

Establishment of the Gonorrhea Mouse Model for Pre-Clinical Testing of Antimicrobial Agents against *Neisseria gonorrhoeae*



Kristie L. Connolly, Ph.D., Thomas Hiltke, Ph.D.,
Carolina Gomez, B.A., Magnus Unemo, Ph.D., & Ann Jerse, Ph.D.

Uniformed Services University
F. Edward Hébert School of Medicine
Department of Microbiology and Immunology
Bethesda, MD, USA

WorldSTI & HIV Congress, Brisbane, 2015



Urgent Need for New Treatments against *Neisseria gonorrhoeae* (Gc) Infections

There is no longer a single class of antibiotics available for treatment of Gc

The extended-spectrum cephalosporins (ESCs) were the last class of antibiotics used for single treatment

- Due to rising MICs, cefixime (CFX) is no longer recommended
- Decreased susceptibility and cases of treatment failure of ceftriaxone (CRO) also reported

Guidelines now recommend dual antimicrobial therapy

- CRO and azithromycin (single 1 g dose administered orally)
 - CRO: injectable cephalosporin; single IM dose (250 mg, CDC, USA)
- Safe and effective treatment for uncomplicated infections at all anatomical sites
 - Uncomplicated urogenital and anorectal infections – 99.2%
 - Pharyngeal infections – 98.9%



Animal Models Can Be Used to Accelerate Product Development

The gonorrhea mouse model is the best characterized animal model of Gc infection and we have used it to test many candidate products, including:

- Antibiotics
- Vaccines
- Vaginal microbicides

Colonization characteristics

- Gc recovered for 10-14 days
- Gc within cervical and vaginal tissue; seen within lamina propria
- Ascending infection in 18-20% of mice

Localized inflammatory response

- Neutrophil influx in 30-80% of BALB/c mice
- Proinflammatory cytokines/chemokines on day 5 of infection

Susceptible to repeat infection with same strain as occurs in humans

- Transient, unremarkable antibody response
- No humoral memory response



Research Needs:

- No *in vivo* breakpoints for known antibiotics established in the gonorrhea mouse model for pre-clinical development of new therapeutics
- The gonorrhea mouse model has not been utilized to test multi-drug resistant Gc strains

Objectives:

- Establish the *in vivo* breakpoint for CRO and CFX of both susceptible and resistant Gc strains
 - At what dose do we see treatment failure?

In Progress:

- Generate pharmacokinetic (PK) data for doses of successful and failed CRO and CFX treatments
 - Can use data to establish method of comparing *in vitro* and *in vivo* efficacy of known antibiotics
 - Use for comparing/predicting success of new therapeutics



MIC Breakpoints for CRO and CFX

In vitro MIC breakpoints

- CLSI considers strains to be susceptible to CRO and CFX when MIC ≤ 0.25 $\mu\text{g}/\text{mL}$
- Definition of decreased susceptibility or resistance to CRO and CFX varies by country and testing method
 - CDC: MICs ≥ 0.5 $\mu\text{g}/\text{mL}$ are considered to have decreased susceptibility

Therapeutic time in humans

- Time where plasma concentration is at least 4 times greater than the MIC₉₀
 - CRO (250 mg, IM): 47 – 76.2 hours
 - CFX (800 mg, oral): 17.1 – 27.3 hours

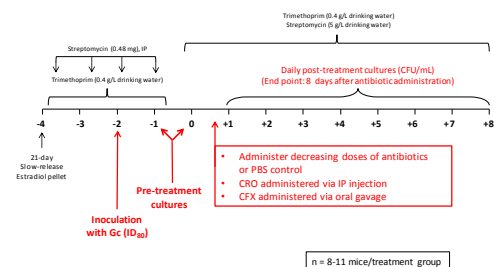
No corresponding dose response information for treatment of infection

- Utilize gonorrhea mouse model to generate *in vivo* clearance and PK data



(Moran & Levine, 1995)

Experimental Design: Dose Response Studies to Determine *in vivo* Efficacy of CRO or CFX



***N. gonorrhoeae* Strain FA1090**

Susceptible to both CRO and CFX (agar dilution assay)

- CRO MIC = 0.0075 µg/mL
- CFX MIC = 0.0075 µg/mL

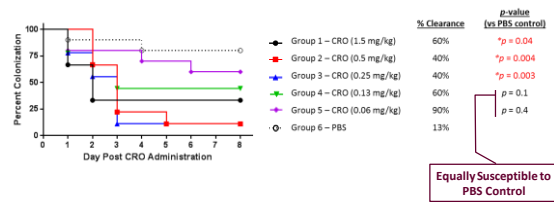
Isolated from a case of disseminated gonococcal infection

- Female patient, 1983
- Naturally streptomycin-resistant

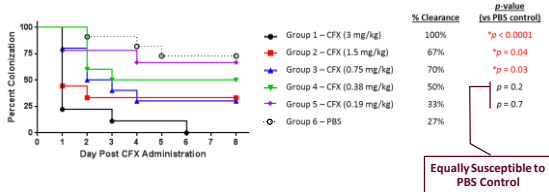
Extensively tested in the experimental male urethral infection model



0.13 mg/kg is the *in vivo* Breakpoint for CRO against FA1090 (CRO^S)



0.38 mg/kg is the *in vivo* Breakpoint for CFX against FA1090 (CFX^S)



***N. gonorrhoeae* Strain H041**

First high-level CRO resistant Gc strain (Ohnishi *et al.* 2011)

- Isolated from pharynx of female commercial sex worker in Kyoto, Japan (2009)
- CRO MIC = 2-4 µg/mL
- CFX MIC = 8 µg/mL

High level of CRO resistance conferred by a unique *penA* mosaic allele

- Mutations in penicillin-binding protein 2 (PBP2)
- Previously correlated with resistance and decreased susceptibility to ESCs



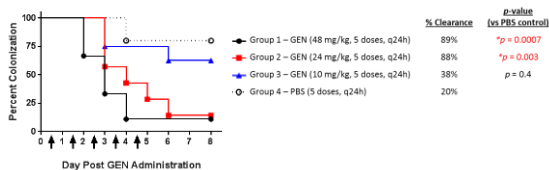
Characterization of H041 Infection in the Gonorrhea Mouse Model

Infectious dose studies

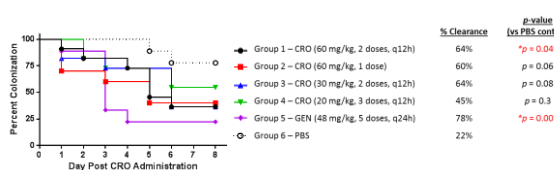
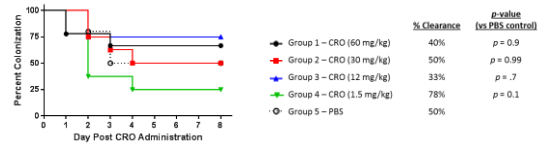
- Inoculation with 10⁴ CFU of H041 yielded infection in 80 – 100% of mice for 14 days

Establishment of a positive control antibiotic

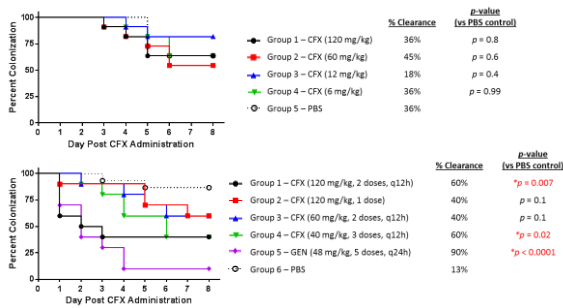
- GEN dose response testing indicated that 5 daily IP doses of 48 mg/kg successfully cleared H041 infection compared to the PBS control



Single Dose of CRO had no Significant Effect on H041 (CRO^R) Colonization



Single Dose of CFX had no Significant Effect on H041 (CFX^R) Colonization



Conclusions and Future Directions

- The gonorrhea mouse model can now be used to test novel antimicrobials against strain H041 *in vivo*
 - GEN was established for use as a positive control
- In vivo* breakpoints were identified for a sensitive Gc strain in the gonorrhea mouse model
 - CRO: 0.13 mg/kg
 - CFX: 0.38 mg/kg
- Delivery of multiple doses of both CRO and CFX significantly reduced the percentage of mice colonized with the CRO^R strain H041
 - Single doses of either antibiotic showed no effect
- PK analysis is underway for both CRO and CFX to be able to relate plasma concentration for each antibiotic to the *in vivo* breakpoint



Acknowledgements

Uniformed Services University (USA)

Ann Jerse, Ph.D.
 Afrin Begum
 Claire Costenoble-Caherty
 *Carolina Gomez
 Isabelle Leduc, Ph.D.
 *Jason Pilligua
 Michelle Pilligua-Lucas
 Nazia Rahman
 Erica Raterman, Ph.D.
 *Rachel Rowland
 Riley Sennett
 Leah Vincent

National Institute of Allergy and Infectious Diseases (NIH, USA)

Thomas Hiltke, Ph.D.
 Ann Eakin, Ph.D.

University of Chapel Hill (USA)

Rob Nicholas, Ph.D.

Örebro University Hospital (Sweden)

Magnus Unemo, Ph.D.

Funding

- Interagency Agreement AA114024-001
- USUHS (USU-DOD MIC73-2493)

