A Hollow-Fiber Infection Model to Evaluate the Prevention of On-Therapy Resistance of *Neisseria gonorrhoeae* to Gepotidacin

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INTRODUCTION

- The World Health Organization estimates 78 million new cases of gonorrhea are aquired each year [1].
- The emergence of multi-drug and extensively drug-resistant *Neisseria gonorrhoeae* has quickly become a global health concern [2].
- One of the major complications surrounding research efforts to combat the spread of drug resistant *N. gonorrhoeae* is its fastidious nature.
  - *N. gonorrhoeae* requires demanding nutrient and atmospheric conditions and frequently auto-lyses after optimal growth.
- Gepotidacin (GSK2140944) is a novel triazaacenaphthylene type IIA topoisomerase inhibitor [3] which inhibits DNA replication against a broad-spectrum of bacterial species, including *N. gonorrhoeae*.
- Herein, we describe the development of a novel *in vitro* hollow-fiber infection model (HFIM) that was utilized to evaluate the exposure of gepotidacin required to prevent the development of on-therapy resistance.

OBJECTIVES

- The objectives of these studies described were the following:
  - To develop a dynamic HFIM which supports the growth of *N. gonorrhoeae*;
  - To complete a series of HFIM studies in which the emergence of a gepotidacin-resistant bacterial subpopulation, as observed in a Phase 2 clinical study, was reproduced; and
  - To identify the exposure of gepotidacin required to prevent the amplification of resistant subpopulations in the HFIM.

METHODS

Antimicrobial Agents and Challenge Isolates

- Gepotidacin was provided by GlaxoSmithKline (Collegeville, PA). Ciprofloxacin and ceftriaxone were purchased from Henry Schein Medical (Melville, NY).
- The *N. gonorrhoeae* clinical isolate (8) evaluated was known to be ciprofloxacin-resistant (minimum inhibitory concentration [MIC]= 2 mg/L GyrA S91F, D95A; ParC D86N), susceptible to ceftriaxone (MIC = 0.004 mg/L), and with a gepotidacin agar/broth MIC of 1/0.5 mg/L. This isolate was collected during the Phase 2 clinical study conducted by GSK and contained the first step gepotidacin mutation, ParC D86N, that was found to be present in the baseline isolates recovered from all three urogenital microbiological failures in that study [4].
- The reference strain, *N. gonorrhoeae* 49226, was purchased from the American Type Culture Collection (ATCC, Manassas, VA).

Susceptibility Testing

- Gepotidacin, ciprofloxacin, and ceftriaxone MIC values were determined in triplicate using Gonococcal agar base as per Clinical and Laboratory Standards Institute guidelines (CLSI) [5].
  - In order to evaluate the MIC of each challenge compound under the liquid conditions utilized in the HFIM, MIC values were also determined using fastidious broth (FB) medium modified to lack agarose.

METHODS

In Vitro Hollow-Fiber Infection Model

- 10 mL of an initial bacterial density of 1.0 x 10^6 CFU/mL was inoculated into the hollow-fiber cartridge (**Figure 1**) (Fibercell Systems, Frederick, MD).
- Assuming a 7-hour half-life for gepotidacin, human free-drug plasma concentration-time profiles were simulated following exposures observed after administration of 0.75 to 12 g of gepotidacin as a single oral dose.
- Ciprofloxacin and ceftriaxone exposures were simulated using half-lives of 3 and 7.5 hours, respectively, simulating observed free-drug plasma profiles following administration of a 0.5 g oral and 0.25 g intramuscular (IM) dose, respectively.
- Samples were collected over the first 48 hours to confirm the simulated concentration-time profiles for each challenge compound via LC-MS/MS.
- Samples for bacterial enumeration were taken at 0 and 4 hours post exposure and on Days 1, 2, 3, 5, and 7.
  - All bacterial samples were washed twice to eliminate drug carryover, and plated on drug-free and agar supplemented with 2 x the agar MIC value of each respective challenge compound.
  - MIC values were determined in duplicate for isolates that were found upon the drug-supplemented agar plates.

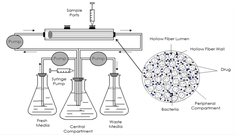


Figure 1. Schematic of the HFIM utilized in the studies described herein

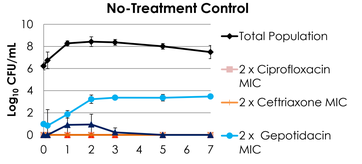
RESULTS

Susceptibility Testing

- Gepotidacin, ciprofloxacin, and ceftriaxone MIC values for the *N. gonorrhoeae* clinical isolate were 0.5, 2, and 0.004 mg/L, respectively.
  - All MIC values for the ATCC reference strain were within CLSI reported values.
  - MIC values were similar between the standard CLSI method and those using FB broth.

In Vitro Hollow-Fiber Infection Model

Figure 2. Change in the total population and resistant bacterial subpopulations over time for the no-treatment control



- As shown in **Figure 2**, the *N. gonorrhoeae* isolate grew well in the HFIM, with the total population reaching a bacterial burden > 8-$\log_{10}$ CFU/mL by Day 1, and slowly declining to 7.5-$\log_{10}$ CFU/mL by Day 7.

RESULTS

- As shown in **Figure 3**, results for the ciprofloxacin and ceftriaxone treatment controls were as expected given the clinical isolate studied was resistant to ciprofloxacin and susceptible to ceftriaxone.
- The gepotidacin exposures evaluated within the system provided a full exposure response from treatment failure to success, with doses ≥ 4.5 g sterilizing the system over the seven-day period (**Figure 4**).

Figure 3. Change in the total population and resistant bacterial subpopulation over time for ciprofloxacin and ceftriaxone treatment controls

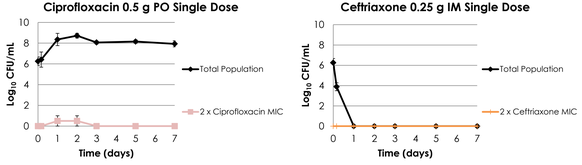
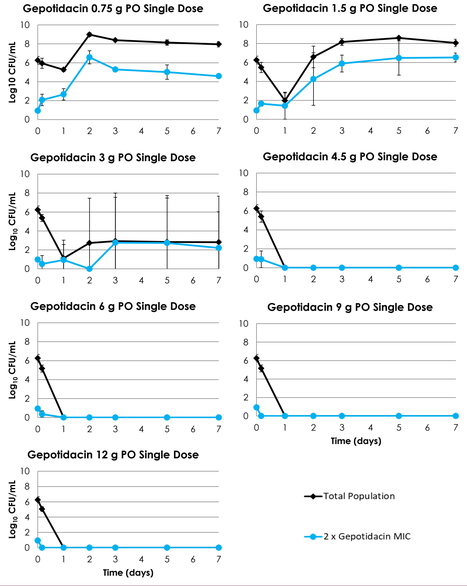


Figure 4. Change in the total population and resistant bacterial subpopulation over time for the seven gepotidacin doses evaluated in the HFIM



RESULTS

- The relationship between change in $\log_{10}$ CFU/mL from bacterial burden of the gepotidacin-resistant subpopulation on Day 7 took on the form of an inverted-U, with doses ≥ 4.5 g preventing amplification of resistance within the system (**Figure 5**).
  - Gepotidacin broth MIC values of 26 isolates collected from the drug-supplemented agar plates increased from the baseline value of 0.5 mg/L to values ranging from 2 to 16 mg/L.
  - When evaluated in the presence of a broad-spectrum efflux pump inhibitor, gepotidacin MIC values decreased two- to three-fold in value for six isolates.
- As shown in **Figure 6**, change in bacterial burden for the gepotidacin 3 g dose replicates was inconsistent.
  - Treatment failure was demonstrated for one of the three replicates.

Figure 5. Relationship between gepotidacin exposure and change in $\log_{10}$ CFU/mL from baseline of the gepotidacin-resistant subpopulation on Day 7

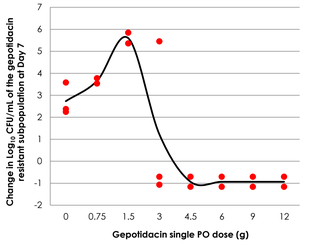
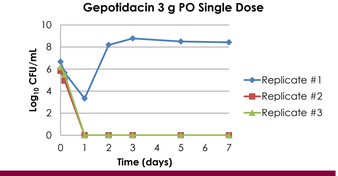


Figure 6. Change in total bacterial burden for the thee gepotidacin 3 g dose replicates



CONCLUSIONS

- Development of this novel HFIM model allowed for the following:
  - *N. gonorrhoeae* growth with minimal autolysis and the opportunity to evaluate gepotidacin drug exposures required to prevent on therapy resistance amplification.
  - Induction of the same type of resistance mutation observed in the Phase 2 study.
- These data will help to guide the design of future dosing regimens for evaluation.

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