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Efficacy of the Novel Fluorocycline TP-271 in a Mouse Model of *Neisseria gonorrhoeae* Infection

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Abstract

Background: The Center for Disease Control and Prevention has recently declared drug-resistant *N. gonorrhoeae* (Gc) as an urgent threat. TP-271 is a novel, broad-spectrum fluorocycline in preclinical development at Tetraphase Pharmaceuticals. To explore its potential for use against Gc infection, TP-271 was tested in a mouse gonorrhea model. **Methods:** The macrolide-resistant isolate Gc strain MS11, originally isolated from a case of cervicitis, was used as the test strain. Female BALB/c mice in the diestrus or anestrus stages of the estrous cycle were treated with 17-β estradiol pellets to induce prolonged susceptibility to Gc infection. Mice were intravaginally inoculated with 2.9 x 10^6 colony forming units and vaginal swabs were quantitatively cultured daily to monitor infection. On day two of infection, antibiotic treatment of culture-positive mice was initiated. TP-271 was administered for 5 days in daily single doses of 6, 12, or 18 mg/kg intraperitoneally (IP). Ceftriaxone (CRO), the positive control, was given at 15 mg/kg as a single IP dose on day 2 post-inoculation and a negative control group was given saline vehicle IP on schedule with TP-271. Vaginal mucus was quantitatively cultured daily on days 3 to 11 post-infection. The MIC values against MS11 for TP-271 and CRO were 0.25 µg/mL and 0.016 µg/mL, respectively. **Results:** After two daily doses, ~40-60% of mice receiving TP-271 cleared infection. By day 6, after 4 daily doses of TP-271 at 6 or 18 mg/kg, 100% of mice in each dose group cleared Gc infection. By day 7, after 5 daily doses of TP-271 at 12 mg/kg, 90% of mice cleared Gc infection. All TP-271- and CRO-treated mice that cleared infection remained culture-negative for 5 days. The kinetics of infection clearance was similar for all three TP-271 groups, while a single dose of CRO at 15 mg/kg completely cleared the infection at 24 hrs post-dose. Untreated controls showed stable levels of Gc colonization up to 11 days post-infection. **Conclusion:** TP-271 was similarly efficacious at all dosages, however, clearance of infection was slower than CRO and may reflect differences in mechanism of antibacterial action. Results warrant further investigation of TP-271 efficacy against drug-resistant isolates of Gc, especially those that are not susceptible to standard of care antibiotics.

Background

- Urethritis in men and endocervicitis in women are serious sexually transmitted diseases caused by *N. gonorrhoeae* infections in which the standard-of-care antibiotics, including tetracyclines, cephalosporins, aminoglycosides and macrolides, have become alarmingly less effective due to the rapid rise in drug resistance. The U.S. Centers for Disease Control and Prevention (CDC) has prioritized drug-resistant *N. gonorrhoeae* as an urgent threat and estimates that there are 246,000 cases of drug-resistant *N. gonorrhoeae* infections annually [1].
- TP-271 is a novel broad-spectrum fluorocycline antibiotic with excellent potency against serious and multidrug-resistant Gram-negative, Gram-positive, anaerobic, and atypical pathogens. TP-271 is active against bacterial isolates expressing the most common tetracycline-specific resistance mechanisms including efflux, ribosomal protection and drug-inactivation mechanisms [2].
- TP-271 was shown to be active *in vitro* against *N. gonorrhoeae*, including penicillin-, ciprofloxacin- and tetracycline-resistant (*tet*(M) and *rpsJ* mutants) clinical isolates [3].
- The purpose of this study was to characterize the efficacy of TP-271 in a mouse model of gonorrhea.

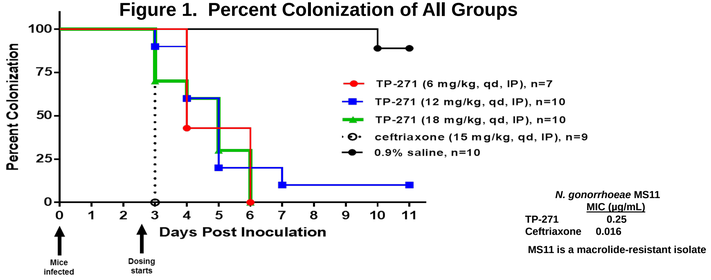
Methods

Bacterial Strain and Culture Conditions. *Neisseria gonorrhoeae* strain MS11 was used as the test strain for the in vivo efficacy study. Strain MS11 is a serum-intermediate strain that was originally isolated from a case of cervicitis [4]. Strain MS11 is a natural *mtr* mutant that exhibits increased resistance to the macrolide antibiotics erythromycin and azithromycin [5] but is susceptible to TP-271 (MIC 0.25 µg/ml). For routine propagation of gonococci and preparation of the inoculum for mouse infection, strain MS11 was cultured on supplemented GC agar [36 g of GC agar powder and 5 g Bacto agar powder were dissolved in 1000 ml water and autoclaved at 121°C for 25 min. Kellogg's supplement I (400 g glucose, 10 g L-glutamine, 20 mg co-carboxylase, H_2O QS I liter) and Supplement II [12 µM $Fe(NO_3)_3$] were added at volume ratios of 1:100 and 1:1000, respectively, before pouring the plates]. GC agar with vancomycin, colistin, nystatin, trimethroprim sulfate (VCNT supplement; Difco) and 100 µg/ml streptomycin sulfate was used to isolate *N. gonorrhoeae* from mouse vaginal swab suspensions. Heart infusion agar (HIA) was used to monitor the presence of facultative aerobic commensal flora during the mouse infection experiment. Incubation conditions *for N. gonorrhoeae* and for commensal bacteria were at 37°C in a humid atmosphere containing 7.5% CO_2 [6].

Gonorrhea Model. Fifty female BALB/c mice in the diestrus or anestrus stages of the estrous cycle were given 21-day slow-release 5 mg 17-β estradiol pellets (Innovative Research of America) to induce susceptibility as per the standard infection protocol described by Jerse, 1999 [6]. Antibiotics (2.4 mg streptomycin sulfate and 0.4 mg vancomycin (SV), intraperitoneally (i.p.) and 0.04 g trimethoprim sulfate per 100 ml drinking water) were also administered to suppress the overgrowth of commensal flora that occurs under estradiol treatment. SV was administered twice daily on days -2 and -1, and streptomycin sulfate only was administered on days 0 and +1. Trimethoprim sulfate was given in the drinking water through day +1, and sterile water was used for the remainder of the infection. Mice were inoculated with *N. gonorrhoeae* strain MS11 suspended in PBS at a dose that establishes infection in 80-100% of mice [2.92 x 10^6 colony forming units (CFU)] at day 0. Vaginal swabs from all mice were quantitatively cultured for *N. gonorrhoeae* on days 1 and 2 post-inoculation by gently inserting a Puritan rayon swab into the vagina. The specimens were suspended in 1 ml of GC broth, and a 1:10 dilution was prepared from this stock to ensure counting accuracy for mice with high levels of colonization. Samples were cultured for *N. gonorrhoeae* on GC-VCNTS agar using the Autoplater 4000 (Spiral Biotech). The recovered colony forming units (CFU) were enumerated using the Spiral Biotech Q-Counter Software. A portion of the swab sample was also inoculated onto HIA agar to isolate commensal flora. After culturing at day +2, mice were randomized into 5 groups, with each group containing 10 mice for antibiotic administration. TP-271 was administered for 5 days in daily single doses of 6 mg/kg (n=7 mice), 12 mg/kg (n=10 mice), or 18 mg/kg (n=10 mice) IP in 0.9% saline, pH 6.5. Ceftriaxone, the positive control, was given at 15 mg/kg (n=9 mice) as a single IP dose on day 2 post-inoculation and a negative control group was given 0.9% saline, pH 6.5, vehicle (n=10 mice) IP on schedule with TP-271. Vaginal mucus was quantitatively cultured daily on days 3 to 11 post-inoculation. The limit of detection was 20 CFU. Differences in the duration of colonization were assessed using a Kaplan Meier survivorship curve. Differences in colonization load were assessed by a 2-way ANOVA using Bonferroni as a post hoc analysis for multiple pair wise comparisons (GraphPad Prism V.6.0). Minimal inhibitory concentration (MIC) assays were conducted as per CLSI standardized methodology [7]. MIC values against MS11 for TP-271 and ceftriaxone were 0.25 µg/mL and 0.016 µg/mL, respectively.

Results

Figure 1. Percent Colonization of All Groups



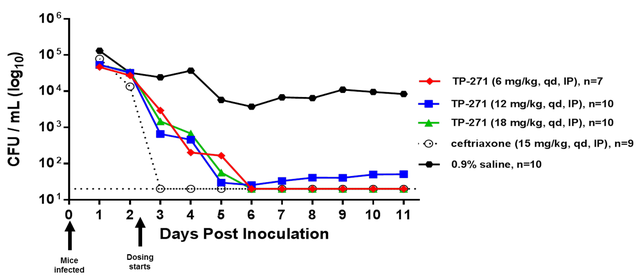
N. gonorrhoeae MS11

	MIC (µg/mL)
TP-271	0.25
Ceftriaxone	0.016

MS11 is a macrolide-resistant isolate

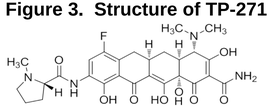
The graph shows total percent colonization of each treatment group over 11 days. Infected mice were given TP-271, ceftriaxone, or 0.9% saline on day 2 of infection. Results for all treatment groups were statistically significantly different from 0.9% saline control ($p < 0.0001$ for all treatment groups).

Figure 2. Geometric Mean CFU/mL of All Groups



The graph shows the geometric mean of $\log_{10}$ CFU/mL of *N. gonorrhoeae* recovered from each experimental group for 11 days post bacterial challenge. The limit of detection (20 CFU; dotted line) was used to calculate the group mean for cultures from which no gonococci were recovered.

Figure 3. Structure of TP-271



Conclusions

- **All 3 dosages of TP-271 (6, 12 and 18 mg/kg) administered over 5 days showed comparable efficacy in clearing *N. gonorrhoeae* infection in this experiment.**
- **After 2 daily doses, ~40-60% of mice receiving TP-271 cleared infection; all TP-271-treated mice, except for one mouse in the 12 mg/kg group, cleared infection after 4-5 daily doses.**
- **The kinetics of infection clearance was similar for all three TP-271 groups, while a single dose of ceftriaxone at 15 mg/kg completely cleared the infection at 24 hrs post-dose. This observation may reflect a difference in antibiotic mechanism.**
- **These results warrant further investigation of TP-271 efficacy against drug-resistant isolates of *N. gonorrhoeae,* especially those that are not susceptible to standard of care antibiotics.**

References

1. CDC Report, Antibiotic Resistance Threats in the United States, 2013; website: http://www.cdc.gov/drugresistance/threat-report-2013
2. Grossman, T., C. Fyfe, W. O'Brien, M. Hackel, J. Sutcliffe. 2012. TP-271 is a Potent, Broad-spectrum fluorocycline with activity against community-acquired bacterial respiratory and biothreat pathogens. Abstr. F-1525. 52nd Interscі. Conf. Antimicrob. Agents Chemother., American Society for Microbiology, Washington, DC.
3. Kerstein, K., C. Fyfe, J. A. Sutcliffe, T. H. Grossman. 2014. TP-271 is a novel fluorocycline active against susceptible and multidrug-resistant Neisseria gonorrhoeae. Abstr. 2445, 114th American Society for Microbiology General Meeting, Boston, MA.
4. Swanson, J., K. Robbins, et al. (1987). Gonococcal pilin variants in experimental gonorrhea. J Exp Med 165(5): 1344-1357.
5. Warner, D. M., W. M. Shafer, et al. (2008). "Clinically relevant mutations that cause derepression of the Neisseria gonorrhoeae MtrC-MtrD-MtrE efflux pump system confer different levels of antimicrobial resistance and in vivo fitness." Molecular Microbiology 70(2): 462-478.
6. Jerse, A. E. (1999). Experimental gonococcal genital tract infection and opacity protein expression in estradiol-treated mice. Infect Immun 67(11): 5699-5708.
7. CLSI, 2012a. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition, in CLSI document M07-A9. Clinical Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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