ACTIVITY OF TOL-463 AGAINST BIOFILMS FORMED BY CANDIDA SPECIES IN AN EX VIVO MURINE VAGINITIS MODEL

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ABSTRACT

Background: TOL-463, a lactobacilli-sparring, boric acid (BA)-based vaginal anti-infective enhanced with EDTA, is in clinical development for the treatment of lower genital tract infections, with established antibacterial/antifungal activity in planktonic and biofilm assays. The objective of this study was to further characterize the antifungal activity of TOL-463 against Candida species employing an ex vivo murine vaginitis model, which has proven to reflect a robust C. albicans biofilm.

Methods: Exptant vaginal tissue from estrogenized mice were pre-treated with penicillin-streptomycin and inoculated with 2.5x10^6 C. albicans or C. glabrata clinical or ATCC isolates and incubated for 24 hours at 37°C to allow for biofilm formation. Thereafter, tissues were treated every 24 hours with vehicle (sterile water), BA 50 mg/mL, or TOL-463 test solutions for up to 3 doses. Fungal burden was assessed by enumeration of CFUs per 500µL of homogenized tissue at 24, 48, and 72 hours after the conclusion of each dose regimen.

Results: TOL-463 was associated with significant reductions in C. albicans and C. glabrata CFUs within 24 hours of the first dose (P<0.0001). By 72 hours following the third dose, >4 log reductions in CFUs were achieved. Greater reduction in CFUs of C. albicans was demonstrated for TOL-463 compared with BA only by the second dose and the difference was sustained through the final test interval (P<0.0001).

Conclusion: TOL-463 was highly effective at inhibiting C. albicans and C. glabrata fungal burden/biofilm formation on vaginal mucosa ex vivo that was superior to BA. These findings are consistent with published data for TOL-463 showing the same advantage, with robust destruction of biofilms formed by C. albicans and G. vaginalis in CCO biofilm reactors. An ex vivo study of TOL-463 against C. vaginalis, a key BV pathogen, is ongoing. TOL-463 holds promise as a non-azole vaginal anti-infective with novel antibiofilm properties.

INTRODUCTION

TOL-463 is a lactobacilli-sparring, BA-based vaginal anti-infective in clinical development for the treatment of lower genital tract infections, with established antibacterial/antifungal activity in planktonic and biofilm assays. Pathogenic biofilms have been implicated as an important virulence attribute of resistance in bacterial vaginosis (BV) and vulovaginal candidiasis (VVC), resulting in high rates of treatment failure and recurrences. Indeed, biofilms have been identified on vaginal biopsy in women with genital tract infections and shown to readily form on vaginal mucosa in vivo and ex vivo in mouse models. Moreover, data show that standard anti-infective agents fail to eradicate these biofilms efficiently in vivo and may explain the poor outcomes encountered clinically. BV and VVC are independently associated with adverse pregnancy outcomes, including premature rupture of membranes, preterm birth and low birth weights. Evidence has also established BV and VVC as important cofactors in HIV transmission and acquisition, including mother-to-child transmission. Because these infections are so widespread, and often chronic and recurring, with increased resistance to traditional agents like fluconazole, the need for novel anti-infective strategies, particularly those targeting the underlying biofilm, are of great interest and promise.

METHODS

Mice: Female C3H/HeN mice, 6 to 8 weeks of age, were purchased from the NCI-Charles River Laboratories and housed in accordance with the procedures outlined by the LUSMHC Institutional Care and Use Committee (IACUC).

Candida strains: C. albicans strain 96113 was obtained from the ATCC. C. albicans strain RMA 22163 and C. glabrata strain RMA 16114 were provided by Diane Citron, Associate Director, RM Alden Research Laboratory (Culver City, CA). Strains were cultured from frozen stocks on Sabouraud dextrose agar at 34°C for 48 h. One colony was added to 10 mL of YPD media and incubated for 18 h at 30°C in a shaking water bath. The stationary phase blastocordias were collected, washed in sterile H2O, and enumerated on a hemocytometer using trypan blue dye exclusion. The concentration of blastocordas was adjusted to 2.5x10^6/mL in sterile H2O prior to tissue inoculation.

Biofilm Model: Tissue CFUs were enumerated at 24, 48 and 72 h after the conclusion of each dose regimen. At each time point, tissues were homogenized in 500 µL of sterile water and serial 10-fold dilutions of homogenates were plated on Sabouraud dextrose agar. Plates were incubated for 48 h at 34°C. Colonies were enumerated and results were expressed as CFU/mL.

Statistical: Treatments differences in the log10 transformed CFUs were compared using t-test from mixed effect ANOVA where organism, days of treatment and hours from last treatment were accounted for in estimates of variability. Significance was defined as P<0.05.

RESULTS

Figure 1. Greater reduction in biofilm load / fungal burden with TOL-463 compared with boric acid (BA) and vehicle (VEH) – C. albicans and C. glabrata pooled results at 72 hrs post treatments 1, 2 and 3

Figure 2. Greater reduction in C. albicans biofilm load / fungal burden with TOL-463 compared with boric acid (BA) and vehicle (VEH) sustained out to 72 hrs post treatment 3

DISCUSSION / CONCLUSIONS

• TOL-463 is a lactobacilli-sparring, BA-based vaginal anti-infective in clinical development for the treatment of BV and VVC, both albicans and non-albicans Candida infections, the latter notoriously resistant to fluconazole and other azole antifungals.

• In this study, TOL-463 was highly effective at inhibiting C. albicans and C. glabrata biofilms grown ex vivo on vaginal tissue explants, with significant reductions in CFUs achieved within 24 hours of the first dose (P<0.0001), and 4 log reductions by 72 hours after the first treatment.

• Greater inhibition of Candida sp. was demonstrated for TOL-463 compared with BA only test solutions by the second dose and the difference sustained through the final test interval (P<0.0001).

• These data are consistent with studies of TOL-463 in CCO biofilm reactors demonstrating the same advantage over BA, with >6 log reductions in pathogenic loads against biofilms formed by C. albicans and G. vaginalis, an initiating BV anaerobe.

• TOL-463 holds promise as a non-azole vaginal anti-infective with established antibacterial/antifungal activity and novel antibiofilm properties.

REFERENCES


