Potent antiviral activity against HSV-1 and SARS-CoV-2 by antimicrobial peptoids

Gill Diamond¹,*, Claudine Herlan², Natalia Molchanova², Erika Figgins¹, Lisa K. Ryan³, Donghoon Chung⁴, Robert Scott Adcock⁴, and Annelise Barron²

¹ Department of Oral Immunology and Infectious Diseases, University of Louisville School of Dentistry, Louisville, KY 40202 USA;  
² Department of Bioengineering, Stanford University, Stanford, CA 94305;  
³ Division of Infectious Diseases and Global Medicine, Department of Medicine, University of Florida School of Medicine, Gainesville, FL 32601 USA  
⁴ Department of Microbiology, Center for Predictive Medicine, School of Medicine, University of Louisville, KY 40202

* Corresponding author: gill.diamond@Louisville.edu
Potent antiviral activity against HSV-1 and SARS-CoV-2 by antimicrobial peptoids

Antimicrobial peptoid

Disruption of viral envelope

Viral inactivation

HSV-1

SARS-CoV-2
Abstract: Most therapeutic strategies for the development of antiviral agents are designed based on targets specific to each virus. We examined the potential broad-spectrum activity of antimicrobial peptoids (AMPs) against two different viruses. AMPs are mimetics of antimicrobial peptides, which are sequence-specific N-substituted glycine oligomers, where their side chains are appended to the backbone amide nitrogens rather than α-carbons. As a result, peptoids are not proteolyzed, and have improved biostability and bioavailability and reduced immunogenicity relative to natural peptides. AMPs exhibit potent in vitro activity against a wide variety of bacteria and fungi via disruption of microbial membranes as well intracellular binding to nucleic acids. Thus, we hypothesized that they also exhibit activity against enveloped viruses. HSV-1 is an enveloped DNA virus that causes topical lesions. Incubation of HSV-1 with a panel of AMPs demonstrates variable inactivation of the virus prior to infection of cultured epithelial cells. Peptoids with the best activity exhibited dose- and time-dependent inactivation of HSV-1. Lead compounds inactivate the virus within 30 minutes at µg/ml concentrations. Transmission electron microscopy shows that these compounds remove the viral envelope, similar to what is seen with detergent treatment. We also tested the compound against SARS-CoV-2, an enveloped RNA virus that causes COVID-19. Our results show a dose-dependent inactivation of this virus prior to infection of target cells. Cytotoxicity assays show little toxic effects when applied to the apical surface of well-differentiated air-liquid interface cultures of airway epithelial cells. These results indicate that AMPs are strong candidates for broad-spectrum antiviral agents.

Keywords: 3 to 5 keywords separated by semi colons
Introduction

• Traditional therapeutic strategies to treat pathogenic viruses involve designing drugs to target specific viral enzymes
  – Acyclovir for Herpes Simplex Virus (HSV)-1
    • Inactivates HSV-specific DNA polymerases
  – Remdesivir for Ebola (and SARS-CoV-2)
    • Inactivates RNA-dependent RNA polymerase

• Problems with this approach
  – Resistance
  – Different drugs for each virus
Introduction

• New approach for antiviral drug development
• Design a drug that targets the overall structure
  – No resistance development
  – Can target multiple viruses
Introduction

• Viral structures:
  – Enveloped vs. non-enveloped
  – RNA vs. DNA

• Goal: Design an antiviral drug based on antimicrobial peptides to target the viral envelope
Introduction

• HSV-1
  – Enveloped DNA virus

• SARS-CoV-2
  – Enveloped RNA virus
Introduction

• Antimicrobial peptides
• Broad-spectrum antimicrobial agents
• Cationic, amphipathic peptides
• Important components of innate immunity
• Mechanism of action is through membrane disruption
• Defensins, cathelicidins, protegrins, magainins
Human cathelicidin LL-37 inactivates KSVH by disrupting the viral envelope

Brice et al., Antiviral Res., 158:25, 2018
Can we use antimicrobial peptides as antiviral therapeutics?

**GOOD**
- Naturally occurring
- Broad-spectrum antimicrobials
- Little resistance
- Low antigenicity

**BAD**
- Protease sensitive
- Expensive to produce and purify
- Often are inactivated by other proteins
Introduction

Design novel antiviral molecules based on antimicrobial peptides

• β-peptoids: \( N \)-substituted glycine polymers
• Resistant to proteases
• Inexpensive to synthesize
• Peptoid helices: \( \alpha \)-chiral side chains stabilize helical structures, \( \sim 3 \) residues per turn, a helical pitch of 6 - 6.7 Å\(^2\)
Synthetic, non-natural peptoid mimics of natural AMPs:
Short, sequence-specific, helical oligo-N-substituted glycines

**Peptoid 1**: $\text{H}-(\text{N}	ext{Lys-Nspe-Nspe})_4-\text{NH}_2$

**Magainin-2**: GIGKFLHSAKKFGKAHFGEIMNS-\text{NH}_2

Chongsiriwatana, N.P. et al. *PNAS*, 2008, 105: 2794-2799.
LL-37 is the single human cathelicidin innate immune peptide, and exhibits potent antiviral activity against enveloped viruses.

_G. Wang, J. Biol. Chem._ (2008) Vol. 283, No. 47, pp. 32637-32643.
Designing an antimicrobial peptoid based on LL-37

Peptoid 1 (MW 1819) (MXB1)

Peptoid 1-C13_4mer (MW 835) (MXB5)

Chongsiriwatana, N.P. et al. 2011, *Antimicrob. Agents Chemother.* 55: 417-420.
Variants with \(N\)-terminal alkylation, \textit{para}-bromination of \textit{Nspe}

- \textit{NLys} \(\text{\textit{N}}\text{-}(4\text{-aminobutyl})\text{glycine}\)
- \textit{Nspe} \(\text{\textit{N}}\text{-}(1\text{-phenylethyl})\text{glycine}\)
- \textit{Ntridec} \(\text{\textit{N}}\text{-}(\text{tridecyl})\text{glycine}\)
- \textit{para-bromo Nspe} \((\text{S})\text{-}(\text{-})\text{-1}\text{-}(\text{4\text{-bromophenylethyl})glycine}\)
### Variants with N-terminal alkylation or para-bromination of Nspe

| Peptoid Sequences | Sequence | Length |
|-------------------|----------|--------|
| MXB1 (Peptoid 1)  | H-(NLys-Nspe-Nspe)$_4$-NH$_2$ | 12mer |
| MXB2              | H-(NLys-Nspe-Nspe(p-Br))$_2$-NH$_2$ | 6mer |
| MXB3              | H-NLys-Nspe-Nspe-NLys-Nspe-Nspe(p-Br)-NH$_2$ | 6mer |
| MXB4              | H-(NLys-Nspe(p-Br)-Nspe(p-Br))$_2$-NH$_2$ | 6mer |
| MXB5 (C13-4mer)   | H-Ntridec-NLys-Nspe-Nspe-NLys-NH$_2$ | 5mer |
| MXB6 (1-11mer)    | H-(NLys-Nspe-Nspe)$_3$-NLys-Nspe-NH$_2$ | 11mer |
| MXB7              | H-(NLys-Nspe-Nspe)$_2$-NH$_2$ (neg. control) | 6mer |
| MXB8              | H-Ndec-(NLys-Nspe-Nspe)$_2$-NH$_2$ | 7mer |
| MXB9              | H-Ndec-(NLys-Nspe-Nspe(p-Br))$_2$-NH$_2$ | 7mer |
| MXB10             | H-Ntridec-(NLys-Nspe-Nspe(p-Br))$_2$-NH$_2$ | 7mer |

Molchanova, N. et al., 2020 Sci. Rep. 10:14805
Results and discussion

Quantify antiviral activity of antimicrobial peptoids

Assay:
- Incubate HSV-1 in the presence of peptoid
- Infect oral keratinocyte cell line OKF6/TERT-1
- Quantify HSV-1 genomic DNA after 24 hours
Results and discussion

Antimicrobial peptoids exhibit potent antiviral activity against HSV-1
Results and discussion
Antimicrobial peptoids exhibit potent antiviral activity against HSV-1
Results and discussion

Antimicrobial peptoids disrupt the HSV-1 viral envelope
Results and discussion

Antimicrobial peptoids inactivate SARS-CoV-2
Results and discussion

Antimicrobial peptoids are not cytotoxic
Conclusions

• Antimicrobial peptoids exhibit potent *in vitro* activity against two different types of enveloped viruses
• No *in vitro* cytotoxicity is observed in differentiated cell cultures
• As with antimicrobial peptides, the activity appears to be through disruption of the viral envelope
• Thus, these peptoids can be developed as broad-spectrum antiviral agents, to treat viral infections including COVID-19
Acknowledgments

Funding:

Thanks to Jillian Cramer, University of Kentucky Electron Microscopy Core