Antiviral Activity of Fattiviracin FV-8 against Human Immunodeficiency Virus Type 1 (HIV-1)

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A novel antiviral agent, fattiviracin FV-8, purified from the culture broth of Streptomyces microflavus strain No. 2445, showed potent antiviral activities against human immunodeficiency virus type 1 (HIV-1), herpes simplex virus type 1 (HSV-1), varicella-zoster virus (VZV), and influenza A and B viruses. The action mechanism of fattiviracin FV-8 against HIV-1 was examined. As a result, the agent was thought to act on HIV-1 particles directly without lysis of the particles, and it afforded the inhibition of viral entry into the host cells.

Key words: Streptomyces microflavus; fattiviracin; antiviral agent; human immunodeficiency virus type 1

In our continuing efforts to isolate novel antiviral agents from soil microorganisms, we have developed a rapid method for detection of antiviral agents using 96-well microtiter plates, and leading to the discovery of the new antiviral agents AH-135Y,10 AH-758,9 AH-1763 Ila,9 and recently fattiviracin A1.5,6 Streptomyces microflavus strain No. 2445 produced many fattiviracin derivatives. The major product of these derivatives is fattiviracin FV-8 (FV-8), which structurally belongs to sugar-fatty acids lactone, consisting of four D-glucose residues and two trihydroxy fatty acids (C25 and C26). The structure was reported in another paper.6 In this paper, we describe the antiviral activity and the action mechanism of FV-8 against HIV-1.

The antiviral activities of FV-8 on HSV-1, VZV, and influenza viruses were measured by a plaque reduction assay.7 Briefly, confluent monolayers of Vero or MDCK cells in 6-well plates were infected with 100 PFU of each virus. After a 1-h adsorption period, the cultures were overlaid with Dulbecco’s modified Eagle’s minimum essential medium (DMEM) containing 2% heat-inactivated fetal calf serum (FCS) including various concentrations of FV-8. The plates were incubated in a CO2 incubator for 3 d, then fixed with formalin and stained with crystal violet in methanol. Infectious virus production was measured by counting plaques caused by virus-induced cytopathic effects. The toxicities to the host cells were examined as described in the preceding paper.5

The antiviral activity of FV-8 on HIV-1 was tested by MAGI (multinuclear activation of a galactosidase indicator) assay.8 MAGI/CCR5 cells (3 × 105 cells) were infected with three types of HIV-1 (IIIB/T+, JR-FL/M-, or 89.6/dual-tropic strains) and incubated for 2 d in growth medium (10% FCS + RPMI 1640) containing different concentrations of FV-8. Individual infected cells were counted in situ with a light microscope by virtue of their blue color after incubation with 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal). In the experiment on the effect of FV-8 treatment time on HIV-1 infection, IIIB strain was used. Several concentrations of FV-8 were added to the culture just before HIV-1 infection, or 1 h or 3 h after HIV-1 infection. To examine the effects of FV-8 on HIV-1 particles, IIIB strain was treated with different concentrations of FV-8 for 30 min at 37°C. After ultracentrifugation (60,000 rpm, 60 min), the precipitate (viral fraction) was suspended in growth medium and infected to MAGI/CCR5 cells. The lysis of the viral envelope and capsid of HIV-1 particles was estimated on the basis of the amount of p24 in the viral fraction measured by HIV-1 p24 capsid enzyme-linked immunosorbent assay (Abbott).9

The antiviral activities and cytotoxicities of FV-8 are shown in Table 1. FV-8 had the similar antiviral activities against IIIB, JR-FL, and 89.6 strains of HIV-1, and these EC50’s were 4.3, 3.5, and 6.1 μg/ml, respectively. The antiviral activities of FV-8 were similar to those of fattiviracin A1, which

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Abbreviations: HIV-1, human immunodeficiency virus type 1; HSV-1, herpes simplex virus type 1; VZV, varicella-zoster virus
Table 1. Antiviral Activities and Cytotoxicities of FV-8

<table>
<thead>
<tr>
<th>Virus</th>
<th>Strain</th>
<th>Cell</th>
<th>Antiviral activity (EC50, µg/ml)</th>
<th>Cytotoxicity (IC50, µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>IIB</td>
<td>MAGI</td>
<td>4.3</td>
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</tr>
<tr>
<td>HIV-1</td>
<td>JR-FL</td>
<td>MAGI/CCR5</td>
<td>3.5</td>
<td>280</td>
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<tr>
<td>HIV-1</td>
<td>89.6</td>
<td>MAGI/CCR5</td>
<td>6.1</td>
<td>280</td>
</tr>
<tr>
<td>HSV-1</td>
<td>KOS</td>
<td>Vero</td>
<td>2.7</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>VZV</td>
<td>Oka</td>
<td>Vero</td>
<td>3.0</td>
<td>&gt;5000</td>
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<tr>
<td>Influenza A</td>
<td>H1N1</td>
<td>MDCK</td>
<td>1.9</td>
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<tr>
<td>Influenza B</td>
<td>B/Lee/40</td>
<td>MDCK</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

1 T-tropic virus strain.  
2 M-tropic virus strain.  
3 Dual-tropic virus strain.  
4 A clone of MAGI cells that express the human chemokine receptor CCR5.  
MAGI cell line is a HeLa cell clone expressing human CD4 and HIV LTR-β-galactosidase.

Fig. 1. Effects of FV-8 Treatment Time on HIV-1 Infection.  
○, Addition of FV-8 just before HIV-1 infection. △, Addition of FV-8 1 h after HIV-1 infection. □, Addition of FV-8 3 h after HIV-1 infection.

Fig. 2. Effects of Fattiviracin FV-8 on HIV-1 Particles.  
○, Antiviral activity of FV-8. △, Amount of p24 in the viral fraction.

had antiviral EC50's of 10.4, 3.9, 3.4, and 2.1 µg/ml against HIV-1, HSV-1, VZV, and influenza A virus, respectively.

The effects of FV-8 treatment time on HIV-1 infection were examined, and are shown in Fig. 1. In the addition just before viral infection, FV-8 had strong antiviral activity (EC50 = 4.3 µg/ml), but 1 h later, the activity was decreased (EC50 = 28.9 µg/ml), and no antiviral activity was observed in the addition 3 h after HIV-1 infection (EC50 > 100 µg/ml). These results suggested that FV-8 does not show the antiviral activity against the HIV-1 that have already invaded host cells, that is, the agent acts on HIV-1 particles directly before infection.

The effects of FV-8 on HIV-1 particles are shown in Fig. 2. Although the antiviral activity of FV-8 increased with the concentration, the amount of capsid protein p24 of HIV-1 was constant. Therefore, FV-8 inactivates HIV-1 particles without lysis of the viral particles, and it inhibits viral entry into the host cells.

The antiviral mechanism of FV-8 is clearly different from those of zidovudine and ritonavir, the antiviral activities of which are inhibitions of reverse transcriptase and protease in the HIV-1 particles, respectively.

In recent studies, it was found that FV-8 did not interact with gp120 binding with CD4 on the surface of host cell, but with trans-membrane gp41 connecting with gp120. We will describe them in our next paper.

References

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