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

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INTRODUCTION

- The emergence of multi-drug and extensively drug-resistant *Neisseria gonorrhoeae* has quickly become a global health concern [1].
- Gepotidacin (GSK2140944) is a novel triazaacenaphthylene type IIA topoisomerase inhibitor [2] 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 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 Isolate

- Gepotidacin was provided by GlaxoSmithKline (Collegeville, PA). Ciprofloxacin and ceftriaxone were purchased from Henry Schein Medical (Melville, NY).
- The *N. gonorrhoeae* clinical isolate (isolate #8) evaluated was known to be ciprofloxacin-resistant (minimum inhibitory concentration [MIC]= 2 mg/L), susceptible to ceftriaxone (MIC = 0.004 mg/L), and with a gepotidacin agar/broth MIC of 1/0.5 mg/L. The 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 [3].

Susceptibility Testing

- Gepotidacin, ciprofloxacin, and ceftriaxone MIC values were determined in triplicate using Gonococcal agar per Clinical and Laboratory Standards Institute guidelines (CLSI) [4].
  - 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.

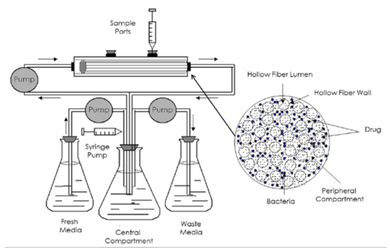
In Vitro Hollow-Fiber Infection Model

- 10 mL of an initial bacterial density of 10^6 colony forming units (CFU)/mL was inoculated into the hollow-fiber cartridge (**Figure 1**) (Fibercell Systems, Frederick, MD), utilizing modified FB medium.

METHODS

- 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 (half-lives of 3 and 7.5 hours, respectively), using free-drug plasma profiles following administration of a 0.5 g oral and 0.25 g intramuscular dose, respectively.
- Samples were collected for observation of simulated pharmacokinetic profiles and enumeration of bacterial burden over the study duration.
  - All bacterial samples were 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* isolate were 0.5, 2, and 0.004 mg/L, respectively. These MIC values, determined using the standard CLSI method, were similar to those using FB broth.

In Vitro Hollow-Fiber Infection Model

- As shown in **Figure 2**, the *N. gonorrhoeae* isolate grew well in the HFIM, reaching a total bacterial burden >8 $\log_{10}$ CFU/mL by Day 1. The ciprofloxacin and ceftriaxone controls performed as expected given that the isolate was resistant to ciprofloxacin and susceptible to ceftriaxone.
- The gepotidacin exposures evaluated provided a full exposure response from treatment failure to success, with doses ≥ 4.5 g sterilizing the system over the seven-day period (**Figure 3**).
- 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 4**).
  - Gepotidacin MIC values of the 26 isolates collected from the agar plates increased from the baseline value of 0.5 mg/L to values ranging from 2 to 16 mg/L.
- As shown in **Figure 5**, the change in bacterial burden for the gepotidacin 3 g dose replicates was inconsistent, with treatment failure observed for one of the three replicates.

RESULTS

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

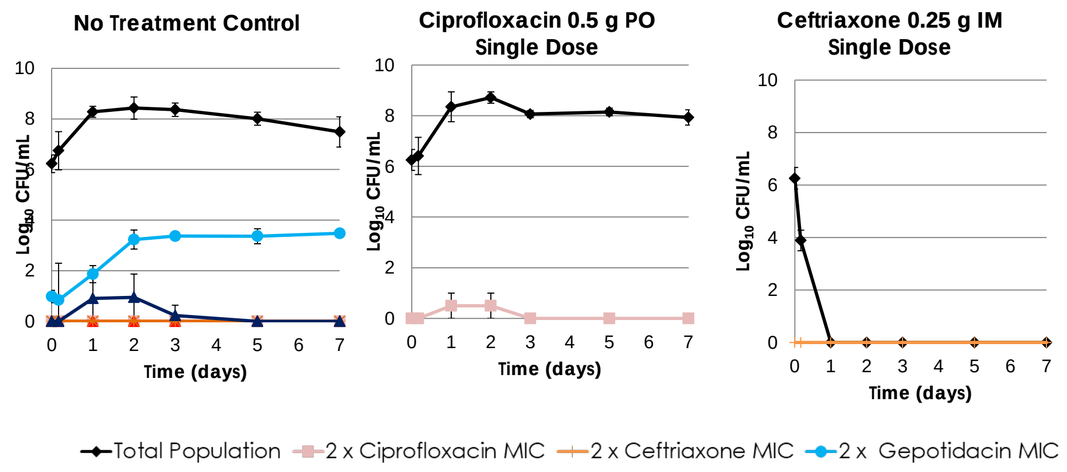


Figure 3. Change in the total population and resistant bacterial subpopulation over time for the seven gepotidacin doses evaluated in the HFIM

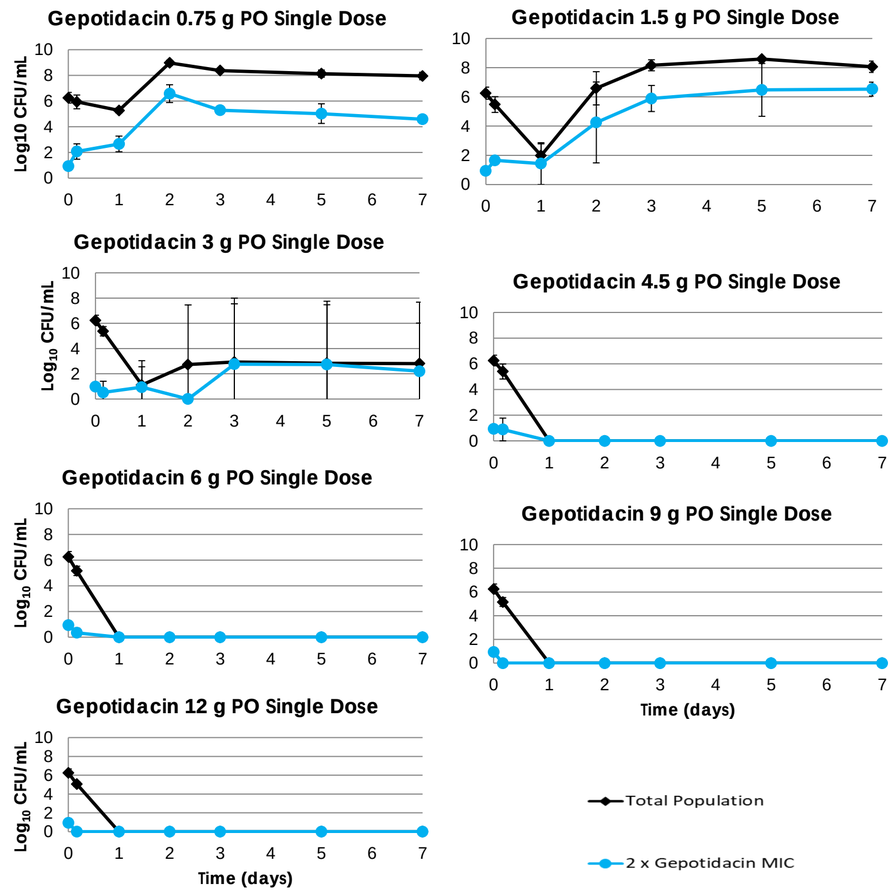


Figure 4. Relationship between gepotidacin exposure and change in $\log_{10}$ CFU/mL from baseline of the gepotidacin-resistant subpopulation on Day 7

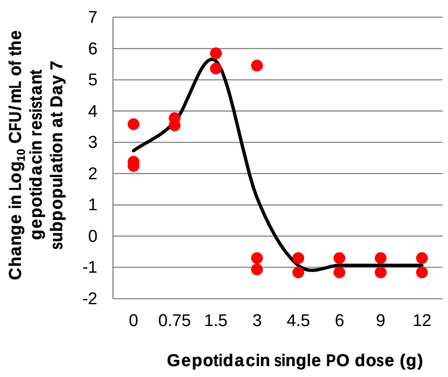
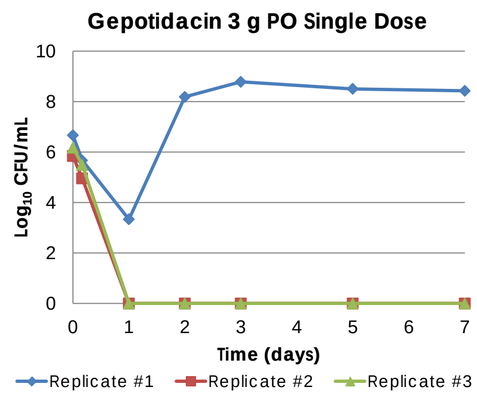


Figure 5. Change in total bacterial burden for the three gepotidacin 3 g dose replicates



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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- This poster will be presented by Dr. Paul G. Ambrose (employee of ICPD); pambrose@ICPD.com

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