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Time Kill Analyses of Concerning Gram-Negative Bacteria with Fosfomycin Alone and in Combination with Select Antimicrobial Agents

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Amended Abstract

Background: ZTI-01 (fosfomycin, FOS, for injection) demonstrates broad-spectrum activity *in vitro*, including multidrug-resistant (MDR) organisms. FOS shows no cross-resistance to other antibiotic classes, and FOS mechanism of action uniquely inhibits an early step in peptidoglycan biosynthesis. Other antibiotic agents in combination with FOS have been proposed to enhance bacterial killing of MDR organisms. Time-kill kinetic analyses (TKK) were performed on select bacteria that demonstrated synergy when tested by checkerboard analysis with FOS and comparator agents.

Methods: Broth microdilution for FOS (Mueller-Hinton broth supplemented with 25 mg/L glucose-6-phosphate) and comparators was performed before performing TKK. TKK employed MIC multiples for FOS, 0.25 and 1X MIC of select comparators, and combinations of FOS and a comparator. TKK were sampled for colony counts at T_0, T_2, T_4, T_8, and T_{24} hours (h). Two *Klebsiella pneumoniae* isolates (1 KPC and 1 ESBL), 2 *Pseudomonas aeruginosa* isolates (non-MDR), and 1 *Acinetobacter baumannii* isolate (MDR) were tested.

Results: FOS was bactericidal when tested against a *K. pneumoniae* (KPC-producer) isolate. A >4 $\log_{10}$ reduction in bacterial growth (colony forming units, CFU) occurred by 4h at 2X MIC. By 24h with FOS (0.5, 1, and 2X MIC), bacterial growth increased approximately 2 $\log_{10}$. Piperacillin-tazobactam (PTZ) at 0.25 and 1X MIC showed little inhibitory activity. At 24h, bacterial growth was similar to growth control. FOS at 0.5, 1, and 2X MIC in combination with PTZ (1X MIC) showed synergy with approximately a 3.8-4.2 $\log_{10}$ reduction at 4h and a 3.4-5.4 $\log_{10}$ reduction at 24h. FOS (1, 2, 4X MIC) showed a slight decrease (1.4-2.2 $\log_{10}$ CFU) at 4h and by 24h, growth was similar to growth control when tested against an ESBL-producing *K. pneumoniae* isolate. For ceftazidime (CAZ) at 0.25 and 1X MIC, there was a slight decrease (0.9-2.1 $\log_{10}$ CFU) by 4h, and at 24h CFU were similar to growth control. FOS (1, 2, and 4X MIC) in combination with CAZ showed synergy with a 3.8-4.3 $\log_{10}$ reduction by 8h (1X CAZ MIC) and at least a 5.1 $\log_{10}$ reduction at 24h (0.25 and 1X CAZ MIC). FOS activity was shown to be synergistic at 24h when tested at 2, 4, and 8X MIC with either 0.25 or 1X CAZ MIC (*P. aeruginosa* #893949) or 0.25 or 1X MIC of meropenem (MEM; *P. aeruginosa* #889839). Against *A. baumannii*, FOS was shown to be synergistic at 24h when tested at 0.5, 1, and 2X MIC with MEM (1X MIC).

Conclusions: The combination of 2 cell wall active agents, FOS plus selected β-lactams, provided enhanced killing and *in vitro* synergy against concerning gram-negative bacteria.

Introduction

- Fosfomycin is a broad-spectrum antimicrobial agent that exhibits a unique mechanism of action against an enzyme target that inhibits an earlier step in bacterial cell wall synthesis compared to other antibacterial agents. Fosfomycin covalently binds to MurA, preventing the first committed step in peptidoglycan biosynthesis
- Activity of fosfomycin has been shown against a wide range of gram-positive and gram-negative bacteria, including concerning multidrug-resistant bacteria
- ZTI-01 (fosfomycin for injection) is currently in clinical development for treating complicated urinary tract infections (https://clinicaltrials.gov/ct2/show/NCT02753946) at a modernized intravenous dosage of 6 g q 8hr
- Multidrug-resistant (MDR) organisms, including those from deep-seated infections, are often treated with combination chemotherapy. Fosfomycin's differentiated mechanism of action has been proposed to enhance killing when combined with other antibiotic agents
- Therefore, the aim of this study was to evaluate the antimicrobial activity of fosfomycin when combined with selected antimicrobial agents and tested against current clinical bacterial strains using time-kill curve methods

Materials and Methods

- A total of 5 isolates were selected based on demonstrating synergy in previous checkerboard testing
- The isolates comprised 2 *Pseudomonas aeruginosa*, 1 *Acinetobacter baumannii-calcoaceticus* species complex (MDR phenotype), and 2 *Klebsiella pneumoniae* (1 carbapenem-resistant [CRE] phenotype and 1 extended-spectrum β-lactamase [ESBL] phenotype)
- Broth used for the time-kill kinetic studies containing fosfomycin was cation-adjusted Mueller-Hinton broth supplemented with 25 mg/L glucose-6-phosphate
- Time-kill concentration tubes were sampled for fosfomycin and comparators at time 0 hours (T0), T2, T4, T8, and T24
- Sample volume was 0.1 mL that was plated directly to appropriate growth agar (tryptic-soy agar with 5% sheep blood) and serially diluted up to 8 times in tubes with 0.9 mL of a 0.85% saline solution before plating to additional agar plates
- Dilution agar plate series were incubated for 24–48 hours at 35°C in a CO_2 environment before quantifying the viable cell count for each tube at the specified times
- Bacterial cell counts were plotted over time to demonstrate the timed kill curves
- Synergy was defined as a ≥2 $\log_{10}$ decrease in CFU/mL between the combination and its most active constituent after 24 hours, and the number of surviving organisms in the presence of the drug combination was ≥2 $\log_{10}$ CFU/mL below the starting inoculum

Results

- ***P. aeruginosa*** (#889839)
  - Results of testing fosfomycin at 4X MIC in combination with 0.25X and 1X MIC of meropenem are located in Figure 1
  - By 8 hours, fosfomycin at 4X MIC reduced the bacterial count (relative to the starting inoculum) 1.8 $\log_{10}$ CFU/mL followed by a rebound in growth at 24h (3.1 $\log_{10}$ CFU/mL increase from starting inoculum concentration)
  - The combination of fosfomycin and meropenem reduced bacterial count >2 $\log_{10}$ CFU compared to the starting inoculum and was >2 $\log_{10}$ CFU lower than fosfomycin alone at 24h
- ***P. aeruginosa*** (#893949)
  - Results of testing fosfomycin at 4X MIC in combination with 0.25X and 1X MIC of ceftazidime are located in Figure 2
  - By 8 hours, fosfomycin at 4X MIC reduced the bacterial count (relative to the starting inoculum) 1.8 $\log_{10}$ CFU/mL followed by a rebound in growth at 24h (2.7 $\log_{10}$ CFU/mL increase from starting inoculum concentration)
  - The combination of fosfomycin with ceftazidime at 1X MIC reduced bacterial count >2 $\log_{10}$ CFU/mL compared to the starting inoculum and was >2 $\log_{10}$ CFU lower than fosfomycin alone at 24h
- ***A. baumannii*** (#920549; MDR phenotype)
  - Results of testing fosfomycin at 2X MIC in combination with 0.25X and 1X MIC of meropenem are located in Figure 3
  - By 8 hours, fosfomycin at 2X MIC reduced the bacterial count (relative to the starting inoculum) 2.5 $\log_{10}$ CFU/mL followed by an increase in growth of 1.9 $\log_{10}$ CFU/mL by 24h
  - The combination of fosfomycin at 2X MIC and meropenem (1X MIC) by 8 hours exhibited a 4.6 $\log_{10}$ CFU/mL decrease from the starting inoculum and by 24h, growth for the combination decreased to below detectable levels
- ***K. pneumoniae*** (#875100; CRE phenotype)
  - Results of testing fosfomycin at 2X MIC in combination with 0.25 and 1X MIC of piperacillin-tazobactam are located in Figure 4
  - Fosfomycin alone at 2X reduced the viable count by >3 $\log_{10}$ CFU/mL by 4h, which rebounded in growth 1.1$\log_{10}$ CFU/mL by 8 hours and by 24h was to 2.3 $\log_{10}$ CFU/mL greater than the initial inoculum (6.0 $\log_{10}$ CFU/mL)
  - By 8 hours, the combination of fosfomycin (2X) when tested with piperacillin-tazobactam (0.25X or 1X MIC) showed a reduced viable count of ≥4 $\log_{10}$ CFU/mL compared to starting inoculum that remained at 24h at least >2 $\log_{10}$ CFU/mL below the level of the starting inoculum
- ***K. pneumoniae*** (#885542; ESBL phenotype)
  - The results of testing fosfomycin at 4X MIC in combination with 0.25X and 1X MIC of ceftazidime are located in Figure 5
  - Fosfomycin at 4X MIC showed a decrease in growth at 4h (2.3 $\log_{10}$ CFU/mL decrease) followed by a rebound in growth at 8h and 24h (2.8 $\log_{10}$ CFU/mL increase from initial inoculum)
  - In combination with 0.25 and 1X MIC ceftazidime, there was a decrease in growth of 3.9-4.2 $\log_{10}$ CFU/mL at 8h that decreased to below detectable levels at 24h (ceftazidime, 1X MIC)
  - Ceftazidime alone at 0.25 and 1X MIC alone showed increased growth at 24h (2.2-2.5 $\log_{10}$ CFU/mL increase from initial inoculum)

Figure 1 Time-kill curve for *P. aeruginosa* isolate 889839 for fosfomycin (with 25 mg/L glucose-6-phosphate) at 4X MIC alone and combined with meropenem at 0.25X and 1X MIC

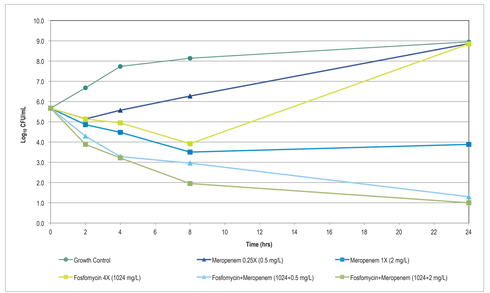


Figure 2 Time-kill curve for *P. aeruginosa* isolate 893949 for fosfomycin (with 25 mg/L glucose-6-phosphate) at 4X MIC alone and combined with ceftazidime at 0.25X and 1X MIC

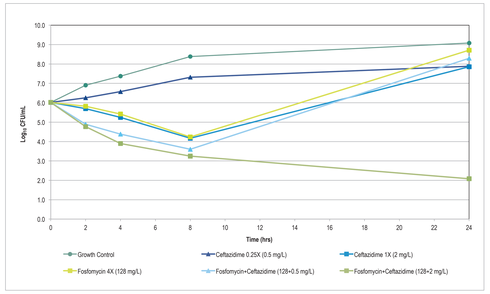


Figure 3 Time-kill curve for *A. baumannii* isolate 920549 (MDR phenotype) for fosfomycin (with 25 mg/L glucose-6-phosphate) at 2X MIC alone and combined with meropenem at 0.25X and 1X MIC

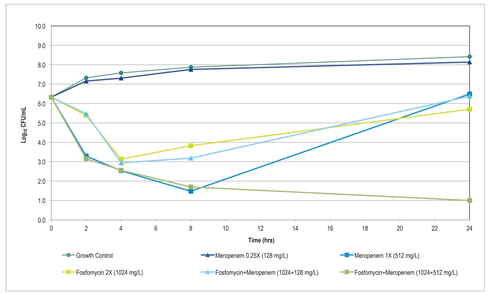


Figure 4 Time-kill curve for *K. pneumoniae* isolate 875100 (CRE phenotype) for fosfomycin (with 25 mg/L glucose-6-phosphate) at 2X MIC alone and combined with piperacillin-tazobactam at 0.25X and 1X MIC

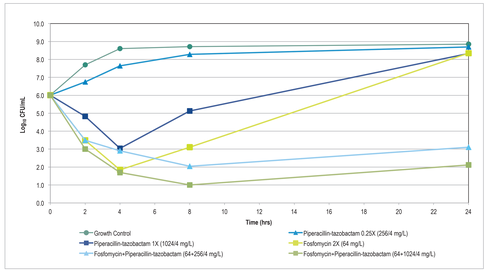
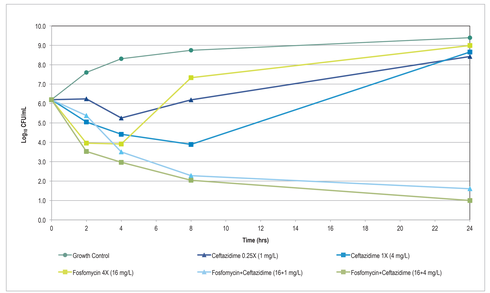


Figure 5 Time-kill curve for *K. pneumoniae* isolate 885542 (ESBL phenotype) for fosfomycin (with 25 mg/L glucose-6-phosphate) at 4X MIC alone and combined with ceftazidime at 0.25X and 1X MIC



Conclusions

- Selected isolates that demonstrated a synergistic response when tested against fosfomycin and another agent in combination in checkerboard experiments confirmed a synergistic response in time-kill kinetic experiments
- Fosfomycin demonstrated synergy (bactericidal activity) in combination with various β-lactam antibiotics (ceftazidime, meropenem, piperacillin-tazobactam) when tested against *K. pneumoniae* (including CRE and ESBL phenotype), *P. aeruginosa*, and *A. baumannii* (including MDR)
- These *in vitro* results demonstrate the potential beneficial effect of using fosfomycin in combination with other classes of antimicrobial agents against concerning MDR organisms

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