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MOLECULAR PHARMACOLOGIC APPROACHES TO THE TREATMENT OF AIDS[1]

Prem S. Sarin

Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892

INTRODUCTION

A human immunodeficiency virus (HIV-1, human T lymphotropic retrovirus, HTLV-III) has been identified as the etiological agent for acquired immune deficiency syndrome (AIDS) and the AIDS related complex (ARC) (1–4). Various therapeutic approaches to controlling the disease, using either inhibitors of reverse transcriptase and virus replication or a vaccine, are currently being investigated. HIV-1 is a cytopathic retrovirus that selectively infects T-helper cells and kills OKT4+ T helper cells, resulting in immune suppression (1–5). HIV-1 contains an RNA directed DNA polymerase (reverse transcriptase) and buds from the cell membrane like other animal retroviruses (6, 7). The replication of virus in the infected cells and further infection of uninfected cells by the newly produced virus can be blocked by chemotherapeutic agents that attack at the various steps in the replication cycle (Figure 1), including virus attachment, reverse transcription, and DNA integration.

The virus identified as the etiological agent of AIDS infects T-helper cells by binding to the cell through a receptor site identified as the T4 receptor or CD4 (8, 9), and the binding of HIV-1 to the T4 cells can be blocked by antibodies to the T4 receptor. Most of the AIDS or ARC patients contain antibodies to the viral antigens that are either not neutralizing or have very

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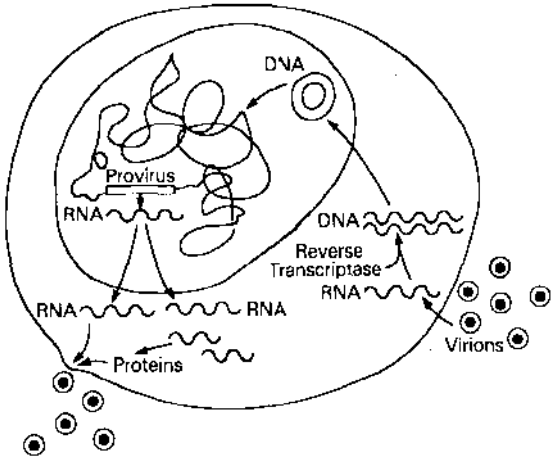

Figure 1 Life cycle of a retrovirus. Various stages in the infection of T cells with HIV-1 and virus replication in infected cells. Targets for therapy include interference with: (*a*) virus attachment to cells; (*b*) reverse transcriptase activity; (*c*) DNA integration; (*d*) DNA transcription and translation; and (*e*) virus assembly and release.

low virus neutralizing activity, and that therefore do protect against virus infection of uninfected T cells. After attachment to the T4 cell through a receptor on the cell surface, the virus enters the cell. Its entry is followed by uncoating of the virus, synthesis of DNA from the viral RNA, and subsequent integration of the viral DNA into the host genome. The viral DNA can also be present in the unintegrated form and can replicate in the cytoplasm. Agents such as amantadine have been used to block infection by the influenza virus (10). It remains to be determined whether similar agents will be useful in preventing HIV-1 infection of T cells and other susceptible cells, such as macrophages, B cells, and monocytes, which can also be infected with HIV-1 to a lesser extent than the other types of cell.

To control HIV-1 infection and to prevent replication of the virus in AIDS and ARC patients, it is important to examine the various steps involved in HIV-1 infection and replication in T cells. HIV-1 infects the target cells by attaching through a specific receptor, entering into the cell, uncoating and exposing reverse transcriptase and viral RNA; there then occurs transcription of the RNA into complementary DNA (cDNA), conversion of the latter into proviral DNA (double stranded DNA, dsDNA), and integration of the proviral

and protein synthesis in the infected cell result in the synthesis of viral RNA and group specific antigen (gag) and envelope proteins, which then attach to the cell membrane in the form of a nucleoid. This event is followed by budding and release of more infectious HIV-1 virus particles.

To date two approaches to controlling this disease with drugs have been explored: (*a*) inhibition of reverse transcriptase, by nucleoside analogue, such as 3'-azido-3'-deoxythymidine (11), suramin (12, 13), phosphonoformic acid (14), or antimoniotungstate (15); and (*b*) inhibition of HTLV-III replication by ribavarin (16), alpha interferon (17, 18), AL-721 (19), D-penicillamine (20), amphotericins (21), synthetic oligonucleotides (22), or the sequiterpenoid quinone avarone and its hydroquinone derivative avarol (23), which were recently identified as cytostatic agents that inhibit the growth of T cell lymphoma lines (24, 25). Structures of some of these compounds are shown in Figure 2; their possible modes of action are shown in Table 1.

BLOCKING VIRUS ATTACHMENT OR ASSEMBLY

Since HIV-1 binds to the T cells through the T4 receptor (8, 9) or through a receptor adjacent to or overlapping with the T4 receptor, it should be possible to block virus attachment to the target cells by blocking virus attachment to

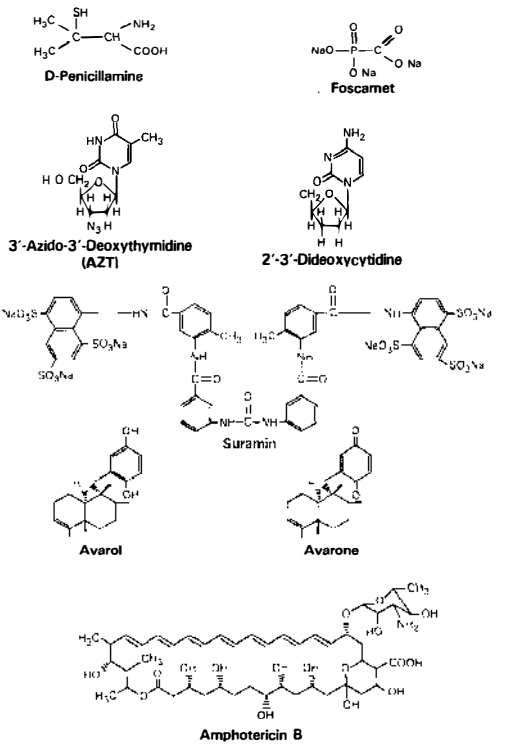

Figure 2 Chemical structures of drugs that inhibit HIV-1 replication.

Table 1 Drugs identified as inhibitors of HIV-1 replication

Drug	References	Possible mode of action	Clinical status
AL721	19	Binding to cholesterol in HVI-1 envelope and HIV-1 infected cells	Early clinical trials at St. Luke's Hospital showed improvement in 5 of 7 patients
Amphotericin analogs	21	Binding to cholesterol in HIV-1 envelope and HIV-1 infected cells	Clinical trials to be initiated
Avarol/Avarone	23	HIV-1 assembly and release inhibitor	Clinical trials to be initiated
D. Penicillamine	20, 26	Binding to cysteine rich viral proteins and T cell receptor	Early clinical trials showed improvement in 60% of patients tested
Foscarnet	14, 46	Reverse transcriptase inhibitor	Clinical trials in progress
Suramin	12, 13	Reverse transcriptase inhibitor	Clinical trials suspended due to toxicity
AZT	52–56	Reverse transcriptase inhibitor, DNA chain termination	Clinical trials in progress. Shows toxic side effects (anemia, neutropenia)
Dideoxynucleosides (Dideoxycytidine)	57	Reverse transcriptase inhibitor, DNA chain termination	Clinical trials in progress
HPA23	63, 64	Reverse transcriptase inhibitor	Clinical trials in progress. Shows toxic side effects (nephrotoxicity and thrombocytopenia)
Ribavarin	58–61	Blocks mRNA capping	Clinical trials in progress. Does not appear to be promising. (CNS toxicity)
Antisense oligodeoxy nucleotides	66	Block DNA transcription and translation	

the receptor through use of monoclonal antibodies that specifically block the receptor site and prevent virus attachment. Monoclonal antibodies made against T4 (especially 4a and 4i) have recently been shown to block the infection of OKT4+ T-helper cells (K. Krohn, A. Ranki, unpublished findings). Synthetic peptides specific for the receptor site to which the virus binds may also be effective in blocking virus infection, as seen in the case of myxovirus and paramyxovirus infections (9). Another approach would be to use agents that destroy the integrity of the viral envelope by puncturing it and thus making it inactive or less infectious. One such agent, AL-721, was recently shown (19) to interfere with HIV-1 infection and replication by extracting cholesterol from the viral envelope, which is composed of glycoproteins, phospholipid, and cholesterol (P. S. Sarin, F. Crews, unpublished findings). In recent clinical trials of this agent on patients with persistent generalized lymphadenopathy (PGL), 5 of the 7 patients showed clinical improvement and a reduction in HIV-1 reverse transcriptase activity (M. Greico, unpublished findings). Further clinical studies with AL-721 on a larger group of patients are planned.

D-Penicillamine

Another compound that has been found to block HIV-1 replication (20) in cell cultures is D-penicillamine (DPA). DPA has been used in the past for the treatment of Wilson's disease, chronic hepatitis, and rheumatoid arthritis and has shown some immunosuppressive activity. In a limited clinical trial on asymptomatic patients with generalized lymphadenopathy, DPA was given normally over a 6-week period (26). All patients had depressed T4/T8 ratios and impaired T cell function. An escalating dose schedule was employed over 2–6 weeks, with doses ranging from 0.5–2gms/day. Ten patients treated for at least 2 weeks showed suppression of HIV-1 replication, and complete inhibition of virus expression occurred in 60% of the patients treated for 6 weeks. HIV-1 expression was inhibited in 60% of the patients, and 2 of the patients have remained virus negative for over 9 months (27). Reversible decrease in lymph node size, absolute lymphocyte counts, and T-cell lymphoproliferative responses were observed in the majority of patients without change in baseline T4/T8 ratios. No significant toxicity was observed in these patients (26, 28). DPA is currently being evaluated in patients with AIDS and ARC. The mechanism of inhibition of HIV-1 by DPA has not yet been defined. DPA is a chelating agent that interacts with proteins and peptides by forming mixed disulfides (29, 30), and that may inhibit HIV-1 replication by binding to HIV-1 viral proteins, such as the cysteine-rich nucleic acid binding protein, envelope glycoprotein, *tat*-3 gene product, and/or the T4 receptor. Large-scale clinical trials on DPA are planned.

Avarol and Avarone

Avarol and avarone are two antimitotic and antimutagenic agents that preferentially inhibit proliferation of leukemic cells (31, 32). Avarol was isolated from *Dysidea avara,* and avarone was obtained by silver oxide oxidation of avarol (Figure 2). The effect of each of these two compounds on HIV-1 replication in H9 cells was studied. In the absence of either drug, uninfected H9 cells performed 1.70 doubling steps during an incubation period of 4 days, whereas HTLV-IIIB infected H9 cells underwent only 0.32 doubling steps. After exposure of H9/HTLV-IIIB cells to 0.1 μg/ml of avarol in one instance and of avarone in the other, the proliferation rate returned to its normal value.

In uninfected H9 cells, no HIV-1 p17 and p24 gag protein expression was detectable by the immunofluorescence assay. However, after exposure of the target H9 cells to HTLV-IIIB virus (500–1000 virus particles per cell), 35% of the target H9 cells expressed HTLV-IIIB p17, and 40% expressed HTLV-III p24 gag protein in the absence of either avarol or avarone. Addition of avarol or avarone at day 0 to H9/HTLV-IIIB cells resulted in a dramatic inhibition of p17 and p24 expression. The optimal dose appears to be 1 μg/ml. At this concentration avarol or avarone causes no inhibitory effect on normal human peripheral blood lymphocytes, cultured in either the absence or presence of the mitogens concanavalin A or pokeweed mitogen (25).

In uninfected H9 cells, no reverse transcriptase activity was detected in the culture medium after an incubation period of 4 days. In contrast, in the medium of H9/HTLV-IIIB infected cells, high enzyme activity could be measured after the same period of time. Incubation of H9/HTLV-III cells in the presence of both avarol and avarone resulted in a substantial decrease in reverse transcriptase activity in the culture supernatant, which suggests that both avarol and avarone are potent inhibitors of HIV-1 replication in H9 cells.

Both avarol and avarone show significant antiviral and cytoprotective effects in the H9/HTLV-IIIB cell system at low concentrations. At these concentrations (0.3 μM), no inhibitory effect is observed in human or murine peripheral blood or spleen lymphocytes (25). At concentrations from 1.5–4 μM of avarol (1.8–2.5 μM of avarone), a 50% reduction in DNA synthesis was observed, as measured by [^{3}H] thymidine incorporation in T-lymphocytes (25). These results may be explained by the earlier observation that free tubulin dimers, formed from microtubules by avarol and avarone, stimulate mitotic growth (32) and modulate the activity of nuclear-envelope triphosphate that is essential for nuclear-cytoplasmic transport of poly $(A)^+$mRNA (33). It remains to be determined whether the stimulation of DNA synthesis observed in B-lymphocytes by low concentrations of avarol and avarone (25) reflects an increase in the immunological activity of these cells.

The molecular mechanisms by which the cytostatic agents avarol and

avarone inhibit HIV-1 replication in vitro have not yet been established. However, at the effective concentrations (0.1 μg/ml), both compounds show no toxicity to H9 cells, which suggests that the observed effect is on HIV-1 infection and replication rather than on the uninfected cells. On the basis of earlier studies (31), it appears that avarol and avarone probably interfere with those cytoskeletal processes involved in the assembly of HIV-1 virus particles and/or the cytopathic effect (4).

These observations strongly indicate that both avarol and avarone are potentially useful in clinical trials on patients with HIV-1 infection because they inhibit HIV-1 virus replication and show a cytoprotective effect on HIV-1 infected cells. In addition, these compounds show "T-lymphotropic" cytostatic activity (25). In vivo, these compounds show: (*a*) low toxicity in mice (25) ($^{LD}10$: 111 mg of avarol or 156 mg of avarone/kg of body weight); (*b*) high therapeutic indexes that are in the range of those determined for cyclophosphamide, daunomycin, and methotrexate, (25) and that can penetrate the blood-brain barrier, a property which may be of great interest in the present context because of HIV-1 infection of brain cells in AIDS patients (34). Clinical trials with avarol and avarone on patients with lymphadenopathy syndrome (LAS), ARC, and AIDS are planned.

Amphotericin B and Analogues

Amphotericin B methyl ester (AME), which is a water soluble derivative of amphotericin B (35), a polyene macrolide antifungal antibiotic, is known to be active against a variety of lipid-enveloped RNA and DNA viruses, several oncogenic retroviruses, and different strains of herpes viruses (35, 36).

AME interacts with sterols and binds to them irreversibly (37). The binding of AME to cholesterol in the membrane of cells causes changes in cell permeability and function, and its binding to sterols of lipid-enveloped viruses decreases infectivity. AME has been shown to be active in inhibiting cell death due to HIV-1 infection and in inhibiting the expression of virus antigens p24 and p17 on infected cells (21). Amphotericin B was also active, whereas candicidin, another polyene macrolide that binds to sterols, was too cytotoxic at the levels needed to inhibit HIV-1 replication (21).

AME was not cytotoxic up to 10 μg/ml but showed significant anti-HIV-1 activity beginning at 1 μg/ml. It significantly inhibited the expression of the virus antigens p24 and p17, as measured by immunofluorescence assay using monoclonal antibodies (21). Protection by AME of H9 cells against the cytopathic effect of HIV-1 resulted in increased survival of cells (21).

The action of these drugs is due generally to their binding to sterols in cell membranes. At high concentration they cause changes in membrane permeability and disruption of the membrane. Sheep red blood cells have been used to assess the cytotoxicity of AME and amphotericin B since disruption

of their membrane can be quantitated by the release of hemoglobin into the fluid phase, which can be measured spectrophotometrically. Sheep red blood cells were incubated with AME and amphotericin B (0.01–100 μg/ml) at room temperature for 1 hr. Neither drug caused lysis of the red blood cells at 10 μg/ml, the concentration at which they are active in inhibiting the replication of HTLV-IIIB in cultures of H9 cells. AME was not cytotoxic even at 100 μg/ml, whereas amphotericin B caused approximately 50% of the red blood cells to lyse.

It appears that AME, at a noncytotoxic dose, can protect target cells against the cytopathic effect of HIV-1. This protection is associated with its apparent inhibition of virus expression. This is not surprising, since AME is known to act directly against the herpes virus, which is an enveloped virus with a lipid membrane containing cholesterol (36). HIV-1 is also an enveloped virus and has been shown to be inactivated by AL-721, which works by extracting cholesterol from the virus envelope (19). Hence HIV-1 should also be sensitive to AME. The inactivation of HIV-1 is probably dependent on the ratio of AME concentration to the quantity of virus particles. It would seem that the antiviral activity of AME, at the concentration used in these experiments, may also be due in part to its action on the target cells whose membrane also contains sterols to which AME can bind. This is consistent with the effect of polyene macrolides on cells of the immune system. In particular, the polyene macrolides are known to modulate the activity of lymphocytes (37). In addition, the antiviral activity of AME was essentially the same whether or not it was preincubated with HIV-1. This finding provides further support for the suggestion that AME can protect cells against HIV-1 by its direct action on the cells as well as by its action on the virus. In antiherpes virus studies (36) using HeLa cells, preincubation of the viruses with AME showed a time and concentration dependency for loss of viral infectivity. Preincubation of the cells with the drug followed by washing also resulted in a significant resistance to viral challenge.

Both AME and its parent compound, amphotericin B, were essentially similar in the protection they gave against cell death, and in their inhibition of expression of virus antigens. The advantages of AME are that it is a water soluble compound, and that it is relatively noncytotoxic to normal cells, as compared to amphotericin B (38).

Candicidin, like amphotericin B, is a polyene macrolide antifungal antibiotic (39) that binds to sterols. However, it is more cytotoxic. This is evident in its action against virus-infected H9 cells (21). At noncytoxic doses it did not protect cells against HIV-1 infection.

Binding to sterols by AME and amphotericin B may well be the key mechanism in their protection of target cells against HIV-1 infection, but clearly not all polyene macrolides sharing this property may be therapeutically

useful. Amphotericin B and its derivative AME are significantly effective in blocking HIV-1 infection and replication in T cells, and hence, they could be potentially useful in the treatment of patients with AIDS or ARC. Clinical studies to evaluate the effectiveness of amphotericin B, AME, and other analogues of amphotericin B with and without liposomal encapsulation, in patients with LAS, ARC, and AIDS will begin soon. In addition, combination of amphotericin B and AME with other drugs may prove to be more useful in the treatment of AIDS, since drugs such as foscarnet and 3'-azido-3'-deoxythymidine (AZT) show a synergistic effect (P. S. Sarin, D. Pontani, D. Sun, unpublished findings).

INTERFERENCE WITH REVERSE TRANSCRIPTION

Inhibition of reverse transcriptase activity of retroviruses has been a major goal of the development of antiviral agents against replication of animal retroviruses (40). Rifamycins (41) and streptovaricins (42) were the compounds first used to inhibit replication of Rauscher murine leukemia virus (RLV) and feline leukemia virus (FeLV) both in vitro and in vivo. In studies in our laboratory (P. S. Sarin, D. Sun, A. Thornton, unpublished findings), some of the rifamycin analogues found to be active against RLV did not inhibit HTLV-IIIB replication in H9 cells. It is difficult to ascertain whether the rifamycin analogues were active against replication of animal retroviruses in early studies but inactive in blocking HIV-1 replication because the compounds were synthesized in the 1970s and had degraded over the years, or whether they are really inactive against HIV-1. Synthetic polynucleotides (43) were also reported to be effective in blocking murine virus replication in vivo and in vitro by interfering with the binding of template primer to murine retrovirus reverse transcriptase. Several compounds, including polymethyl-C have recently been reported by Chandra and co-workers to block HIV-1, reverse transcriptase (44, 45).

Foscarnet

A potent inhibitor of reverse transcriptase is foscarnet (phosphonoformate) (15, 46). The chemical structure of foscarnet and the mechanism of action of this compound is shown in Figure 3. This compound was first identified for treatment of cytomegalovirus (CMV) infections and has since been used to control CMV infections (B. Oberg, personal communication). Foscarnet completely inhibits reverse transcriptase activity at a concentration of 150 μM, and the concentration of it required for complete inhibition of HTLV-III replication in H9 cells is from 150–300 μM (15). The drug does not show any toxicity to the cells in culture up to a concentration of 750 μM. Since the effect of the drug persists for 4 days to a week after it has been added to cell

cultures, it may be worthwhile to examine the efficacy of this drug when it is administered according to an intermittent dose schedule (1–2 doses per week) rather than by continuous infusion. Foscarnet is currently undergoing clinical trials in AIDS and ARC patients in various countries. This compound is relatively nontoxic and is given to the patients by continuous infusion. A slow release oral form of this compound that could be prescribed for AIDS/ARC outpatients would be useful and of interest to researchers.

Suramin

Suramin is an HIV-1 reverse transcriptase inhibitor (47, 48) that has been used to treat trypanosomiasis in Africa. In clinical trials, suramin showed transient reduction in virus expression and severe toxic side effects, including anemia, proteinurea, hepatic failure, and agranulocytosis (49, 50). After treatment with suramin was discontinued, virus expression returned to pre-treatment levels. Due to its toxic side effects, clinical trials of this compound have been suspended.

Azidothymidine (3'Azido-3'-deoxythymidine, AZT)

AZT is an analogue of thymidine. It is an active reverse transcriptase inhibitor that blocks DNA synthesis by chain termination. AZT has been shown to inhibit murine and feline retrovirus replication in cell culture and subsequently was found to inhibit HIV-1 replication in cell culture (52, 53). AZT

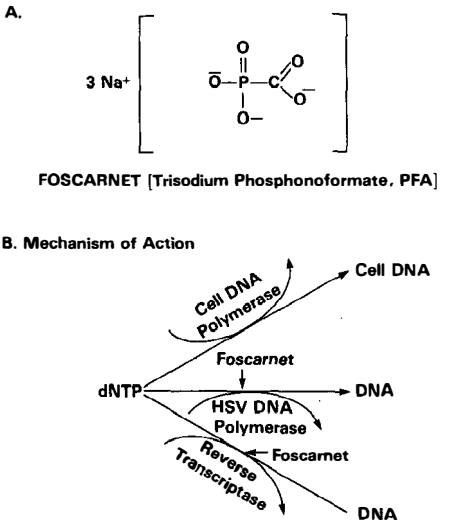

Figure 3 Structure and mechanism of action of foscarnet. (A) Chemical structure of foscarnet. (B) Mechanisms of action of foscarnet against cellular and viral DNA polymerases.

was found to be very effective in early clinical trials (54, 55) and has been approved by the Food and Drug Administration for general use on AIDS patients with pneumocytis carinii pneumonia (PCP) who fit the criteria established by the agency. The drug is generally given intravenously but can also be given orally. AZT has a half-life of approximately 60 minutes. The peak plasma levels of approximately 1.8 μM are obtained by infusion of 1mg/kg AZT or by administration of twice that quantity orally. Immunological improvement and weight gain have been seen in some patients, but other groups of patients have shown toxic side effects (55). Bone marrow suppression, anemia requiring blood transfusions, nausea, insomnia, severe headaches, and neutropenia have been commonly seen in patients treated with this drug. Until better and more potent drugs are available, AZT will probably continue to be used on a limited basis for treatment of AIDS patients.

2'-3'-Dideoxycytidine

Other dideoxynucleosides are being examined for their effectiveness in the treatment of AIDS. One of the analogues, 2'-3'-dideoxycytidine, has recently been shown to be a potent inhibitor of HIV-1 replication in cell culture (56). Like AZT, 2'-3'-dideoxynucleotides are reverse transcriptase inhibitors that block DNA synthesis by chain termination. Clinical trials of these analogues are in progress. The behavior of these analogues in clinical trials may parallel the results obtained with AZT.

Ribavarin

Ribavarin has been used to block replication of murine retroviruses both in vitro and in vivo and in the treatment of viral infections in humans. More recently, it was shown to block the replication of HIV-1 in vitro (57, 58). Ribavarin is considered to block virus replication by interfering with 5' capping of viral mRNA. Ribavarin has been used in the past for the treatment of respiratory syncytial virus (59) and influenza virus (60) infections. This compound is undergoing clinical trials in AIDS and ARC patients in a number of countries, but the results so far look less than promising. In our studies, ribavarin did not inhibit HTLV-III replication in H9 cells (P. S. Sarin, Y. Taguchi, D. Sun, unpublished findings). Recent studies indicate that in combination with AZT, ribavarin acts as an antagonist (61).

HPA-23

Ammonium antimony tungstate (HPA-23) is another inhibitor of HIV-1 reverse transcriptase (62) that has recently been used in clinical trials in France on patients with AIDS and ARC (63). Transient reduction in HIV-1 circulating in peripheral blood lymphocytes was observed, but the compound was used at doses showing toxic side effects. In our studies (P. S. Sarin, D.

Sun, A. Thornton unpublished findings), HPA-23 did not show any significant reduction in HIV-1 replication in cell culture systems at concentrations that are not toxic to the cultured cells. Further expanded clinical trials of HPA-23 are currently in progress and will show whether this compound will eventually be useful in the treatment of AIDS or ARC.

Cyclosporin

Cyclosporin is another compound that has recently been investigated by French researchers studying the treatment of AIDS patients. The structure of cyclosporin and its possible mechanism of action show that this compound is

Table 2 Inhibition of HIV-1 replication by various drugs

NSC number	Drug	ID50 (μg/ml)
103-627	Azacytidine	>300
253-272	Caracemide	>300
241-240	Carboplatin	>300
145-668	Cycloclytidine HCl	>300
126-849	Deazuridine	>300
261-036	Desmethylmisonidazole	>300
132-313	Diandydrogalactitol	>300
118-994	Diglycoaldehyde	>300
264-880	Dihydroazacytidine KCl	>300
	Doxorubicin HCl	>20
134-490	Emofolin	>300
296-961	Ethiofos	>300
312-887	Fludarabine phosphate	>300
PFA	Foscarnet	>10
	HPA-23	>100
301-467	Hyroxyethyl-nitroimidazole acetamide	>300
169-780	ICRF-187	>300
129-943	ICRF-159	>300
132-319	Indicine N-oxide	>300
8806	Melphalan	>300
261-037	Misonidazole	>300
224-131	PALA-Disodium	>300
118-742	Pentamcthylamine-2HCl	>300
218-321	Pentostatin	>300
135-758	Piperazindedione-bis chlorpiperidyl HCl	>300
192-965	Spirogermanium HCl	>300
314-055	SR-2555	>300
148-958	Tegafut	>300
286-193	Tiazofurin	>300
281-272	5-Azacytosine arabinoside	>300
	Ansamycin	>300

immunosuppressive. In our studies of the inhibition of HTLV-IIIB replication in H9 cells, we found that this compound did not inhibit replication of HIV-1, nor that of HTLV-I or HTLV-II. HTLV-I is a retrovirus associated with the cause of adult T cell leukemia (ATL), and HTLV-II is a related retrovirus isolated from patients with hairy cell leukemia (2, 3).

A list of some of the approximately 500 compounds that we have examined in our laboratory is given in Table 2.

INHIBITORS OF DNA AND RNA TRANSCRIPTION (ANTISENSE OLIGONUCLEOTIDE INHIBITORS)

Another approach to inhibiting retrovirus replication that has been explored is the use of antisense oligonucleotide inhibitors (synthetic oligonucleotides) designed to bind to specific target sites of the viral genome. Zamecnik and coworkers (64) used synthetic oligonucleotides (chain length 13–15) in the inhibition of Rous sarcoma virus in cell culture. These synthetic oligonucleotides interfered with virus replication at various steps of the replication cycle (Figure 4). Since the complete nucleotide sequence of the HIV-1

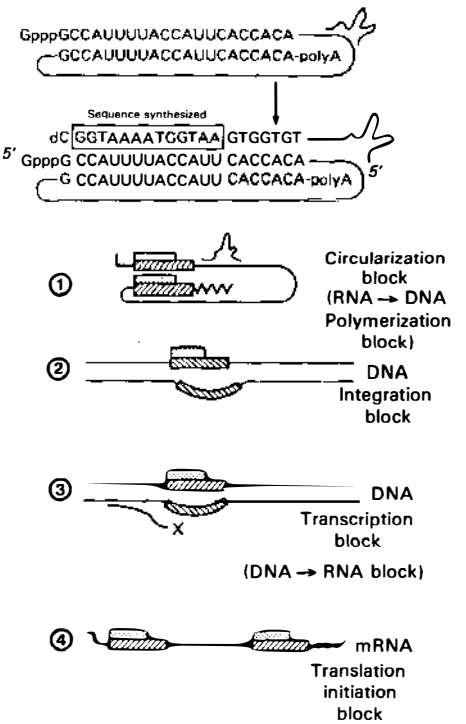

Figure 4 Possible target sites for antisense RNA therapy.

genome is known, synthetic oligonucleotides specific for regions adjacent to the primer binding site and *tat*-3 gene splice acceptor and donor sites were examined for their effect on HIV-1 replication in H9 cells, as well as for their effect on syncytia inhibition in Molt 3 cells. Syncytia assay involves the culture of Molt 3 cells infected with HIV-1 in the presence or absence of drugs. A reduction in the number of syncytia in the presence of a drug is indicative of the inhibitory effect of the drug on HIV-1 replication. As shown in Table 3, oligonucleotides having a chain length of 20 were found to be most active in inhibiting virus replication (65). The greatest inhibition of virus replication was due to oligonucleotides specific to the *tat*-3 gene splice acceptor and donor sites. Approximately 8% of the oligonucleotides added to cell culture are taken up by the cells; thus a concentration is achieved within the cell that is high enough to be effective (65). Whether these compounds will prove to be useful in the treatment of AIDS or ARC patients remains to be determined.

FUTURE DIRECTIONS

Further studies need to be carried out to examine the mechanism of action of the drugs that inhibit HIV-1 replication. In addition, efforts should be directed

Table 3 Inhibition of HIV-1 replication by oligodeoxynucleotides

Sequence	Oligomer length	Concentration μg/ml	HIV-1 binding site	Percent inhibition RT	Percent inhibition p24
CCCCAACTGTGTACT	15	5		0	0
CCCCAACTGTCTACT	15	10		0	0
CTGCTAGAGATddt	12	5	5'-vicinal to PBS	30	17
CTGCTAGAGATddt	12	10	5'-vicinal to PBS	36	50
CTGCTAGAGATddt	12	20	5'-vicinal to PBS	40	36
CTGCTAGAGATTTTCCACAC	20	50	5'-vicinal to PBS	50	50
CTGCTAGAGATTTTCCACAC	20	10×3*	5'-vicinal to PBS	50	75
TTCAAGTCCCTGTTC-GGGCGCAAAA	26	50	at PBS	80	NT
GCGTACTCACCAGTCGCCCGC	20	50	splice donor site	85	60
CTGCTAGAGATTA	14	50	5'-vicinal to PBS	75	NT
ACACCCAATTCTGAAAATGG	20	50	splice acceptor site	67	90

*Daily addition of oligomer for 3 days. NT = not tested

toward identifying other agents that can inhibit reverse transcriptase activity, such as template binders, substrate analogues, or enzyme binders and antimetabolites that can modify proteins. Water soluble or dispersible semisynthetic polyene macrolides and their methyl esters, as well as the glycerophospholipid conjugates of selected antiretroviral agents, need to be examined further to determine their ability to inhibit HIV-1 replication. The glycerophospholipid conjugates are of special interest, since they may be able to cross the blood brain barrier. The use of antisense RNA appears to be very promising and efforts should be expanded to obtain synthetic oligonucleotides that will penetrate the cell membrane, survive attack by nucleases within the cell, and selectively hybridize to specific sites of the HIV-1 genome. Methyl phosphonate and thiophosphate analogues have recently been found to be resistant to nuclease attack, and they can enter the cells more effectively than native oligonucleotides. These studies should provide us with effective antiviral agents that may be useful, either alone or in combination with immunomodulators, in the treatment of AIDS. At this stage of research, it appears that no single agent is capable of successfully countering AIDS. It is therefore important to search for drugs with synergistic effects for use in combination therapy along with immunomodulators that may be able to reconstitute the immune system. Several drugs appear to be promising, and the clinical trials with these agents and other new drugs will provide the basis for treatment of patients with HIV infections.

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