P0054

Paper Poster Session I

Issues in prevention and management of biofilm and foreign-body infection

TP-076 is active *in vitro* against biofilms formed by a panel containing fluoroquinolone-resistant and susceptible uropathogenic *Escherichia coli* clinical isolates

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Background: Bacteria can persist as biofilms in chronic and device-related infections. TP-076 is a novel tetracycline-class antibiotic with potent antibacterial activity against Gram-negative pathogens, including carbapenem-resistant Enterobacteriaceae. TP-076 was evaluated *in vitro* against biofilms formed by a panel of ten uropathogenic *Escherichia coli* clinical isolates versus levofloxacin, a standard of care for complicated urinary tract infections.

Methods: The minimal inhibitory concentration (MIC) of compounds was determined according to CLSI guidelines except that tryptic soy broth/yeast extract (TSB/YE) medium was used. Biofilm assays were done in at least triplicate. Cultures were grown in TSB/YE for 2 hours at 35° C, diluted to ~10^6 colony forming units (CFU) in TSB/YE, and 500 µL of culture was added to 5 mL polystyrene tubes and allowed to form biofilms at 35°C for 24 hrs, without shaking. At 24 hrs, planktonic cells were aspirated and biofilms were fed with either 600 µL of fresh TSB/YE, or TSB/YE containing TP-076 (2 or 20 µg/mL) or levofloxacin (20 or 200 µg/mL), and incubated for an additional 24 hrs at 35°C. For staining biofilms, planktonic cells were aspirated, tubes were rinsed with water and stained with 0.1% crystal violet (CV). For biofilm quantification, planktonic cells were aspirated, tubes were washed with saline, cells were released from biofilms by sonication in saline and plated for CFUs. The % CFU reduction, versus the initial biofilm inoculum, was calculated. For three levofloxacin-resistant isolates whose 24 hr biofilms appeared less robust by CV staining, 24 hr biofilms were re-fed with fresh media and grown for an additional 24 hours prior to testing with drug.

Results: All ten isolates were highly susceptible to TP-076, with MIC values ranging from 0.031 to 0.13 µg/mL. Seven of the ten strains were highly levofloxacin-resistant, with levofloxacin MIC values ranging from 32 to 128 µg/mL; the three levofloxacin-susceptible isolates had levofloxacin MIC values ranging from 0.031 to 0.13 µg/mL. TP-076 at 2 µg/mL effectively cleared biofilms from all isolates, reducing biofilm CFUs to >98% of the initial biofilm inoculum. Levofloxacin at 20 µg/mL effectively cleared biofilms formed by the three susceptible isolates (>99% CFU reduction), but failed to clear the biofilms of the seven levofloxacin-resistant isolates. For the levofloxacin-resistant isolates, 200 µg/mL of levofloxacin produced >99% CFU reduction for four levofloxacin resistant isolates, >95% reduction for two isolates and failed to reduce biofilm CFUs for one isolate. The TP-076 and levofloxacin susceptibilities of 24 hr and 48 hr biofilms were similar for the three isolates tested under both conditions, confirming that the activity of TP-076 was not an artifact of an initially fragile biofilm.

Conclusion: This *in vitro* activity of TP-076, if confirmed *in vivo*, would support its potential use in the clinical treatment of chronic biofilm infections.