SCREENING FOR INHIBITORS OF AVIAN MYELOBLASTOSIS VIRUS REVERSE TRANSCRIPTASE AND EFFECT ON THE REPLICATION OF AIDS-VIRUS

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Reverse transcriptase plays an important role in the natural cycle of retroviruses including human immunodeficiency virus (HIV), a causative agent of aquired immune deficiency syndrome (AIDS) and AIDS related complex (ARC), especially in the early stage of integration of viral genomes into cellular DNA[1,2]. Since HIV features not only reverse transcription but also cytopathic effect on OKT4$^+$ T-cells[3], natural hosts of the virus, it seems acceptable that the inhibitors of this enzyme are of potential therapeutic use against AIDS and ARC. HPA 23[4] and suramin[5], both the active inhibitors of reverse transcriptases of various species origin, have been applied to AIDS or ARC patients. Recently, the clinical application of azidothymidine was reported[6]. The reverse transcriptase catalyzed chain elongation of DNA was presumably terminated by the incorporation of azidothymidine triphosphate formed by cellular kinases.

The inhibition of reverse transcriptase by antibiotics such as rifamycin derivatives[7], adriamycin[8], daunomycin[8], distamycin A[9], sakyomicin A[10] and streptonigrin[11,12] has been reported. Most of these works were, however, carried out many years ago and under different assay conditions. In order to revaluate systematically these observations and to extend them to the wide range of antibiotics, we have been conducting mass survey for inhibitors of reverse transcriptase. According to the recent observations[13], it is suggested that the properties of reverse transcriptases of various species origin are closely related. Therefore, we used commercially available avian myeloblastosis virus reverse transcriptase as a model enzyme. For the *in vitro* assay of viral replication, the cytotoxicity of a test sample should be as low as enough not to mask the effect on viral replication. For this purpose, antibiotics which showed strong inhibition of reverse transcriptase were tested for their effects on the growth of murine lymphosarcoma L5178Y cells, selecting those suitable for the *in vitro* assay of the replication of AIDS-virus.

The details of assay method for reverse transcriptase and culture conditions for L5178Y cells were described previously[11,14]. The re-

Table 1. Biological properties of peptide group antibiotics.

Antibiotic	% Inhibition (RT)		ID_{50} (μg/ml) (L5178Y)	Applied to HIV assay
	40 μg/ml	10 μg/ml		
Actinomycin D	6	0	NT	No
Janiemycin	80	59	>4.0	Yes
Colistin	89	49	>4.0	Yes
Enduracidin A	67	50	>4.0	Yes
Enduracidin B	70	28	>4.0	Yes
Luzopeptin A	100	89	0.0003	No
Luzopeptin B	96	97	0.016	No
Luzopeptin C	100	100	0.8	Yes
Echinomycin	11	0	0.003	No
Triostin A	33	10	NT	No

RT: Reverse transcriptase. NT: Not tested.

Table 2. Biological properties of bleomycin group antibiotics.

Antibiotic	% Inhibition (RT)		ID_{50} (μg/ml) (L5178Y)	Applied to HIV assay
	40 μg/ml	10 μg/ml		
Bleomycin A_2	51	38	0.006	No
Bleomycin B_2	23	12	0.005	No
Pepleomycin	59	13	0.119	No
Platomycin A	77	70	0.0009	No
Tallysomycin A	63	31	0.028	No

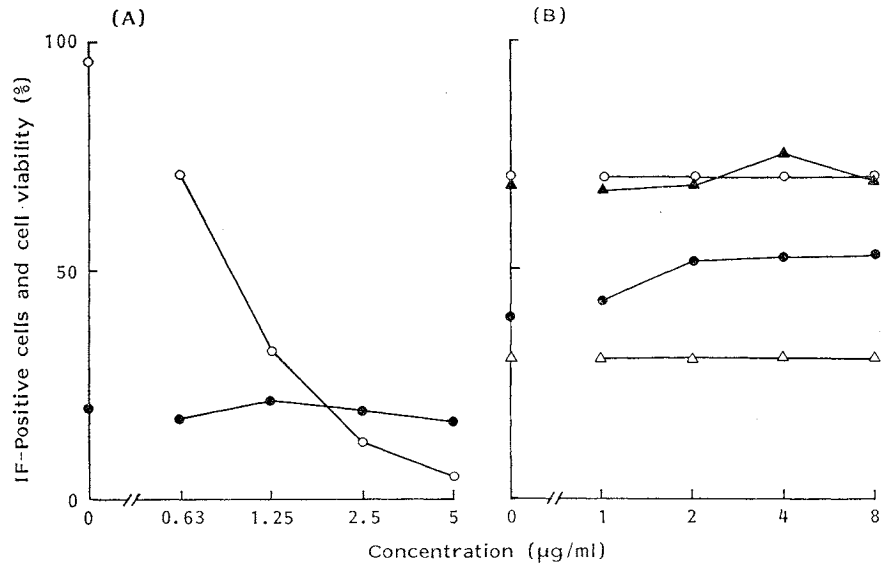
RT: Reverse transcriptase.

Fig. 1. Effects of luzopeptin C, janiemycin and colistin on the replication of HIV in MT-4 cells.

The cells infected with HIV were cultured in the presence of luzopeptin C, janiemycin or colistin at 37°C for 3 days. The viral antigen in methanol-fixed cells was stained by the indirect immunofluorescence method and the cell viability was measured by the trypan blue dye exclusion test[15].

(A) ○ Luzopeptin C, IF-positive cells; ● luzopeptin C, cell viability.

(B) ○ Janiemycin, IF-positive cells; ● janiemycin, cell viability; △ colistin, IF-positive cells; ▲ colistin, cell viability.



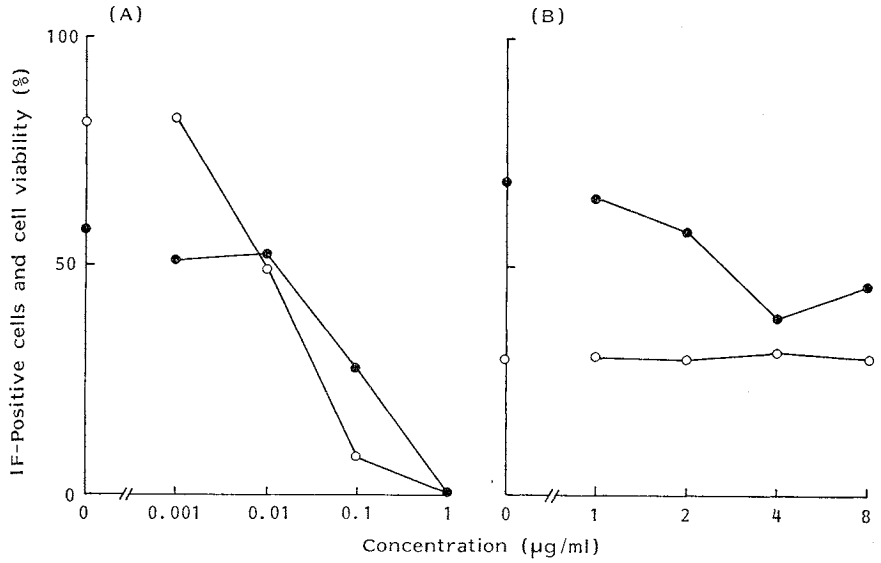
plication of AIDS-virus was assayed by the method of HARADA *et al.*[15]. Briefly, $HIV_{HTLV-III}$ was propagated in MT-4 cells in the presence of antibiotic and the expression of viral specific antigens was assayed 3 days after viral infection by the indirect immuno-fluorescence technique. The results shown in Tables 1 and 2 were presented as the percent inhibition of reverse transcriptase in the presence of either 40 or 10 μg/ml antibiotic and the concentration of each antibiotic giving 50% inhibition of cell growth. As a rule, the inhibition of reverse transcriptase over 70% at 40 μg/ml or 50% at 10 μg/ml was defined to be significant. When ID_{50} of the antibiotic selected by the enzyme assay against the growth of L5178Y cells was higher than 0.5 μg/ml, it was applied to the *in vitro* viral assay. On the basis of these criteria, 15 antibiotics were selected out of 150 antibiotics tested. Further, sakyomicin A[10], adriamycin[16] and luzopeptin C (this paper) were finally proven to be effective against the replication of AIDS-virus. In this paper, the part of results obtained with the antibiotics classified in the peptide and bleomycin groups and the streptonigrin derivatives are described.

Though the inhibition of reverse transcriptase has not yet been reported by the peptide group

Fig. 2. Effects of streptonigrin amide (STN-NH_2) and the glycine derivative (STN-Gly) on the replication of HIV in MT-4 cells.

The cells infected with HIV were cultured in the presence of STN-NH_2 (A) or STN-Gly (B) at 37°C for 3 days.

○ IF-positive cells, ● cell viability.



antibiotics with the exception of actinomycin D[17], the strong inhibition of the enzyme was observed by janiemycin, colistin and enduracidins, and in particular by luzopeptins. Luzopeptins A and B, bis and mono acetates of luzopeptin C, respectively, however, showed marked cytotoxicity against L5178Y cells in well accordance with the previous report[18]. The results shown in Fig. 1A clearly demonstrate that the replication of HIV in MT-4 cells is suppressed by luzopeptin C at higher concentrations (2.5~5.0 μg/ml), while the viability of MT-4 cells infected with HIV is not significantly affected in the same range of concentrations. In contrast, janiemycin and colistin lacked the ability to suppress the replication of HIV at concentrations up to 8 μg/ml (Fig. 1B) as well as enduracidins A and B (data not shown). The marked variation in the control values of %IF-positive cells is mainly due to the cytopathic effect of AIDS-virus and the difficulty in adjusting the rate of viral replication constant in the different experiments. In spite of the structural similarity to luzopeptins, the quinoxaline antibiotics, triostin A and echinomycin, were inactive against reverse transcriptase.

Reverse transcriptase was significantly inhibited by some of the bleomycin group antibiotics as exemplified by the results with platomycin A, the antibiotics of this group gave such a profound damage to L5178Y cells that they were not applied to the AIDS system. In addition, no significant effect on the reverse transcriptase was observed by actinomycin D under the assay conditions employed in this work.

Streptonigrin (STN-OH) is one of the most potent inhibitors of reverse transcriptase among more than 150 antibiotics tested in our screening. Although the growth of L5178Y cells was tremendously suppressed by this antibiotic, we observed that the amide derivatives at the carboxyl group were far less toxic than STN-OH without being accompanied by any marked decrease in inhibition of reverse transcriptase[11]. As examples, streptonigrin amide (STN-NH_2) and the glycine derivative (STN-Gly) were tested for their effects on the replication of HIV, since both belonged to the compounds with marginal cytotoxicity. It is evident from the results shown in Fig. 2 that the inhibition of viral replication by STN-NH_2 is secondary to the decrease in cell viability and no significant

effect on both viral replication and cell viability is observed by STN-Gly at concentrations up to 8 μg/ml. We reported previously the marked defect of STN-Gly in membrane transport[19] and this might account for the present observations.

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