COMPARATIVE STUDIES OF THE INHIBITORY PROPERTIES OF ANTIBIOTICS ON HUMAN IMMUNODEFICIENCY VIRUS AND AVIAN MYELOBLASTOSIS VIRUS REVERSE TRANSCRIPTASES AND CELLULAR DNA POLYMERASES

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The inhibition of human immunodeficiency virus (HIV) reverse transcriptase by certain antibiotics and related compounds was studied in comparison with that of avian myeloblastosis virus (AMV) reverse transcriptase and cellular DNA polymerases α and β. In general, compounds that inhibited HIV reverse transcriptase also inhibited AMV reverse transcriptase. For example, 10 μg/ml of the isoquinoline quinones used in this study inhibited approximately 80% of the activity of reverse transcriptases of HIV and AMV, but did not inhibit the activity of DNA polymerases α and β even at 50 μg/ml. AMV enzyme was more sensitive than HIV enzyme to colistin, enduracidins A and B, janiemycin, glysperin A, and thielavins A and B. The streptonigrin alkyl esters, however, inhibited HIV reverse transcriptase only. Sakyomicin A, luzopeptins, ellagic acid and suramine inhibited the activities of reverse transcriptases and cellular DNA polymerases.

Reverse transcription, a step catalyzed by the unique DNA polymerase, reverse transcriptase, is a pivotal step in the replication of retroviruses and in the stable inheritance of viral genome. An effective inhibitor of reverse transcriptase may selectively block viral replication and therefore, the enzyme has been one of the molecular targets in antiviral studies for many years. We have undertaken a search for inhibitors of avian myeloblastosis virus (AMV) reverse transcriptase among antibiotics and related compounds. Human immunodeficiency virus (HIV), a causative agent for acquired immune deficiency syndrome (AIDS), like other retroviruses, possesses reverse transcriptase. The recent development of recombinant DNA techniques made large amounts of HIV reverse transcriptase available. We wished to compare the characteristics of HIV and AMV reverse transcriptases from the viewpoint of susceptibility to the inhibitors. The selectivity of inhibitors was further studied among DNA polymerases including DNA polymerases α and β, because many properties of cellular DNA polymerases are shared by reverse transcriptase.

Materials and Methods

Materials

AMV reverse transcriptase was purchased from Seikagaku Kogyo Co., Ltd. HIV reverse trans-

criptase was isolated using the recombinant DNA method[1]. The coding sequence for the "pol" region of HIV I was inserted into a derivative of the bacterial PAS1 expression vector. The enzyme expressed in *Escherichia coli* was purified using affinity chromatography[1].

DNA polymerases α and β were isolated from leukocytes of a patient with acute myelogeneous leukemia. They were purified by successive chromatography using DEAE-cellulose, phosphocellulose and single-stranded DNA-cellulose columns according to the procedures described previously[2,3]. The purified enzymes were free of cross contamination and resemble respective viral and cellular DNA polymerases in that they displayed normal preferences of template/primer and monovalent and divalent cations, and sensitivities to certain inhibitors. Poly(dA) and poly(rA) were purchased from Sigma Chemical Company. Oligo$(dT)_{12-18}$ was obtained from Pharmacia Fine Chemicals Co. [*Methyl*-^{3}H]TTP was a product of New England Nuclear. All other chemicals were commercial products of the analytical grade.

Assay Methods

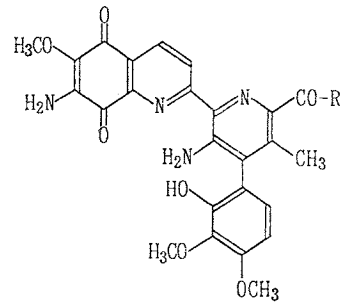
The detailed assay methods for DNA polymerases α and β were described by ALLAUDEEN *et al.*[2], and ALLAUDEEN and BERTINO[3]. AMV reverse transcriptase was assayed by the previous method[4,5]. Assay of HIV reverse transcriptase was carried out employing the reaction conditions reported by HOFFMAN *et al.*[6] with some modifications[7]. Briefly, a reaction mixture (100 μl) consisting of Tris-HCl 50 mM (pH 7.8), dithiothreitol (DTT) 5 mM, $MgCl_2$ 5 mM, KCl 150 mM, Triton X-100 0.05%, glutathione 0.3 mM, ethylene glycol bis(2-aminoethyl ether) tetraacetic acid (EGTA) 0.5 mM, poly(rA) 50 μg/ml, oligo$(dT)_{12-18}$ 12 μg/ml, [^{3}H]TTP 10.0 μM (4.8 Ci/mmol) and enzyme was incubated at 37°C for 60 minutes. Acid insoluble fractions were collected on a MF-Millipore filter paper (HA, 0.45 μm), washed several times with 5% TCA containing 2 mM sodium pyrophosphate and once with 70% ethanol and air dried. The radioactivity remaining on the filter paper was determined in a liquid scintillation counter.

Results

The results are shown in Tables 1 to 5, in which the inhibition exceeding 70% at the high dose (50 or 40 μg/ml) and/or 50% at the low dose (10 μg/ml) was defined to be significant. In a primary screening using AMV reverse transcriptase, those compounds listed in Table 1 did not show significant inhibition. Based on the inhibitory activities against HIV reverse transcriptase and DNA polymerases α and β, the compounds are further classified into three categories as follows; 1) selective inhibitors of AMV reverse transcriptase (Table 2), 2) compounds which inhibit both AMV and HIV reverse transcriptases without affecting cellular DNA polymerases (Table 3), and 3) non-specific inhibitors (Table 4).

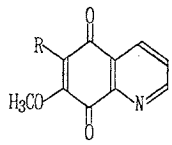
Streptonigrin and luzopeptins A, B and C are among the most potent inhibitors of AMV reverse transcriptase. Previously, we observed that the streptonigrin C-2′ amide derivatives such as the glycine derivative (STN-7) inhibited AMV reverse transcriptase to the same extent as streptonigrin[4], whereas streptonigrin methyl

Fig. 1. Structures of streptonigrin derivatives.

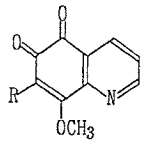


Streptonigrin	R=OH
STN-1	R=OCH_3
STN-2	R=$O(CH_2)_3CH_3$
STN-3	R=$OCH(CH_3)_2$
STN-4	R=$O(CH_2)_3CH_3$
STN-5	R=$O(CH_2)_2N(CH_3)_2$
STN-6	R=$O(CH_2)_3N(CH_3)_2$
STN-7	R=$NHCH_2COOH$
STN-8	R=$NHCH(CH_3)COOH$
STN-9	R=$NH(CH_2)_2N(CH_3)_2$
STN-10	R=$NH(CH_2)_3N(CH_3)_2$

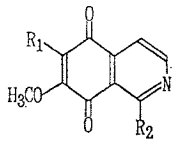
Fig. 2. Structures of quinoline quinones and indole quinone.



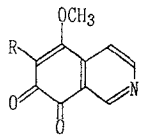
QQ-1 R=H
QQ-2 R=CH_3



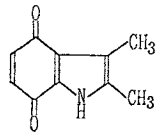
QQ-3 R=H
QQ-4 R=CH_3



QQ-5 R_1=R_2=H
QQ-6 R_1=H R_2=CN
QQ-7 R_1=CH_3 R_2=H
QQ-8 R_1=CH_3 R_2=CN
QQ-9 R_1=R_2=CH_3



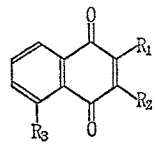
QQ-10 R=H
QQ-11 R=CH_3



IQ-1

ester (STN-1) did not inhibit the enzyme activity[4,8]. As well as STN-7, the other streptonigrin amide derivatives, STN-8, 9 and 10, inhibited the reverse transcriptases from AMV and HIV to an equal extent (Table 3). Four of the ester derivatives of streptonigrin, STN-1, 2, 3 and 4, inhibited only the reverse transcriptase of HIV but the other three enzymes were not inhibited by them (Table 5). This observation is consistent with the report by CHIRIGOS *et al.*[8] who showed that STN-1 did not inhibit the AMV reverse transcriptase activity. It is worth noting that the parent compound, streptonigrin, inhibited the activity of AMV reverse transcriptase but not cellular DNA polymerases examined[6]. Although the ester derivatives, STN-1 to STN-4, lacked the ability to inhibit AMV reverse transcriptase, the dimethylamino group endowed the ester derivatives with inhibitory activity against not only AMV reverse transcriptase but also HIV enzyme (see the results for STN-5 and 6 in Table 3). We have included the quinoline quinones in our study because of their structural resemblance to streptonigrin[9~12]. As in the case of streptonigrin, they are potent inhibitors of both AMV and HIV reverse transcriptases, but not cellular DNA polymerases (Table 3). For example, the quinones used in this study, at 10 μg/ml, inhibited the reverse transcriptases of HIV and AMV approximately 80%; they did not inhibit the DNA polymerases α and β activities even at 50 μg/ml.

Fig. 3. Structures of naphthoquinones.



NQ-1 R_1=R_2=R_3=H
NQ-2 R_1=R_2=H R_3=OH
NQ-3 R_1=R_2=Cl R_3=H
NQ-4 R_1=CH_3 R_2=R_3=H
NQ-5 R_1=C_2H_5 R_2=R_3=H
NQ-6 R_1=OH R_2=R_3=H
NQ-7 R_1=OH R_2=$(CH_2)_2COOH$ R_3=H
NQ-8 R_1=OH R_2=$(CH_2)_2COOC_2H_5$ R_3=H

Sakyomicin A showed moderate inhibition of AMV reverse transcriptase[13]; the inhibition values at 40 and 10 μg/ml were 63 and 26%, respectively. The role of naphthoquinone moiety as the minimum requisite for this activity was confirmed previously[14]. Besides AMV reverse transcriptase, HIV enzyme was inhibited by sakyomicin A and the naphthoquinone derivatives such as NQ-1, 2, 3

Table 1. Antibiotics and other compounds not showing significant inhibition of AMV reverse transcriptase.

Peptides	Ezomycins A1 and A2	DNA Gyrase Inhibitors
Actinomycin D	Formycin A	Cumermycin A1
Aculeacin A	Herbicidin A	Nalidixic acid[a]
Amidinomycin	Minimycin	Norfloxacin[a]
Bacitracin	Neplanomycins A, B, C, D and F	Noboviocin[a]
Capreomycin	Nucleocidin	
Echinomycin	Oxamicetin	Miscellaneous
Empedopeptin	Polyoxins A and C	Azomycin A
Ilamycin	Tubercidin	Cerexin A
Pyridomycin		Chartreucin
Siomycin A	Ansamycins	Chicamycin
Triostin A	Ansamitocin	Chloramphenicol
	Geldanamycin	Chromostin
Aminoglycosides	Rifampicin	Deoxyfrenolicin
Butirosine A		Glysperins B and C
Glebomycin	Macrolides	Histidinomycin
Inosamycins A, C and D	Cirramycin A	Isohematinic acid
Neomycin B	Erythromycin	Julymycin
Paromomycin	Kitasamycin	Lincomycin
Sorbistin A1		Miharamycin A
Xylostatin	Bleomycins	Mitomycins A and C
	Bleomycins A2, A5 and B2	Mycoplanecin
Anthracyclines	Peplomycin	Novomycin
Aclarubicin	Tallysomycins A and B	Pholipomycin
Baumycin		Pyrrolnitrin
Daunorubicin	Polyenes	Showdomycin
Figaroic acid	Trichomycin	Streptolydigin
Marucellomycin		Tomaymycin
	Pluramycins	Trindamycin
Tetracyclines	Neopluramycin	Vancomycin
Chlortetracycline	Pluramycin	α-Naphthoflavone[a]
	Neothramycin	2′-Hydroxychalcone[a]
Nucleosides		Betulin[b]
Amicetin	Polyethers	D-(+)-Catechin[b]
Cytarabine[a]	Dianemycin	Curucumin[b]
Blasticidin S	Leusermycin	Glycyrrhizin[b]
Bredinin	Lonomycin A	Ursolic acid[b]
Cadeguomycin	Moyukamycin	

[a] Synthetic compounds, [b] compounds of plant origin.

and 4 (Table 4).

Peptide group antibiotics showed a variety of responses. Colistin, enduracidins A and B, and janiemycin inhibited AMV reverse transcriptase selectively (Table 2), whereas luzopeptins A, B and C[15] inhibited all the enzymes tested potently and non-specifically (Table 4). In spite of structural resemblance to luzopeptins, echinomycin and triostin A were not inhibitory against AMV reverse transcriptase (Table 1).

Suramin as well as ellagic acid inhibited all the four enzymes (Table 4). Doxorubicin[16], glysperin A, and thielavins A and B were inhibitory to only AMV reverse transcriptase (Table 2).

Table 2. Antibiotics and other compounds inhibiting preferentially AMV reverse transcriptase.

	Inhibition (%)									
	DP α		DP β				HIV-RT		AMV-RT	
	poly(dA)/oligo(dT)[a]		poly(dA)/oligo(dT)		poly(rA)/oligo(dT)		poly(rA)/oligo(dT)		poly(rA)/oligo(dT)	
	50[b]	10	50	10	50	10	50	10	40	10
Doxorubicin	31	1	0	6	21	0	27	0	70	30
Colistin	0	0	0	0	0	0	21	17	89	49
Enduracidin A	0	0	0	6	0	0	32	13	67	50
Enduracidin B	0	0	5	1	0	0	27	1	70	28
Glysperin A	0	0	6	0	0	0	6	0	74	60
Janiemycin	0	0	7	2	0	0	25	0	80	29
Thielavin A	24	0	0	0	0	0	13	4	89	56
Thielavin B	0	0	0	0	0	0	8	0	80	56
1,4-Benzoquinone[c]	78	60	40	0	92	20	15	0	95	30
NQ-5[c]	40	40	17	4	0	0	0	2	85	80
Fisetin[d]	69	43	27	6	89	11	23	10	100	94

Abbreviations: DP, DNA polymerase; HIV-RT, HIV reverse transcriptase; AMV-RT, AMV reverse transcriptase.
[a] Template/primer, [b] concentration (μg/ml), [c] synthetic compounds, [d] compound of plant origin.

Table 3. Antibiotics and other compounds inhibiting preferentially AMV and HIV reverse transcriptases.

	Inhibition (%)									
	DP α		DP β				HIV-RT		AMV-RT	
	Poly(dA)/oligo(dT)[a]		Poly(dA)/oligo(dT)		Poly(rA)/oligo(dT)		Poly(rA)/oligo(dT)		Poly(rA)/oligo(dT)	
	50[b]	10	50	10	50	10	50	10	40 (20)	10
Streptonigrin	44	27	0	0	29	3	87	81	93	84
STN-5	37	35	0	0	6	0	93	83	84	75
STN-6	32	38	0	0	8	0	83	84	91	71
STN-7	29	28	0	0	0	0	88	86	93	91
STN-8	34	23	0	0	16	11	84	79	(81)	76
STN-9	21	26	27	16	17	0	90	88	(61)	49
STN-10	21	21	19	12	12	6	90	88	(77)	67
QQ-1	43	28	26	7	25	22	92	92	87	85
QQ-2	25	19	4	0	17	8	87	83	81	67
QQ-3	43	33	1	8	48	23	88	88	90	85
QQ-4	38	28	20	3	47	26	89	88	90	89
QQ-5	0	0	3	0	4	0	86	84	85	81
QQ-6	0	0	0	0	11	0	88	87	82	83
QQ-7	0	0	0	0	6	5	93	85	75	60
QQ-8	14	0	0	0	16	23	91	90	84	73
QQ-9	0	0	0	0	13	11	89	67	68	54
QQ-10	14	0	0	0	23	14	89	85	91	77
QQ-11	25	16	0	0	30	25	92	82	92	88
Sakyomicin A	40	23	7	7	25	18	59	0	63	26
NQ-1	59	41	2	0	34	9	92	89	81	65
NQ-2	56	45	0	0	40	12	95	85	94	82
NQ-3	66	23	0	0	16	0	85	73	76	34
NQ-4	44	32	0	0	16	13	70	30	77	32

Abbreviations: See Table 2.

STN-5~STN-10: Streptonigrin derivatives, QQ-1~QQ-11 and NQ-1~NQ-4: synthetic compounds.

[a] Template/primer, [b] concentration (μg/ml).

Table 4. Antibiotics and other compounds inhibiting DNA polymerases non-specifically.

	Inhibition (%)									
	DP α		DP β				HIV-RT		AMV-RT	
	Poly(dA)/oligo(dT)[a]		Poly(dA)/oligo(dT)		Poly(rA)/oligo(dT)		Poly(rA)/oligo(dT)		Poly(rA)/oligo(dT)	
	50[b]	10	50	10	50	10	50	10	40	10
Luzopeptin A	93	66	91	62	94	74	85	68	100	89
Luzopeptin B	100	100	100	99	98	98	100	95	96	97
Luzopeptin C	98	100	100	99	99	99	100	99	100	100
Ellagic acid[c]	81	0	59	0	99	30	75	36	94	95
Suramin[c]	71	31	0	0	98	30	100	84	96	81
NQ-6[d]	22	27	3	0	0	0	0	0	0	5
NQ-7[d]	26	5	22	16	18	9	0	0	0	11
NQ-8[d]	16	6	16	8	7	0	0	0	1	5
IQ-1[d]	40	2	0	0	15	17	22	9	18	0

Abbreviations: See Table 2.
[a] Template/primer, [b] concentration (μg/ml), [c] compounds of plant origin, [d] synthetic compounds.

Table 5. Streptonigrin derivatives inhibiting HIV reverse transcriptase exclusively.

	Inhibition (%)									
	DP α		DP β				HIV-RT		AMV-RT	
	Poly(dA)/oligo(dT)[a]		Poly(dA)/oligo(dT)		Poly(rA)/oligo(dT)		Poly(rA)/oligo(dT)		Poly(rA)/oligo(dT)	
	50[b]	10	50	10	50	10	50	10	40	10
STN-1	37	29	22	7	13	0	68	23	12	0
STN-2	17	8	18	13	9	8	66	24	18	0
STN-3	30	20	17	17	13	11	74	7	18	6
STN-4	38	30	0	0	3	0	67	0	0	0

Abbreviations: See Table 2.
[a] Template/primer, [b] concentration (μg/ml).

Discussion

Many compounds used in this study inhibited the reverse transcriptases of HIV and AMV. However, some compounds listed in Table 2 inhibited preferentially AMV reverse transcriptase; the ester derivatives of streptonigrin, on the other hand, inhibited HIV reverse transcriptase only. Such differences between the reverse transcriptases of HIV and AMV and the structure activity relationship data obtained using these compounds may be exploited in designing better and more selective inhibitors.

As for quinone compounds such as streptonigrin and sakyomicin A, we have proposed the existence of a specific site of interaction, referred to as a "quinone pocket", on AMV reverse transcriptase[17]. On the basis of the results shown in Table 3, we postulate that HIV reverse transcriptase also possesses a "quinone pocket" which is concerned with the sensitivity of this enzyme to quinone compounds of both synthetic and natural origins.

Among the peptide group antibiotics, luzopeptins showed a significant inhibition of HIV reverse transcriptase; however they also inhibited the other enzymes used in this study. The effects of luzopeptin C, janiemycin and colistin on the *in vitro* replication of HIV have been reported earlier[15]. The replication of HIV in MT-4 cells was suppressed in the presence of luzopeptin C at the concentrations not affecting cell viability. Meantime, no effect on viral replication and cell viability was observed in the case of the other antibiotics. Which function of luzopeptin C plays a crucial role in the inhibition of *in vitro* HIV replication, however, remains to be clarified.

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