The Effect of Antimicrobial Agents on Foreskin Epithelial Integrity

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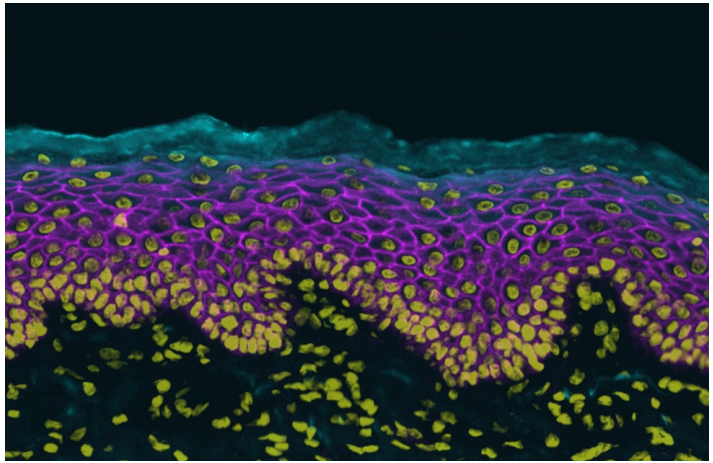

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Introduction

HIV-Associated Bacteria

Specific taxa of anaerobic bacteria in the penile microbiome are associated with increased susceptibility to Human Immunodeficiency Virus-1 (HIV) in heterosexual men[1]. These bacteria include *Peptostreptococcus anaerobius, Prevotella bivia, Prevotella disiens, Dialister propionicifaciens,* and *Dialister micraerophilus*[2], which have been shown to increase pro-inflammatory cytokine production, recruit CD4+ T cells to the inner foreskin, and associate with HIV acquisition[2] (**Figure 1**).

Mechanisms of Increased HIV Susceptibility

HIV infects cells expressing the CD4 and CCR5 co-receptors, including T cells, macrophages, and dendritic cells. Anaerobe-induced inflammation resulting in the recruitment of CD4+ T cells to the tissue surface by may therefore explain in part how these bacteria seem to drive HIV acquisition. However, these anaerobes may also increase HIV susceptibility by disrupting foreskin epithelial barrier function. Compromised barrier integrity may more readily allow HIV to traverse the epithelium and access target cells in the underlying dermis.

The Epithelium as a Barrier to Infection

The stratified, squamous epithelium of the foreskin has several physical barriers to HIV infection. The first is the *stratum corneum,* a layer of dead cells held in a rigid structure with keratin filaments. The rest of the epithelium consists of tightly-packed epithelial cells held together by tight junctions **(Figure 2)**, adherens junctions, and desmosomes. These junctions can limit viral diffusion between epithelial cells.

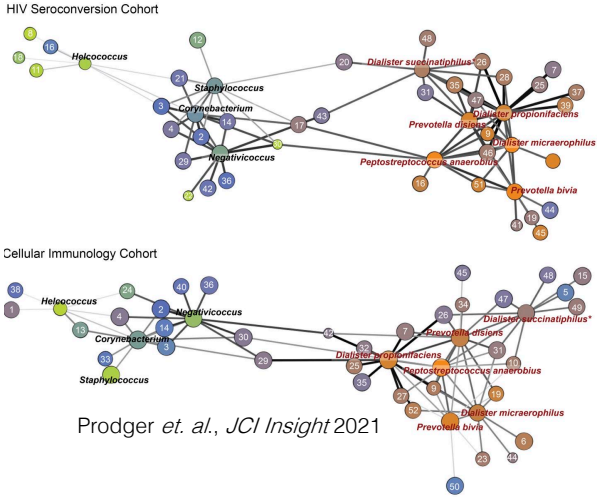

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Figure 1. Penile anaerobes associated with HIV seroconversion, inflammation, and cytokines. Correlation between species is denoted by the spatial location of nodes and edge color; node size displays species prevalence. Species-level correlations with subpreputial IL-8 are displayed by node color. Correlations were assessed by Spearman's test.

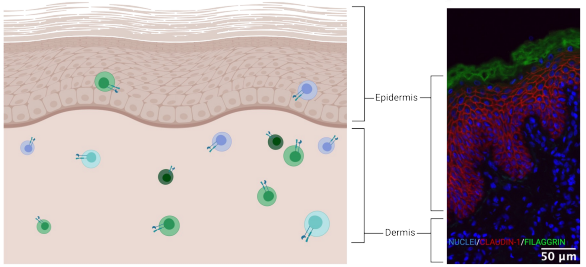

Figure 2. The Epidermis as a Barrier to HIV Infection. Virions need to traverse the *stratum corneum* (green) and tightly-packed epithelial cells held together by epithelial junction proteins (red) to reach the dermis where HIV target cells primarily reside[4].

Removing Penile Anaerobes with Antimicrobial Treatments

To test the hypothesis of whether penile anaerobes disrupt foreskin epithelial barrier function, we assessed the effect of antimicrobial treatments on epithelial integrity metrics as part of a randomized controlled trial conducted in Entebbe, Uganda[3].

Methods

Study Design

HIV-negative men (n = 125) undergoing elective penile circumcision were randomized to either a control group which underwent circumcision immediately, or to defer circumcision for 4 weeks and receive either oral tinidazole, or topical metronidazole, clindamycin, or hydrogen peroxide.

Immunofluorescence Microscopy

Foreskin tissues were stained for the protein filaggrin to mark the keratin layer, and epithelial junction proteins E-Cadherin, Desmoglein-1, and Claudin-1. Whole-tissue fluorescence microscopy images **(Figure 3)** were analyzed for the following epithelial integrity metrics: keratin thickness, epithelial thickness, and epithelial junction protein expression.

Image Analysis

Apical and basal edges of the epidermis and were manually traced along with the *stratum corneum* to measure *stratum corneum* and epithelial thicknesses **(Figure 4A)** . Epithelial junction protein expression was measured as described in **Figure 4**.

Soluble (Cleaved) E-cadherin

Soluble E-cadherin was quantified from penile swabs collected immediately prior to circumcision using ELISA.

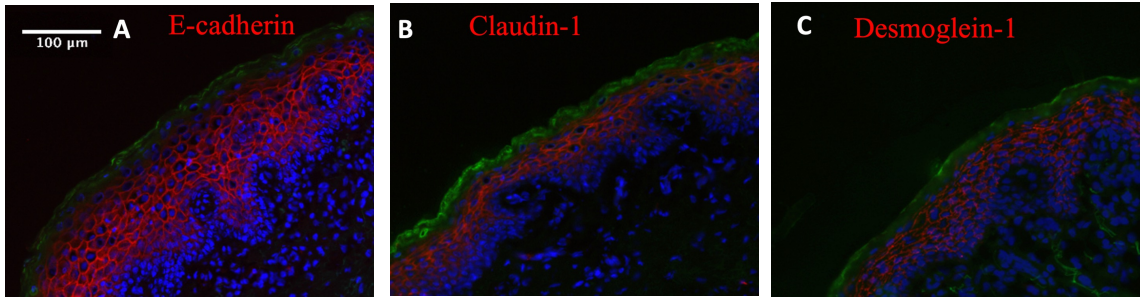

Figure 3. Epithelial Junction Protein Staining in Human Foreskin Tissue. Representative immunofluorescence images of human foreskin tissue stained for epithelial junction proteins and filaggrin. Junction proteins (A) E-cadherin, (B) Claudin-1, and (C) Desmogelin-1, are shown in red, while the stratum corneum protein filaggrin is visualized with green in all images. DAPI (blue) was used as a counterstain for the visualization of cell nuclei and the basal layer of the epidermis. Images shown are example regions of whole tissue sections scanned in at 20× magnification (scale bar 100 µm).

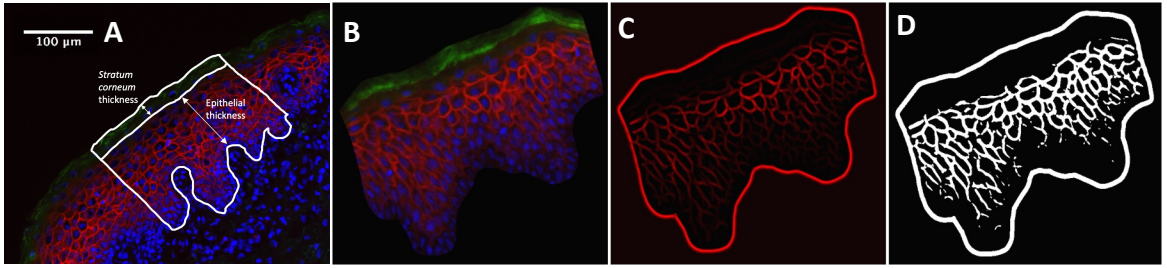

Figure 4. Epithelial Integrity Analysis. Whole tissue sections scanned at 20× magnification; apical and basal edges of the epidermis and the *stratum corneum* were manually traced (A) and processed into smaller images to facilitate workflow (B). These raw images were processed in a contrast-independent approach called a neuriteness function in MATLAB (C), accounting for varying staining intensity without making erroneous breaks[5]. An intensity threshold was applied to these processed images; binary images were created from positive staining above this threshold (D). Mean fluorescence intensity of the original image in areas identified by the binary net structure was analyzed as a measurement of junction marker expression. Scale bar 100 µm.

Results

Topical Antimicrobial Treatments were Associated with Increased Tissue E-Cadherin Expression

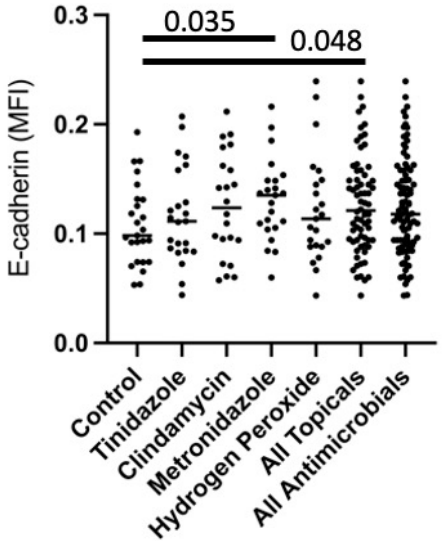

Figure 5. The Effect of Antimicrobial Treatments on Expression of E-cadherin in Inner Foreskin Tissue. E-cadherin expression was quantified using immunofluorescence and expressed as mean fluorescence intensity (MFI). Men receiving topical antimicrobials (n = 68) had significantly higher E-cadherin expression than control men (n = 25, p = 0.048). This was especially true for men receiving topical metronidazole (n = 23, p = 0.035). Student's unpaired T Test.

Topical Antimicrobial Treatments were Associated with Decreased Soluble E-Cadherin

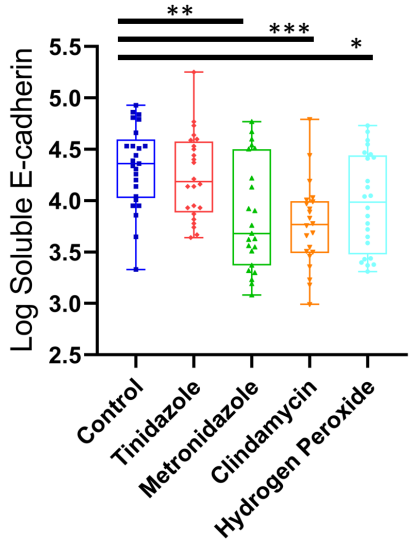

Figure 6. The Effect of Antimicrobial Treatments on Soluble E-cadherin. Soluble E-cadherin was measured from subpreputial swabs collected immediately prior to circumcision. Men receiving topical metronidazole (n = 23, p < 0.001), topical clindamycin (n = 22, p < 0.0001), or topical hydrogen peroxide (n = 23, p < 0.01) had significantly lower soluble E-cadherin than control men (n = 25). Student's unpaired T Test.

Topical Clindamycin and Oral Tinidazole were Associated with a Thinner Keratin Layer

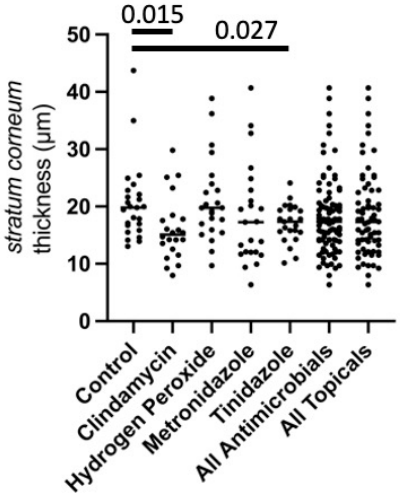

Figure 7. The Effect of Antimicrobial Treatments on *stratum corneum* Thickness. *Stratum corneum* thickness was assessed from manually traced immunofluorescence images. Men receiving topical clindamycin (n = 22, p = 0.015) or oral tinidazole (n = 23, p < 0.027), had a significantly thinner *stratum corneum* than control men (n = 25). Student's unpaired T Test.

Other Measured Outcomes

No significant differences were observed between treatment groups for epithelial thickness, claudin-1 expression, or desmoglein-1 expression.

Conclusion & Discussion

Some Antimicrobial Treatments may Cause *Stratum Corneum* Thinning

Oral tinidazole and topical clindamycin may have adverse effects on foreskin barrier integrity by causing thinning of the *stratum corneum*. Other treatments may be more suitable as HIV prevention therapies.

Penile Anaerobes may be Causing Proteolytic Cleavage of the Adherens Junction Protein E-Cadherin

The negative correlation between soluble and tissue E-cadherin **(Figure 8)** combined with the observed treatment effects **(Figures 5 & 6)** suggests that these bacteria may be causing proteolytic cleavage of E-cadherin, directly or indirectly through inflammation. Furthermore, this effect appears reversible through antimicrobial treatments which presumably remove the bacteria responsible for this cleavage.

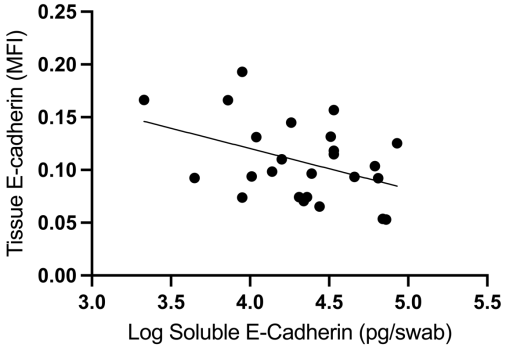

Figure 8. Correlation of Inner Foreskin Tissue E-cadherin expression (Mean Fluorescence Intensity) and soluble E-cadherin in control men. n = 25, p = 0.040, Pearson's Correlation Test.

Future Directions

Analyzing penile swabs for microbiome composition will allow us to determine whether the treatments removed anaerobes as expected, and whether the observed treatment results were due to the reduction of specific HIV-associated bacteria. Further research is required to explore the mechanism by which penile anaerobes may be causing proteolytic cleavage of E-cadherin and whether this results in a barrier more permeable to HIV virions.

Conflict of Interest Statement

The presenter and co-authors have nothing to declare.

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