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

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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, the only human cathelicidin and an effective spermicide on human sperm, has broad antimicrobial activity including in vitro activity against HIV. 17BIPHE2 is a mimic of 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. We, therefore, hypothesize that 17BIPHE2 will be a potent inhibitor of HIV infection.

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. Alternatively, HIV was incubated with 17BIPHE2 prior to infection of target cells. Infection was quantified by luciferase activity in an HIV reporter TZM-bl cell line or by p24 ELISA in activated CD4+ T cells.

Results: 17BIPHE2 inhibited HIV replication in stimulated ACH-2 cells in a dose dependent manner, while this was not observed with LL-37. Pre-incubation with 17BIPHE2 decreased the ability of HIV to infect TZM-bl cells in a dose-dependent manner across multiple titers of HIV. Preliminary results demonstrated that when HIV was incubated with 17BIPHE2 before infecting CD4+ T cells, HIV infection decreased with increasing amounts of 17BIPHE2.

Conclusion: Initial results show that 17BIPHE2 can reduce the ability of HIV to infect relevant target cells. The mechanisms of this activity and the specific stages of HIV replication where 17BIPHE2 exerts anti-HIV activity remain to be established. This project provides the groundwork to study 17BIPHE2 in other cells/tissues of the female reproductive tract and eventually in in vivo models of HIV infection.
Introduction

HIV Is A Global Health Issue
- HIV disproportionately affects young women\(^1\)
- 19.6 million women infected worldwide\(^2\)
- New infection rates of up to 3 per 100 person years in areas most affected\(^3\)
- Continues despite established approaches to prevent HIV infection including the availability of prophylactic therapies\(^4\)

Antimicrobial Peptides (AMPs)
- Natural host defense peptides against microbial attacks
- Broad microbial effects on Gram-positive and Gram-negative bacteria, yeast, and enveloped viruses\(^5\)
- Bind negatively charged phospholipids on microbial surfaces resulting in microbial membrane permeabilization and cell death\(^6\)
- Sperm surface is endowed with anionic molecules, making it possible for AMPs to act as spermicides in an analogous manner\(^7,5\)

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\(^8,7\)
- 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)

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)\(^13\)

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

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

- 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\(^8,7\)
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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\(^9,10,11,12\)
- Dose-dependent inhibition of HIV infection in both PBMC and CD4+ T cells occurs after pre-incubation of cells with LL-37\(^9\)
- The central fragment of LL-37 appears to be responsible for its anti-HIV activity\(^10\)
- LL-37 inhibits recombinant HIV-1 reverse transcriptase and protease\(^11\)
- LL-37 enhances HIV infection in Langerhans cells, but is protective in dendritic cells\(^12\)
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

ACH-2 are a chronically infected T-cell clone containing one copy of integrated proviral HIV-1 and can be stimulated to induce viral replication using phorbol myristate acetate (PMA).

2. Determining whether AMPs can inhibit HIV from infecting target cells

TZM-bl cells are a luciferase reporter cell line derived from HeLa cells that express CD4, CCR5, and CXCR4.
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-bi Cells

Highest dose of both peptides reduced HIV infection. (* p<0.05, One-way ANOVA n=5)

2B. PBMC and CD4+ T Cells

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.

17BIPHE2 appeared to slightly decrease p24 production of activated PBMC at 5 days although this was not statistically significant. (One-way ANOVA ns n=3)

Preliminary data indicate a decrease in p24 production when HIV_NL4.3 was pre-incubated with 17BIPHE2 before infecting activated CD4+ T cells. (n=2)
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.

• LL-37 and 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 culture.

• When 17BIPHE2 was pre-incubated with HIVCs204A and present during the two hour infection there was a trend toward a decrease in infection, but the decrease was not statistically significant.

• Preliminary results demonstrated that 17BIPHE2 used in HIVNL4.3 pre-treatment and present during infection decreased HIV infection in activated isolated CD4+ T cells.

Future Directions

• Determine whether 17BIPHE2 exerts anti-HIV activity by inducing changes to the target cells that make them less susceptible to infection. Target cells (PBMC and CD4+ T cells) will be pre-incubated with the peptide before infection then assessed for p24 production.

• Assess CD4+ T cells pretreated with 17BIPHE2 by flow cytometry for changes of expression of cell surface markers, which are involved in HIV infection. These include HIV co-receptors CCR5 and CXCR4, as well as activation markers such as CD69 and HLA-DR.

• Perform experiments to determine whether 17BIPHE2/LL-37 can inhibit HIV infection of other target cells in the female reproductive tract including vaginal epithelial cells, Langerhans cells, and dendritic cells.

Final Thoughts

• Development of AMPs into MPT agents should help overcome unintended pregnancies as well as the rise of sexually transmitted infections.

• This project will provide foundation for mechanistic studies within physiologically relevant models of HIV infection.

References