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

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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 KSHV by disrupting the viral envelope

Brice et al., Antiviral Res., 158:25, 2018
Introduction

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: α-chiral side chains stabilize helical structures, ~3 residues per turn, a helical pitch of 6-6.7 Å²
Synthetic, non-natural peptoid mimics of natural AMPs: 
*Short, sequence-specific, helical oligo-N-substituted glycines*

**Peptoid 1:** H–(NLys–Nspe–Nspe)_4–NH₂

**Magainin-2:** GIGKFLHSAKKFGKAFVGEIMNS–NH₂

LL-37 is the single human *cathelicidin* innate immune peptide, and exhibits potent antiviral activity against enveloped viruses.

Designing an antimicrobial peptoid based on LL-37

Peptoid 1 (MW 1819) (MXB1)

Peptoid 1-C13_4mer (MW 835) (MXB5)

Nspe  Nssb  Ntridec  Npm  Nlys

Variants with \( N \)-terminal alkylation, \textit{para}-bromination of \textit{Nspe}

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

<table>
<thead>
<tr>
<th>Peptoid Sequences</th>
<th>Peptoid Sequence</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MXB1 (Peptoid 1)</td>
<td>( \text{H}-(\text{NLys-Nspe-Nspe})_{4}-\text{NH}_2 )</td>
<td>12mer</td>
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<tr>
<td>MXB2</td>
<td>( \text{H}-(\text{NLys-Nspe-Nspe(p-Br)})_{2}-\text{NH}_2 )</td>
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<td>MXB3</td>
<td>( \text{H}-\text{NLys-Nspe-Nspe}-\text{NLys-Nspe-Nspe(\text{p-Br})-NH}_2 )</td>
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<td>MXB4</td>
<td>( \text{H}-(\text{NLys-Nspe(p-Br)-Nspe(p-Br)})_{2}-\text{NH}_2 )</td>
<td>6mer</td>
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<tr>
<td>MXB5 (C13-4mer)</td>
<td>( \text{H}-\text{Ntridec-NLys-Nspe-Nspe-NLys-NH}_2 )</td>
<td>5mer</td>
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<tr>
<td>MXB6 (1-11mer)</td>
<td>( \text{H}-(\text{NLys-Nspe-Nspe})_{3}-\text{NLys-Nspe-NH}_2 )</td>
<td>11mer</td>
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<tr>
<td>MXB7</td>
<td>( \text{H}-(\text{NLys-Nspe-Nspe})_{2}-\text{NH}_2 ) (neg. control)</td>
<td>6mer</td>
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<tr>
<td>MXB8</td>
<td>( \text{H}-\text{Ndec-(NLys-Nspe-Nspe)}_{2}-\text{NH}_2 )</td>
<td>7mer</td>
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<tr>
<td>MXB9</td>
<td>( \text{H}-\text{Ndec-(NLys-Nspe-Nspe(p-Br))}_{2}-\text{NH}_2 )</td>
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<td>MXB10</td>
<td>( \text{H}-\text{Ntridec-(NLys-Nspe-Nspe(p-Br))}_{2}-\text{NH}_2 )</td>
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</tbody>
</table>

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

![Graph showing the inactivation of SARS-CoV-2 by peptoids at different concentrations. The x-axis represents concentration (µg/mL), and the y-axis represents % control. Two lines are shown: TM4 in blue and TM9 in red. The graph indicates a decrease in control percentage as concentration increases.]
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
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