Antiviral Research, 15 (1991) 205–216

ADONIS 016635429100146C

AVR 00470

Antiretroviral activities of anthraquinones and their inhibitory effects on reverse transcriptase

Hirotaka Higuchi[1], Kazuya Mori[1], Akihisa Kato[2], Tetsuo Ohkuma[1], Tohru Endo[1], Hideko Kaji[2] and Akira Kaji[3]

[1]*Research Institute for Molecular Genetics, Tsumura and Co., Ibaraki, Japan;* [2]*Department of Pharmacology, Jefferson Medical College, Philadelphia, Pennsylvania, U.S.A. and* [3]*Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.*

(Received 12 July 1990; accepted 26 November 1990)

Summary

Alizarin complexone (AC), alizarin Red S (ARS) and various other anthraquinones were evaluated for their inhibitory effects on Rous-associated virus 2 reverse transcriptase (RAV-2 RT). Some 1,2-dihydroxyanthraquinones were active against this enzyme and AC was the most potent inhibitor among these compounds [50% inhibitory concentration (IC_{50}): 3.8 μg/ml]. AC slightly inhibited Rous sarcoma virus RT (RSV RT) and human immunodeficiency virus type 1 RT (HIV-1 RT) (IC_{50}: 100 μg/ml and 45 μg/ml, respectively). However, AC efficiently inhibited focus formation by Rous sarcoma virus (RSV) and cytopathogenicity of human immunodeficiency virus type 1 (HIV-1). Simultaneous administration of AC with RSV to newborn chickens also delayed tumor induction by RSV.

Alizarin complexone; Reverse transcriptase; Retrovirus

Introduction

The search for antiretroviral agents has been widely carried out using retrovirus-associated reverse transcriptase (RT). To date, various compounds such as antibiotics (Wu et al., 1980; Okada et al., 1986; Inouye et al., 1987b), nucleoside analogues (Chen and Oshana, 1987; Cheng et al., 1987; Clair et al., 1987), alkaloids and tannins (Sethi, 1983, 1985; Kakiuchi et al., 1985), pyrophosphate analogues (Vrang and Öberg,

Correspondence to: A. Kaji, Dept. of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6076, U.S.A.

1986; Wondrak et al., 1988) and anionic dyes (Balzarini et al., 1986) have been found to inhibit this enzyme. Among these compounds, the following have been reported to be effective against avian myeloblastosis virus (AMV) RT: streptonigrin (Chirigos et al., 1973), which contains a quinoline quinone moiety in the skeleton; sakyomycin A and juglon (Inouye et al., 1987a) which belong to a family of naphthoquinones; daunomycin and adriamycin (Dhananjaya and Antony, 1987) which are anthracycline antibiotics. These results indicate that the compounds containing a paraquinone moiety in the skeleton appear to be active against RT. However, anthraquinones such as natural dyes and related compounds have not been studied extensively.

In this communication, we report that from over fifty anthraquinones tested, alizarin complexone (AC) was the most potent inhibitor of Rous-associated virus 2 (RAV-2) RT. This compound exhibited antiviral activities against Rous sarcoma virus (RSV) and human immunodeficiency virus type 1 (HIV-1).

Materials and Methods

Materials

Alizarin complexone (MW=385) and alizarin (MW=240) were purchased from Katayama Chemical Industries. Alizarin Red S (MW=342) was from Wako Pure Chemical Industries. Quinalizarin (MW=272) and purpurin (MW=256) were from Tokyo Kasei Kogyo. RAV-2 RT was purchased from Takara Shuzo. DNA polymerase I Minimal Nuclease (*E. coli* CM5197), Poly(rA)·pdT$_{12-18}$, unlabeled deoxynucleoside triphosphates and dideoxythymidine triphosphate (ddTTP) were obtained from Pharmacia. Poly(rA)·pdT$_{15}$ was purchased from Boehringer Mannheim and [^{3}H]thymidine triphosphate was from Amersham International. The plasmid expressing HIV-1 RT was kindly provided by Dr Hughes (National Cancer Institute – Frederick Cancer Research Facility). Foscarnet (trisodium phosphonoformate) was kindly provided by Dr Öberg (Astra Alab AB Research and Development Laboratories Antiviral Chemotherapy).

Cells and viruses

Chicken embryo fibroblast (CEF) culture used in these experiments was prepared as described previously (Vogt, 1969). MT-4 cells, a human CD4+ cell line carrying human T-cell lymphotropic virus type 1 (Miyoshi et al., 1982) and Sup-T1 cells, a human malignant T-lymphoid cell line (Smith et al., 1984) were used for the anti-HIV-1 assay. MT-4 cells were kindly provided by Dr J.A. Levy (University of California, San Francisco, CA) and Sup-T1 cells were kindly provided by Drs F. Gonzalez and J.A. Hoxie (University of Pennsylvania, Philadelphia, PA). These cells were grown in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco). HIV-1 (strain HTLV-IIIB) was prepared from culture supernatant of H9/HTLV-IIIB cells (American Type Culture Collection, ATCC) (Popovic et al., 1984). The virus titer of the supernatant was 10^4 TCID$_{50}$ on MT-4 cells.

Enzyme assays

The reaction mixture (50 µl) for the RAV-2 RT assay contained 50 mM Tris·HCl (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 3 mM dithiothreitol (DTT), 0.5 µg of poly-(rA)pdT_{12-18}, 10 µM [^{3}H]TTP (30 GBq/mmol) and 0.1 U of RAV-2 RT. Where indicated, test compounds were added at various concentrations as described in each experiment. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by cooling to 0°C followed by addition of 20 µl of 0.1 M EDTA (pH 8.0). Incorporation of [^{3}H]TTP into polymers was determined with the use of DE81 Whatman ion exchange filter papers as previously described (Lindell et al., 1970). Test compounds were generally dissolved in DMSO, and the DMSO solution containing various concentrations of each compound was added to the reaction mixture before the addition of the enzyme. For a negative control, only DMSO was added to the reaction mixture. In all cases, the final concentration of DMSO was 5% in the reaction mixture.

HIV-1 RT assay was performed essentially according to the method previously reported (Hizi et al., 1988). HIV-1 RT was prepared with the *E. coli* expression system as described by Hizi et al. (1988). RSV RT assay was performed as follows: the reaction mixture (100 µl) contained 50 mM Tris·HCl (pH 8.1), 50 mM NaCl, 3 mM DTT, 5 mM $MgCl_2$, 100 µM [^{3}H]TTP (37 GBq/mmol), 1% Triton X-100, 2 µg of poly-(rA)dT_{15} and 30 µl of RSV [2.3×10^6 focus forming units (FFU)/ml]. The reaction mixture was incubated at 37°C for 1 h. Duplicate aliquots (45 µl) of each reaction mixture were spotted onto Whatman 3MM filter papers, and the trichloroacetic acid-insoluble radioactivity was measured with a Beckman scintillation counter.

DNA polymerase assay was performed essentially according to the method reported by Hanajima et al. (1985).

Assay of RSV focus formation and RSV production

To establish the in vitro cytotoxic effects of various test compounds against CEF cells, the cells were seeded at 8.75×10^4 cells/0.1 ml/well into a 96-well microplate (Coster) and incubated with various test compounds at 37°C for 6 h in a 5% CO_2 atmosphere. Medium was removed and the cells were washed with PBS (phosphate buffer saline: 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.4). Medium, 90 µl (Ham's F-10 supplemented with 5% FBS and 1% chicken serum) and test compounds which dissolved in water or DMSO (10 µl) were added to each well. At the same time, color blank wells which contained several diluted solutions of compounds without the cells were prepared. The microplate was incubated for 4 days at 37°C in a 5% CO_2 atmosphere. Viable CEF cells were measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method, as previously reported (Mosmann, 1983; Pauwels et al., 1988).

For the in vitro antiviral test against RSV, CEF cells (3.5×10^5 cells/1 ml/well) were incubated in a 24-well microplate (Falcon) at 37°C for 6 h in a 5% CO_2 atmosphere. The cells were infected with RSV [multiplicity infection (MOI): 0.0001] for 2 h in the presence of test compounds. The focus assay was performed as described previously

(Vogt, 1969). Each experimental value was obtained from the average of four sets of data, and the experiments were repeated at least twice.

The inhibitory effect of various compounds on the production of RSV was tested essentially as described above except for slight modifications. Briefly, CEF cells were infected with RSV (MOI: 0.01) simultaneously with addition of test compounds. RSV was removed from the medium 2 h postinfection (p.i.), followed by the addition of fresh medium containing test compounds. The infected cells were exposed to test compounds for 24 h at which time the first samples were taken for the focus assay. At this point, the medium was changed for fresh medium without test compounds and the cells were further incubated. The second samples for the focus assay were taken at 48 h p.i. The test compounds in the virus solution were sufficiently diluted to have no influence on the focus assay.

Measurement of RSV-induced tumor formation

The effect of AC on RSV tumor formation was examined with SPAFAS chicken according to the method reported by Gyles et al. (1967). One group consisted of 4 chickens (3 days old). AC was dissolved in saline and the pH was adjusted to 7.4 by NaOH solution. Each chicken was inoculated subcutaneously (s.c.) in the wing web with 0.1 ml of virus solution (1×10^3 FFU/ml) containing various concentrations of AC. AC was administered s.c. consecutively for 6 days after virus inoculation. Tumor size was measured once daily from day 1 to day 14 p.i., and the mortalities of the chickens were recorded.

Assay of anti-HIV activities and cytotoxic activities of various compounds

The procedure for measuring anti-HIV activity in MT-4 cells has been described (Pauwels et al., 1988).

HIV-1 solution adjusted to 20 $TCID_{50}$ well was used. Anti-HIV activities and cytotoxicities of the compounds with Sup-T1 cells were determined by the similar procedure for MT-4 cells at day 7 p.i.

Results

Inhibitory effects of anthraquinones on reverse transcriptase from various sources

As shown in Table 1, among over fifty anthraquinones tested, AC, ARS, quinalizarin, alizarin and purpurin were effective against RAV-2 RT. Their IC_{50} ranged from 3.8 to 17.5 μg/ml. Among these compounds, AC was the most potent inhibitor of RAV-2 RT. These active compounds were further examined for their inhibitory effects on HIV-1 RT, RSV RT and *E. coli* DNA polymerase I. The inhibitory effects of AC against these three enzymes were not as pronounced as their activity against RAV-2 RT. The most potent inhibitors of HIV-1 RT and RSV RT were purpurin (IC_{50}: 4.7 μg/ml) and quinalizarin (IC_{50}: 20.0 μg/ml), respectively. It is clear from Table 1

TABLE 1

Inhibitory effects of various compounds on reverse transcriptases and *E. coli* DNA polymerase I

Compound	IC_{50} (μg/ml)[a]			
	RAV-2 RT	HIV-1 RT	RSV RT	DNA pol I
ddTTP	0.14	0.04	0.97	33
Foscarnet	10.0	0.13	16.0	440
AC	3.8	45.0	100.0	230
ARS	7.0	8.5	40.0	58
Quinalizarin	7.7	9.3	20.0	27
Alizarin	12.5	19.0	>300.0	20
Purpurin	17.5	4.7	80.0	20

[a]Effectiveness of inhibitors is expressed as concentrations causing 50% inhibition. The reverse transcriptase and DNA polymerase I activities were measured as described in Materials and Methods.

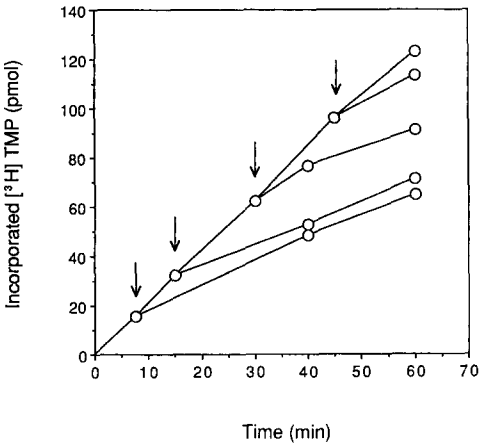


Fig. 1. Effect of delayed addition of AC in the RAV-2 RT reaction. AC (4 μg/ml) was added at the time indicated by the arrows. The assay conditions are described in Materials and Methods.

that RAV-2 RT, HIV-1 RT and RSV RT had different sensitivities to the anthraquinones.

Analysis of the inhibitory effects of AC

In the experiment described in Fig. 1, the effect of AC on RAV-2 RT was examined after the reaction was initiated. It is clear from this figure that the reaction was linear over 30 min and DNA synthesis was inhibited at all indicated times after the onset of the reaction. This result suggests that after the enzyme has formed a complex with the template-primer and the substrate, AC can still exert an inhibitory action.

Anti-RSV effects of anthraquinones in vitro

Inhibitory effects of AC, ARS, quinalizarin, alizarin and purpurin on CEF cell growth and RSV focus formation were studied. As shown in Table 2, the CC_{50}/IC_{50}

TABLE 2

Effects of various inhibitors on focus formation of RSV

Compound	IC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	Ratio (CC_{50}/IC_{50})
AZT	2.0	30.0	15.0
AC	8.5	180.0	21.2
ARS	40.0	180.0	4.5
Alizarin	NE[c]	125.0	–
Quinalizarin	NE	250.0	–
Purpurin	NE	60.0	–

[a]See legend to Table 1.
[b]CC_{50} is defined as the inhibitor concentration causing 50% inhibition of cell growth by MTT assay, as described in Materials and Methods.
[c]No effect of test compound was shown under the condition where cytotoxic effect was not observed.

TABLE 3

Inhibition of RSV production by AC

Compound (μg/ml)	Virus titer at hours postinfection (FFU[a]/10 μl)			
	0–24 h	% inhibition	24–48 h	% inhibition
Control	14.8 ± 4.3[b]	0	1190.0[b]	0
AZT 5.0	1.5±0.5	89.9	12.5 ± 1.3	99.0
10.0	<1.0	>93.2	<1.0	>99.0
AC 15.6	3.5 ± 2.1	76.4	185.0 ± 19.0	84.5
31.3	3.7 ± 0.5	75.0	86.7 ± 44.6	92.7
62.5	1.0	93.2	16.8 ± 9.3	98.6

[a]Focus forming units.
[b]Results are expressed as means ± SD.

ratio indicates the effectiveness of these agents as possible antiviral drugs. AC appears to be the most effective agent, followed by AZT. Despite some anti-RT activities, quinalizarin, alizarin and purpurin had no antiviral activities in this system. In a separate experiment a concentration-dependent inhibition of RSV focus formation by AC without cytotoxic effect was observed (data not shown).

Table 3 shows the inhibitory effect of AC on RSV production. In this experiment, CEF cells were infected with RSV at an MOI of 0.01 in the presence of various concentrations of AC. The amounts of virus released into the medium at 24 h and at 48 h p.i. were determined by the focus assay as described in Materials and Methods. It can be seen from Table 2 that at 62.5 μg/ml AC led to 93% inhibition of virus production from 0–24 h and 99% between 24 and 48 h p.i. A strong inhibitory effect on virus production was also observed with AZT.

To examine whether AC exerted a direct effect on virus particles or not, RSV was incubated at 4°C for 3 h in the presence or absence of AC (31.3 μg/ml). Virus suspensions were then diluted to the extent that AC did not influence focus formation as determined in the preceding experiment. No appreciable direct effect of AC was observed on RSV in this experiment (data not shown).

Delayed tumor development due to AC treatment

Antiviral activity of AC in vivo was determined with chickens infected with RSV. It can be seen from Fig. 2 that the administration of AC delayed the development of a tumor on the wing web. At the dosage of 200 mg/kg per day, tumor development was delayed by as much as 6 days. Each chicken was followed until it died due to the tumor. The mean survival time of the control group without the treatment was 14.0±1.4 days, while treatment with AC prolonged this survival time to 18.5±2.4 days. The same treatment with uninfected chickens did not cause any deleterious effect.

Anti-HIV activity of AC and ARS in vitro

As shown in Table 4, AC inhibited the cytopathogenicity of HIV-1 on Sup-T1 cells. The effect, however, was not marked, in that the maximum protection was only 37.0% at 50 μg/ml. There was no appreciable anti-HIV activity of ARS against infected Sup-T1 cells. AZT had a potent inhibitory effect on the cytopathogenicity of HIV-1 in Sup-T1 cells. In the case of the MT-4 cell system, the protective effects of AC and ARS were significant. Thus, AC inhibited HIV-1-induced cytopathogenicity by 50% at 19.0 μg/ml, and ARS did so at 47.5 μg/ml. In any case AC and ARS were much less active than AZT. Alizarin, purpurin and quinalizarin had no effect on the cytopathogenicity of HIV-1 (data not shown).

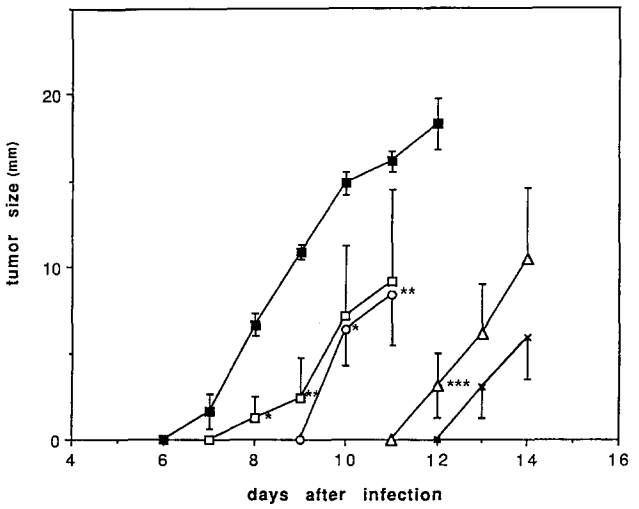


Fig. 2. Delayed tumor development in chickens due to AC treatment. AC was administered simultaneously with virus infection at the wing web. For an additional 6 days, AC was further administered daily as described in Materials and Methods. Tumor size was calculated by [the longest diameter of tumor (mm) + the shortest diameter of tumor (mm)]/2. *P* values were determined by Student's *t* test. *, $P<0.01$; **, $P<0.05$; ***, $P<0.001$. Each error bar on points was expressed as means ± SE of four chickens. Dosages of AC were: ■, 0 mg/kg; □, 3.13 mg/kg; ○, 12.5 mg/kg; Δ, 50.0 mg/kg; ×, 200 mg/kg.

TABLE 4

Inhibitory effects of AC and ARS on cytopathogenicity (CPE) of HIV-1 in Sup-T1 cells and MT-4 cells

Compound	Cells	IC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	Ratio (CC_{50}/IC_{50})
AZT	SUP-T1	0.475	>250	>526
	MT-4	0.0014	4.8	3428.6
AC	SUP-T1	–[c]	64.5	<1
	MT-4	19.0	79.0	4.2
ARS	SUP-T1	NE[d]	21.5	<1
	MT-4	47.5	120.0	2.5

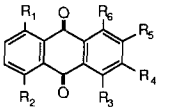
Data represent mean values for two to three separate experiments.

[a]50% Inhibitory concentration, achieving a 50% protection of Sup-T1 cells and MT-4 cells against the cytopathic effect of HIV-1.

[b]50% Cytotoxic concentration: required to reduce the viability of normal uninfected Sup-T1 cells and MT-4 cells by 50%.

[c]At 50 μg/ml AC inhibited cytopathogenicity by 37%.

[d]NE: No effect.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
AC	H	H	H	$CH_2N(CH_2COOH)_2$	OH	OH
ARS	H	H	H	SO_3Na	OH	OH
Quinalizarin	OH	OH	H	H	OH	OH
Alizarin	H	H	H	H	OH	OH
Purpurin	H	H	OH	H	OH	OH

Discussion

During the search for inhibitors of RAV-2 RT, it was found that anthraquinones with hydroxy groups corresponding to R_5 and R_6 groups were active against this enzyme (Table 1 and Fig. 3). Most of those which did not have hydroxy groups corresponding to R_5 and R_6 (for example, anthraquinone and anthrarufin) were not active (data not shown). AC, which is the most potent inhibitor of RAV-2 RT among the anthraquinones tested, is known to be a chelating reagent for various metal ions and its chelating activity with cerium (III) was used for the determination of the fluoride ion (Belcher et al., 1959; Leonard and West, 1960). However, the chelating action of AC did not play a role in the inhibitory effect of this compound on RAV-2 RT. Thus, inhibitory effect of AC was not influenced by increasing concentrations of Mg^{2+} ion up to 100 μM (data not shown).

Comparison of the inhibitory effects of active compounds on RTs from various sources revealed interesting features. RAV-2 RT was much more sensitive to AC

(IC_{50}: 3.8 μg/ml) than RSV RT or HIV-1 RT (IC_{50}: 100 μg/ml and 45 μg/ml, respectively). On the other hand, purpurin had a much higher activity on HIV-1 RT (IC_{50}: 4.7 μg/ml) and quinalizarin was the most potent inhibitor of RSV RT (IC_{50}: 20 μg/ml). The fact that an inhibitory agent could influence the various RTs in a much different manner has already been reported. For example, foscarnet inhibits HIV-1 RT sixteen times more than AMV RT (Vrang and Öberg, 1986) and TIBO derivatives are specifically inhibitory to HIV-1 RT but not to any other retrovirus RT (Pauwels et al., 1990). On the other hand, ddTTP is 100-fold more inhibitory to HIV-1 RT than AMV RT, and 1000-fold more inhibitory to HIV-1 RT than to Moloney murine leukemia virus RT (White et al., 1989). These results, together with our present data, indicate that the search for anti-HIV agents should be performed with HIV-1 RT (or HIV-2 RT) and not other retrovirus RTs.

Among the anthraquinones shown in Table 1, AC and ARS inhibited RSV focus formation (IC_{50}: 8.5 μg/ml and 40 μg/ml, respectively, see Table 2). However, the inhibitory concentration of AC on focus formation was approximately one tenth of that required to inhibit RSV RT. Thus, this inhibitory effect on focus formation might not be entirely due to inhibition of RSV RT. As shown in Table 3, AC inhibited RSV production at the concentration which inhibited focus formation, suggesting that the same mechanism may operate for inhibition of focus formation as well as for inhibition of RSV production. Consistent with the inhibitory action of AC on focus formation as well as virus production, AC delayed tumor induction by RSV in vivo. It should be noted that AC only affected tumor induction. Once the tumor had appeared, daily administration of AC did not influence the growth rate of the tumor. This is consistent with the concept that AC interferes with the replicative cycle of RSV and not tumor growth itself. In fact, AC had no effect if administration of this compound was delayed to 24 h after virus inoculation (data not shown). Of the anthraquinones tested, AC was the most potent inhibitor of HIV-1 cytopathogenicity in MT-4 cells [IC_{50}: 19 μg/ml (Table 4)].

Inhibitory concentrations of AC for HIV-1 cytopathogenicity were lower than those for HIV-1 RT, suggesting that the inhibitory action of AC on HIV-1 pathogenicity might not be entirely due to inhibition of RT. This notion was further supported by the finding that some compounds, such as quinalizarin and purpurin, had no effect on RSV focus formation while they had some inhibitory effects on RSV RT (Tables 1 and 2). Recently, a dimeric anthraquinone (hypericin) has been reported to be an active agent against murine leukemia viruses. The action of hypericin was ascribed to inhibition of viral assembly (Meruelo et al., 1988; Lavie et al., 1989). It is possible that AC may act in similar fashion to hypericin. The exact mechanism of the anti-HIV-1 action of AC remains to be elucidated.

Although the anti-HIV activity of AC is much less pronounced than that of AZT, it appears possible that chemical modifications of this compound may lead to more active and more specific inhibitors of HIV. In addition, the anti-HIV potency of the compounds may vary according to the nature of the target cells used. In fact, several target cells of HIV have been used by various laboratories for the screening of anti-HIV agents.

Anti-HIV activity of AZT varies to a great extent depending on the target cells [i.e.,

MT-4 and ATH-8 (Herdewijn et al., 1987)]. Despite the wide clinical use of AZT, it has a number of shortcomings. In particular, it causes bone marrow toxicity which makes the long-term use of this drug rather difficult (Richman et al., 1987). Furthermore, long-term use of AZT also leads to the emergence of AZT-resistant HIV strains (Larder et al., 1989). Therefore, new anti-HIV agents with a completely different mode of action from AZT would seem desirable. Our findings suggest that AC may be a lead compound from which potentially more potent and selective anti-HIV agents could be derived. Studies with various AC derivatives for their effectiveness against HIV-1 are in progress.

Shortly after this paper was completed, a paper describing similar effects of alizarin complexone with a different system appeared: Schinazi et al. (1990). Anthraquinones as a new class of antiviral agents against human immunodeficiency virus. Antiviral Res. 13, 265–272.

Acknowledgements

We thank Masahiko Tanaka for valuable suggestions regarding the preparation of recombinant HIV-1 RT and Amy Kaji for assistance in the preparation of the manuscript. This work was supported in part by a grant from Japan Health Science Foundation, Ministry of Health and Welfare.

References

Balzarini, J., Mitsuya, H., De Clercq, E. and Broder, S. (1986) Comparative inhibitory effects of suramin and other selected compounds on the infectivity and replication of human T-cell lymphotropic virus (HTLV-III)/lymphadenopathy-associated virus (LAV). Int. J. Cancer 37, 451–457.

Belcher, R., Leonard, M.A. and West, T.S. (1959) Submicro-methods for the analysis of organic compounds. Part X: determination of fluorine. J. Chem. Soc. 3577–3579.

Chen, M.S. and Oshana, S.C. (1987) Inhibition of HIV reverse transcriptase by 2′,3′-dideoxynucleoside triphosphates. Biochem. Pharmac. 36, 4361–4362.

Cheng, Y.-C., Dutschman, G.E., Bastow, K.F., Sarngadharan, M.G. and Ting, R.Y.C. (1987) Human immunodeficiency virus reverse transcriptase. J. Biol. Chem. 262, 2187–2189.

Chirigos, M.A., Pearson, J.W., Papas, T.S., Woods, W.A., Wood, H.B., Jr. and Spahn, G. (1973) Effect of streptonigrin (NSC-45383) and analogs on oncornavirus replication and DNA polymerase activity. Cancer Chemother. Rep. 57, 305–309.

Clair, M.H.St, Richards, C.A., Spector, T., Weinhold, K.J., Miller, W.H., Langlois, A.J. and Furman, P.A. (1987) 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. Antimicrob. Agents Chemother. 31, 1972–1977.

Dhananjaya, K. and Antony, A. (1987) Inhibition of avian myeloblastosis virus reverse transcriptase and its associated activities by daunomycin and adriamycin. Indian J. Biochem. Biophys. 24, 265–270.

Gyles, N.R., Miley, J.L. and Brown, C.J. (1967) Size, score and speed of development of tumors in chickens in response to subcutaneous inoculations with Rous sarcoma virus. Poult. Sci. 46, 789–795.

Hanajima, S., Ishimaru, K., Sakano, K., Roy, S.K., Inouye, Y. and Nakamura, S. (1985) Inhibition of reverse transcriptase by limocrocin. J. Antibiot. 38, 803–805.

Herdewijn, P., Balzarini, J., De Clercq, E., Pauwels, R., Baba, M. Broder, S. and Vanderhaeghe, H. (1987) 3′-Substituted 2′,3′-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. J. Med. Chem. 30, 1270–1278.

Hizi, A., Mcgill, C. and Hughes, S.H. (1988) Expression of soluble, enzymatically active, human immunodeficiency virus reverse transcriptase in *Escherichia coli* and analysis of mutants. Proc. Natl. Acad. Sci. USA 85, 1218–1222.

Inouye, Y., Oogose, K., Take, Y., Kubo, T. and Nakamura, S. (1987a) Role of single-electron reduction potential in inhibition of reverse transcriptase by streptonigrin and sakyomycin A. J. Antibiot. 40, 702–705.

Inouye, Y., Take, Y., Nakamura, S., Nakashima, H. Yamamoto, N. and Kawaguchi, H. (1987b) Screening for inhibitors of avian myeloblastosis virus reverse transcriptase and effect on the replication of AIDS-virus. J. Antibiot. 40, 100–104.

Kakiuchi, N., Hattori, M., Namba, T. Nishizawa, M., Yamagishi, T. and Okuda, T. (1985) Inhibitory effect of tannins on reverse transcriptase from RNA tumor virus. J. Natl. Prod. 48, 614–621.

Larder, B.A., Darby, G. and Richman, D.D. (1989) HIV with reduced sensitivity to Zidovudine (AZT) isolated during prolonged therapy. Science 243, 1731–1734.

Lavie, G., Valentine, F., Levin, B., Mazur, Y., Gallo, G., Lavie, D., Weiner, D. and Meruelo, D. (1989) Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. Proc. Natl. Acad. Sci. USA 86, 5963–5967.

Leonard, M.A. and West, T.S. (1960) Chelating reactions of 1,2-dihydroxyanthraquinon-3-ylmethylamine-NN-diacetic acid with metal cations in aqueous media. J. Chem. Soc. 4477–4486.

Lindell, T.J., Weinberg, F., Morris, P.W., Roeder, R.G. and Rutter, W.J. (1970) Specific inhibition of nuclear RNA polymerase II by α-amanitin. Science 170, 447–449.

Meruelo, D., Lavie, G. and Lavie, D. (1988) Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. Proc. Natl. Acad. Sci. USA 85, 5230–5234.

Miyoshi, I., Taguchi, H., Kubonishi, I., Yoshimoto, S., Ohtsuki, Y., Shiraishi, Y. and Akagi, T. (1982) Type C virus-producing cell lines derived from adult T cell leukemia. Gann Monogr. 28, 219–228.

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Okada, H., Mukai, H., Inouye, Y. and Nakamura, S. (1986) Biological properties of streptonigrin derivatives II: inhibition of reverse transcriptase activity. J. Antibiot. 39, 306–308.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J. and De Clercq, E. (1988) Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20, 309–321.

Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaeckers, A., Van Gelder, J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E. and Janssen, P.A.J. (1990) Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343, 470–474.

Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. (1984) Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224, 497–500.

Richman, D.D., Fischl, M.A., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Hirsch, M.S., Jackson, G.G., Durack, D.T., Nusinoff-Lehrman, S. and the AZT Collaborative Working Group (1987) The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. 317, 192–197.

Sethi, M.L. (1983) Enzyme inhibition VI: inhibition of reverse transcriptase activity by protoberberine alkaloids and structure-activity relationships. J. Pharm. Sci. 72, 538–541.

Sethi, M.L. (1985) Comparison of inhibition of reverse transcriptase and antileukemic activities exhibited by protoberberine and benzophenanthridine alkaloids and structure-activity relationships. Phytochem. 24, 447–454.

Smith, S.D., Shatsky, M., Cohen, P.S., Warnke, R., Link, M.P. and Glader, B.E. (1984) Monoclonal antibody and enzymatic profiles of human malignant T-lymphoid cells and derived cell lines. Cancer Res. 44, 5657–5660.

Vogt, P.K. (1969) Focus assay of Rous sarcoma virus. In: K. Hable and N.P. Salzman (Eds), Fundamental techniques of virology. pp. 198–211. Academic Press, Inc., New York.

Vrang, L. and Öberg, B. (1986) PPi Analogs as inhibitors of human T-lymphotropic virus type III reverse

transcriptase. Antimicrob. Agents Chemother. 29, 867–872.

White, E.L., Parker, W.B., Macy, L.J., Shaddix, S.C., McCaleb, G., Secrist III, J.A., Vince, R. and Shannon, W.M. (1989) Comparison of the effect of carbovir, AZT, and dideoxynucleoside triphosphates on the activity of human immunodeficiency virus reverse transcriptase and selected human polymerases. Biochem. Biophys. Res. Commun. 161, 393–398.

Wondrak, E.M., Löwer, J. and Kurth, R. (1988) Inhibition of HIV-1 RNA-dependent DNA polymerase and cellular DNA polymerases α, β and γ by phosphonoformic acid and other drugs. J. Antimicrob. Chemother. 21, 151–161.

Wu, R.S., Wolpert-DeFilippes, M.K. and Quinn, F.R. (1980) Quantitative structure-activity correlations of rifamycins as inhibitors of viral RNA-directed DNA polymerase and mammalian α and β DNA polymerases. J. Med. Chem. 23, 256–261.