Transcytosis as a Mechanism of HIV Entry into Endocervical Tissue: Evaluating Data in Stages

Angela J. Fought
Background:

• During male-to-female transmission, HIV needs to cross the mucosal epithelium of the female reproductive tract
  • To gain access to underlying target cells
• Previously, we illustrated that HIV can penetrate intact columnar and squamous genital epithelia
  • In ex vivo and in vivo systems
  • Carias et al. 2013
  • We were only able to illustrate that virus enters the squamous epithelium in a diffusion-based mechanism
Focus for this study:

ENDOCYTOSIS
Background:

- Utilizing an ex vivo system, we investigated transcytosis as another possible mechanism of HIV penetration
  - Transcytosis, the transfer of molecules through cells

- Utilizing explant tissues:
  - From 11 women
  - We investigated two transcytosis inhibitors
    - To examine if virus entry was impeded compared to controls
Control (A) and the inhibitors (B-C)
Methods:

• Model A (Count of Virions)
  • Evaluated if there are differences in the count of HIV virions for controls compared the tissue treated with the inhibitors

• Model B (Virion Penetration)
  • Compared the proportion of virions penetrating for controls and the two inhibitors

• Model C (Depth of Virions)
  • Compared if there are differences in the depths for controls and the inhibitors

• Notes
  • Analyses performed in SAS 9.4
  • Using a Bonferroni corrected $\alpha=0.0167$ for pairwise tests
Methods Model A (Count):

Model A: Count of Virions

- Negative Binomial (NB)
  - If over dispersion

- Poisson
  - If mean ≈ variance

Check if Zero Inflation (ZI)

- Generalized Estimating Equation (GEE) Model using GENMOD Procedure
  - Only repeated statement

- GEE Model using NLMIXED Procedure
  - Repeated statement and ZI code
Methods Model A (ZI Details):

• Run GENMOD procedure
  • Including ZI
  • Not accounting for repeated measures

• Run NLMIXED procedure
  • Including ZI
  • Not accounting for repeated measures

• Run NLMIXED procedure
  • Including ZI and repeated measures
  • Check if ZI is needed
Methods Model A (ZI Details):

• If ZI is needed
  • Use NLMIXED procedure
  • Including ZI and repeated measures

• If ZI is not needed
  • Use the GENMOD procedure
  • Accounting only for repeated measures
Results Model A (Count):

- NB GEE using GENMOD
- No ZI
- Inhibitor 2 was different than the others (both $p<0.001$), while the control and inhibitor 1 were not
Results Model B (Virion Penetration):

Model B: Virion Penetration
• Binomial GEE model
• Performed using GENMOD

Results:
• The Control group was different from inhibitors 1 and 2 (both $p<0.001$)
• Inhibitors 1 and 2 were not different
Results Model B (Virion Penetration):

Estimated Mean Proportions

- Control: 0.42
- Inhibitor 1: 0.21
- Inhibitor 2: 0.2
Results Model C (Virion Depth):

- Selected the best distribution for depth
  - Gamma GEE model
- Performed using
  GENMOD

Results:
- All three groups are different from each other (all $p<0.01$)
Results Model C (Virion Depth):

Estimated mean depth (bar) overlaid on a scatter plot of the individual depth values.
Conclusions:

• Although the analysis initially sounded simple the process was an educational opportunity in a myriad of ways

• We illustrated that HIV entry into endocervical cells occurs via transcytosis
Additional Research:

• We are investigating particle transport in mucus
  • How it is affected by
    • HIV infected women vs controls
    • Phase of cycle
    • Menopause
    • Bacterial Vaginosis
    • Microbiome
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