Anti-HIV activity of the modified human antimicrobial peptide 17BIPHE2

Ana Vera-Cruz1,2, Stephanie Burke-Schinkel2, Nongnuj Tanphaichitr2,3, Jonathan B. Angel1,2,4
1. Department of Biochemistry, Microbiology, & Immunology, University of Ottawa, Ottawa, ON, Canada
2. Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada,
3. Department of Obstetrics/Gynecology, University of Ottawa, Ottawa, ON, Canada
4. Department of Infectious Diseases, The Ottawa Hospital, Ottawa, ON, Canada

Conflict of Interest Disclosure: The presenter has no conflicts of interest

Background: Unwanted pregnancies and sexually transmitted infections (STIs) are major health concerns of women worldwide. These concerns have prompted efforts to develop Multipurpose Prevention Technologies (MPTs), which simultaneously provide contraception and prevent STIs, including HIV. LL-37, an effective spermicide on human sperm, has broad antimicrobial activity including in vitro activity against HIV. 17BIPHE2 is a truncated LL-37 peptide, engineered to contain 5 unnatural residues, thus limiting its protease degradation by vaginal fluid. Hence, this AMP represents a promising MPT agent.

Methods: PMA-stimulated ACH-2 cells, a chronically HIV-infected T cell line, were incubated with LL-37 or 17BIPHE2, and HIV replication was evaluated by p24 concentration in the supernatant via ELISA. In addition, HIV was incubated with 17BIPHE2 prior to infection of various target cells for HIV infection. Alternatively, target cells were incubated with 17BIPHE2 prior to HIV infection. Infection was quantified by luciferase activity in an HIV reporter TZM-bl cell line and by p24 ELISA in activated PBMC and CD4+ T cells.

Results: In ACH-2 cells, there was significant reduction in p24 production when cells were treated with 17BIPHE2, but not LL-37. When 17BIPHE2 was pre-incubated with HIV prior to infection and present during infection, viral replication decreased in the TZM-bl reporter cell line, but this result was not recapitulated in the primary activated cells, PBMCs nor isolated CD4+ T cells. Conversely, pre-incubation of 17BIPHE2 with target cells prior to infection significantly inhibited HIV infection in a dose-dependent manner. Initial mechanistic studies involving evaluation of cell-surface markers of activation and co-receptor expression indicated no change between untreated and 17BIPHE2-treated cells.

Conclusion: 17BIPHE2 may act on the cell or on the cell/virus interaction rather than on the virus itself to inhibit HIV infection and presents a promising anti-HIV therapy that may be developed into an effective MPT.
Introduction

Antimicrobial Peptides (AMPs)
- AMPs are part of the natural host defense peptides against microbial attacks.
- They have broad microbicidal effects on Gram-positive and Gram-negative bacteria, yeast, and enveloped viruses.
- AMPs bind negatively charged phospholipids on microbial surfaces resulting in microbial membrane permeabilization and cell death.
- Sperm surface is enriched in anionic sulfoglycolipid, making it possible for AMPs to act as spermicides in an analogous manner.

HIV Is A Global Health Issue
- HIV disproportionately affects young women.
- 19.6 million women infected worldwide.
- New infection rates of up to 3 per 100 person years in areas most affected.
- HIV infection continues despite established approaches to prevent HIV infection including the availability of prophylactic therapies.

Multipurpose Prevention Technology (MPT)
What?
- World Health Organization has prompted the development of MPT, a single product or a combination of products administered in one device to prevent unintended pregnancy and sexually transmitted infections (STIs).

Why?
- Global unmet need for contraception due to limited choices.
- 1 million sexually transmitted infections every day.
  ▪ These STIs can lead to poor health outcomes if left untreated.

Anti-HIV activity of LL-37 and its engineered truncated peptides should be further studied
- LL-37 has been reported to have anti-viral activity against some enveloped viruses, but studies on its anti-HIV activity are limited with some conflicting results.
- Dose-dependent inhibition of HIV infection in both PBMC and CD4+ T cells occurs after pre-incubation of cells with LL-37.
- The central fragment of LL-37 appears to be responsible for its anti-HIV activity.
- LL-37 inhibits recombinant HIV-1 reverse transcriptase and protease.
- LL-37 enhances HIV infection in monocyte-derived Langerhans cells, but is protective in monocyte-derived dendritic cells.

LL-37 and its engineered truncated peptide, 17BIPHE2
- LL-37 is the only human AMP in the cathelicidin family and is released by the innate immune system in response to microbial attacks and has spermicidal activity.
- 17BIPHE2 is a truncated LL-37 engineered to have five unnatural amino acids making it more resistant to protease degradation. 17BIPHE2 has similar spermicidal activity to LL-37 (Our unpublished work).

LL-37 may exert its anti-HIV effect in CD4+ T-cells by interfering with various steps of the HIV life cycle

- LL-37 may exert its anti-HIV effect in CD4+ T-cells by interfering with various steps of the HIV life cycle.

- LL-37 competes with gp120 for co-receptor binding.
- LL-37 inhibits RT, preventing DNA synthesis.
- LL-37 inhibits protease-mediated cleavage of polyproteins.
- LL-37 increases Type I interferon anti-viral response.
- LL-37 increases NLR expression.
- LL-37 decreases antiviral lipids thereby depleting lipids from the viral envelope leading to improper formation of the viral envelope.

- LL-37 damages HIV viral envelope preventing the virus from infecting the host cell.

- LL-37 increases NLR expression.

- LL-37 inhibits recombinant HIV-1 reverse transcriptase and protease.

- LL-37 enhances HIV infection in monocyte-derived Langerhans cells, but is protective in monocyte-derived dendritic cells.

- World Health Organization has prompted the development of MPT, a single product or a combination of products administered in one device to prevent unintended pregnancy and sexually transmitted infections (STIs).

- LL-37 may exert its anti-HIV effect in CD4+ T-cells by interfering with various steps of the HIV life cycle.
Hypothesis
It is predicted that LL-37 and 17BIPHE2 have anti-HIV activity.

1. Determining whether LL-37 and 17BIPHE2 reduce HIV replication in chronically infected cell line

2. Determining whether LL-37 or 17BIPHE2 act directly on HIV, making it less able to infect target cells

A)

B)
Results

1. ACH-2 Cells

17BIPHE2 decreased HIV replication in a dose-dependent manner in ACH-2 cells, whereas LL-37 did not. (* p<0.05, One-way ANOVA, n=3)

2A. TZM-bl Cells

Dose-dependent decrease in luciferase expression after the virus was pre-incubated with 17BIPHE2 and when virus and peptide were simultaneously added.  

\[ n=3, \text{One-way ANOVA} \  \star \ P \leq 0.05 \  \star \star \ P \leq 0.01 \]

2B. PBMC

No significant in reduction in p24 production when the virus was pre-treated with 17BIPHE2

\[ n=3, \text{One-way ANOVA} \  \star \star \star \ P \leq 0.001, \  \star \star \star \star \ P \leq 0.0001 \]

3. PBMC

A significant dose-dependent reduction in p24 when activated PBMCs or activated CD4+ T cells were pre-incubated with 17BIPHE2 prior to HIV infection

\[ \text{Left: Raw data; Right: Normalized to untreated group} \]

CD4+ T Cells

A significant dose-dependent reduction in p24 when activated PBMCs or activated CD4+ T cells were pre-incubated with 17BIPHE2 prior to HIV infection

\[ \text{Left: Raw data; Right: Normalized to untreated group} \]

17BIPHE2 did not adversely affect viability of PBMC or CD4+ T cells as measured by exclusion of propidium iodide (PI) and intracellular Calcein fluorescence following 2h incubation with PI and Calcein AM.
Conclusions

Summary of Results

• 17BIPHE2 decreased HIV replication in ACH-2 cells whereas LL-37 did not, possibly due to preferential protease degradation of LL-37. This led us to focus on 17BIPHE2.

• 17BIPHE2 decreased HIV infection in TZM-bl in a dose-dependent manner when the peptide was incubated with the virus before infecting cells and also present during 48h incubation.

• Pre-incubation of HIV with 17BIPHE2 did not have a significant impact on infection, but pre-incubation of 17BIPHE2 with cells prior to infection did.

• The peptide may not act on the virus itself, but act on the cells or the virus/cell interaction to decrease susceptibility to HIV infection.

Future Directions

• Study 17BIPHE2 in other cells types that represent initial targets of HIV eg. dendritic cells and Langerhans cells.

• Mechanistic studies to determine how LL-37/17BIPHE2 inhibits HIV.

• Humanized mouse studies to determine if LL-37/17BIPHE2 can prevent HIV infection in vivo.

Implications of Findings

• Development of 17BIPHE2 into an anti-HIV prophylactic/preventative agent.

• Multipurpose prevention technology (MPT) development
  • 17BIPHE2 also possesses spermicidal activity and is microbicidal against other sexually transmitted pathogens (Lee et al., submitted to Human Reprod)15

• Improve women's health, potentially allowing them more options to control their sexual and reproductive health.

References