

NEW ANTIFUNGAL ANTIBIOTICS,
 BENANOMICINS A AND B
 INHIBIT INFECTION OF T-CELL
 WITH HUMAN IMMUNODEFICIENCY
 VIRUS (HIV) AND SYNCYTIUM
 FORMATION BY HIV

Sir:

New antifungal antibiotics, benanomicins A and B, have been isolated from the culture filtrate of *Actinomyces* sp. MH193-16F4.^{1,2)} Benanomicins A and B have a benzo[*a*]naphthacene quinone skeleton and are active against fungi and some Gram-positive bacteria. No acute toxicities of benanomicins A and B were observed at 600 mg/kg and 100 mg/kg, respectively, when these compounds were injected into mice intravenously. We found that these antibiotics inhibited *de novo* infection of human T-cells with human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS).^{3,4)} They also inhibited syncytium formation of human T-cells after cocultivation with HIV-1-producing cells, suggesting that benanomicins A and B inhibit the attachment of HIV-1 to human T-cells.

Benanomicins A and B were dissolved in DMSO at 10 mg/ml and stored at 4°C before use. For assay of their anti-HIV activities, the stock solutions were diluted with Ca²⁺ and Mg²⁺-free phosphate-buffered saline and added to Costar 48-well culture plates.

The MT-4 human T-cell line susceptible to HIV-1⁵⁾ was used for detection of anti-HIV activities. At first, effects of benanomicins A and B and DMSO (solvent control) on growth of MT-4 cells were examined. MT-4 cells (1×10^5 cells/ml) were seeded into the 48-well plates in an amount of 0.5 ml/well. Then 50 μ l of benanomicin A or B or DMSO was added at the final concentrations indicated in Fig. 1. After cultivation for 4 days, viable cells were counted by the dye exclusion method. Growth of MT-4 cells was slightly inhibited by 100 μ g/ml of benanomicins A and B. At the concentration of 100 μ g/ml of the test compounds, the cultures contained DMSO, used as the solvent, at 1%. DMSO also affected the growth of MT-4 cells at this concentration. Viabilities of MT-4 cells treated with benanomicins and DMSO were similarly high, although parts of MT-4 cells, especially small parts adjoining other cells, that

had been treated with benanomicin B at 100 μ g/ml became reddish. When we took the effects of DMSO into account, we concluded that benanomicins A and B had little effect, if any, on growth of MT-4 cells at concentrations up to 100 μ g/ml.

Next the effects of benanomicins A and B on HIV infectivity were examined. MT-4 cells were seeded and benanomicins A and B were added as described above. Two hours later MT-4 cells were infected with 50 μ l of the HTLV-III_B strain⁶⁾ of HIV-1 at the multiplicity of infection (m.o.i.) of 0.025~0.05. After 4 days MT-4 cells were smeared onto slide glasses, dried and fixed with acetone. The presence of HIV-1 antigen-positive cells were detected by the indirect immunofluorescence assay (IFA) as described elsewhere.⁷⁾ DMSO did not affect infection with HIV-1 and more than 90% of MT-4 cells became immunofluorescent upon IFA (data not shown). Infection with HIV-1 was almost completely inhibited by treatment with benanomicin A at 30~100 μ g/ml or benanomicin B at 10~100 μ g/ml (Fig. 2). Benanomicin B was slightly more inhibitory in this assay than benanomicin A. Thus Figs. 1 and 2 indicated that benanomicins A and B inhibited infection with HIV-1 at concentrations not toxic to MT-4 cells.

Fig. 1. Effects of benanomicins A and B on growth of MT-4 cells.

MT-4 cells (1×10^5 cells/ml) were seeded into Costar 48-well plates and benanomicins A (○) and B (□) were added at the indicated concentrations. DMSO (△) was the solvent control. 4 days later viable cells were counted by the dye exclusion test.

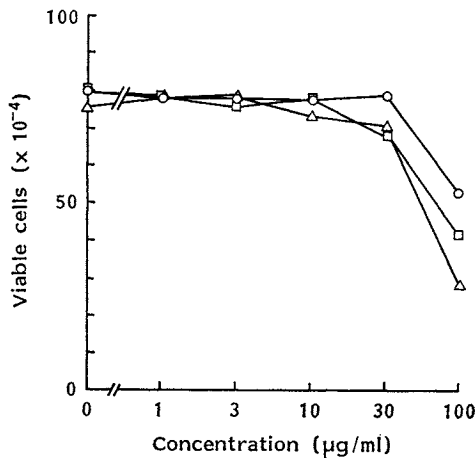
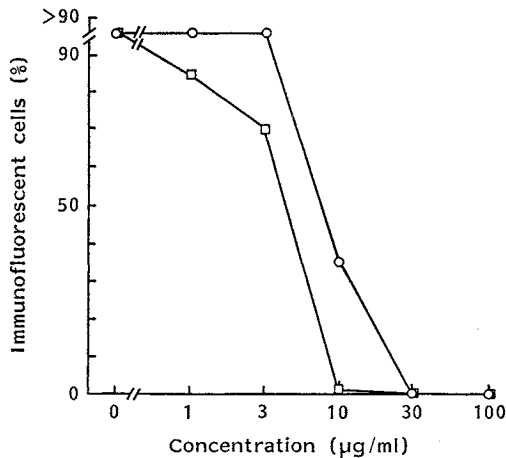


Fig. 2. Effects of benanomicins A and B on infection of MT-4 cells with HIV-1.

MT-4 cells were treated with benanomicins A (○) and B (□) as in Fig. 1.

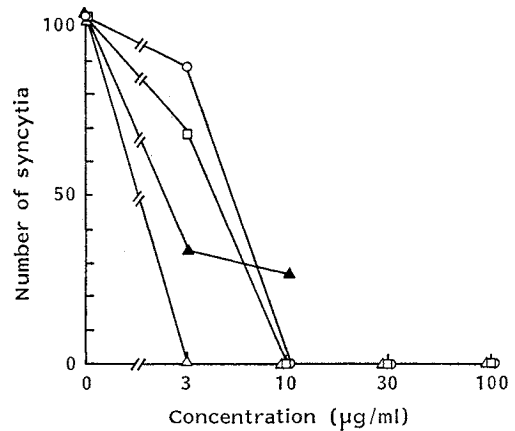


The cells were infected with HIV-1 2 hours later. HIV-1 antigen-positive cells were examined after cultivation for 4 days. Percentages of viral antigen-positive cells as detected by IFA were shown.

We then examined whether the benanomicins inhibited syncytium formation induced by HIV-1. For this, the Molt-4 human T-cells⁹⁾ were seeded into Costar 48-well plates in an amount of 1×10^5 cells/well. Benanomicins A and B were added at the concentrations indicated in Fig. 3. As the controls, azidothymidine (AZT) and dextran sulfate were added in a similar way. After 2 hours, Molt-4 cells persistently infected with HIV-1 were added at 1.5×10^4 cells/well. The number of syncytia or multinucleated giant cells formed in 5×5 mm space in each well after cultivation for 24 hours was counted under a microscope. Cells whose diameters were approximately 5 times or more as large as those of the Molt-4 cells not co-cultivated with HIV-1-producing Molt-4 cells were considered to be syncytia. Benanomicins A and B inhibited syncytium formation at concentrations of 10~100 µg/ml (Fig. 3). AZT did not markedly inhibit syncytium formation of Molt-4 cells induced by HIV-1 as expected (Fig. 3), although *de novo* infection of MT-4 cells with HIV-1 was almost completely inhibited by AZT at the concentration of as low as 0.3~1 µg/ml (data not shown). Dextran sulfate, which has been reported to inhibit binding of HIV-1 to the cells⁹⁾ inhibited

Fig. 3. Effects of benanomicins A and B, AZT and dextran sulfate on syncytium formation of Molt-4 cells.

Benanomicins A and B (○ and □), AZT (▲), dextran sulfate (△).



Molt-4 cells were co-cultivated with HIV-1-producing Molt-4 cells in the presence of the test compounds for 24 hours. Numbers of syncytia in 5×5 mm space were counted.

syncytium formation efficiently.

Many syncytia of Molt-4 cells were detected within 24 hours. Formation of proviral DNA was not necessary for syncytium formation under the assay conditions described above. Compounds such as AZT that specifically inhibits formation of proviral DNA¹⁰⁾ do not inhibit syncytium formation. Substances such as dextran sulfate or monoclonal antibodies against CD4 that inhibit adsorption of HIV-1 to the cells^{11,12)} inhibit syncytium formation. Fig. 3 suggested that benanomicins A and B inhibited adsorption of HIV-1 to the cells like dextran sulfate or anti-CD4 monoclonal antibodies. Benanomicins A and B mainly act on earlier steps of HIV-1 infection, although there is still a possibility that these compounds inhibit formation of proviral DNA at later steps of infection. It remains to be determined whether benanomicins A and B interact with HIV-1 or some molecule such as the CD4 molecule present on human T-cells.

Benanomicins A and B have antifungal activities against *Candida*, *Cryptococcus* or *Aspergillus*.¹³⁾ These fungi have been frequently detected in patients with AIDS. Thus, if further analyses show that benanomicins can be used as

an antiviral agent for AIDS, their administration may be especially advantageous for patients with AIDS or AIDS-related complex who are infected with fungi or are at risk for fungal infection.

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