Poster # D03

IMPACT OF ANTIMICROBIALS ON PENILE IMMUNOLOGY: A RANDOMIZED CLINICAL TRIAL

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Background

- The foreskin is the main site of HIV acquisition in heterosexual uncircumcised men[1]
- Anaerobic bacteria in the foreskin prepuce, particularly Bacteria Associated with Seroconversion, Inflammation and Cells (BASIC) species that include *Prevotella* and *Dialister* spp., enhance penile HIV susceptibility [2,3].
- The ability of local (topical) or systemic antimicrobial treatments to alter penile immunology has not been defined.
- We performed a clinical trial to examine the impact of antimicrobials on *ex vivo* foreskin HIV susceptibility and preputial soluble immune factors.
- Details of the clinical trial protocol and endpoints have been published previously[4].

Methods

This open label clinical trial randomized 125 HIV-uninfected, STI-free Ugandan men requesting voluntary penile circumcision (PC) to one of five arms (n=25 each).
The control group received immediate PC, while four intervention groups deferred PC for 4 weeks and received either:
(1) oral tinidazole for 2 days;
(2) penile topical metronidazole twice daily for one week then biweekly until PC;
(3) topical clindamycin twice daily for one week then biweekly until PC;
(4) topical hydrogen peroxide twice daily for one week then biweekly until PC.

The primary endpoint was *ex vivo* clade A HIV pseudovirus entry into foreskin-derived CD4+ T cells following PC.
Secondary endpoints were:
(1) Safety and tolerability.
(2) Impact on 9 soluble immune factors in prepuce swabs at 1 week (Visit 2) and 4 weeks (Visit 3), assayed using the MesoScale Discovery (MSD) multiplex ELISA.

Results

- 125 participants were enrolled through the Circumcision Clinic at Entebbe General Hospital, Uganda between 12/2017-11/2018
- 116 (93%) participants completed the protocol.
- The median participant age was 24 years (range, 18-49 years).

STUDY ENDPOINTS

- All antimicrobial treatments were well tolerated.

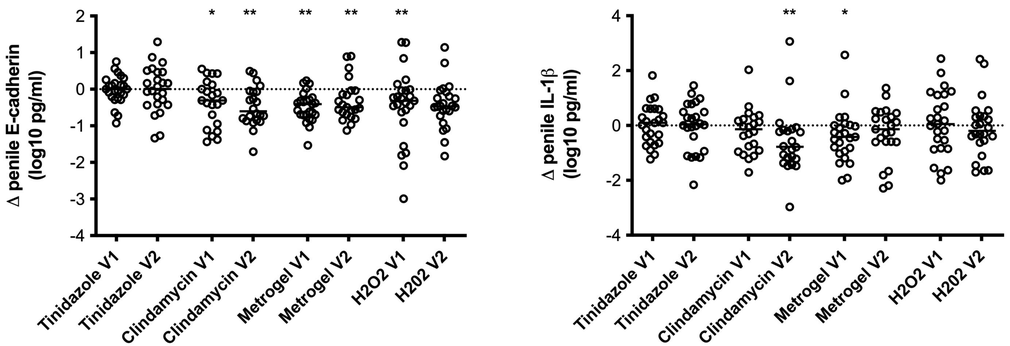
- Oral tinidazole reduced HIV virus entry into foreskin-derived CD4+ T cells vs. controls (9.3% vs. 13.1%, p=0.017), with a trend to lower CCR5 expression (36.8% vs. 44.5%, p=0.09).
- Oral tinidazole had no effect on soluble immune factors in the penile prepuce.

- Topical treatments had no impact on foreskin T cell subsets or virus entry, but all three treatments reduced preputial levels of soluble E-cadherin (p<0.01), suggesting improved epithelial integrity.
- Clindamycin and metronidazole reduced prepuce IL1β levels (p<0.05).

- Immunofluorescent staining of inner foreskin tissues confirmed that cellular adherens junctions were enhanced by topical antimicrobials (p<0.05), most strongly by topical metronidazole (p=0.035).

- Topical antimicrobials enhanced epithelial integrity in the coronal sulcus, and reduced some inflammatory cytokines.
- Oral tinidazole reduced virus entry into foreskin-derived CD4+ T cells, analogous to oral metronidazole after BV treatment.

Figure 1: Impact of antimicrobials on soluble immune factors in the coronal sulcus.



- **Figure 1** shows the log10 change from baseline in coronal sulcus levels of soluble E-cadherin (a biomarker of epithelial damage) and IL-1β (an inflammatory cytokine) after daily x7d (V1) and biweekly x 3 weeks (V2) administration of antimicrobials; differences from baseline were assessed using a paired Mann Whitney test. *p<0.05; **p<0.01
- Oral tinidazole had no impact on either biomarker.
- All topical antimicrobials reduced sEcadherin, with the effects most notable for topical clindamycin and metronidazole; these also reduced IL-1β

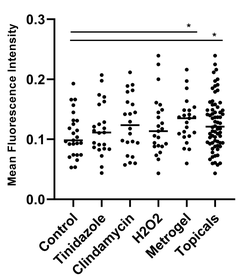


Figure 2: Impact of antimicrobials on tissue E-cadherin expression.

- Immunofluorescent staining of inner foreskin tissues at the time of surgery (V2) validated soluble E-cadherin as a biomarker of epithelial disruption
- Specifically, IF analysis confirmed that cellular adherens junctions were enhanced by topical antimicrobials (p<0.05), most strongly by topical metronidazole (p=0.035)
- Filaggrin yellow + E-Cadherin magenta + DAPI Cyan

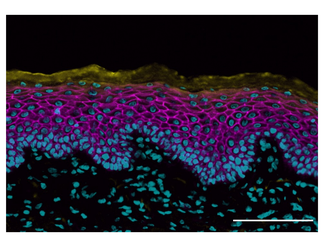
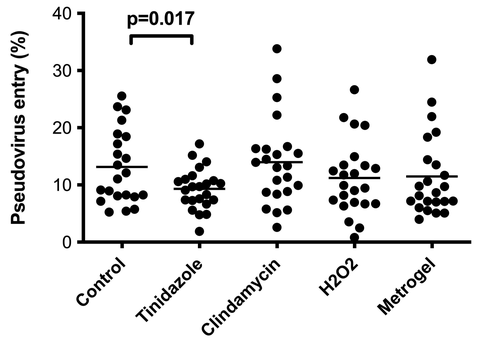


Figure 3: Impact of antimicrobials on entry of a clade A pseudovirus into foreskin-derived CD4+ T cells.



- **Figure 3** shows % clade A pseudovirus entry into foreskin-derived CD4+ T cells at the completion of antimicrobial treatment, or without any antimicrobial treatment in the control group.
- Virus entry into foreskin-derived CD4 cells was only reduced in the oral tinidazole group, with no effect of topical antimicrobials.

Summary and Conclusions

- All the antimicrobials studied in this pilot clinical trial had significant effects on penile immunology and/or HIV pseudovirus entry into foreskin-derived CD4+ T cells.
- The distinct immune effects of oral vs. topical agents echo similar findings after treatment of bacterial vaginosis[5].
- In future work we hope to correlate inter-individual and inter-agent heterogeneity in immune outcomes with: (a) treatment-induced changes in the penile microbiome, and (b) with the nature and location of immune cell subsets in the epithelium of the inner foreskin.

References

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