INHIBITION OF HIV REPLICATION BY 19-*O*-*n*-PENTYLDAMAVARICIN Fc *IN VITRO*

Sir:

Damavaricin Fc (Fig. 1) is an atropisomeric mixture of two isomers which were produced from streptovaricin C by alkaline degradation[1]. We have investigated the biological activities of several derivatives of damavaricin Fc (DvFc) that have different alkyl ether linkages at the C-19 position of the naphthoquinone ring of the molecule. One of those derivatives, 19-*O*-*n*-pentyldamavaricin Fc (*n*-pentyl-DvFc) (Fig. 1) has shown inhibitory activity against focus formation by the mouse sarcoma virus/mouse leukemia virus complex and is known as an inhibitor of reverse transcriptase[1]. Recently, we also found that *n*-pentyl-DvFc seemed to act on sulfhydryl groups of the cell membrane in HTLV-I-infected T-cells[2]. In this report, we describe the effect of *n*-pentyl-DvFc on HIV replication.

MT-4 cells were exposed to HIV at a multiplicity of infection of 0.006 for 1 hour at 37°C. The HIV had been obtained from the culture supernatant of HIV-infected Molt-4 cells. After washing, cells were resuspended in RPMI-1640 supplemented with 10% fetal calf serum (FCS) to give a concentration of 3×10^5 cells/ml, and were incubated in the presence or absence of various concentrations of *n*-pentyl-DvFc in a CO_2 incubator.

H-9, HUT-78 (HTLV-I-negative T-cell line) or U937 clone 16 (monocytoid) cells were suspended at a concentration of 1×10^5 cells/ml in 1 ml of RPMI-1640 supplemented with 10% FCS containing various concentrations of *n*-pentyl-DvFc. The cells were mixed with 1 ml of HIV suspension of which the titer has been adjusted to give about 50% cells expressing HIV-antigen after incubation for 6 days.

Indirect immunofluorescence (IF)[3] was used to evaluate the expression of HIV-specific antigen and the frequency of antigen-positive cell was calculated.

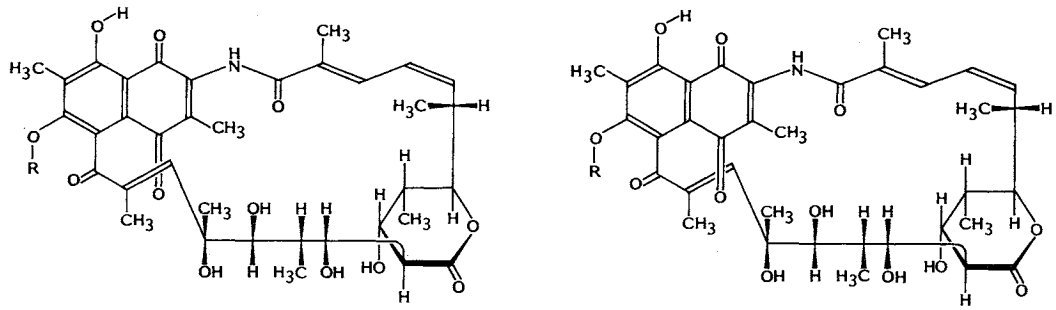
When MT-4 cells were used as host, only 19% of cells expressed HIV-antigen in the presence of *n*-pentyl-DvFc (2.5 μg/ml) whereas no-drug control cells showed 81% positive on the third day after infection (Fig. 2). However, the MT-4 cell line is one of the HTLV-I-transformed cell lines which have been known to be highly sensitive to cytotoxicity of *n*-pentyl-DvFc[2]. In fact, MT-4 cells seemed to be almost in a static state under this condition (2.5 μg/ml).

Thus, we examined the anti-HIV effect of *n*-pentyl-DvFc using the HTLV-I-negative cell lines, H-9, HUT-78 or U937 as permissive host cells of HIV. In these cells, the 50% cytotoxic dose (CD_{50}) of *n*-pentyl-DvFc was in the range of 9 μg/ml (U937) to 16 μg/ml (HUT-78), and was higher than that of MT-4 cells (2 μg/ml).

n-Pentyl-DvFc suppressed the expression of HIV-antigen in dose dependent manner in these three cell lines (Fig. 3). About 50% inhibition (ED_{50}) was observed at a concentration of 3 μg/ml. 3′-Azido-3′-deoxythymidine (AZT), used as a positive control, showed ED_{50} values ranging from 0.013 μg/ml (H-9) to 0.024 μg/ml (HUT-78).

The mechanism of the inhibitory effect of *n*-pentyl-DvFc on HIV replication is not clear at the present moment. Selective efficacy (ratio of CD_{50} to ED_{50}) of *n*-pentyl-DvFc was in the range of about 3 to 5 in these three cell lines, suggesting that this drug acts on the host cell metabolism rather than on the virus itself.

Fig. 1. The structures of DvFc and *n*-pentyl-DvFc.



DvFc R=H
n-Pentyl-DvFc R=*n*-Pentyl

Fig. 2. Effect of *n*-pentyl-DvFc on HIV replication.

MT-4 cells were infected with HIV at a multiplicity of infection of 0.006 and incubated in the absence (●) or presence (○ 1 μg/ml, ▲ 2.5 μg/ml) of *n*-pentyl-DvFc. For the expression of HIV-specific antigens, indirect immunofluorescence was done.

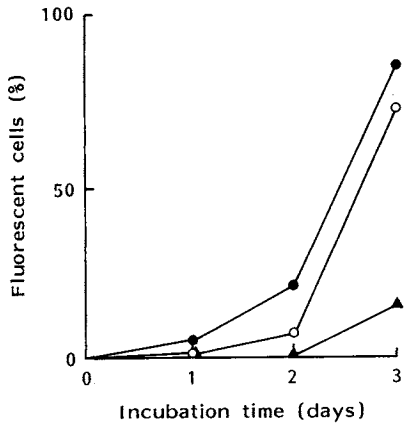
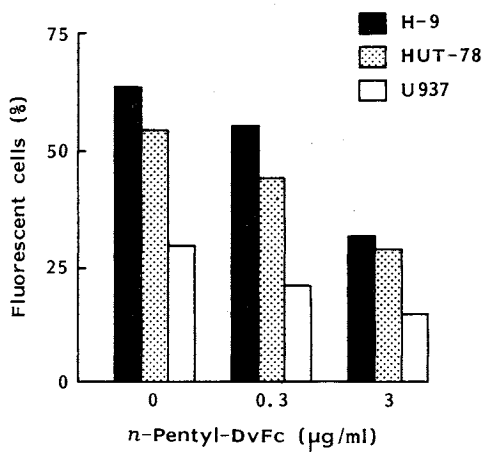


Fig. 3. Effect of *n*-pentyl-DvFc on HIV replication in HTLV-I-negative cell lines.

H-9, HUT-78, or U937 cells were infected with HIV and incubated in the presence of *n*-pentyl-DvFc. After 6 days, cells were subjected to immunofluorescence.



Although the HIV-specificity is low as compared to that of HIV-selective inhibitors including AZT, *n*-pentyl-DvFc might be useful for the therapy of HIV-infected diseases in combination with anti-HIV drugs.

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