HIV Inhibition and Variation in Anti-Microbial Peptides Associated with Intercourse

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Introduction

- Anti-microbial peptides (AMP) in vaginal fluid play a key role in vaginal immune responses to sexually transmitted infections including HIV
- AMP levels are influenced by inflammation, semen, and menstrual cycle but the influence of penile-vaginal intercourse (PVI) has not been studied
- Variation in AMP levels associated with PVI could affect HIV inhibition and risk of infection

Aims

- Assess whether levels of trappin, human beta-defensin 2 (HBD2), lactoferrin, secretory leukocyte protease inhibitor (SLPI), and lysozyme are associated with PVI compared to non-sexual quiet time shared with a partner
- Assess whether levels of trappin, HBD2, lactoferrin, SLPI, and lysozyme effect *in-vitro* HIV inhibition

Methods

- 40 healthy women (ages 18- 39 years)
- Vaginal fluid collected 1hour before and 15 minutes after two sex partner interaction conditions, separated by 28 days
  - Condition 1: condom-protected PVI and collect vaginal fluid before intercourse and 15 minutes following PVI
  - Condition 2: 1 hour of quiet time with partner, without sexual interaction
- Vaginal fluid collected with a polywick tampon worn for 1hr
- Tampons frozen in 1 ml of PBS until collected for processing

Vaginal AMP Assay

- hβd-2, trappin-2/elafin, SLPI, lysozyme and lactoferrin measured by ELISA
- Values normalized to total protein to control for inter-sample dilution variation

***In Vitro* HIV Infection**

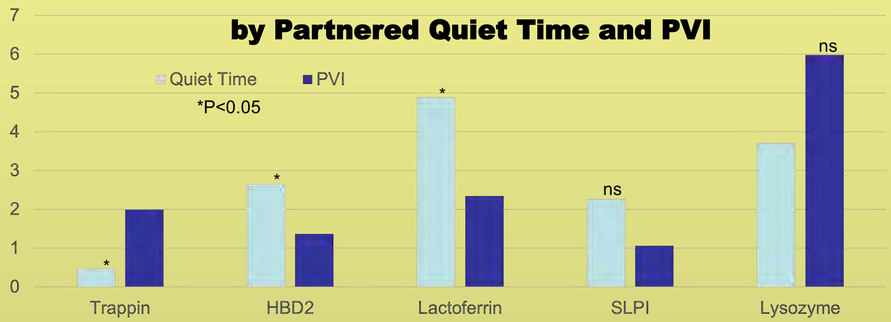
- Infection reporter assay: TZM-bl cells expressing CD4, CXCR4, and CCR5
- Infected with macrophage tropic HIV-1 (laboratory adapted subtype-B) in 96 well plates ($1.25x10^4$ cells/well)
- Filtered vaginal fluid added in 10% concentration
- % HIV inhibition measured by luciferase activity relative to controls
- Random-effects logistic regression and multilevel mixed effects linear regression to account for within-person comparisons; $p<0.05$ used to define statistical significance

Results

AMP and sexual activity

1. Trappin-2/elafin levels (ng/ml) were significantly higher after PVI than after partnered quiet time
   - Mean trappin levels were significantly higher with PVI than partnered quiet time when controlling for levels of HBD2 and lactoferrin
2. HBD2 and lactoferrin levels (ng/ml) were significantly higher after partnered quiet time than after PVI
   - Mean HBD2 remained significantly higher after partnered quiet time than after PVI when controlling for trappin and lactoferrin levels
3. Mean SLPI levels appeared elevated after partnered quiet time but the difference was not statistically significant (p=0.08)
4. Mean lysozyme level appeared elevated after PVI but the difference was not significant (p=0.07)

Figure 1. Trappin, HBD2, Lactoferrin, SLPI, Lysozyme, by Partnered Quiet Time and PVI



AMP and in-vitro HIV Inhibition

1. % inhibition was lower after partnered quiet time in follicular phase (Table 1)
2. Both HBD2 and lysozyme were significantly associated with % HIV inhibition (Table 2)
   - For every 18 unit (ng/ml) increase in HBD2, there was a 1% increase in HIV inhibition.
   - For every 1.1 unit (ng/ml) increase in lysozyme, there was a 1% increase in HIV inhibition.

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Table 1. % HIV Inhibition by Vaginal Fluid, by Phase

	Menstrual Cycle Phase	
	Follicular	Luteal
Partnered quiet time		
Before	36.3 (25.6, 47.0)	35.4 (22.8, 47.9)
After	26.7 (15.6, 37.8)	35.1 (22.0, 48.2)
PVI		
Before	39.7 (24.7, 54.7)	37.6 (23.2, 52.0)
After	26.3 (5.8, 46.8)	38.9 (25.0, 52.8)

Table 2. Multilevel mixed-effects linear regression model for % HIV Inhibition*

	Coefficient (ng/ml)	95% CI	P-value
Trappin	-0.8	-3.5, 1.9	0.566
HBD2	18.1	3.4, 32.9	0.016
Lactoferrin	-0.2	-1.1, 0.6	0.613
SLPI	0.2	-0.8, 1.3	0.691
Lysozyme	1.1	0.6, 1.6	<0.001

*Adjusted for phase and PVI

Conclusions

- PVI is associated with increases or decreases in vaginal AMP, depending on AMP
  - PVI associated with increased trappin levels, compared to partnered quiet time
  - PVI associated with decreased in HBD2 and lactoferrin, compared to partnered quiet time.
- Changes could be associated with sexual arousal rather than mechanical aspects of PVI
- In vitro HIV inhibition is associated with both menstrual cycle phase and AMP levels, especially HBD2 and lysozyme
- These data describe a potential role for intercourse-associated changes in vaginal mucosal immunity that has implications for understanding of HIV infection as well as topical HIV prevention products such as microbicides.

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