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# Antibacterial activities of a fosfomycin/tobramycin combination: a novel inhaled antibiotic for bronchiectasis

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***Objectives*: To compare the *in vitro* and *in vivo* activities of a 4:1 (w/w) fosfomycin/tobramycin combination (FTI) with those of fosfomycin and tobramycin alone against cystic fibrosis (CF) and non-CF bronchiectasis pathogens.**

***Methods*: Clinical isolates of CF *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex, *Escherichia coli* and *Klebsiellia* spp. were evaluated by MIC, MBC, post-antibiotic effect (PAE), synergy, time–kill, a rat pneumonia model and spontaneous mutation frequency (SMF).**

***Results*: FTI showed high activity against *E. coli*, *H. influenzae*, *S. aureus* and *Klebsiella* spp. For the *S. aureus* strains, 75% of which were methicillin resistant (MRSA), FTI had a lower $MIC_{90}$ than tobramycin. For *P. aeruginosa*, FTI had a lower $MIC_{90}$ than fosfomycin, but tobramycin was more active than either. Synergy studies showed no antagonism between fosfomycin and tobramycin, and 93% of the isolates demonstrated no interaction. FTI was rapidly bactericidal and exhibited concentration-dependent killing in time–kill studies. In the rat pneumonia model, FTI and tobramycin demonstrated bactericidal killing of *P. aeruginosa*; both were more active than fosfomycin alone. The SMF for *S. aureus* resistance to FTI was 2–4 $\log_{10}$ lower than that for tobramycin and 2–7 $\log_{10}$ lower than that for fosfomycin. For *P. aeruginosa*, the FTI SMF was 2–3 $\log_{10}$ lower than that for fosfomycin and 1–2 $\log_{10}$ lower than that for tobramycin.**

***Conclusions*: FTI is a broad-spectrum antibiotic combination with high activity *in vitro* and *in vivo*. These data suggest FTI could be a potential treatment for respiratory infections caused by Gram-positive and Gram-negative aerobic bacteria.**

Keywords: *P. aeruginosa*, *S. aureus*, respiratory infections

## Introduction

Cystic fibrosis (CF) and non-CF bronchiectasis patients are predisposed to chronic respiratory infections caused by a variety of bacteria including *Pseudomonas aeruginosa*, *Staphylococcus aureus* and non-typeable *Haemophilus influenzae. Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* complex are also frequent pathogens in CF,[1,2] and *Moraxella catarrhalis*, *Streptococcus pneumoniae* and enteric Gram-negative bacilli are seen in non-CF bronchiectasis.[3] Extensive use of intravenous, oral and inhaled antibiotics has improved the survival of CF patients,[4,5] but has also led to the development of bacterial resistance.[6,7] There is a clear need for new antibiotics, and novel approaches including combination drugs should be explored.

An ideal therapy would be delivered directly to the lungs, kill a broad spectrum of bacteria, have a favourable safety profile and reduce the development of resistance. A combination antibiotic consisting of fosfomycin and tobramycin may be an appropriate addition to the current treatments for the management of respiratory infections. Fosfomycin is a phosphonic acid antibiotic[8] with

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little reported human toxicity when administered parenterally.[9] It is active against both Gram-positive[10,11] and Gram-negative bacteria,[12] and inhibits the first step of peptidoglycan biosynthesis in the bacterial cell wall.[8,9] Both oral (fosfomycin calcium and fosfomycin trometamol) and intravenous (fosfomycin disodium) formulations are available, but only oral fosfomycin trometamol is approved in the USA for treating uncomplicated urinary tract infections.[9,13] Parenteral administration of fosfomycin is sporadically used in the UK to treat CF bacterial respiratory infections,[6,14] but an aerosol formulation deliverable directly to the lungs has not yet been developed.

Tobramycin is an aminoglycoside and is highly potent against Gram-negative bacteria, in particular *P. aeruginosa*.[15] Tobramycin is rapidly bactericidal and acts by inhibiting bacterial protein synthesis.[16] Aerosolized tobramycin (TOBI®) is used for the management of *P. aeruginosa* respiratory infections in CF patients.[5] Nephrotoxicity and ototoxicity are adverse reactions associated with tobramycin therapy.[17] The greatest risk factor for development of toxicity is cumulative exposure to large doses of tobramycin. Bronchiectasis patients may be at increased risk of developing tobramycin toxicity because they receive prolonged and repeated antibiotic therapies over their lifetime.[18]

The purpose of this study was to evaluate the *in vitro* and *in vivo* activities of a 4:1 (w/w) fosfomycin/tobramycin combination (FTI) against bacterial respiratory pathogens seen in the CF and non-CF bronchiectasis populations, and to compare them with those of fosfomycin and tobramycin, individually. *S. aureus* and *P. aeruginosa* were the major focus of this work, both because of their frequency and virulence in bronchiectasis infections and because, as resistance increases in these two organisms, treatment becomes problematic.

## Materials and methods

### *Bacterial strains*

*P. aeruginosa* strains from patients with CF ($n=100$) were obtained from the Therapeutics Development Network Center for CF Microbiology at the Children's Hospital and Regional Medical Center (Seattle, WA, USA). *B. cepacia* ($n=20$) complex strains were obtained from the University of British Columbia (Vancouver, BC, Canada). Non-CF (respiratory, bloodstream, skin/soft tissue) *P. aeruginosa* ($n=60$), *Enterococcus faecalis* ($n=5$), *E. coli* ($n=22$), *H. influenzae* ($n=16$), *Klebsiella* spp. ($n=22$), *M. catarrhalis* ($n=5$), *S. maltophilia* ($n=17$), *S. aureus* ($n=16$), *S. pneumoniae* ($n=8$) and *Streptococcus pyogenes* ($n=5$) strains were obtained from The Jones Group Laboratories (North Liberty, IA, USA) and The Clinical Microbiology Institute (Wilsonville, OR, USA). *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212 and *H. influenzae* ATCC 49247 served as quality control and reference strains.[19] An animal-passaged derivative of *P. aeruginosa* ATCC 27853 (C177) was used in the rat pneumonia studies.

### *Antibiotics*

Fosfomycin disodium, tobramycin sulphate and vancomycin hydrochloride were obtained from Sigma-Aldrich (St Louis, MO, USA). Ciprofloxacin hydrochloride was obtained from Cellgro (Herndon, VA, USA). FTI consisted of a 4:1 ratio (w/w basis) of fosfomycin and tobramycin. Glucose-6-phosphate (Sigma-Aldrich) was added to the media at a final concentration of 25 mg/L for all *in vitro* evaluations of fosfomycin and FTI.[19,20]

### *MICs and MBCs*

MICs were determined by agar plate dilution and broth microdilution methods.[19,20] The MIC was defined as the lowest concentration of antibiotic that prevented visible growth after incubation at 35°C for 18–24 h. FTI MIC values were expressed as the concentration of both drugs (example, FTI MIC of 8 mg/L = 6.4 mg/L fosfomycin + 1.6 mg/L tobramycin). Vancomycin and ciprofloxacin MICs were determined only for the *S. aureus* isolates. MBCs were determined according to CLSI (formerly NCCLS) guidelines.[21] The MBC was defined by a $\geq 3$ $\log_{10}$ decrease in cfu/mL of the original inoculum.

### *Chequerboard synergy*

Interactions between fosfomycin and tobramycin were determined by the broth microdilution chequerboard method.[22] Two-fold serial dilutions bracketing the expected MIC value of both antibiotics were evaluated. The fractional inhibitory concentration (FIC) was calculated as the MIC of compound 1 in combination with a second compound, divided by the MIC of compound 1 alone. An FIC index (FICI) was calculated for each drug combination as the sum of the individual FICs of compounds 1 and 2. Synergy was defined as an FICI of $\leq 0.5$, no interaction as an FICI $>0.5$ and $\leq 4$, and antagonism as an FICI $>4$. The lowest FICI was used for final interpretation of drug interactions.

### *Post-antibiotic effect (PAE)*

PAE values were determined by the viable plate count method.[23] Bacteria were incubated with antibiotic at 2× the MIC for 1–2 h in a shaking 35°C water bath. Growth controls were included in each experiment. Following exposure, the cultures were diluted 1:1000 and the cfu/mL determined hourly. The PAE was defined as $T-C$, where $T$ is the time required for the viable counts of an antibiotic-exposed culture to increase 1 $\log_{10}$ cfu/mL above the counts determined immediately after dilution and $C$ is the corresponding time for the growth control.

### *Time–kill studies*

Time–kill experiments were performed according to CLSI standards.[21] Antibiotics were evaluated at multiples of the MIC in cation-adjusted Mueller–Hinton broth (CAMHB) (Remel, Lenexa, KS, USA). A no-drug control was run in each assay. Bacterial cultures and antibiotic were incubated at 35°C in a shaking water bath and killing activity assessed at 1, 2, 4, 6 and 24 h. Antibiotic concentrations that reduced the original inoculum by $\geq 3$ $\log_{10}$ cfu/mL were considered bactericidal.

### *Animal efficacy studies*

Animals were handled according to the Guidelines for the Care and Use of Laboratory Animals.[24] All animal protocols were approved by an IRB/Ethics Committee. Male Sprague–Dawley rats (180–200 g) were obtained from Charles River Laboratories (Hollister, CA, USA) and acclimatized for 5 days prior to use. Animals were housed individually in ventilated cages, fed Purina Lab Diet *ad libitum* and allowed free access to water.

Antibiotic efficacy was determined using a rat bacterial pneumonia model.[25] Rats were anaesthetized with isoflurane, and $\sim 10^3$ cfu of *P. aeruginosa* C177 in a 2% agar solution were instilled into the lungs with an oral gavage needle. The inoculum was deposited at the first bifurcation and distributed throughout the lungs by inspiration. Animals were allowed to recover for 18 h post-infection. Each experiment consisted of pre-treatment ($n=5-7$), saline control ($n=5-7$) and antibiotic ($n=5-7$) groups. Rats were anaesthetized with isoflurane, and 100 μL of antibiotic solution or saline was instilled into the trachea using a Microsprayer™ (Penn-Century Inc., Philadelphia, PA, USA). Antibiotics were administered intratracheally twice daily for 3 days.

Animals were euthanized by intraperitoneal administration of sodium pentobarbital. The pre-treatment control group was harvested 18 h post-infection, and the saline and treatment groups 18 h after the last antibiotic exposure. Lungs were removed aseptically, homogenized in sterile normal saline and viable bacteria determined by the colony count method. Statistical differences between the saline control group and treatment groups were evaluated by the Mann–Whitney Rank Sum Test using GraphPad Prism® software package version 3.03 (GraphPad Software, Inc., San Diego, CA, USA).

### *Single-step resistance*

Development of resistance after a single exposure to antibiotic was determined using four clinical and one reference strain of *S. aureus* and *P. aeruginosa*. Late log-phase cultures ($10^9-10^{10}$ cfu) were spread onto Mueller–Hinton agar (BBL, Sparks, MD, USA) plates containing 4× the MIC of each antibiotic. The culture plates were incubated at 35°C for 48 h and the number of colonies on each plate was enumerated manually. The frequency of resistance was calculated by dividing the number of bacteria growing at the defined antibiotic concentration by the number of bacteria in the inoculum.[26] MIC values were calculated for three representative spontaneous mutants and compared with those for the parental strain.

## Results

### *MICs*

Table 1 summarizes the MICs at which 50% ($MIC_{50}$) and 90% ($MIC_{90}$) of the clinical isolates were inhibited. FTI had high activity against the 16 random *S. aureus* strains, and moderate activity against *S. pneumoniae*, *S. pyogenes* and *E. faecalis*. Twelve of the 16 *S. aureus* strains were categorized as methicillin-resistant (MRSA). The FTI $MIC_{50}$ value (2 mg/L) was nearly identical to that of vancomycin (1 mg/L) and was superior to that of ciprofloxacin (>4 mg/L) for *S. aureus*. FTI was also active against single linezolid-resistant (C059) and glycopeptide-intermediate *S. aureus* (GISA) (C060) isolates, with MICs of 2 and 1 mg/L, respectively.

Among the Gram-negative organisms examined FTI had the lowest $MIC_{50}$ for *E. coli* (0.5 mg/L), *H. influenzae* (0.5 mg/L), *Klebsiella* spp. (1 mg/L) and *P. aeruginosa* (non-CF, 4 mg/L; and CF, 8 mg/L) strains. FTI also had high activity against *M. catarrhalis* strains, but poor activity against *S. maltophilia* and *B. cepacia* complex. Against tobramycin-resistant and high fosfomycin MIC (≥128 mg/L) strains, FTI had MICs comparable to that of the most active single antibiotic component. Tobramycin had the lowest $MIC_{50}$ and $MIC_{90}$ values for the CF (2 and 16 mg/L) and non-CF *P. aeruginosa* (1 and 128 mg/L) strains. Fosfomycin had potent activity against *S. aureus*, *H. influenzae*, *E. coli* and *Klebsiella* spp. It showed moderate activity against *P. aeruginosa* and *S. maltophilia*, and poor activity against *B. cepacia* complex.

### *MBCs*

FTI and tobramycin were bactericidal against the *S. aureus* (100%), *S. pneumoniae* (100%), *P. aeruginosa* (100%), *E. coli* (100%), *Klebsiella* spp. (100%) and *H. influenzae* (83% and

**Table 1.** MICs of FTI, tobramycin and fosfomycin for Gram-positive and Gram-negative bacteria

| | MIC (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | FTI | | tobramycin | | fosfomycin | |
| Organism (no. of strains) | range | $MIC_{50}$ ($MIC_{90}$) | range | $MIC_{50}$ ($MIC_{90}$) | range | $MIC_{50}$ ($MIC_{90}$) |
| *S. aureus*[a] (16) | 0.5–16 | 2 (8) | 0.125–512 | 0.5 (256) | 0.125–16 | 2 (4) |
| *S. pneumoniae*[a] (8) | 4–32 | ND | 16–64 | ND | 8–32 | ND |
| *S. pyogenes*[b] (5) | 16–32 | ND | 16–64 | ND | 16–64 | ND |
| *E. faecalis*[a] (5) | 32 | ND | 8–512 | ND | 32 | ND |
| *E. coli*[a] (22) | 0.125–1 | 0.5 (1) | 0.5–1 | 1 (1) | 0.25–4 | 0.5 (2) |
| *H. influenzae*[a] (16) | ≤0.13–4 | 0.5 (2) | 0.5–1 | 1 (1) | 0.25–4 | 0.5 (2) |
| *Klebsiella* spp.[b] (22) | 0.5–16 | 1 (8) | 0.13–>512 | 0.13 (16) | 0.5–16 | 4 (16) |
| *M. catarrhalis*[a] (5) | 0.5–1 | ND | 0.5–1 | ND | 4–16 | ND |
| *P. aeruginosa*[a], non-CF (60) | 1–256 | 4 (128) | 0.13–>512 | 1 (128) | 1–>512 | 32 (128) |
| *P. aeruginosa*[a], CF (100) | 1–128 | 8 (64) | 0.25–>512 | 2 (16) | 4–>512 | 64 (512) |
| *S. maltophilia*[a] (17) | 8–256 | 64 (128) | 2–>512 | 64 (256) | 32–512 | 64 (128) |
| *B. cepacia complex*[a] (20) | 0.5–>512 | 512 (>512) | 1–>512 | 64 (512) | 512–>512 | >512 (>512) |

ND, not determined due to the small number of isolates examined.
[a]MICs were determined by the agar dilution method.
[b]MICs were determined by the broth microdilution method.

**Table 2.** MBC/MIC ranges of clinical strains

| Organism (no. of strains) | Range of MBC/MIC[a] | | |
|---|---|---|---|
| | FTI | tobramycin | fosfomycin |
| *S. aureus* (10) | 1–2 | 1–2 | 1–8 |
| *S. pneumoniae* (7) | 1–4 | 1–2 | 1–16 |
| *P. aeruginosa*, non-CF (10) | 1–4 | 1–2 | 2–8 |
| *P. aeruginosa*, CF (8) | 1–4 | 1–4 | 2–>16 |
| *E. coli* (10) | 1–4 | 1–4 | 1–8 |
| *Klebsiella* spp. (5) | 1 | 1–4 | 1–4 |
| *H. influenzae* (6) | 1–8 | 1–2 | 1–8 |

[a]The MBC/MIC ratio was calculated by dividing the MBC (mg/L) by the MIC (mg/L).

100%, respectively) strains (Table 2). Fosfomycin was bactericidal against *S. aureus* (80%), *S. pneumoniae* (86%), *P. aeruginosa* (78%), *E. coli* (90%), *Klebsiella* spp. (100%) and *H. influenzae* (83%) strains. FTI and tobramycin had MBC/MIC ratios $\leq$8, suggesting that both antibiotics work by killing bacteria rather than by inhibiting bacterial growth.

### *Chequerboard synergy*

No antagonism was seen between fosfomycin and tobramycin by the chequerboard method for any of the 27 strains tested: *S. aureus*, $n=4$; *P. aeruginosa*, $n=17$; *E. coli*, $n=5$; and *H. influenzae*, $n=1$. The combination was categorized as no interaction for 25 of the 27 strains (93%), and synergistic for 1 *P. aeruginosa* strain and 1 *E. coli* strain.

### *PAE*

FTI had the longest PAE for the three type strains, followed by tobramycin and fosfomycin (Table 3). FTI had the longest PAE with *S. aureus* (3.8 h) followed by *P. aeruginosa* (3 h) and *E. coli* (2.8 h).

**Table 3.** PAE of FTI, tobramycin and fosfomycin for *S. aureus* *P. aeruginosa* and *E. coli*

| Organism | Antibiotic | MIC (mg/L) | PAE (h) |
|---|---|---|---|
| *S. aureus*[a] ATCC 29213 | FTI | 2.0 | 3.8 |
| | tobramycin | 0.5 | 2.8 |
| | fosfomycin | 2.0 | 1.3 |
| *P. aeruginosa*[b] ATCC 27853 | FTI | 4.0 | 3.0 |
| | tobramycin | 0.5 | 2.0 |
| | fosfomycin | 4.0 | 1.0 |
| *E. coli*[b] ATCC 25922 | FTI | 1.0 | 2.8 |
| | tobramycin | 0.5 | 2.5 |
| | fosfomycin | 2.0 | 1.0 |

[a]PAE was determined after a 2 h exposure to 2× the MIC of antibiotic.
[b]PAE was determined after a 1 h exposure to 2× the MIC of antibiotic.

### *Time–kill studies*

FTI and tobramycin killed in a concentration-dependent fashion while fosfomycin killed in a time-dependent fashion against *S. aureus* and *P. aeruginosa* (Figure 1). Against a methicillin-susceptible *S. aureus* (MSSA) strain (ATCC 29213), FTI and tobramycin were bactericidal at 2 mg/L (2× MIC) and 1 mg/L (2× MIC), respectively. FTI was bactericidal against the MRSA strain (C354) at 1 mg/L (data not shown). FTI and tobramycin were rapidly (1–2 h) bactericidal against *P. aeruginosa* ATCC 27853 at 4 mg/L (1× MIC) and 0.5 mg/L (1× MIC), respectively. FTI and tobramycin maintained bactericidal killing at 24 h, while cultures exposed to fosfomycin experienced regrowth.

### *Animal efficacy studies*

In the absence of antibiotic treatment, cfu/lung decreased $<1 \log_{10}$ at days 4 and 7 post-infection. Intratracheal administration of FTI showed progressively greater killing of *P. aeruginosa* with increasing dose (Figure 2). In subsequent experiments, complete eradication of the C177 infection was seen with 5 and 12.5 mg/kg FTI. Tobramycin showed $3 \log_{10}$ bacterial killing at 2.5 mg/kg. Administration of tobramycin doses higher than 3 mg/kg resulted in complete eradication of the *P. aeruginosa* infection, while doses $\leq$0.5 mg/kg did not result in bacterial killing. A reduction in cfu/lung was not observed after administration of $\leq$10 mg/kg fosfomycin.

### *Single-step resistance*

Table 4 shows the frequencies of spontaneous single-step mutations leading to antibiotic resistance. Against the five *S. aureus* strains, FTI had a mutation frequency $2-4 \log_{10}$ less than tobramycin and $2-7 \log_{10}$ less than fosfomycin. Against *P. aeruginosa*, FTI was superior to tobramycin, but the differences were $\leq 2 \log_{10}$. Relative to the parent strains, MIC values of the spontaneous mutants increased 4-, 16- and 32- to 128-fold for FTI, tobramycin and fosfomycin, respectively.

## Discussion

This study investigated the *in vitro* and *in vivo* antibacterial activities of FTI, a novel inhaled antibiotic combination. Fosfomycin was selected as the major component because it is active against both Gram-positive[10,11] and Gram-negative bacteria,[12] is bactericidal[10] and has a good safety profile.[8] However, fosfomycin kills in a time-dependent fashion,[10] is only moderately active against *P. aeruginosa* and some of the more resistant Gram-negative organisms,[12] and has a high mutation frequency resulting in bacterial resistance *in vitro*.[26,27] Tobramycin is rapidly bactericidal, exhibits concentration-dependent killing activity,[16] is highly active against many resistant Gram-negatives[15] and has a low prevalence of bacterial resistance. Tobramycin constitutes the minor component because of the benefits of reducing the lifetime accumulation of aminoglycoside toxicity,[18] which can be minimized by lowering the dose of tobramycin.

Polymicrobial respiratory infections are a significant cause of morbidity and mortality in CF and non-CF bronchiectasis patients[2,3] as well as other diseases characterized by chronic

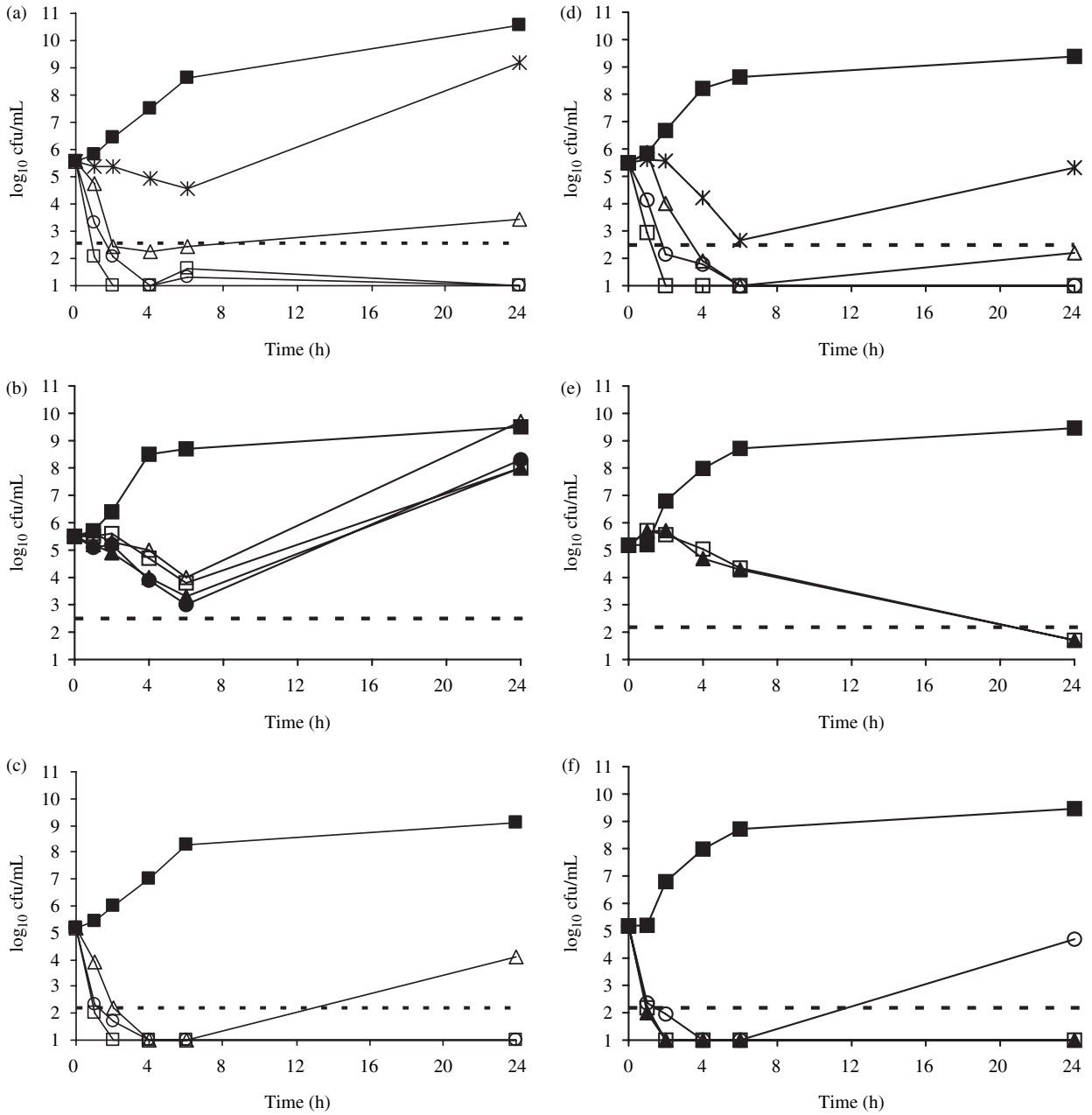


**Figure 1.** Time–kill curves for *P. aeruginosa* ATCC 27853 and (a) FTI, (b) fosfomycin and (c) tobramycin, and for *S. aureus* ATCC 29213 and (d) FTI, (e) fosfomycin and (f) tobramycin. Antibiotics were tested at concentrations of 0.5× MIC (asterisks), 1× MIC (open triangles), 2× MIC (open circles), 4× MIC (open squares), 8× MIC (filled triangles) and 16× MIC (filled circles). Closed squares, no drug; broken line, bactericidal line.

airway infection. Important pathogens common to both populations include *S. aureus*, *P. aeruginosa* and non-typeable *H. influenzae*. FTI demonstrated excellent activity against both *S. aureus* and *H. influenzae. S. aureus* may be particularly pathogenic and the prevalence of MRSA is increasing.[1] We demonstrated that FTI had high activity against ciprofloxacin- and methicillin-resistant *S. aureus*, suggesting that it might be a good therapeutic agent for these infections. Neither CF nor non-CF *P. aeruginosa* was found to be as susceptible to FTI as it was to tobramycin when susceptibility testing was conducted by CLSI standards.[19] However, *in vitro* antibiotic activity does not always correlate with *in vivo* activity, particularly in CF because CF sputum has been shown to inhibit the activity of aminoglycosides.[28,29] FTI demonstrated relatively poor activity against other CF pathogens, particularly *S. maltophilia* and *B. cepacia* complex. However, it was active against other non-CF bronchiectasis pathogens including *M. catarrhalis*, *E. coli*, *Klebsiella* and *S. pneumoniae*.

Examination of synergy by the chequerboard method demonstrated no interaction between fosfomycin and tobramycin for the majority of clinical *P. aeruginosa*, *E. coli* and *S. aureus* strains. Antagonism was not observed, while synergy was detected in 7% of the strains. To our knowledge this is the first report describing the interactions between fosfomycin and

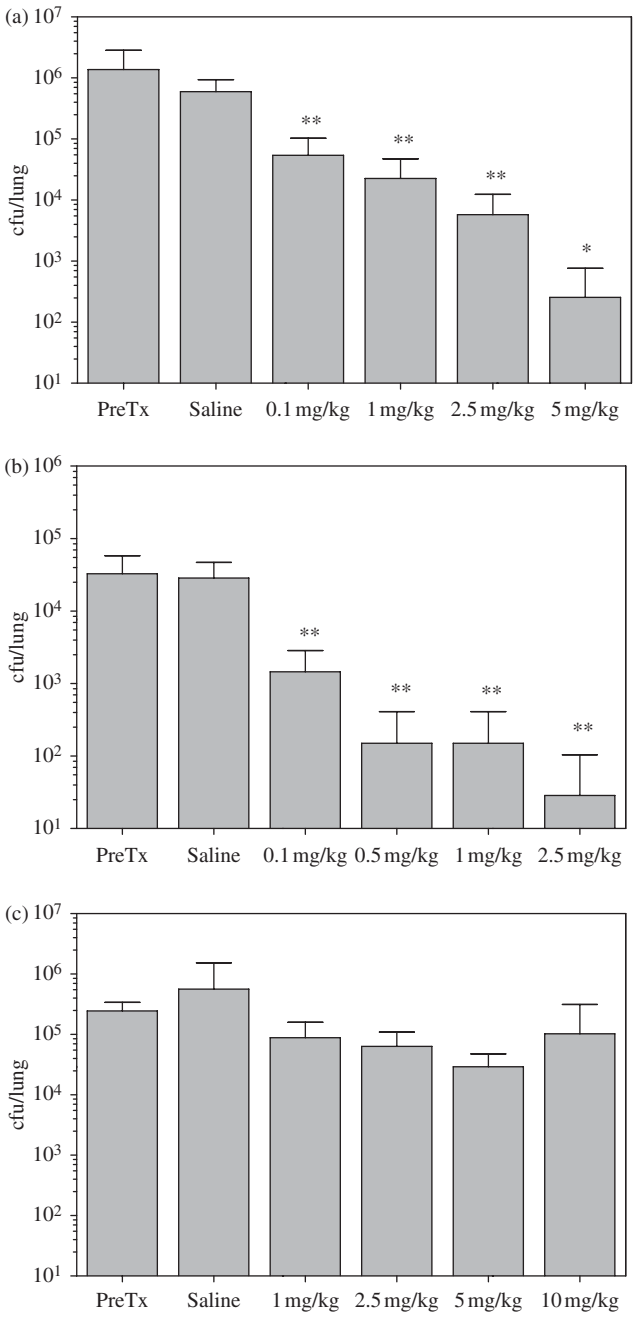


**Figure 2.** Reduction of *P. aeruginosa* (strain C177) cfu in the rat lung after intratracheal administration of antibiotic twice daily for 3 days. (a) FTI. (b) Tobramycin. (c) Fosfomycin. Data are expressed as means ± SD. *$P < 0.05$; **$P < 0.01$. PreTx, pre-treatment.

tobramycin. Our results were consistent with those reported between fosfomycin and other aminoglycosides.[30,31] The PAE of FTI was superior to that of fosfomycin and tobramycin for all three bacterial species. These data were consistent with time–kill kinetics, which also demonstrated slower bacterial regrowth with FTI compared with tobramycin or fosfomycin.

Time–kill experiments were conducted to evaluate the rate and degree of bacterial killing. FTI and tobramycin were rapidly bactericidal against *P. aeruginosa* and *S. aureus*. FTI killed in a concentration-dependent fashion, which is somewhat surprising because fosfomycin, the major component of the combination, killed in a time-dependent fashion.[10] Tobramycin killed in a concentration-dependent fashion like other aminoglycoside antibiotics.[16] We also demonstrated that fosfomycin was bactericidal against *S. aureus*, which is consistent with previous studies.[10] Fosfomycin's mechanism of killing against *P. aeruginosa* is not well characterized. This study demonstrates that bactericidal killing is not reached at concentrations $\leq 16\times$ the fosfomycin MIC for *P. aeruginosa* ATCC 27853. MBC experiments also confirmed that FTI reached bactericidal killing against clinical *P. aeruginosa*, *S. aureus* and *E. coli* strains.

**Table 4.** Spontaneous mutation frequency resulting in development of antibiotic resistance

| Organism (strain) | Frequency | | |
|---|---|---|---|
| | FTI | fosfomycin | tobramycin |
| *S. aureus* | | | |
| C051 | $<1.8\times10^{-10}$ | $3.0\times10^{-5}$ | $3.5\times10^{-6}$ |
| C053 | $<1.8\times10^{-10}$ | $7.7\times10^{-3}$ | $2.0\times10^{-7}$ |
| C055 | $<4.3\times10^{-9}$ | $2.5\times10^{-6}$ | $3.8\times10^{-7}$ |
| C057 | $1.0\times10^{-9}$ | $2.1\times10^{-5}$ | $1.1\times10^{-6}$ |
| ATCC 29213 | $<3.1\times10^{-10}$ | $2.6\times10^{-8}$ | $1.6\times10^{-7}$ |
| *P. aeruginosa* | | | |
| C002 | $5.0\times10^{-6}$ | $6.5\times10^{-3}$ | $1.1\times10^{-5}$ |
| C003 | $1.1\times10^{-6}$ | $1.1\times10^{-6}$ | $4.2\times10^{-5}$ |
| C013 | $1.2\times10^{-7}$ | $9.2\times10^{-3}$ | $1.4\times10^{-6}$ |
| C014 | $3.4\times10^{-6}$ | $1.4\times10^{-4}$ | $1.3\times10^{-6}$ |
| ATCC 27853 | $4.6\times10^{-7}$ | $7.2\times10^{-4}$ | $3.0\times10^{-5}$ |

The *in vitro* studies with FTI were supported by animal efficacy experiments in rats. Lung cfus were stable over the treatment period (3 days) and demonstrated that bacterial killing was due to the activity of the antibiotics (data not shown). *P. aeruginosa* colony counts in rat lung dropped at least 3 $\log_{10}$ with treatment and the organisms were eradicated with higher doses of FTI. While the *in vivo* activity of FTI is slightly less than that of tobramycin on a weight basis, tobramycin accounts for only 20% of FTI. The tobramycin MIC for C177 (0.5 mg/L) is 8-fold less than the FTI MIC (4 mg/L) and may explain the slight difference in activity. Fosfomycin alone has very little activity against *P. aeruginosa in vivo* and confirms the *in vitro* data presented in this study.

Development of antibiotic resistance is of particular concern in bronchiectasis patients. Antibiotic options are limited and bacterial isolates, particularly those from CF individuals, are resistant to many of the currently approved antibiotics.[2] Combinations of antibiotics administered independently (parenteral or parenteral + oral) are commonly used to treat multidrug-resistant *P. aeruginosa* during exacerbations,[31,32] and to slow the development of resistance.[2] Therefore, it would also seem promising to combine fosfomycin and tobramycin in the same aerosol formulation if the combination shows a clinically relevant benefit such as slowing the development of resistance. The spontaneous mutation frequency resulting in resistance after a single exposure was dramatically less than the frequencies of fosfomyin or tobramycin for *S. aureus* and *P. aeruginosa*.

Fosfomycin resistance occurred very rapidly after a single *in vitro* exposure, in our studies. This finding is consistent with previous reports.[26] However, evidence for the *in vivo* development of fosfomycin resistance is lacking. Fosfomycin has been extensively used for >20 years in Japan and Europe for the treatment of urinary tract infections. Despite this, the reported fosfomycin resistance in urinary *E. coli* isolates remains <2%.[13]

Antibiotic options for CF or non-CF bronchiectasis patients are limited. FTI, a novel antibiotic combination, has many desirable properties. Pre-clinical research demonstrates that FTI is active against important CF and non-CF respiratory pathogens including *S. aureus*, *H. influenzae*, *M. catarrhalis*, coliforms and multidrug-resistant *P. aeruginosa*. FTI was rapidly bactericidal and had activity comparable to that of tobramycin. Moreover, FTI reduced the development of antibiotic resistance. Fosfomycin, the major component of FTI, has a very favourable safety profile when administered parenterally.[9] Additionally, several studies have shown that fosfomycin reduces aminoglycoside-induced nephrotoxicity.[33–35] Since tobramycin constitutes 20% of FTI on a weight basis, the cumulative toxic effects due to tobramycin could also be reduced. The comparative safety of FTI should ultimately be evaluated in future studies in the affected populations. These data suggest that FTI should be investigated further for the treatment of CF and non-CF bronchiectasis infections.

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## Transparency declarations

D. L. M., T. F. K and W. R. B. are currently employed by Gilead Sciences, Inc. D. L. M., W. R. B. and T. F. K. own stock and options in Gilead Sciences, Inc. J. L. B. serves as a consultant for Gilead Sciences, Inc.

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