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Inhibitory Effect of Laidlomycin on Human Immunodeficiency Virus Replication

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Laidlomycin is a polycyclic polyether antibiotic which possesses an inhibitory activity against various mycoplasma species[1], and is effective in controlling coccidiosis in chickens[2]. It is also known as selectively inhibiting the migration of hemagglutinin glycoprotein from Golgi apparatus to the cell surface in measles virus[3].

We previously reported the inhibitory activities of various polyethers on human immunodeficiency virus type 1 multiplication[4]. The compounds caused concentration-dependent inhibition of HIV replication in primary infected cultures. Some of them were also effective against chronically infected U937 cells. Herein, we report the inhibitory effect of laidlomycin for HIV-1 replication.

Laidlomycin was prepared at the Institute of Microbial Chemistry and the structure is shown at Figure 1. The experiment analyzing effects on primary infection was performed as follows. H9 cells (5×10^6 cells/ml) were pretreated with serially diluted laidlomycin at 37°C for 30 minutes and infected with HIV-1 IIIB at 100TCID50. The cells were incubated for an additional 90 minutes to permit adsorption of viral particles to cells, then diluted with fresh media (10% FBS/RPMI) to a final concentration of 1.5×10^5/ml and culture in a 96 well plate. On day 6, viral replication was monitored by reverse transcriptase (RT) assay of culture supernatant, and cytotoxicity was examined by MTT (3-4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide) assay[5]. As shown in Fig. 2, laidlomycin treatment resulted in a concentration dependent inhibition of HIV replication.

To exclude the possibility that laidlomycin directly inhibits reverse transcriptase in this assay system, we added laidlomycin directly to RT assay. No change in RT values was observed at the concentration as high as 100 μg/ml (data not shown).

We had previously classified the polyethers into two groups by their inhibitory actions against primary infection. In order to examine the mechanism of action of laidlomycin, we investigated the various treatment periods. Laidlomycin was added to H9 cells (i) 30 minutes prior to infection, (ii) 2 hours after initial exposure to virus. As shown in the data in Figure 3, laidlomycin had the same activity when H9 cells were pretreated with the compound before inoculation of HIV-1 as they did when they were added to H9 cells during the virus adsorption period. This result suggests that laidlomycin interferes with events after virus adsorption period as the virus goes through its successive replication cycle.

To determine the effect of laidlomycin on HIV gene transcription, we analyzed the expression of HIV *env* in cells by reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted from the cells (2×10^6) treated with/without laidlomycin (1 μg/ml) 3 days after infection, then 200 ng of each RNA was reverse transcribed with Omniscript (Qiagen), and subjected to PCR using specific oligonucleotide primers. We used the primer

Fig. 1. Chemical structure of laidlomycin.

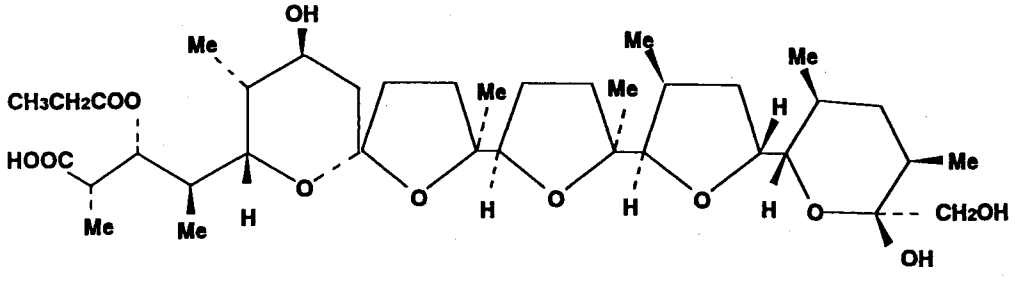
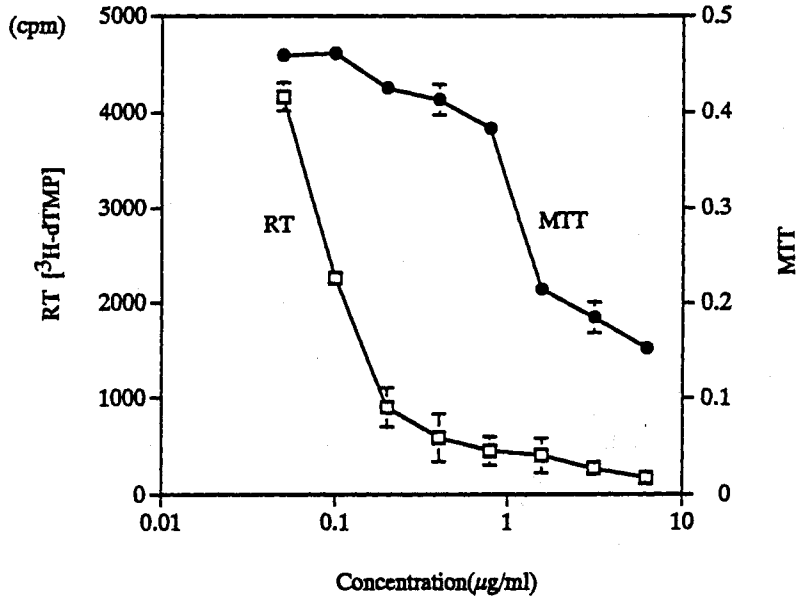
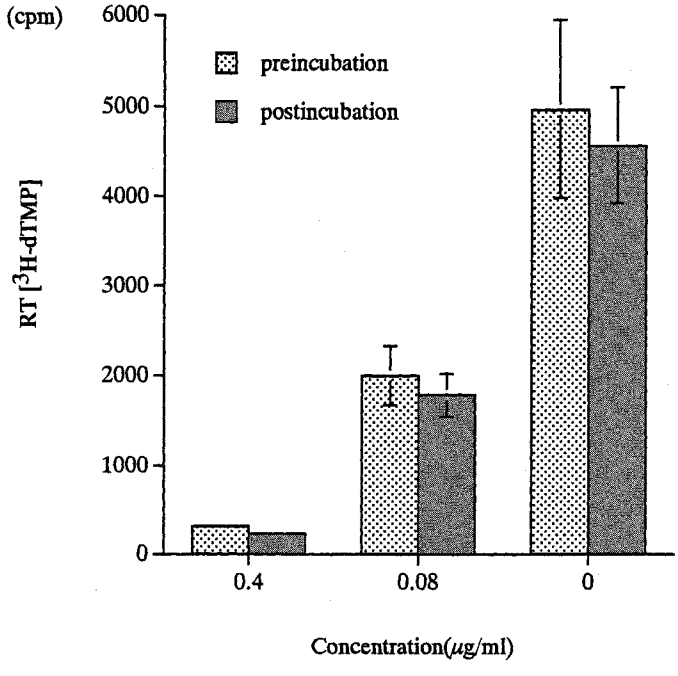


Fig. 2. Inhibitory effect of laidlomycin on HIV replication.



The data represents the means and standard deviation of triplicate experiments.

Fig. 3. Inhibitory effect of laidlomycin during virus adsorption period.



The data represents the means and standard deviation of triplicate experiments.

Fig. 4. Expression of HIV *env* mRNA in laidlomycin treated H9 cells.

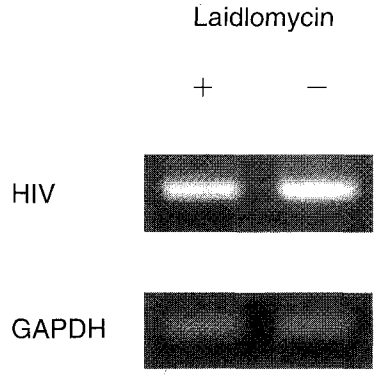
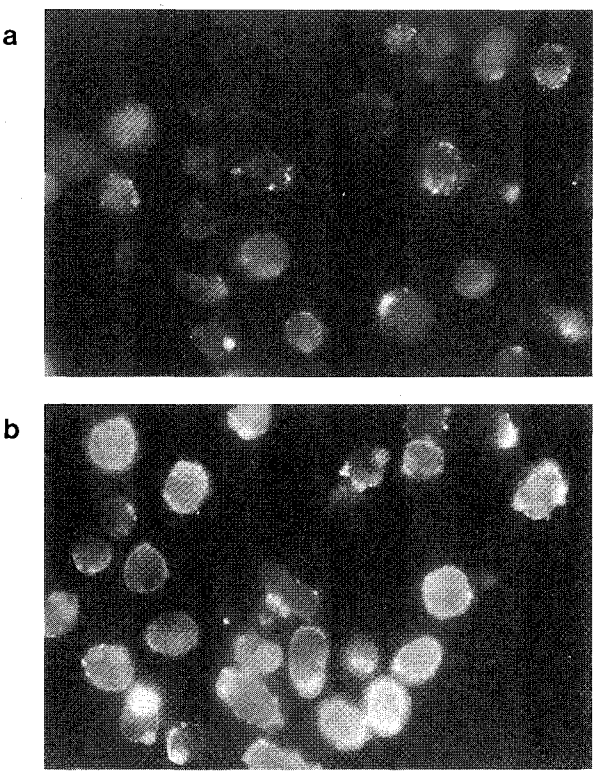


Fig. 5. Immunofluorescent images for confirmation of the presence of HIV-1 in cells treated with laidlomycin.



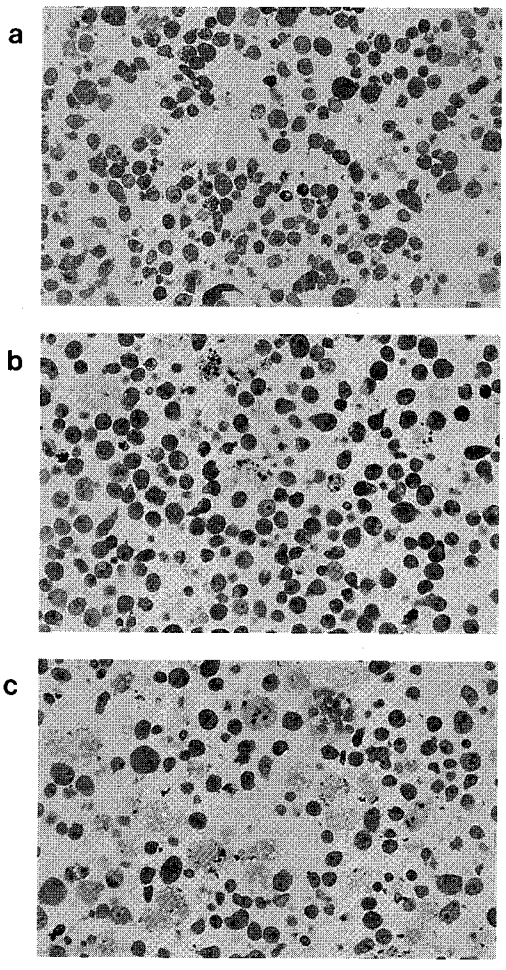
a) HIV-1 infected H9 cells treated with laidlomycin.
b) Untreated HIV-1 infected H9 cells.

pair, designed to flank the commom splice donor and acceptor site of the *env*. The sequence of *env* primers were 5′-TAGTACTGCAGTCTCGACGCAGGACTCGGC-3′ and 5′-GAATCTAGATCCCAAGGAGCATGGTGCC-3′, as previously reported by DAVIS *et al.*[6]. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was coamplified by RT-PCR as a inner control, and primers used were 5′-GAAGGTGAAGGTCGGAGTC-3′ and 5′-GAAGATGGTGATGGGATT-3′ (TaqMan PCR Reagent Kit Protocol, PE Applied Biosynthesis). Reaction mixtures were heated to 95°C for 5 minutes, and then cycled 22 times; each cycle consisted of denaturation of 95°C 30 seconds, annealing at 62°C for 30 seconds, and strand elongation at 72°C for 30 seconds. The amplified products resulting from PCR were analyzed by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining. Figure 4 shows that integrated HIV-1 proviruses are transcriptionally activated regardless of treatment of laidlomycin.

We next analyzed HIV *env* protein expression by immunofluorescence technique. H9 cells primarily infected with HIV-1 IIIB in the presence of laidlomycin (1 μg/ml) on day 4 were fixed with 1% paraformaldehyde/0.1M sodium phosphate (pH 7.2), washed with PBS, and placed on poly(L-lysine)-coated glass slides. After incubation for 30 minutes at room temperature with blocking solution, cells were incubated with mouse anti HIV gp120 monoclonal antibody[7]. After extensive washing in PBS, the cells were labeled for 1 hour at room temperature with fluorescein isothiocyanate (FITC) - conjugated goat anti mouse IgG. The cells were examined with fluorescent microscope. As shown in Fig. 5, in contrast to the strong staining on the plasma membrane in untreated cells (Fig. 5 b), gp120 expression was markedly reduced in the cells treated with laidlomycin (Fig. 5 a)

We further examined the effect of laidlomycin on the syncytium formation by IIIB infected H9 cells. Syncytium assays were performed by mixing 4×10^5 CEM cells with 4×10^4 chronically IIIB infected H9 cells in the presence of laidlomycin or monensin in a 6 well plate. The plates were incubated at 37°C for 48 hours and the number of giant cells was determined by microscopic examination. As shown in Figure 6 a~c, the syncytia was completely blocked for the cells treated with 1 μg/ml laidlomycin compared to untreated cells, and monensin showed a partial inhibition for syncytium formation at that concentration. Inhibition of syncytium formation tends to suggest that the viral glycoproteins in laidlomycin treated cells were not transported to the cell surface from Golge apparatus, and therefore not available for the binding to the CD4 molecule. It is supported by immunofluorescein staining which proved

Fig. 6. Inhibitory effects of laidlomycin and monensin on syncytium formation.



a) H9/IIIB cells cocultured with CEM in the presence of 1 μg/ml of laidlomycin.
b) H9/IIIB cells cocultured with CEM in the presence of 1 μg/ml of monensin.
c) Untreated control culture.

the reduction of viral protein on the cell surface treated with laidlomycin regardless of normal transcription of viral message.

The chemical structure of monensin is very similar to that of laidlomycin, and monensin has shown to block endoproteolytic cleavage and secondary glycosylation steps of glycoprotein gp160, which results in a reduction in syncytium formation[8,9]. Therefore, it is very possible that laidlomycin has same mechanism of action against HIV replication. The results of present studies demonstrate that laidlomycin is a potent inhibitor of HIV replication and syncytium formation in T-cell lineage cell lines.

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