INHIBITORY EFFECT OF CURROMYCIN A AND B ON HUMAN IMMUNODEFICIENCY VIRUS REPLICATION

Sir:

Two triene-β-lactone antibiotics[1] recently isolated from the culture of *Streptomyces* sp. MJ213-62F4 resembling *Streptomyces melanosporofaciens* were identical with curromycins A and B[2], respectively, produced by a genetically modified strain of *Streptomyces hygroscopicus*. In the present communication, we report their effect on human immunodeficiency virus replication in both acute and chronic infection.

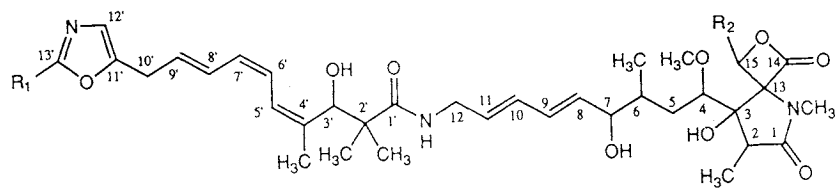
The experiment analyzing effects on primary infection was performed according to the following. H9 cells[3] were pretreated with serially diluted agents at 37°C for 30 minutes and then infected with HIV-1 IIIB at a multiplicity of 0.05. The cells were incubated at a density of 1.5×10^6/ml for 90 minutes at 37°C to permit adsorption of viral particles and then diluted with fresh media 1 : 10 for culturing in a 96 well plate. On day 6, the culture fluid was harvested for reverse transcriptase (RT) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays. The RT assay[4] was performed to estimate the concentration of viral particles in the culture supernatant. In brief, 10 μl of supernatant containing HIV particles was disrupted with 10 μl of detergent solution [50 mM Tris-HCl pH 8, 10 mM dithiothreitol (DTT), 300 mM KCl, 0.5% TritonX-100] in a 96-well round bottom plate. Following 15 minutes incubation at 4°C, add 25 μl of RT reaction buffer containing 50 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 5 mM DTT, 0.25 U/ml poly(rA)oligo(dT)$_{12\sim18}$ (Pharmacia), 15 μCi/ml [^{3}H]dTTP. The reaction mixtures were incubated at 37°C for 18 hours and 15 μl of mixture was then spotted onto DE81 Whatmann filter paper, dried, washed with 2 × SSC, and rinsed with ethanol. Radioactivities of these filters were counted by a scintillation counter. The MTT assay[5], based on the mitochondrial reduction of MTT, was utilized to measure the number of living cells as follows; 100 μl of cell suspension was taken and transfered to a new 96 well plate. 10 μl of 5 mg/ml MTT-phosphate-buffered saline (PBS) was then added and incubated at 37°C for 4 hours. To lyze cells and solubilize MTT formazan, add 100 μl of 10% SDS and incubate overnight, then measure absorbance on a microplate reader with a test wavelength of 550 nm and reference wavelength of 630 nm.

As shown in Fig. 2, curromycins A and B treatment resulted in a concentration dependent inhibition of HIV replication in this acute assay system. The 50% effective concentrations (EC_{50}) of curromycins A and B for HIV replication were 2.5 μg/ml and 5 μg/ml, respectively. AZT treatment also showed concentration dependent inhibition in this assay system.

To exclude the possibility that curromycins directly inhibit reverse transcriptase in this RT assay system, we monitored p24 antigen by capture ELISA to study the production of HIV antigens with treatment of curromycins. OD values which represent the antigen expression exhibited the same tendency with RT values (data not shown), therefore, these agents did not have effect on RT directly. The 50% inhibitory concentration (IC_{50}) values of MTT were at 6.5 μg/ml for curromycin A and 20 μg/ml for curromycin B.

We next examined the antiviral activity of curromycins in chronically infected U937 cells[6]. 2×10^5/ml cells were seeded in a 96 well plate in the presence of serially diluted curromycins. Culture of each well was harvested at 96 hours for RT and MTT assays. The result demonstrated that curromycins also has anti HIV activity in chronically infected cells (Fig. 3). Their selectivity index (ratio of IC_{50} to EC_{50}) was greater than 10. It suggests that curromycins are more effective in chronically infected monocytoid lineage than primary infected

Fig. 1. Chemical structures of curromycin A and curromycin B.



Curromycin A $R_1 = CH_3$ $R_2 = CH_3OCH_2$
Curromycin B $R_1 = CH_3$ $R_2 = CH_3$

Fig. 2. The effect of curromycins A and B and AZT on primary infected human lymphoid cells.

(A) Triedimycin A, (B) Triedimycin B, (C) AZT, open: % inhibition of RT, closed: % inhibition of MTT.

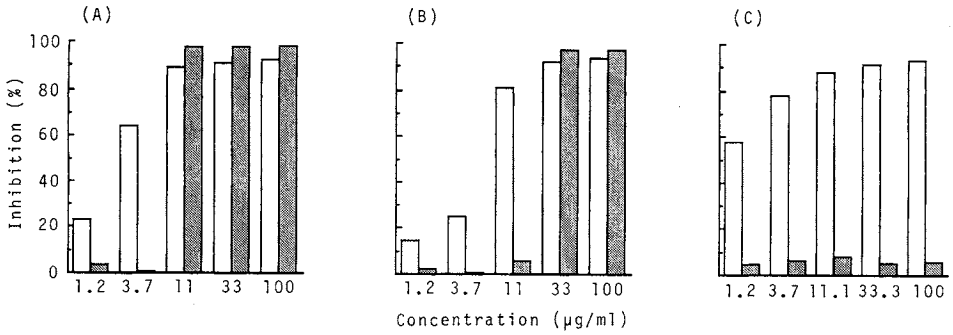
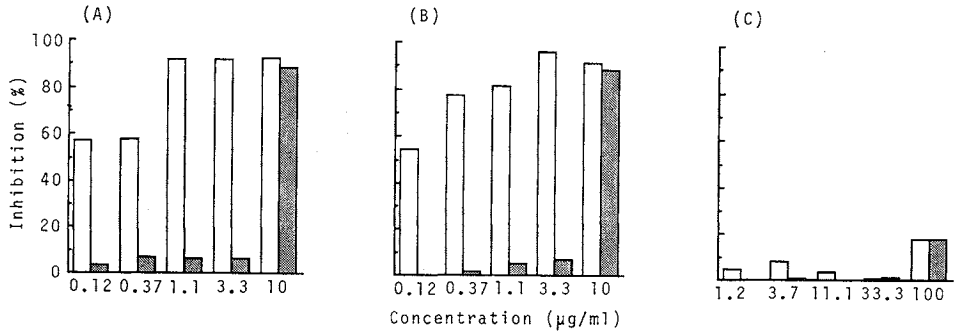


Fig. 3. The effect of curromycins A and B and AZT in chronic replication assay.

(A) Triedimycin A, (B) Triedimycin B, (C) AZT, open: % inhibition of RT, closed: % inhibition of MTT.



lymphoid lineage. Less effect could been observed with AZT in this chronic assay system. The results obtained in the chronic assay indicates that curromycins affect a late step in the virus replication cycle. This may provide a therapeutic strategy that is useful for both acute and chronic aspects of virus infection.

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MARIKO NAKAMURA
HITOSHI HONMA
MINORI KAMADA
TSUNEYA OHNO*
SETSUKO KUNIMOTO†
YOKO IKEDA†
SHINICHI KONDO†
TOMIO TAKEUCHI†

The Jikei University School of Medicine,
3-25-8 Nishi-shinbashi, Minato-ku,
Tokyo 105, Japan
† Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

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