

Anti-HIV activity of the human antimicrobial peptide, LL-37, and its truncated peptide, 17BIPHE2

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Background: Lack of access to contraception is a key contributor to unwanted pregnancies. Concurrently, sexually transmitted infections (STIs) such as HIV are a major health concern worldwide. Together, these issues prompted the development of Multipurpose Prevention Technologies (MPT), capable of providing contraception and preventing STIs. One potential MPT is the human antimicrobial peptide, LL-37, and its truncated and modified version, 17BIPHE2, both known to have spermicidal activity.

Methods: Increasing concentrations of HIV were incubated with LL-37 or 17BIPHE2 prior to infection of target cells. In an HIV luciferase reporter TZM-bl cell line, infection was quantified by luciferase activity. In PBMC and CD4+ T cells, infection was measured by concentration of p24 via ELISA in the supernatant. These experiments were repeated with cells pre-incubated with peptide prior to HIV infection. The effect of the peptides on cell phenotype and viability, measured by Sytox Green staining, were evaluated by flow cytometry.

Results: Co-incubation with LL-37 decreased the ability of HIV to infect TZM-bl in a dose-dependent manner across multiple titers of HIV. When LL-37 was incubated with HIV before infecting PBMC or CD4+ T cells, p24 concentration increased with increasing amounts of LL-37. Infection also increased when PBMC were incubated with LL-37 prior to infection with HIV. Effect of peptides on cell death was inconclusive, but HLA-DR and CCR5 expression on CD4+ T cells was increased when PBMC were incubated with LL-37.

Conclusion: LL-37 can reduce infectiousness of HIV but is also capable of activating cells, increasing their susceptibility to infection, indicating that the anti-HIV activity of LL-37 may be dependent on cell type and/or culture conditions. 17BIPHE2 will also be evaluated as, given its modified nature, it may retain anti-HIV activity without activating cells. This could provide the foundation for studies of the activity of these peptides in other cells/tissues of the female reproductive tract.

Introduction

Multipurpose Prevention Technology (MPT) refers to a contraceptive that can also prevent at least one sexually transmitted infection (STI).

- Women and young women lack access to contraception and are seeking more options especially in a world in which the population is growing extensively.
- Alongside unwanted pregnancies, STIs continue to be a worldwide health concern, specifically sexually transmitted HIV, which disproportionately affects young women despite the availability of prophylactic interventions.
- These issues together prompted the development of MPT.

Antimicrobial Peptides (AMPs)

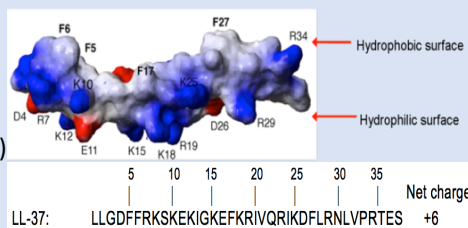
MPT Development Approach: test known antimicrobial agents for spermicidal activity

- AMPs: Host defense peptides against microbial attacks
- Broad microbicidal effects on Gram-positive and Gram-negative bacteria, yeast, and enveloped viruses
- AMPs can be used as spermicidal agents

LL-37 and its truncated peptide, 17BIPHE2

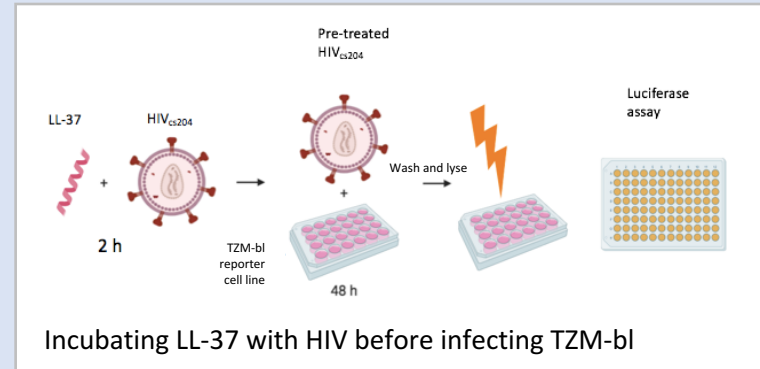
- LL-37 is a human anti-microbial peptide released by the innate immune system in response to microbial attacks and has spermicidal activity.
- It has been reported to have anti-viral activity against some enveloped viruses, but its anti-HIV activity is incompletely understood.
- 17BIPHE2 is a truncated and modified version of LL-37 that is more resistant to protease degradation and has similar spermicidal activity

Objective: Determine if LL-37 and 17BIPHE2 can inhibit HIV in different tissue models of infection.

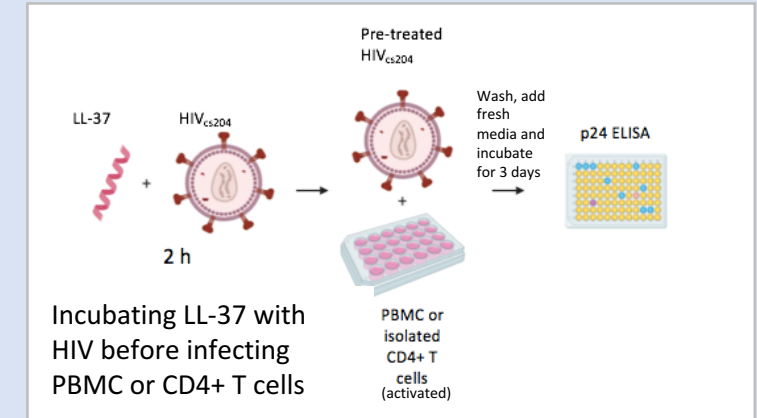


Methods

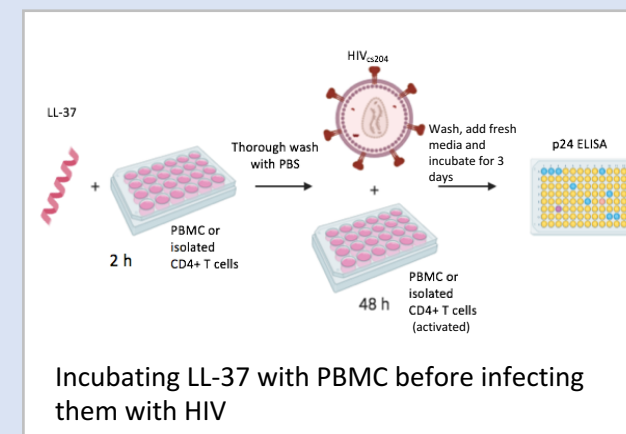
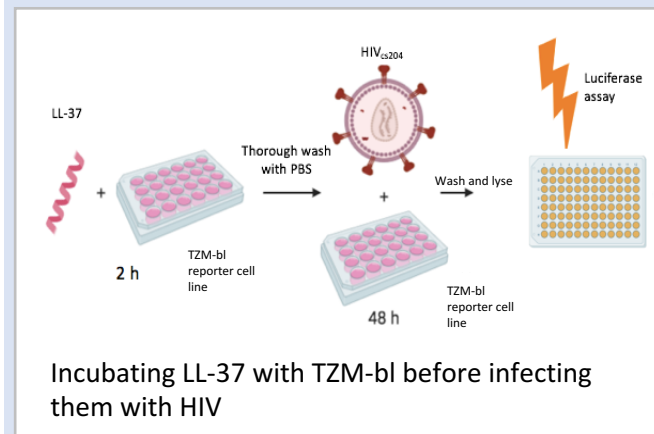
Determining whether LL-37 exerts anti-HIV activity by acting on the virus



TZM-bl are a reporter cell line derived from He-La cells that express CD4, CCR5, and CXCR4.

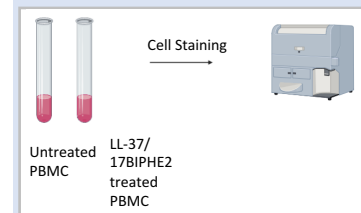


Determining whether LL-37 exerts anti-HIV activity by changing target cells, making them less susceptible to infection



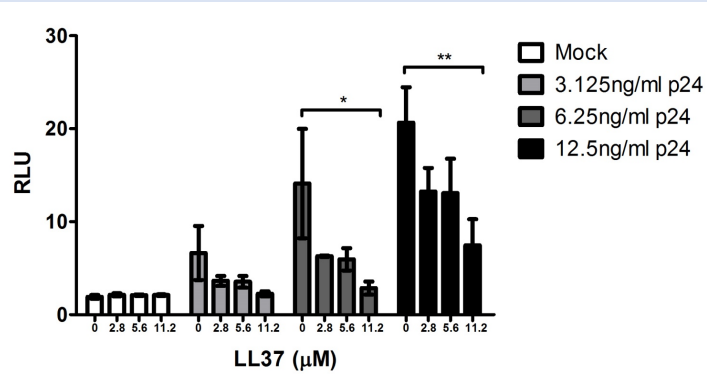
PBMC were treated with water (0μM AMP) or treated with LL-37 or 17BIPHE2 for 24 hours. Cells were then incubated with Sytox Green for 15 minutes before measurement of Sytox Green incorporation via Flow Cytometry.

Flow Cytometry was also used to stain markers for cell activation (HLA-DR) and CCR5 expression in activated CD4+ T cells.

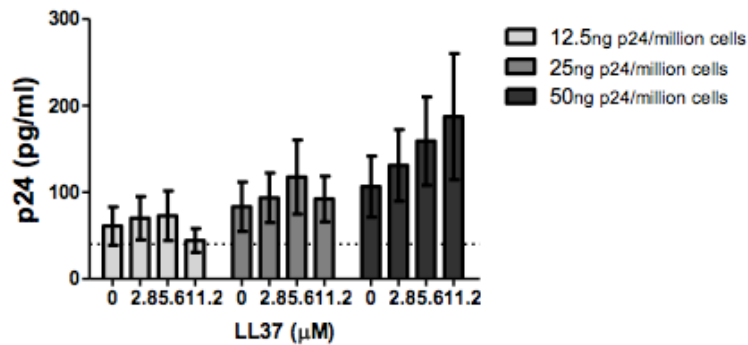


Results

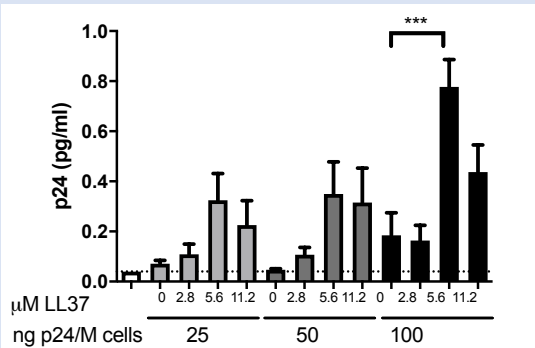
Determining whether LL-37 exerts anti-HIV activity by acting on the virus



After pre-incubating HIV_{CS204} with LL-37 before infecting TZM-bl cells, the highest dose of LL-37 (11.2 μM) caused a significant reduction in HIV infection as measured by luciferase production when cells were infected with 6.25ng/ml or 12.5ng/ml of p24 (* p<0.05, ** p<0.01, two-way ANOVA with Bonferroni posttest). n=5

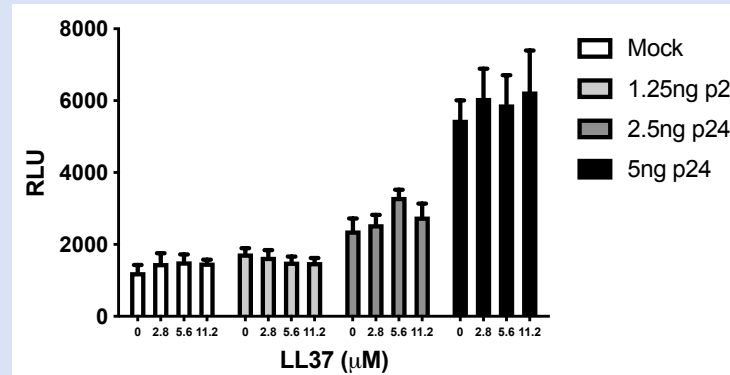


There were no significant differences in p24 production from PBMC after HIV_{CS204} was incubated with varying concentrations of LL-37 before infecting PBMC (two-way ANOVA with Bonferroni posttest) n=4

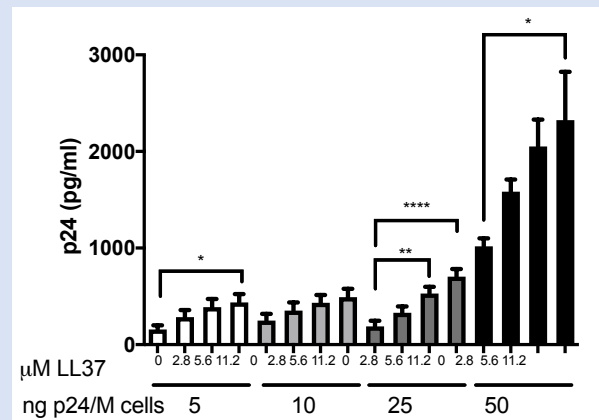


Increased LL-37 trended toward an increase in p24 production when LL-37 was pre-incubated with HIV_{NL4.3} before infecting CD4+ T cells (*** p<0.001, two-way ANOVA with Bonferroni posttest). n=4

Determining whether LL-37 exerts anti-HIV activity by changing target cells, making them less susceptible to infection



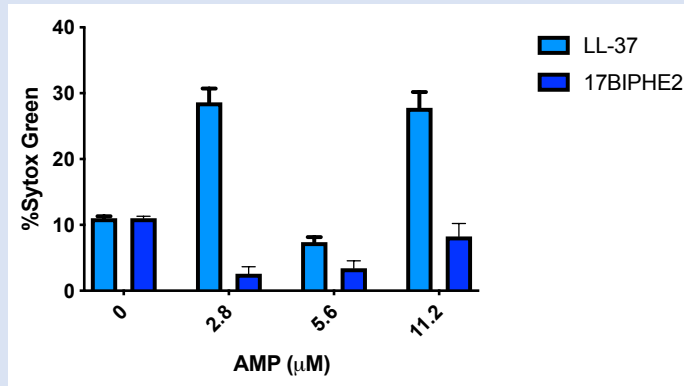
When TZM-bl were incubated with different concentrations of LL-37 before HIV_{CS204} infection, no significant changes in luciferase production after (two-way ANOVA with Bonferroni posttest). n=3



Increasing amounts of LL-37 led to an increase in p24 production when PBMC were pre-incubated with LL-37 prior to infection with HIV_{CS204} (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 two-way ANOVA with Bonferroni posttest). n=3 (50ng/M), n=4 (5 and 10ng/M), or n=7 (25ng/M)

Results

Determining whether LL-37 or 17BIPHE2 increases cell permeability

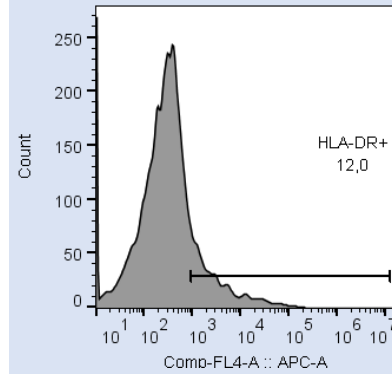


PBMC treated with LL-37 had increased Sytox Green incorporation compared to untreated cells and cells treated with 17BIPHE2. n=2

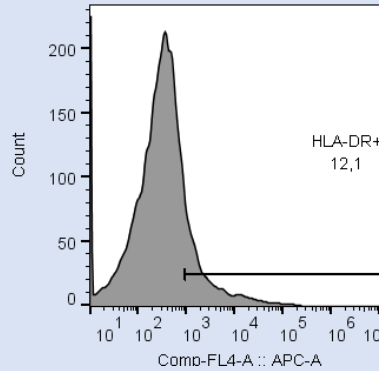
Activated CD4⁺ T cells were incubated with various concentrations of LL-37 for 24 hours. Cells were then stained for a marker of activation (HLA-DR+) and for CCR5 expression and evaluated by Flow Cytometry.

LL-37 increased cell activation and CCR5 expression in a dose-dependent manner.

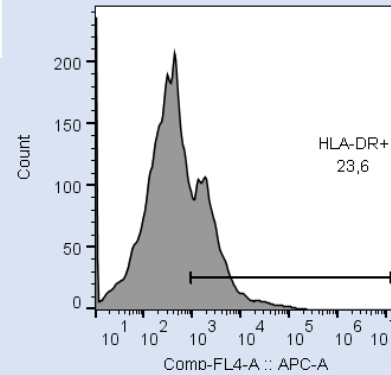
LL-37 increases cell activation in a dose dependent manner:



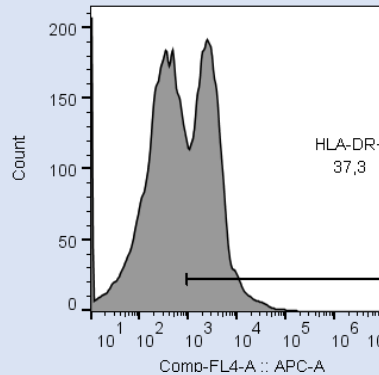
No LL37



2.8 μM

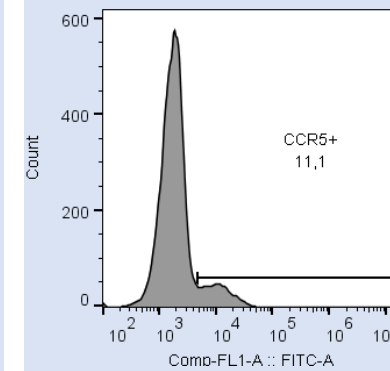


5.6 μM

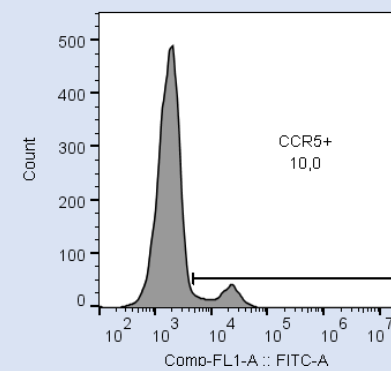


11.2 μM

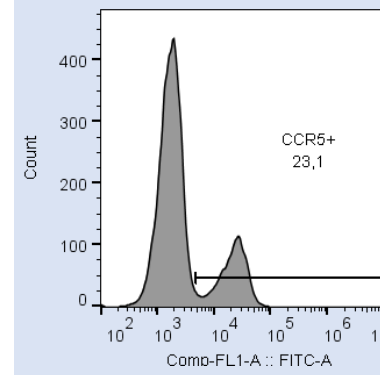
LL-37 increases CCR5 expression in a dose dependent manner:



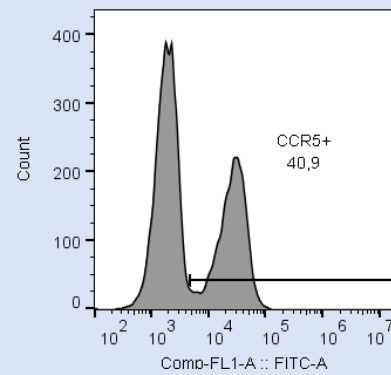
No LL37



2.8 μM



5.6 μM



11.2 μM

Conclusions

Summary of Results

- LL-37 decreased HIV infection in TZM-bl in a dose-dependent manner when the peptide is incubated with the virus before infecting cells. There were no significant differences when TZM-bl were incubated with LL-37 before infection.
- LL-37 increased infection in PBMC and CD4 T-cells which could be due to the immunomodulation of HLA-DR and CCR5.
- LL-37 appeared to increase cell permeability, but these findings need to be confirmed other well-established stains for hematopoietic cell death such as propidium iodide and Annexin-V.
- Preliminary results demonstrate that 17BIPHE2 does not increase cell permeability and is a promising candidate for MPT.

Future Directions

- All experiments involving LL-37 will be repeated using 17BIPHE2. Since 17BIPHE2 is a truncated and modified form of LL-37, its possible that it does not exhibit as many immunomodulatory effects as LL-37.
- Another model for HIV infection is the cell line, ACH-2. ACH-2 cells are a T-cell clone that are chronically infected with HIV. Using this model, peptides can be administered to determine if they demonstrate anti-viral activity.

Final Thoughts

- The growing population continues to be burdened by the rise of sexually transmitted infections like HIV.
- MPT and the development of AMPs can be used to combat these issues especially in the developing world.
- This project will provide foundation for mechanistic studies within physiologically relevant models of HIV infection.

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