
TBRA MIC > 128, [§] %	7	8	6	16	0.30
≥84 d of inhaled tobramycin in previous 12 mo, %	84	88	84	83	0.84

Definition of abbreviations: MIC = minimum inhibitory concentration; TBRA = tobramycin.

Number of subjects in each group, baseline characteristics, and exposure to inhaled tobramycin in the prior year are shown. Among subjects not reporting azithromycin use, those randomized to inhaled tobramycin were slightly younger than those randomized to inhaled aztreonam lysine. No other significant differences were identified between groups.

*P value corresponds to analysis of variance test for normally distributed variables and Chi-square test for frequencies. For frequencies smaller than 5 subjects, Fisher exact test was used.

[†]Mean (SD).

[‡]Parental MIC breakpoint.

[§]Inhaled MIC breakpoint.

Table 2. Change in FEV₁% over one and three courses of inhaled antibiotics

	Tobramycin	Tobramycin + Azithromycin	Aztreonam	Aztreonam + Azithromycin
FEV ₁ % Day 0–28				
Mean estimate* (absolute change in FEV ₁ %)	7.93 (3.62)	−0.835 (−0.59)	10.20 (4.68)	8.59 (3.40)
95% CI	2.81, 13.06	−4.15, 2.48	5.34, 15.06	5.26, 11.93
SE	2.62	1.69	2.48	1.70
P value†	0.005		0.0002	<0.0001
FEV ₁ % Day 0–140				
Mean estimate* (absolute change in FEV ₁ %)	7.07 (3.16)	−1.95 (−1.25)	7.81 (3.41)	6.23 (2.49)
95% CI	2.14, 12.01	−5.2, 1.31	3.24, 12.39	2.97, 9.50
SE	2.52	1.66	2.33	1.66
P value†	0.0028		0.0007	0.0005

Definition of abbreviation: CI = confidence interval.

The relative change in FEV₁% predicted over one and three courses of inhaled antibiotics for each group is shown, with 95% CI and SE. The absolute change in FEV₁% is shown in parentheses. The group with combined azithromycin and inhaled tobramycin use had significantly less improvement in FEV₁% over either one or three courses of inhaled antibiotic (28 and 140 days) when compared with each of the other three groups. No significant difference was present between the other three groups.

*Estimates are the relative change in FEV₁% predicted from linear regression model adjusting for average baseline FEV₁% of 52.3. The absolute change in FEV₁% predicted is shown in parentheses.

†P values reflect comparison with group receiving tobramycin and azithromycin.

subjects with all existing information and outcome measures requested. Azithromycin was recorded as a concomitant medication by self-report at enrollment.

Outcome Measures and Statistical Analyses

For normally distributed variables, mean and SE were reported. Comparisons across groups were made with two-sample *t* tests and analysis of variance for normally distributed data, and Chi-square tests for frequency data. The outcomes of interest were regressed on group, age, sex, and available baseline values where appropriate using multiple linear regression. The primary outcome of interest was change

in FEV₁% predicted over one and three courses of inhaled antibiotic. The secondary outcomes of interest were time to intravenous or additional inhaled antibiotics, change in CF Questionnaire-Revised Respiratory Domain Symptom Score (CFQ-R RSS), and change in sputum *P. aeruginosa* density after one and three courses of inhaled antibiotics. For analysis of crossover data, linear mixed models were used. For time to antibiotic use, Cox proportional hazard models were used. Missingness at random was examined with frequencies across group. All analyses were performed with SAS v9.3 and graphed in GraphPad Prism v6.0. Additional details are available in the online supplement.

Flow Cell Biofilm Culture

Fifteen isolates of *P. aeruginosa* were analyzed, each having been collected from a unique study subject randomized to inhaled tobramycin who reported concomitant azithromycin use. Bacteria were cultured and exposed to antibiotics in three-channel flow cells as previously described (Technical University of Denmark, Lyngby, Denmark) (17). Antibiotic concentration used: tobramycin (40 µg/ml), azithromycin (20 µg/ml), and aztreonam lysine (900 µg/ml), alone or in combination, for 22-hour constant flow exposure. Syto9 (5 µM) and propidium iodide (15 µM) were added to the medium to stain all cells and only dead cells, respectively. Biofilms were imaged and analyzed to quantify live and dead biomass as described (17, 19). The change in mean percent decrease in live biomass volume provided an estimate of the antibiofilm effects of each drug regimen. Additional details are available in the online supplement.

Results

Baseline Characteristics of Defined Groups

Baseline demographic and clinical data for the four defined groups are shown in Table 1. Overall, concomitant use of oral azithromycin was reported by 71% of the 128 subjects randomized to receive inhaled tobramycin and 67% of the 135 subjects randomized to aztreonam lysine. Age was similar between those randomized to inhaled tobramycin or inhaled aztreonam lysine, (25.1 vs. 25.8 yr, *P* = 0.52) or between those with and without azithromycin use (25.6 vs. 25.2 yr, *P* = 0.76). Among those not reporting azithromycin use, subjects in the aztreonam group were significantly

Table 3. Prevalence of decline in FEV₁% while receiving inhaled antibiotic

	Tobramycin	Tobramycin + Azithromycin	Aztreonam	Aztreonam + Azithromycin	P Value*
28-d FEV ₁ % decline from Day 0	12/37 (32.4%)	44/82 (53.7%)	11/42 (26.2%)	24/82 (29.3%)	0.003
140-d FEV ₁ % decline from Day 0	10/33 (30.3%)	43/75 (57.3%)	13/38 (34.2%)	25/75 (33.3%)	0.007

The prevalence of a decline in FEV₁% predicted while using inhaled antibiotics was quantified and compared between groups. The group with combined azithromycin and inhaled tobramycin had a greater prevalence of decline in FEV₁% (54% and 57% of subjects) after one and three courses of inhaled antibiotics, compared with the other groups, who were similar (26–34%).

*P value corresponds to Chi-square test.

older than those in the tobramycin group (difference of 5.3 yr, $P < 0.05$ from Tukey honestly significant difference test). In analyzing missingness of data, no significant differences were found across groups for number of subjects who did not complete the study (Fisher exact test P value = 0.49). Similarly, no differences were found in number of subjects without FEV₁ or cfu data for all three treatment cycles.

Baseline FEV₁% predicted was not significantly different across defined groups, with mean values ranging from 51 to 55% predicted (Table 1; $P = 0.58$). There was a nonsignificant trend toward slightly lower FEV₁% among subjects reporting azithromycin use, which was incorporated in regression analyses for other outcomes. Sputum density of *P. aeruginosa* and *in vitro* antibiotic resistance to tobramycin were also similar at baseline. Using proposed minimum inhibitory concentration breakpoints for inhaled tobramycin treatment (128 ug/ml) (20, 21), less than 10% of subjects randomized to tobramycin were identified as resistant to inhaled tobramycin before the first treatment course.

Exposure to inhaled tobramycin over the 12 months preceding trial participation was captured and quantified based on use of three or more 28-day courses (≥ 84 d). This prior exposure to inhaled tobramycin was similar in all four of our defined groups, with 83 to 88% reporting use of three or more courses.

Primary Outcome of Interest, Lung Function Measured by FEV₁%

In the primary outcome of interest, change in FEV₁ (% predicted), those using azithromycin who were randomized to tobramycin showed a decline over the 28 days (one course) and 140 days (three courses) of inhaled antibiotic (Table 2). This contrasts with subjects not reporting azithromycin use, who experienced significantly greater increases in FEV₁% at both time points (Figures 1A and 1C; $P < 0.01$). Subjects receiving aztreonam lysine also showed improved FEV₁ while on inhaled antibiotic, which was not associated with azithromycin use status (Figures 1B and 1C). Change in FEV₁% was similar between subjects randomized to aztreonam lysine, with or without azithromycin use, and those without azithromycin use in the tobramycin study arm.

Figure 1. (a) Both absolute and relative change in FEV₁% predicted during the first course of inhaled antibiotic (Day 0–28) are shown for subjects randomized to inhaled tobramycin, with or without azithromycin use. (b) Those groups receiving inhaled aztreonam lysine had similar improvement in FEV₁%, regardless of azithromycin status. Tobramycin-azithromycin (TBRA-AZM) group significantly differed in absolute change in FEV₁% predicted from TBRA group ($P = 0.0021$), aztreonam lysine (AZTR) group ($P < 0.0001$), and AZTR-AZM group ($P = 0.0002$). (c) Relative change in FEV₁% over three courses of inhaled antibiotics (Day 0–140), including all four groups analyzed. TBRA-AZM group significantly different from TBRA group ($P = 0.0028$), AZTR group ($P = 0.0007$), and AZTR-AZM group ($P = 0.0005$). Columns represent mean (SEM). Regression model adjusted for baseline FEV₁.

Recognizing that data presented only as group means can underestimate the variability in individual subject response, we analyzed the proportion of subjects in each group who experienced a decline in FEV₁% while receiving inhaled antibiotic. Some subjects in each group showed both increase and decrease in FEV₁% over one and three courses of inhaled antibiotic, highlighting the heterogeneity in individual changes associated with each tested intervention. Approximately 55% of subjects in the combined azithromycin-tobramycin group experienced a decline below baseline lung function at Day 28 or Day 140, compared with 26 to 34% of subjects in the other three groups (Table 3; $P < 0.01$).

Secondary Outcomes

When averaged across the three courses included in this trial, subjects randomized to tobramycin who reported azithromycin use had a nonsignificant trend ($P < 0.10$) toward lower improvement in disease-related quality of life while receiving inhaled antibiotic, compared with inhaled tobramycin alone. The other groups had similar improvement beyond the minimal clinically significant threshold (5.5 points) for the CFQ-R RSS while receiving inhaled antibiotic over one or three courses (Figures 2A and 2B).

The need for additional intravenous or inhaled antibiotics outside of the study protocol was used to capture likely pulmonary exacerbations in this trial. When considering azithromycin use, we found that those in the tobramycin arm who reported concomitant azithromycin required additional antibiotic treatment sooner and more often compared with all other groups after adjusting for age, sex, and baseline FEV₁% (Figure 3; $P = 0.01$ for tobramycin versus tobramycin-azithromycin).

In this study population, the group with combined azithromycin and inhaled tobramycin use had little change in sputum *P. aeruginosa* density while receiving inhaled antibiotic over three courses ($-0.02 \log_{10}$ cfu/g; 95% CI, -0.35 to 0.32). A nonsignificant trend toward greater reduction in sputum bacteria was present in those receiving inhaled tobramycin without azithromycin ($-0.48 \log_{10}$ cfu/g; 95% CI, -0.92 to -0.05 ; $P = 0.068$). This was similar to groups randomized to inhaled aztreonam, with or without

Figure 2. Self-report of disease related quality of life using the Cystic Fibrosis Questionnaire-Revised Respiratory Domain Symptom Score (CFQ-R RSS) was included in this study. (a) Change in CFQ-R RSS during first course of inhaled antibiotic. (b) Average change over three courses of inhaled antibiotics. Subjects with combined azithromycin and inhaled tobramycin failed to reach the clinically meaningful threshold of 5.5 points during the first course or averaged over the three courses of inhaled antibiotics. The other three groups had similar improvement in this outcome, beyond 5.5 points after one or averaged over three courses. After controlling for known baseline characteristics, the difference between groups randomized to inhaled tobramycin, based on azithromycin use, was not statistically significant but trended in favor of those subjects without azithromycin use ($P < 0.10$ over three courses); model covariates include age, sex, baseline FEV₁%; columns represent mean (SEM). AZM = azithromycin; AZTR = aztreonam lysine; TBRA = tobramycin.

azithromycin, who had a change of -0.55 and $-0.69 \log_{10}$ cfu/g, respectively.

Additionally, the group of 49 subjects reporting azithromycin use who crossed over from inhaled tobramycin to inhaled aztreonam lysine as part of an extension study showed a reduction in sputum *P. aeruginosa* similar to the other three groups (Figure 4A, far right column). Figure 4B shows the effect of azithromycin use on change in sputum *P. aeruginosa* density over the study duration among those randomized to tobramycin. Note the expected decrease in cfu in subjects without azithromycin ($P = 0.026$). This contrasts with virtually no change in cfu in those with

concomitant azithromycin ($P = 0.862$). Additional analysis of only those subjects reporting azithromycin use who crossed over from inhaled tobramycin to inhaled aztreonam lysine can be found in the online supplement. After crossover to inhaled aztreonam lysine, significant improvement was seen in FEV₁% ($P < 0.001$), whereas reported quality of life and sputum bacterial density did not reach statistical significance (see Figures E1A–E1C). In the 15 subjects randomized to inhaled tobramycin and not using azithromycin who participated in the extension aztreonam lysine phase, no significant difference was observed in the FEV₁% or other outcome measures.

Figure 3. Time to need additional antibiotics for *Pseudomonas aeruginosa* was analyzed as a surrogate for pulmonary exacerbation. In our analysis, we find that the group with combined azithromycin and inhaled tobramycin required additional antibiotic treatment significantly sooner when compared with all other groups. The tobramycin-azithromycin (TBRA-AZM) group was significantly different from TBRA group ($P = 0.0116$), AZTR group ($P < 0.0001$), and aztreonam lysine (AZTR)-AZM group ($P = 0.0011$). P value from Cox proportional hazards model adjusted for age, sex, and baseline FEV₁%.

In Vitro Biofilm Testing with Study Isolates

Using an *in vitro* biofilm culture model with constant flow antibiotic exposure, we analyzed 15 isolates of *P. aeruginosa* collected from distinct subjects with CF enrolled in this trial. In these studies, concentrations of azithromycin that are likely relevant to the CF airway antagonized the antimicrobial effect of tobramycin for 6 of 15 isolates tested (22). The mean reduction in biomass was 39.3% with tobramycin alone and 14.3% with tobramycin plus azithromycin. Differences in the effect of combined azithromycin-tobramycin were observed from one *P. aeruginosa* isolate to the next. Six of the 15 isolates showed clear antagonism, defined as greater than 20% increase in biomass compared with tobramycin alone. Three showed additive effect (>20% reduction in biomass), and three showed no effect. For three additional isolates, technical quality was limited, and the results were thus indeterminate. We then tested the combination of aztreonam lysine and azithromycin in the six isolates in which antagonism between tobramycin and azithromycin was observed. None showed antagonism with aztreonam lysine, and additive effect with reduction in biofilm biomass was observed in two of six isolates. Representative images from biofilm analyses are available in Figure E2.

Discussion

Our data from this secondary analysis of a recent CF clinical trial and from our *in vitro* culture model indicate that azithromycin may reduce the ability of tobramycin to kill *P. aeruginosa* in the airways of some patients, impairing the benefits to pulmonary health. These findings, which are consistent with recent *in vitro* and animal investigations (17), may explain the diminishing effects of inhaled tobramycin observed in published trials since azithromycin therapy was introduced and widely adopted into CF clinical practice. The original study of inhaled tobramycin in subjects with CF, published in 1999, demonstrated an increase in FEV₁ (% predicted) of approximately 10%, which was sustained over three 28-day courses of medication use (6). A CF trial comparing tobramycin solution and dry powder formulations, published in 2011, showed an improvement of 3 to 5% over three courses of treatment (23). The active comparator study published in 2012, from which our secondary analyses are derived, found a paradoxical decline in FEV₁% among subjects receiving three courses of inhaled tobramycin (5).

Chronic azithromycin therapy in CF began in the early to mid-2000s. By 2005, more than 50% of U.S. patients with *P. aeruginosa* who were otherwise

candidates for this drug therapy were prescribed azithromycin (24). In 2013, we find that 75% of patients with CF in the United States patient registry who are prescribed inhaled tobramycin are also prescribed azithromycin. These registry data cannot guarantee adherence or actual use but strongly indicate that inhaled tobramycin and oral azithromycin are now frequently used in combination and at a similar prevalence to that reported in the clinical trial reanalyzed herein. Collectively, these data show that the improvement in FEV₁ observed in sequential clinical trials of inhaled tobramycin has lessened over a period of time during which chronic azithromycin treatment has been introduced and widely incorporated into clinical practice.

Clearly, other factors may also contribute to diminishing response to a therapy over a decade of use. Specifically, the emergence of antibiotic resistance to tobramycin has long been recognized as a potential consequence of chronic use. It should be emphasized that subjects participating in this trial had prior exposure to inhaled tobramycin but not aztreonam lysine on enrollment. However, widespread high-level tobramycin resistance has not been demonstrated to date, and *in vitro* resistance to tobramycin has not correlated well with clinical response (25, 26). Thus, we are not aware of any other convincing explanation for the apparent diminishing effects of inhaled tobramycin.

In our analyses, the association between lack of lung function improvement and combined use of tobramycin with azithromycin appears clear. We also noted significantly earlier and greater need for additional antibiotic treatment and less improvement in quality of life. An unfavorable trend in sputum bacterial density was observed, but this did not reach statistical significance. However, *in vitro* antagonism in 40% of the clinical *Pseudomonas* isolates tested, contrasting with no antagonism between azithromycin and aztreonam lysine, further supports our concern over a selective, adverse drug interaction. Our findings also indicate that the previous assertion of inhaled aztreonam lysine's clinical superiority to inhaled tobramycin in patients with CF who were previously treated with inhaled tobramycin may not apply to all such patients (5). Specifically, the 31% of subjects not using azithromycin appear

Figure 4. The average reduction in sputum *Pseudomonas aeruginosa* density reported in cfu $\log_{10}$ /g is shown. (a) Mean change while receiving inhaled antibiotics during each of the three courses included in this trial. Virtually no change was observed in the group with combined azithromycin and inhaled tobramycin. The tobramycin-azithromycin (TBRA-AZM) group was significantly different from aztreonam lysine (AZTR)-AZM group ($P = 0.008$) and AZTR group ($P = 0.004$) and approached the predefined cutoff for significance when compared with the TBRA group ($P = 0.0675$). The far right column shows the average reduction in cfu density in subjects reporting azithromycin who crossed over to an extension phase with inhaled aztreonam lysine, after receiving inhaled tobramycin in the primary study. More direct comparisons of only these subjects participating in the crossover extension phase are presented in the online supplement. Regression model adjusted for age, sex, and baseline sputum bacterial density. (b) Group means for sputum *P. aeruginosa* density across three courses of inhaled antibiotic (from Day 0–140) in subjects randomized to inhaled tobramycin. A significant reduction was observed in the group without azithromycin use, and no change occurred in the group with azithromycin use. The difference in this change, when compared between the two groups and controlled for known covariates, was not statistically significant ($P = 0.08$).

to have benefitted similarly with either inhaled tobramycin or aztreonam lysine in this trial.

We and other research groups have published nonhuman studies identifying a potential antagonism between

azithromycin and tobramycin (17, 27, 28). In an animal model of *P. aeruginosa* infection at a cutaneous burn wound site, we found that a single dose of azithromycin greatly diminished the antibacterial effect of subsequent tobramycin while showing

neutral or additive effects with ciprofloxacin (17). Others have demonstrated this drug–drug interaction in complementary models of biofilm culture conditions in laboratory and limited CF clinical strains of *P. aeruginosa* (27, 28). Burn wounds and the CF airway share a robust neutrophilic host inflammatory response and propensity to become infected with *P. aeruginosa* biofilms. The unexpected findings in our burn wound model motivated the investigations reported in this paper. This report provides the first direct human data indicating a drug–drug interaction between azithromycin and tobramycin.

The concentrations of tobramycin and azithromycin delivered to the site of *P. aeruginosa* in the CF airway are largely unknown. Therefore, it is difficult to determine how directly relevant the concentrations used in our *in vitro* model are to the human condition. The primary purpose of these experiments was to test the effect of adding azithromycin in a biofilm growth model with partial antimicrobial activity using traditional antipseudomonal antibiotics. Future planned studies will test alternative drug concentrations to determine if such an approach can overcome the apparent antagonism observed. Additionally, we used our biofilm model to determine if antagonism could be seen without any impact on host response. This too limits our ability to draw correlation between our *in vitro* findings and treatment in CF lung disease. Nevertheless, these data are largely consistent with the clinical outcomes data. The heterogeneity observed between different subject samples in the biofilm culture model is interesting and somewhat mirrors the heterogeneity present in the clinical outcomes analyses. Bacteria samples were selected from subjects categorized as both having and not having antagonism between azithromycin and tobramycin. However, *in vitro* growth patterns did not correlate with change in the clinical outcome measures. Therefore, we see similar patterns of potential drug interaction in both clinical and laboratory testing but do not now have a test to accurately predict such an effect in individual subjects.

Among the macrolides, azithromycin has a unique dicationic structure. As a polycation, azithromycin is believed to use self-promoted uptake to bind to and

gain entry into *P. aeruginosa* (29, 30). In this process, antibiotic molecules displace elemental cations (i.e., Mg^{+} and Ca^{+}) that crosslink lipopolysaccharide molecules in the outer membrane of gram-negative bacteria, thereby disrupting this barrier and allowing access into the bacterium (31). Aminoglycosides (i.e., tobramycin) are also believed to be dependent on self-promoted uptake to kill *P. aeruginosa*, using the same polycationic binding sites as azithromycin to gain entry into gram-negative bacteria (31–33). Adding excess Mg^{+} or Ca^{+} to cultures of *P. aeruginosa* impairs the antimicrobial effects of tobramycin or azithromycin against *P. aeruginosa* (29). Other macrolides, which are not polycationic, do not use self-promoted uptake (34). This property of azithromycin provides biological plausibility to support a potential and selective drug–drug interaction, suggesting a mechanism whereby azithromycin may competitively inhibit tobramycin in the CF airway. Aztreonam does not use this mechanism to penetrate bacteria, which would explain why subjects randomized to inhaled aztreonam lysine were not impacted by azithromycin use in our analyses, as hypothesized. Alternatively, azithromycin may selectively induce aminoglycoside-resistance mechanisms such as the MexXY efflux pump, as indicated in an *in vitro* analysis of the transcriptional effects of azithromycin on *P. aeruginosa* biofilms (35).

Azithromycin concentrates in macrophages and neutrophils at 100- to more than 1,000-fold serum levels and in CF may selectively deposit in the airway via neutrophil influx and apoptosis. Azithromycin can thus persist in the airways for weeks after discontinuation of drug use (22, 36). Airway leukocytosis is a central component of CF lung disease, in which bacterial infection (e.g., *P. aeruginosa*) is nearly always sequestered within the airway. Therefore, the combined properties of azithromycin and pathophysiology of CF lung disease likely result in high azithromycin concentrations at the site

of *P. aeruginosa* infection, where it may reduce the clinical efficacy of inhaled tobramycin.

Retrospective analyses of datasets from trials not specifically designed to measure the variables of interest can be misleading. Criteria to help determine the validity of such analyses have been published (37). Our analyses of azithromycin use in the recent active comparator trial satisfy most of these validation criteria. We observe consistent patterns of effect in related outcome measures, and the statistical test of interaction (38, 39) between azithromycin and tobramycin for our primary outcome of interest (change in FEV₁%) is significant. We believe these data are provocative and warrant further investigation. However, we also wish to highlight the trend toward lower baseline lung function in groups with concomitant azithromycin use. Similarly, we cannot be certain that these subjects have not also had greater exposure to aminoglycoside antibiotics in prior years or that they do not have overall greater disease severity. Differences in drug exposure may alter variables not measured in these analyses (e.g., airway microbiome or other forms of microbial adaptation). A relatively small number of subjects with azithromycin use who crossed over from inhaled tobramycin to inhaled aztreonam lysine in the extension study showed improvements in FEV₁ and sputum bacterial density after crossover. This suggests that such subjects may not be more severely affected or refractory to inhaled antibiotic therapy, but such subjects were relatively few. When considering our findings, it is important to recognize that both inhaled tobramycin and oral azithromycin have been shown beneficial as single agents in larger, dedicated, prospective CF clinical trials (6, 10). We also note that the study subjects in this trial, as well as the two previous trials of inhaled tobramycin referred to above (6, 23), were relatively older, with mean age in the low to mid 20s and mean FEV₁% in the 50s % predicted. At present, it is not known if similar associations between

azithromycin use and response to inhaled tobramycin would be seen in younger subjects or those with less impaired pulmonary function.

Subgroup analyses of data from the original CF trial of azithromycin in the United States found that among the just over 50 subjects also reporting inhaled tobramycin use, FEV₁% increased, but to a lesser degree than was observed in those not using inhaled tobramycin (40). However, the relative risk of pulmonary exacerbation was less with combined azithromycin and inhaled tobramycin as compared with azithromycin alone (40). These data are partially inconsistent with what we observe when reanalyzing a more recent clinical trial—particularly in the risk of pulmonary exacerbation. Presently, it is unclear to what extent the methodological differences, years of medication use, or other unknown factors contribute to this inconsistency.

CF consensus guidelines for chronic therapy have recently been updated and rate the certainty of net benefit as “high” for both inhaled tobramycin and azithromycin in patients with *P. aeruginosa* infection (16). We recognize both the limitations in our current analyses and the potential for a proven drug–drug interaction to impact clinical care recommendations for half or more of all patients with CF over a lifetime. Therefore, a prospective clinical trial, controlling for limitations inherent in this *post hoc* analysis, is necessary. Such a trial, which is currently in the planning stage, will also provide an opportunity to collect bacterial isolates and other clinical specimens from subjects in support of future mechanistic studies. Until more definitive data are available, we are not advocating that patients with CF stop using these medications in combination. However, clinicians need to be aware of the potential for this adverse drug–drug interaction in individual patients with CF. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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