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A combination microbicide using the lipid-ether 1-octyl-sn-glycerol (OG) (3 mM) and peptide LSA5 (9 μM) synergistically inactivated six clinical isolates of herpes simplex virus type 2 (HSV-2) by 30- to 100-fold and five of six HSV-1 isolates by 10-fold more than the sum of OG and LSA5 used individually within 30 min. OG plus LSA5 inactivated all HSV clinical isolates by ≥1,000-fold in 10 to 40 min.

Two questions remained unanswered at the end of our previous study. The first was whether clinical isolates of HSV-1 and HSV-2 would show the same susceptibilities to inactivation by mixtures of lipid and peptide. The second was whether replacing D2A21 (molecular weight, 2,775.5) with a more potent peptide, LSA5, would decrease the time required to inactivate clinical isolates of HSV-2. LSA5 is a 28-residue arginine- and valine-rich antimicrobial peptide that demonstrates broad antimicrobial activity in bacterial/host cell culture models.

The present study demonstrates the increased efficacy of LSA5 (molecular weight, 3,819) (5, 7, 8) against HSV-2 compared to that of D2A21 (2) when used by itself and in combination with the antimicrobial lipid 1-octyl-sn-glycerol (OG) (1, 3).

Clinical isolates of HSV-1 and HSV-2 were obtained from Jeanne Jordan at the Magee Women’s Hospital (Pittsburgh, PA) using an institutional review board-approved protocol. In response to a physician order, independent of this protocol, swabs were collected from patients and put in M-4 medium, and samples not needed for HSV diagnosis were frozen at −20°C and shipped to our laboratory, coded but without identifiers, as outlined in our institutional review board protocol. HSV-1 and HSV-2 were grown in Vero and CV-1 cells, respectively, and assayed as previously described (6, 9). Microbicides, individually or in combination, were incubated at 37°C with clinical HSV isolates for the times indicated (Fig. 1 and 2) and at 37°C in the presence of 1% fetal bovine serum. The incubation mixtures were then titered for remaining viral infectivity (5). Each experiment was performed in triplicate, and all data points were the mean ± standard deviation of at least three separate experiments. Human serum was not used in these experiments, because immune-system-related elements present in

### TABLE 1. Time required to reduce HSV-1 and HSV-2 titers by ≥3 log_{10}^{	ext{a}}

<table>
<thead>
<tr>
<th>Samples</th>
<th>Initial titer</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSV-1</td>
<td>HSV-2</td>
</tr>
<tr>
<td></td>
<td>OG alone (3 mM)</td>
<td>LSA5 alone (9 μM)</td>
</tr>
<tr>
<td>p1</td>
<td>10^{5.92}</td>
<td>&gt;60</td>
</tr>
<tr>
<td>p2</td>
<td>10^{8.04}</td>
<td>60</td>
</tr>
<tr>
<td>p3</td>
<td>10^{7.76}</td>
<td>&gt;60</td>
</tr>
<tr>
<td>p4</td>
<td>10^{6.17}</td>
<td>&gt;60</td>
</tr>
<tr>
<td>p5</td>
<td>10^{6.71}</td>
<td>&gt;60</td>
</tr>
<tr>
<td>p6</td>
<td>10^{5.94}</td>
<td>&gt;60</td>
</tr>
</tbody>
</table>

|         | HSV-2         |            |
| p7      | 10^{8.33}     | 60         | >60        | 50       | 30       | 10       |
| p8      | 10^{8.33}     | >60        | >60        | 60       | 40       | 20       |
| p9      | 10^{4.66}     | >60        | >60        | 60       | 30       | 20       |
| p10     | 10^{4.42}     | >60        | >60        | >60      | 30       | 30       |
| p11     | 10^{4.42}     | >60        | >60        | >60      | 30       | 30       |
| p12     | 10^{4.21}     | >60        | >60        | >60      | 30       | 30       |

### Notes

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The results presented in this table are derived from Fig. 1 and 2.
most human serum will inactivate HSV and compromise the ability to monitor the direct effects of OG and LSA5.

The time required to reduce the titers of clinical HSV-1 isolates (Fig. 1 and Table 1) by \( \geq 3.0 \log_{10} \) units (1,000-fold) when OG was combined with 9 \( \mu M \) LSA5 varied from 10 to 40 min. Isolates with the lowest initial titers were inactivated more rapidly than higher-titer isolates. Clinical isolates p1 and p2, with the two lowest initial titers of \( 10^{2.92} \) and \( 10^{3.04} \), respectively, were also reduced in titer by 3 \( \log_{10} \) units using a combination of OG and 3 \( \mu M \) LSA5. The three clinical isolates of HSV-1 with titers of \( 10^{2.92} \) (p1), \( 10^{3.04} \) (p2), and \( 10^{3.79} \) (p3) were completely inactivated in 40 min by 3 mM OG plus 6 \( \mu M \) LSA5, whereas the HSV-1 isolates with titers of \( 10^{4.17} \), \( 10^{4.71} \), and \( 10^{5.04} \) (p4, p5, and p6) were reduced by 3 \( \log_{10} \) units in 30 to 40 min, but their titers then exhibited a lower rate of decrease between 40 and 60 min.

In contrast to HSV-1, the time required to inactivate the six clinical HSV-2 isolates (Fig. 2) by \( \geq 3 \log_{10} \) units was dependent on the particular isolate and not on the initial viral titer (Table 1). Isolate p12, with an initial titer of \( 10^{6.2} \), was reduced in titer by 1,000-fold within 10 min by 3 mM OG combined with 3 \( \mu M \) LSA5, whereas isolate p10 (initial titer, \( 10^{4.42} \)) required 30 min to reach the same decrease in titer when OG was combined with 6 or 9 \( \mu M \) LSA5. All clinical isolates of HSV-2 were completely inactivated in 60 min by a combination of 3 mM OG and 6 \( \mu M \) LSA5 and within 20 to 50 min when the LSA5 concentration in the mixture was raised to 9 \( \mu M \).

The levels of antiviral activity produced by mixtures of OG (3 mM) and LSA5 (6 or 9 \( \mu M \)) suggested a greater-than-additive, possibly synergistic, response to all 12 HSV clinical isolates.
isolates. However, defined statistical analysis of this response was not further addressed. This is especially true at the shorter exposure times, e.g., 30 min, where, with the exception of HSV-1 isolate p1, which had the lowest initial titer, mixtures of 3 mM OG and 9 μM LSA5 inactivated HSV-1 isolates (e.g., p4) at least 10-fold more than the sum of OG and LSA5 used individually at 3 mM and 9 μM, respectively. OG and LSA5 mixtures inactivated all HSV-2 isolates by 30- to 100-fold more than the sum of OG and LSA5 used individually. For example, following 30 min of incubation, the titers of HSV-2 clinical isolates p11 and p12 (Fig. 2) were reduced by >100,000-fold (p12), depending on the isolate. HSV-1 isolates p5 and p6, which were not more susceptible to inactivation by OG than by LSA5, had the highest titers among the HSV-1 isolates. These results are in accord with our previous study using laboratory strains (2), which showed that at a similar titer, HSV-1 was more susceptible to OG inactivation than HSV-2, and this can be clearly seen in the present study by comparing HSV-1 isolate p2 and HSV-2 isolate p7. HSV-2 isolates were more sensitive to inactivation by LSA5 than HSV-1 isolates, and this increased sensitivity was strain and not titer dependent. This study also shows that LSA5 decreases the titer of HSV-2 by 100- to 1,000-fold more than the previously used antimicrobial peptide, D2A21 (2), in 60 min.

The use of multiple active components targeting two points

![Graph](https://example.com/graph.png)

FIG. 2. Inactivation of HSV-2 clinical isolates p7 to p12 by mixtures of OG and LSA5. Each point is the mean ± the standard deviation for three separate experiments.
on the HSV envelope extends the time of protection provided over that provided by utilizing a single active component. This is accomplished in two ways: first, by using two different mechanisms of action, which increases the ability to effectively inhibit viral replication, and second, by maintaining persistent antiviral activity as the concentration of each active component diminishes over time below its effective microbicidal level when used alone. A microbicide with a single active component requires a higher concentration of the active component and has a narrower spectrum of activity. The present study also indicates that a mixture of compounds may make the use of an effective but expensive antiviral agent feasible by combining it with a less expensive active component, which can potentiate its activity at a decreased concentration. The use of multiple active compounds is both cost and time efficient.

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