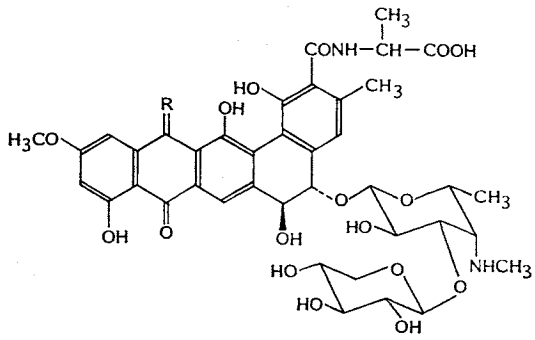
INHIBITORY EFFECT OF NEW ANTIBIOTIC, PRADIMICIN A ON INFECTIVITY, CYTOPATHIC EFFECT AND REPLICATION OF HUMAN IMMUNODEFICIENCY VIRUS *IN VITRO*

Sir:

Pradimicin A (Fig. 1) has been found as a new antifungal antibiotic in the culture filtrate of *Actinomadura hibisca* sp. nov. Strain P157-2 (ATCC 53557).[1~3] In this communication, we describe that the antibiotic and its imino derivative efficiently inhibit the human T-lymphotropic virus type IIIB (HTLV-IIIB)-induced cyctopathic effect of MT-4 cells and the cell fusion by cell to cell infection and syncytia formation in the cocultures of MOLT-4 and MOLT-4/human immunodeficiency virus $(HIV)_{HTLV-IIIB}$ cells.

The number of viable MT-4 cells infected with HIV at a multiplicity of infection of 0.002 decreased during the cultivation and almost all cells were dead by 6 days after infection as determined by a trypan blue dye exclusion method.[4] When pradimicin A (**1**) and imino derivative (**2**) were added to the culture, the cell damage induced by HIV was significantly suppressed at the concentrations more than 3.5 μg/ml of compounds (Fig. 2). With regard to cytotoxicity of **1** and **2**, almost no growth inhibition of MT-4 cells was observed at the concentrations up to 30 μg/ml. Higher concentrations of **1** and **2** showed only weak growth inhibition against MT-4 cells.

Fig. 1. Chemical structures of pradimicin A and its imino derivative.



Pradimicin A (**1**) R=O
13-Imino derivative (**2**) R=NH

Fig. 2. Effect of pradimicin A (A: day 3, B: day 6) and imino derivative (C: day 3, D: day 6) on the cell growth of MT-4 cells (open bars), and inhibitory effect on the virus-induced cytopathic effects in HIV-infected MT-4 cells (shadow bars).

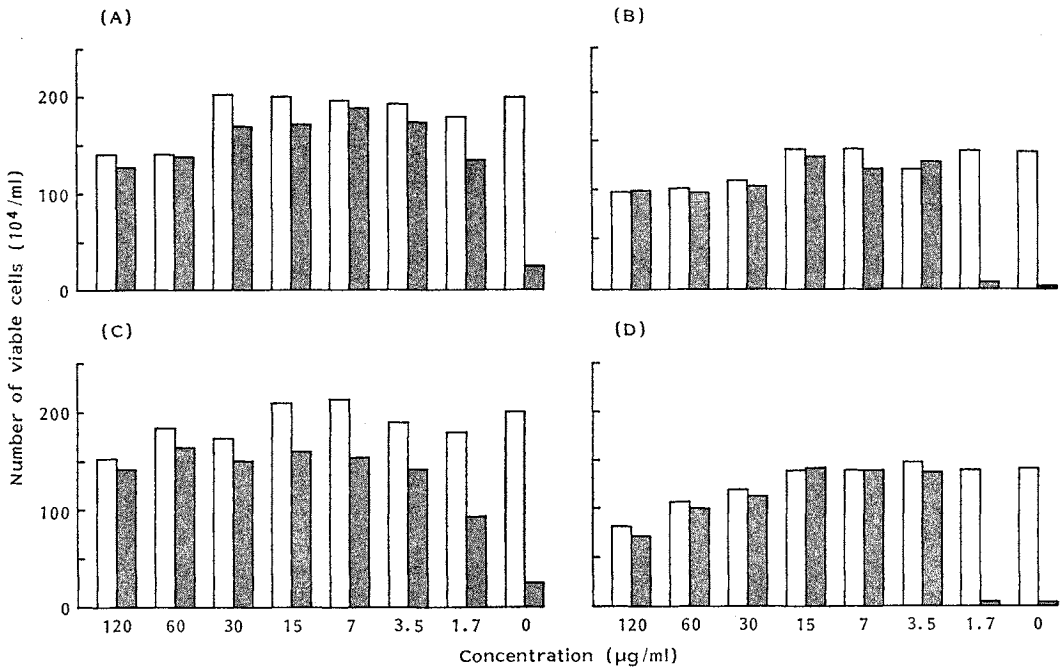
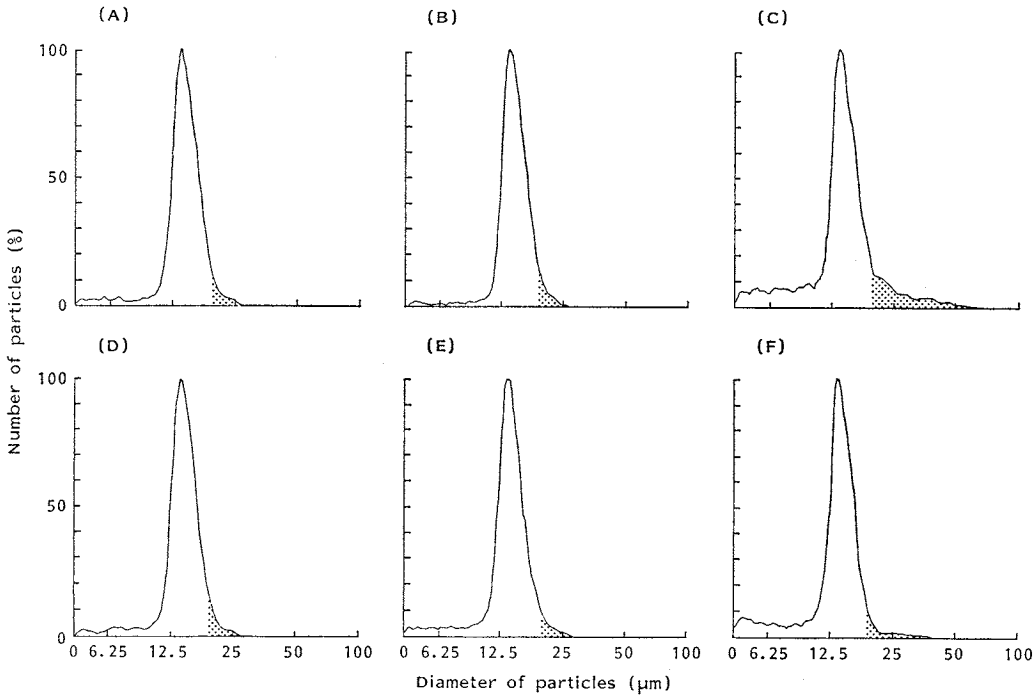


Fig. 3. Effect of pradimicin A and its imino derivative on HIV-induced multinucleated giant cell formation in cocultures.



Cell size distribution of MOLT-4 (A), MOLT-4/$HIV_{HTLV-IIIB}$ (B) and coculture of both cell lines (C) was analyzed by a cell multisizer. A portion of particles with more than 20 μm in diameter was dotted. Effect of dextran sulfate (D), pradimicin A (E) and imino derivative (F) was examined at the concentrations of 50 μg/ml, 25 μg/ml and 25 μg/ml, respectively.

Experssion of HIV antigen as revealed by immunofluorescence was significantly suppressed when HIV-infected MT-4 cells were cultured in the presence of **1** and **2**, and positive cells were only less than 1% at the concentrations more than 3.5 μg/ml of both **1** and **2** (data not shown).

We next inquired the effect of these compounds on the multinucleated giant cell formation. MOLT-4 and MOLT-4/$HIV_{HTLV-IIIB}$ cells were mixed in a 1 : 1 ratio in proportion of cell number and incubated for 20 hours at 37°C. Then the size of particles was measured and the diameter was plotted on the horizontal axis by a cell multisizer.[5] Fig. 3 shows the results obtained by a cell multisizer in cocultures added at 25 μg/ml of **1** and **2** or 50 μg/ml of dextran sulfate used as a positive control. The percentage of cell particles with more than 20 μm was only 2.7% and 2.4% in MOLT-4 cells and MOLT-4/$HIV_{HTLV-IIIB}$ cells, respectively. On the other hand, in the coculture of both cell lines the frequency of cell particles with more than 20 μm in diameter was 11.8%. When **1** and **2** were added to cocultures, giant cell formation was apparently suppressed at the concentrations more than 12.5 μg/ml.

The viable cells in each culture were also counted and the fusion index (FI) value was calculated as described previously.[6] Control culture without compound showed the FI value of 1.71. The FI value of the culture containing 50 μg/ml of dextran sulfate, which completely inhibitied the giant cell formation, was 0.12 and the culture containing 50 μg/ml and 25 μg/ml of **1** and **2** showed the FI value of less than 0.2 (data not shown).

1 and **2** did not inhibit avian myeloblastosis virus (AMV) reverse transcriptase (1 U) even at the concentration of 100 μg/ml (data not shown).

From the results **1** and **2** showed the anti-HIV activity at the concentrations more than 3.5 μg/ml in the cell-free infection with MT-4 cells. More importantly, they showed inhibitory activity to the HIV-induced giant cell formation at the concentrations more than 12.5 μg/ml in coculture

system. In the presence of **1** or **2** size distribution pattern of cells in the cocultures was similar to that in the culture with dextran sulfate used as a positive control (Figs. 3D~3F). On the other hand, these compounds did not inhibit the AMV reverse transcriptase. These results strongly suggest that **1** and **2** exert their anti-HIV activity on the step of viral adsorption.

1 and **2** are shown to have antiviral activity against influenza virus.[2] Very recently, TAKEUCHI *et al.* also reported that benanomicin,[7] which has closely related structure to **1**, was active against fungi and some Gram-positive bacteria. At present we do not know the reason why **1** or benanomicin is active against such various species of microorganisms.

1 and **2** scarcely inhibited the growth of MT-4 cells and chemotherapeutic indices showed relatively large. Therefore, it is worthy to study these compounds more extensively in terms of chemotherapeutic and/or prophylactic point of view against AIDS and ARC.

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