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Review Article

Drug delivery approaches for anti-HIV drugs

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Summary

Several approaches are currently being followed in the development of strategy for the treatment of HIV infection. Evaluation of the effect of drug delivery systems on the efficacy and toxicity may improve the anti-HIV treatment. This review focuses on various approaches used in the development of drug delivery systems for the controlled, sustained and site-targeted delivery of dideoxynucleosides for these treatments.

Introduction

The human immunodeficiency virus (HIV) has been known to be the etiologic agent responsible for the acquired immunodeficiency syndrome (AIDS). The virus is classified as a member of a rare, but highly organized, group of retroviruses (Wells and Poiesz, 1990) that possess their own set of regulatory elements, in addition to *trans*-acting cellular genes. After invading the human body, HIV gradually erodes the ability of the immune system to resist various pathogens, thus making the patient increasingly vulnerable to a number of opportunistic infections and cancers. Without an effective treatment, death from AIDS occurs within 2–4 years following clinical diagnosis

A schematic representation of HIV is depicted in Fig. 1. Two glycoproteins, gp120 and gp41, are embedded in the lipid bilayer of the outer coat. The gp120, which is the major extracellular envelope glycoprotein, is located on the surface of the outer coat, whereas the gp41, a transmembrane glycoprotein, passes through the outer coat. Inside the HIV cell there is a protein core containing two proteins, p24 and p18, and enclosing two strands of viral RNAs and the reverse transcriptase (RT) enzyme (St. Georgiev and McGowan, 1990).

How cellular immune function is impaired by HIV is only partially understood. The virus is known to eliminate gradually the subset of T cells

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that bear CD4 receptors. Such a loss would be expected to debilitate the cell-mediated immune response, because CD4-positive (CD4 +) T cells, which are also known as helper T cells, control the activity of many other components of the cellular immune response. Nevertheless, some immune dysfunction is often present even before a decline in CD4 + T cells becomes evident. This observation means that the loss of helper T cells is not the sole cause of the impairment.

The development of a particular type of opportunistic infections is related to the blood concentration of CD4 + T cells (Table 1). Healthy individuals have about 1000 cells in every milliliter of blood. In the HIV-infected individuals, the number declines by an average of about 40–80 cells a year. When the helper T cell count falls to the range of 200–400 cells/ml, the first infections, which are relatively benign but annoying, usually appear on the skin and mucous membranes. Among these may be thrush (painful sores of the mouth caused by the fungus *Candida albicans*), shingles (infection of the nerves and skin by varicella zoster virus), unusually severe athlete's foot (caused by several types of fungi) and oral hairy leukoplakia (whitish patches on the tongue caused by Epstein-Barr virus). Once such symptoms appear, a person is often said to have the AIDS-related complex (ARC). The same is true for individuals suffering from chronic, unexplained fevers, diarrhea, night sweats and/or weight loss. As immunity wanes still further, serious AIDS-defining opportunistic infections usually develop. These often include three major killers: *P. carinii* pneumonia, cryptococcal meningitis (caused by a fungus) and toxoplasmosis (a parasitic infection of the brain) (Table 1) (Mills and Masur, 1990).

Both CD4% and rate of change of CD4% in an individual has significant prognostic value in determining AIDS-free survival time (Burcham et al., 1991). In an azidothymidine-based antiretroviral therapy, nearly all deaths occurred in patients with blood CD4 level less than 50 cells/ml

TABLE 1

Opportunistic infections frequently found in people infected with HIV, which typically arise in a sequence related to the blood concentration of CD4 + T lymphocytes

Year after onset of HIV infection	Decrease in CD4 + T lymphocytes per mm^3	Infection	Organism	
Eight	~ 425 to ~ 250	skin infections	bacterium	
		shingles (skin)	virus	
		thrush (mouth and tongue)	fungus	
Ninth	~ 250 to ~ 100	severe athlete's foot	fungus	
		oral hairy leukoplakia	virus	
		tuberculosis	bacterium	AIDS-defining infections
		Pneumocystis carinii pneumonia	parasite	
		histoplasmosis (disseminated)	fungus	
		coccidioidomycosis (disseminated)	fungus	
		cryptococcal meningitis	fungus	
		toxoplasmosis (brain)	parasite	
		herpes simplex infections (skin, mucous membranes, esophagus)	virus	
		Cryptosporidium infections (intestine)	parasite	
Tenth	~ 100 to zero	cytomegalovirus infections (retina, esophagus, colon)	virus	
		Mycobacterium avium complex infections (disseminated)	bacterium	

Normal CD4 + T lymphocyte count is ~ 1 000 per mm^3; death occurs at zero CD4 + T lymphocytes per mm^3.
The cell count, which declines over time, reflects the degree of immune impairment caused by HIV.

(Yarchoan et al., 1991). The CD4 + T-lymphocyte count has been found to increase at low ddI concentrations; the extent of this increase was found to be directly proportional to the patient's CD4 count at the start of therapy (Drusano et al., 1992). These findings have implications in the antiretroviral therapy of HIV, in which CD4 count may be used as an end point for clinical trials.

A decade has passed since the identification of the human immunodeficiency virus type 1 (HIV-1) as the etiologic agent of AIDS. In this decade, even though a significant progress has been made in our understanding of this complex retrovirus, the progress in the development of antiviral agents has been rather slow. To achieve an optimal therapy of HIV-1 infection, it is likely that one or more steps in the viral replication cycle need to be inhibited and, therefore, combinations of antiviral agents, which act at different stages in the virus life cycle, may be used to achieve an additive or synergistic effect (Fig. 2 and Table 2

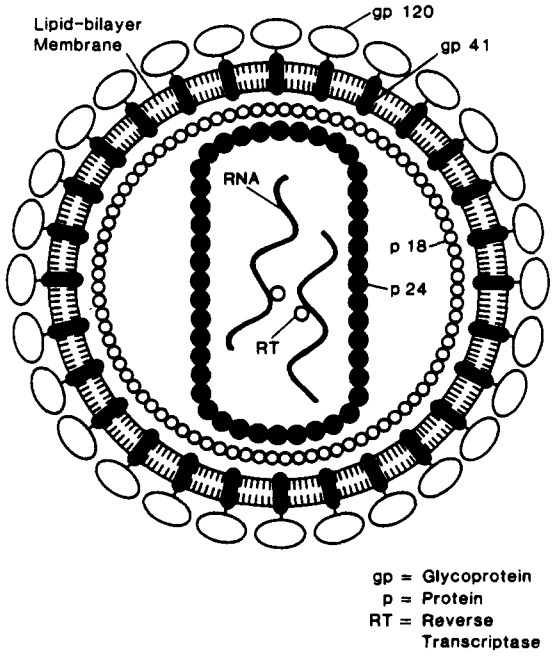

Fig. 1. Structural composition of AIDS-causing HIV (modified from Gallo, 1987).

(Johnson and Schooley, 1989; Mitsuya et al., 1991).

Antiretroviral agents (Polsky, 1989; Sandstrom, 1989; Yarchoan et al., 1989a,b; Broder, 1990; Broder et al., 1990; De Clercq, 1990, 1991; Hirsch, 1990) thus include:

- Inhibitors of viral binding and cell entry
- Inhibitors of reverse transcription
- Agents which act at other sites of viral replication

However, the antiretroviral agents developed to date have been primarily the inhibitors of the reverse transcriptase in HIV. The HIV RT has been one of the most intensively studied viral targets in the development of anti-HIV drugs. This enzyme is an attractive target since its specificity for HIV replication is unique compared to analogous proteins found in the human cell. Also, a large amount of information is available on the RT of other animal retroviruses or human cells (Tisdalé et al., 1989; Goff, 1990; Kraus et al., 1990). Knowing the differences between the viral and cellular enzymes would lead to the design of virus-specific drugs that are less toxic to the host cell (St. Georgiev and McGowan, 1990).

A broad family of 2′,3′-dideoxynucleoside analogs have been identified to have potent antiretroviral activity, since they can be metabolized to form potent chain-terminating inhibitors of RT (Waqar et al., 1984). The dideoxynucleosides are of special interest because they prove that a simple chemical modification of their sugar moiety can predictably convert a normal substrate for nucleic acid synthesis into a potent inhibitor for the replication of AIDS virus and its cytopathic effect (Chien and Wearley, 1989).

It has been found that a number of deoxynucleoside analogues, in which the 3′-hydroxy group is replaced by a hydrogen, an azido, or other group that does not form phosphodiester linkage, are potent inhibitors of HIV replication in vitro (Fig. 3). These compounds, called dideoxynucleosides, are phosphorylated by the enzymes in mammalian cells to 5′-triphosphate. It is believed that the dideoxynucleoside 5′-triphosphate produced, in turn, inhibits the activity of RT, by acting as a chain terminator as well as a competitive inhibitor of the physiologic deoxynucleoside

5′-triphosphates (Waqar et al., 1984; Mitsuya and Broder, 1986, 1987; Starnes and Cheng, 1987; Hao et al., 1988; Yarchoan et al., 1989b). Dideoxynucleosides are active against HIV in human monocyte/macrophages as well as in T cells. In addition to these dideoxynucleosides, a number of other nucleoside analogues have also been found to have some anti-HIV activities (Yarchoan et al., 1990a).

An important issue is the prevention and/or elimination of the integration of HIV provirus within the host genome. As the dideoxynucleoside-type anti-HIV drugs do not eliminate the provirus, chronic use of these drugs is usually necessary, because viral DNA may remain integrated within the patient's cells for an indefinite duration (Folks et al., 1986). A critical factor that needs to be considered, therefore, is the toxicity of the chemotherapeutic agent during a long-term treatment (Bouchard et al., 1988).

It is likely that anti-HIV therapy will have to be maintained for the life of the patient. Given the problems of resistance development and cumulative toxicity, it is unlikely that a single agent will be able to provide the optimal therapy throughout the course of treatment. Taking a cue from the development of effective therapies for certain cancers or tuberculosis, however, it is reasonable to expect that combinations of drugs might provide more effective therapy than any single drug used alone (Yarchoan et al., 1990b).

A combination of AZT with ddC (Spector et al., 1989; Basham et al., 1991; Meng et al., 1992) or FLT (Harmenberg et al., 1990) or ddI (Dornsife et al., 1991) has been found to yield a reduction in toxicity as well as in resistance compared to either drug used alone. Anti-HIV agents may be found to have synergistic effect, particularly if they act at different steps of the HIV life cycle (e.g., Johnson et al., 1989; Anand et al., 1990; Hayashi et al., 1990; Pincus and Wehrly, 1990; Baba et al., 1991). Furthermore, studies on the substrate specificity of human deoxycytidine kinase toward antiviral 2′,3′-dideoxynucleoside

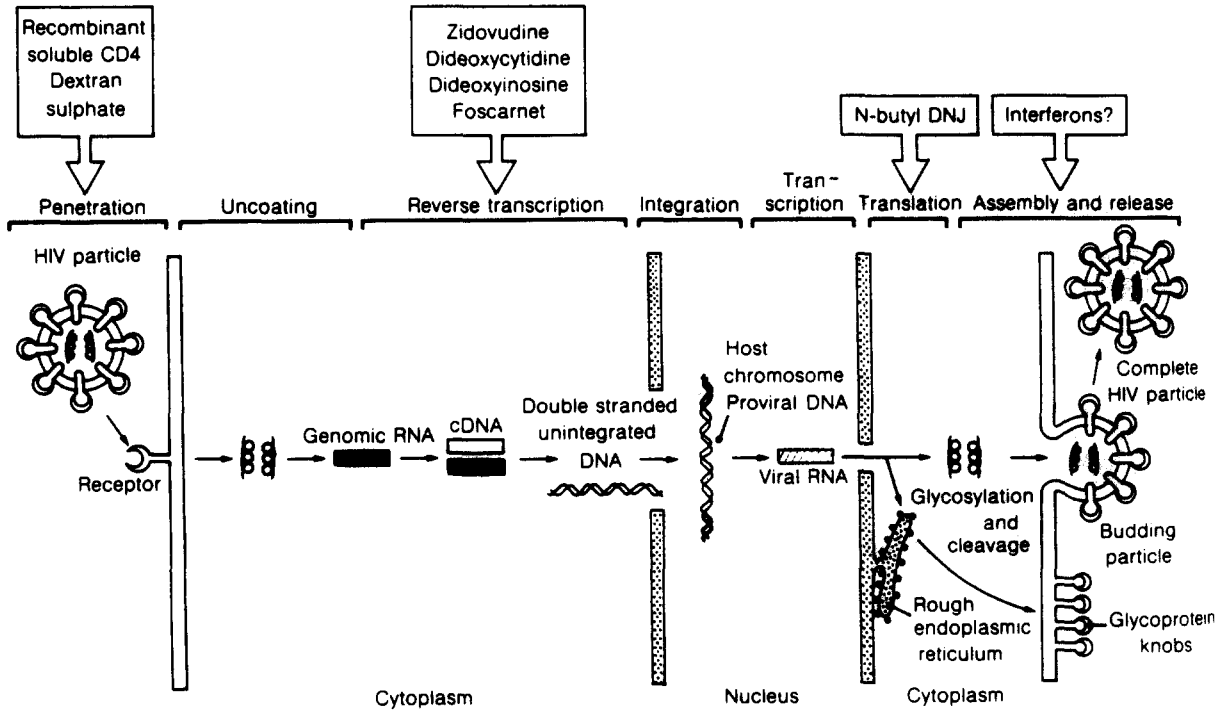

Fig. 2. Schematic representation of the life cycle of HIV-1 and the sites of action for various antiretroviral agents (reproduced from Johnson and Schooley, 1989).

analogs have demonstrated that cytosine- and purine-containing ddNs are transported and activated by independent pathways and, therefore, have important implications for anti-HIV therapy in that pyrimidine and purine ddNs might be used in combination for the treatment of AIDS (Kierdaszuk et al., 1992).

Certain opportunistic infections (e.g., herpes viruses) may activate HIV replication, and suppression of such infections may in turn reduce HIV replication. This has provided a rationale for studying the simultaneous administration of AZT and acyclovir (Surbone et al., 1988). Taking another approach, erythropoietin or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Hammer and Gillis, 1987; Baldwin et al., 1989; Perno et al., 1989; Pluda et al., 1990), the agent that stimulates the growth of bone marrow, might ameliorate the toxicity associated with AZT; GM-CSF may also act to enhance the activity of AZT in monocyte/macrophages. Finally, there is a renewed interest in combining the antiretroviral

TABLE 2

Stages in the HIV replicative cycle which may serve as targets for therapeutic intervention

Stage	Possible intervention
Binding to target cell	Antibodies to HIV or cellular receptors; CD4 analogs; calcium channel antagonists may rescue neuronal cells from possible gp120-induced cytocidal effect
Fusion to target cell	Agents that block the gp41 fusogenic domain function
Entry, uncoating of RNA, and functional release of HIV RNA	Agents that can block viral entry or uncoating; hypericin and pseudohypericin may block functional HIV RNA release
Transcription of RNA to DNA by RT	RT inhibitors (e.g., AZT and other dideoxynucleoside analogs TIBO compounds and dipyridodiazepinone derivatives, etc.)
Accumulation of unintegrated HIV in acute infection	Any intervention described above may suppress the cytopathic effect of HIV (if the DNA accumulation of unintegrated HIV DNA relates to premature cell death)
RNA degradation by RNase H activity	Specific blocking agents for HIV RNase H inhibitors
Migration of viral DNA to nucleus	Agents that block this step (to be identified)
Integration of HIV DNA into host genome (mediated by In protein)	Agents that inhibit *pol*-encoded integrase (In protein) function (to be identified)
Transcription and translation	Inhibitors of Tat or Rev activity; mutant Tat molecules; TAR inhibitors; TAR decoys; Rev protein inhibitors; HIV mRNA-specific destruction by ribozymes
Translation	Antisense constructs against regulatory HIV genes: *rev* gene
Ribosomal frameshifting	Inhibitors for ribosomal frameshift (to be identified)
Gag-Pol polyprotein cleavage	HIV protease inhibitors, e.g., transition-state mimetics
Myristoylation and glycosylation by cellular enzymes	Drugs (e.g., castanospermine and inhibitors of trimming glucosidase)
Dimerization, binding of lysine tRNA	Inhibitors for these stages (to be identified)
Packaging	Antisense contructs against the packaging ψ sequence (to be identified)
Viral budding	Interferons or interferon inducers; antibodies to viral antigens that block the release of virus
Extracellular processing of Gag-Pol polyproteins	HIV protease inhibitors

Source: Mitsuya et al. (1991).

therapy with immunostimulation (or immune reconstitution) (Lane et al., 1990; Yarchoan et al., 1990a).

In a normal individual, macrophages ingest an invading microbe and then are activated by gamma interferon and other cytokines (secreted by T cells) to destroy the microbe. But in patients infected with HIV, T cells are damaged and gamma interferon is in short supply, and so the parasite is ingested but not destroyed. As a result, some researchers have been exploring the value of adding gamma interferon (or alpha A interferon) to a standard therapeutic regimen (Hartshorn et al., 1987; Vogt et al., 1988; Berman et al., 1989; Kovacs et al., 1989; Krown et al., 1990; Mills and Masur, 1990). Although evidence of short-term effects has been found, the combination has shown no evidence of lasting antiviral activity beyond that achieved with AZT alone in patients with advanced HIV-1 infection (Edlin et al., 1992).

However, not all drug combinations have favorable interactions against HIV-1 (Baba et al., 1987; Balzarini et al., 1990, 1991; Busso and Resnick, 1990). The combination of AZT and ribavirin has been found to be antagonistic (Vogt et al., 1987); the mechanism for the observed antagonism appears to be attributed to the fact that the elevation of deoxythymidine triphosphate levels induced by ribavirin could produce a feedback which adversely inhibits cytosolic thymidine kinase in the host cell, which is required for the phosphorylation of AZT to its active triphosphate form (Johnson and Hirsch, 1990). Nevertheless, even though multiple dose-effect analysis has revealed a strong antagonism between ganciclovir and either AZT or ddI, at drug concentrations that were well below the cytotoxic range, at higher drug concentrations, the combination of ganciclovir and AZT was synergistically cytotoxic, and gangciclovir and ddI were additively cytotoxic (Medina et al., 1992).

Thus, combination regimens should be tested in vitro prior to clinical trials, particularly for those agents that may share the common metabolic pathways (Johnson and Hirsch, 1990).

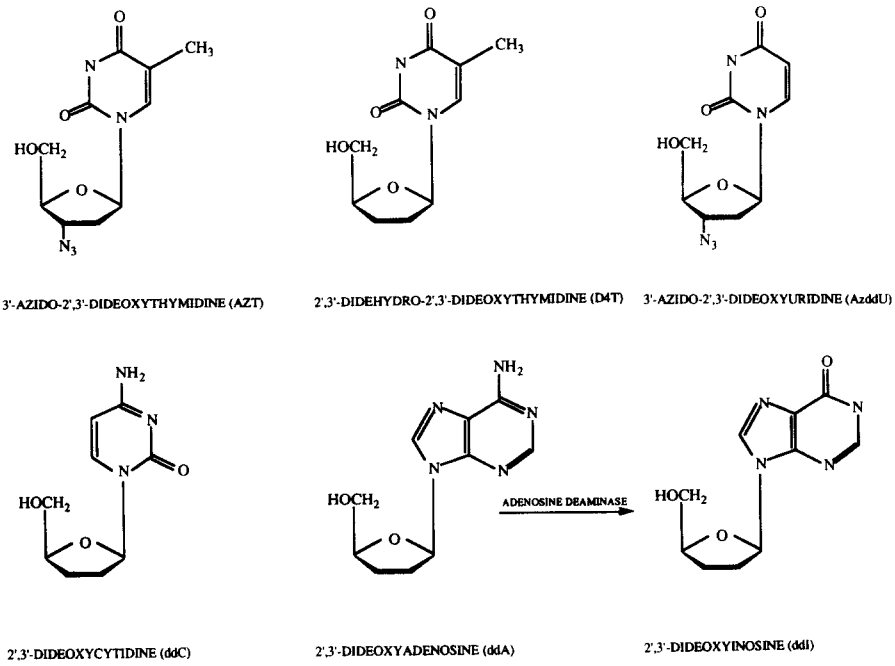

Fig. 3. Structure of dideoxynucleosides that have been in clinical trial. ddA is rapidly metabolized to ddI by adenosine deaminase (modified from Yarchoan et al., 1990a).

Solubilization and stabilization

The performance of a drug is a function of its physicochemical properties, such as aqueous solubility and drug stability. Studies have shown that the acidic lability of dideoxynucleosides is significantly increased upon removal of the 2′,3′-hydroxyl groups, the solution stability of dideoxynucleosides has become a significant concern in dosage form development. Moreover, the low solubility of some dideoxynucleosides may also pose problems, particularly in the development of solution-type dosage forms. Methods for improving the solubility and acidic stability of dideoxynucleosides may therefore have practical utility (Darrington et al., 1990).

The effect of complexation on the acid-catalyzed hydrolysis of ddA, a model dideoxypurine nucleoside, and the relationships between the state of ionization of ddA, the energies of complex formation, and the reactivity of ddA within the complexes, has been investigated. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) was chosen over β-CD as the model cyclodextrin for these studies since it has a much greater water solubility and lower toxicity than β-CD. The acid-catalyzed hydrolysis of ddA is completely inhibited in HP-β-CD complexes. Due to the small binding constants of both the protonated and neutral complexes, however, the maximum stabilization attainable in a 0.1 M HP-β-CD solution is 5-fold at pH 5 and only 2-fold at pH 2. Therefore, cyclodextrin complexation is not highly effective in stabilizing ddA in an acidic environment. Nevertheless, the approach may have utility in stabilizing other dideoxynucleosides if their binding affinities are significantly greater (Darrington et al., 1990).

Both ddA and ddI are unstable at low pH, dissociating into 2′,3′-dideoxyribose and the free base (adenine and hypoxanthine, respectively). Adenine is subsequently metabolized to 2,8-dihydroxyadenine, which is insoluble and can cause renal failure. By contrast, hypoxanthine is handled better by the body; it is catabolized to uric acid and subsequently excreted. Because of the toxicity of adenine, ddI is the preferred form for oral administration. Even with ddI, the issue of acid instability must be adressed when this drug is given by the oral route, e.g., by administering the drug with antacids or buffers (Yarchoan et al., 1990a; Hartman et al., 1991; Back et al., 1992).

2-Chloro-2′,3′-dideoxyadenosine (Cl-ddA) is a member of the family of ddA nucleoside analogs. It has been determined to have a relatively low solubility (1.01 mg/ml) in water. The desired solubility for formulation purposes is > 5 mg/ml. Due to its low apparent pK_a value, it may be possible to improve the aqueous solubility by formulating the compound at a pH lower than its pK_a. However, this compound is unstable in an acidic solution (at pH 2.0, $t_{1/2} = 23$ min). The solubility studies suggested that by using a mixed solvent system consisting of 40% propylene glycol, 10% ethanol, 50% phosphate buffer (0.05 M, pH 8.0), a solution formulation containing 5 mg/ml of Cl-ddA (or 10 mg/ml at 60 : 10 : 30) can be prepared. pH 8.0 was chosen, because it achieved maximum stability. It should be noted that these vehicles, especially the one with high concentration of propylene glycol, are likely to be irritating if directly administered to patients (Al-Razzak and Stella, 1990).

The major degradation product obtained in the solution formulation of Cl-ddA is 2-chloroadenine. The solubility studies of 2-chloroadenine in various solvents suggested that it is significantly less soluble than the corresponding nucleoside. Although the solubility of adenine was significantly enhanced in the presence of ddA, which could have resulted from the self-association of purine nucleosides through stacking interactions, only a slight increase in the solubility of 2-chloroadenine in the presence of Cl-ddA was obtained in the mixed solvent systems. The poor solubility of 2-chloroadenine could potentially limit the shelf-life of a liquid formulation of Cl-ddA (Al-Razzak and Stella, 1990).

AZT (4 mg/ml) in admixtures with 5% dextrose injection or 0.9% sodium chloride injection stored in polyvinyl chloride infusion bags has been found to be stable for up to 192 h (8 days) at

room temperature and under refrigeration (Lam et al., 1991).

Systemic drug delivery systems

Although several 2′,3′-dideoxypyrimidine nucleoside analogues have been investigated as anti-AIDS agents, rapid metabolism, low bioavailability and a number of severe side effects have been reported in AIDS patients undergoing treatment. Since either these nucleoside analogues work as metabolic antagonists, or their anti-viral effects can be time-dependent, a sufficient inhibitory concentration should be maintained in the body in order to produce the anticipated anti-viral action and to avoid undesirable side effects, which are attributable to excessive plasma concentration of drug (Kawaguchi et al., 1990b).

Drug coadministration

Probenecid The effect of the interaction between AZT and probenecid, a uricosuric agent, on pharmacokinetics has been evaluated in rats (Galinsky et al., 1991), rabbits (Hedaya and Sawchuk, 1989; Sawchuk and Hedaya, 1990; Wong et al., 1992), monkeys (Cretton et al., 1991; Qian et al., 1991) and humans (De Miranda et al., 1989; Hedaya et al., 1990). Concomitant administration of probenecid has been found to greatly alter the pharmacokinetics of AZT and G-AZT, resulting in a prolongation of their apparent elimination half-lives, an increase in their plasma concentrations, and a marked reduction in their renal clearance. In addition, the CSF/plasma concentration ratios for AZT and its catabolites were also greatly increased, suggesting that probenecid inhibits the efflux of AZT and its catabolites from CSF to plasma.

Dipyridamole Dipyridamole, a commonly used coronary vasodilator and antithrombotic drug, has been shown to potentiate the activity of AZT (Szebeni et al., 1989; Betageri et al., 1990) and ddC (Szebeni et al., 1989; Patel et al., 1991) against HIV-1 in human monocyte-macrophages in vitro. In the uninfected monocyte and macrophages, dipyridamole was noted to significantly inhibit the cellular salvage of thymidine and deoxycytidine, respectively. However, it did not affect the salvage of AZT and ddC. The observed inhibition of the salvage of competing physiological nucleosides may explain or contribute to the potentiating effect of dipyridamole on the antiviral activity of dideoxynucleoside drugs.

Methadone Intravenous drug users are the second largest population of individuals with HIV infection and AIDS in the United States and Europe, and represent a growing population in certain developing countries. Long-term methadone maintenance treatment is the most prevalent form of opiate addiction treatment. Pharmacokinetic parameters for methadone and AZT, alone and in combination, were determined in HIV-infected individuals including former intravenous drug users who were receiving methadone maintenance therapy. While AZT had no effect on methadone maintenance, a subset of methadone-maintained patients exhibited a significant alteration in their handling of AZT, resulting in elevated serum levels of the antiviral agent. The mechanism for this effect and its clinical consequences remain unclear (Schwartz et al., 1992).

Prodrugs

In an attempt to alleviate the drug-related toxicity of AZT in patients with AIDS, a pro-drug of AZT, 5′-{(1,4-dihydro-1-methyl-3-pyridinylcarbonyl)oxy}-3′-azido-2′,3′-dideoxythymidine (DP-AZT), has been synthesized and evaluated. Cellular uptake of DP-AZT by H9 cells and peripheral blood lymphocytes (PBL) was found to achieve at least a 50% greater intracellular concentration than AZT within 2 h. DP-AZT was observed to be significantly less toxic to murine bone marrow cells than AZT. The ED_{50} for inhibiting the production of HIV-specific p24 antigen was 0.05 μM for DP-AZT, compared to 0.125 M for AZT. These results demonstrated that DP-AZT has a higher therapeutic ratio than AZT as an anti-HIV agent (Gogu et al., 1989).

2′,3′-Dideoxy-2′,3′-didehydrothymidine (D4T) is a potent inhibitor for the RT in the HIV. In order to improve its delivery and/or to overcome any of its side effects, five novel ester-type prodrugs of D4T have been synthesized. A marked

increase in the partition coefficients (chloroform/water system at 25°C) was achieved through this prodrug modification. The rate of hydrolysis of D4T and its esters was determined at four solution pH values and at 40°C. Despite the fact that hydrolysis to D4T occurs at pH 10, all of the esters have been found to be rather stable under neutral or acidic conditions (pH 2–7) with D4T and/or thymine as the degradation products. At pH 2–10, D4T degrades slowly to yield thymine as a product. The ester-type prodrugs of D4T have shown considerable chemical stability but they are susceptible to enzyme hydrolysis (Kawaguchi et al., 1990a).

Ten AZT aliphatic ester-type prodrugs were synthesized, and the enzymatic regeneration of AZT from these prodrugs was investigated both in vitro and in vivo. The enzymatic hydrolysis rate of the AZT esters in the presence of various mouse tissues (plasma, liver, intestine and kidney) was observed to be highly dependent upon the lengths of the alkyl chains in the prodrugs. The caprate or caprylate of AZT showed the highest rate of enzymatic hydrolysis in three of the four tissues studied; beyond these esters, either the decrease or the increase in the alkyl chain length resulted in the decrease in the rate of enzymatic hydrolysis. AZT and three of its prodrugs (acetate, caprate, and stearate) were further studied in mice by intraperitoneal administration, and the plasma concentrations of AZT and its prodrugs were measured. The AZT concentrations in plasma following the administration of AZT-acetate rapidly decreased with a half-life of only 14.5 min, which is similar to that following the administration of AZT itself. On the other hand, the concentrations following the administration of caprate or stearate were observed to decrease slowly and maintained for as long as 4 h after dosing. The plasma concentrations of prodrug were below the detection limit (0.01 μg/ml), except for acetate. The absence of the caprate and stearate in plasma may be attributed to the high hydrophobicity or favorable tissue distribution of these esters (Kawaguchi et al., 1990b).

The plasma concentrations of AZT following the oral administration of AZT and its prodrugs in water and olive oil to rats has also been studied. A persisting plasma concentration of AZT was observed following the oral administration of AZT-acetate (a solution in oil) and AZT-stearate (a suspension in water and a solution in oil), which may be attributable, at least in part, to their relatively low specificity to enzymatic hydrolysis and possible slower release of the lipophilic prodrugs from the oil vehicle. Similar to the results by intraperitoneal administration, no prodrugs were detected in the plasma samples. The observation suggests possible deposition of the prodrugs into certain tissues, such as erythrocytes or fatty tissues, and/or rapid hepatic 'first-pass' bioconversion to AZT (Kawaguchi et al., 1991).

Membrane-soluble phosphate triester derivatives of AZT have been prepared, as the prodrugs of bioactive nucleotides, and evaluated against HIV-1 in vitro. It has been found that simple dialkyl phosphate triesters of AZT are inactive as antiviral agents, whereas phosphorodiamidate (P-N linked) derivatives display a potent anti-HIV effect (Devine et al., 1990). It was hoped that the 5′-phosphorodiamidates might act as membrane-soluble prodrugs of the bioactive nucleotides of AZT. Five different amino acids were employed, covering a range of structures and polarities, to prepare several 5′-phosphorodiamidates. These derivatives were tested in a human lymphoblastoid cell line for their inhibitory effect on HIV-1 proliferation. The results indicated that the amino acid derivatives are potent inhibitors of viral proliferation, and small changes in structure have led to a marked change in activity (Jones et al., 1991). A series of phosphorodiamidate derivatives of AZT has been prepared as membrane soluble prodrugs and evaluated in vitro against HIV-1. The terminally substituted alkyl amines were found to have a pronounced anti-HIV effect, but this effect declined upon increasing the length of the methylene spacer. The results were consistent with a mechanism of action involving intracellular cleavage of the phosphoramidate bond, and release of the nucleotide, or its derivative (Curley et al., 1990). If the phosphorus center carries a trichloro- (or trifluoro-) ethyl group and a carboxyl-protected amino-linked amino acid, the compounds display potent anti-HIV activity with low host toxicity,

but this activity does not increase with the introduction of a haloalkyl moiety. The trichloroethyl methoxyalaninyl compound is exceptional, in which the activity is enhanced by 50-fold with the addition of trichloroethyl group (McGuigan et al., 1991, 1992).

The failure of dideoxynucleosides to isolate HIV from mononuclear cells in the peripheral

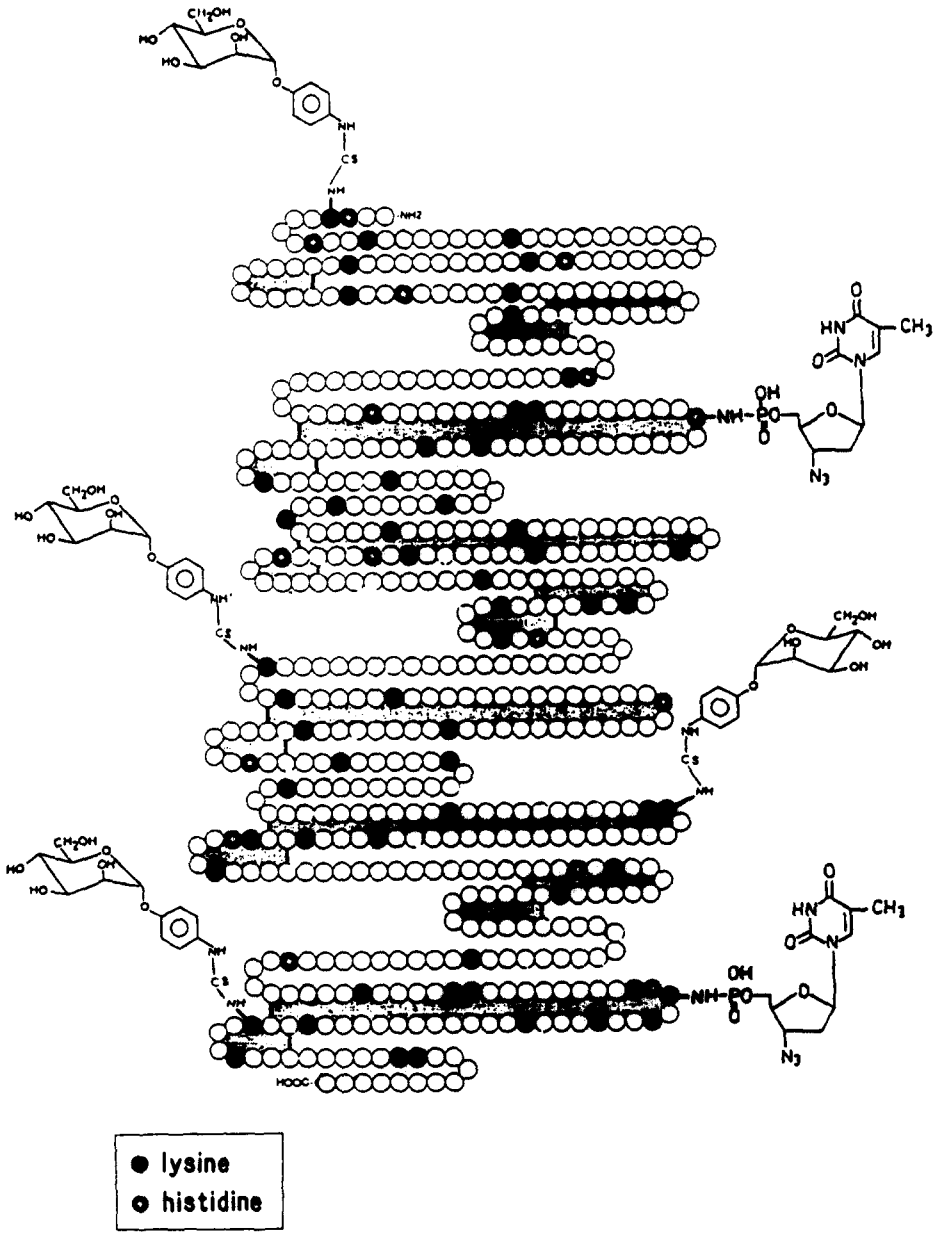

Fig. 4. Schematic representation of a neoglycoprotein-AZT-MP conjugate, in which azidothymidine-monophosphate (AZT-MP) is bound to lysine group of HSA through the coupling with *p*-aminophenyl-mannose. Circles represent amino acids (reproduced from Molema et al., 1990).

blood may be due to the inadequate inhibition of virus production by macrophages, which is a major reservoir of HIV infection. It has been observed that cells of the macrophage lineage take up large amounts of liposomal material administered parenterally. Based on this observation, phosphatidyl-AZT, AZT diphosphate dipalmitin, phosphatidyl-ddC and phosphatidyl-ddT, etc., novel phospholipid prodrugs, which are readily incorporated into lipid bilayers, have been synthesized. It is anticipated that by forming liposomes, these antiretroviral prodrugs will be taken up in greater proportion by macrophages. This property would appear to make phospholipid prodrugs the promising candidates with a potential to target dideoxynucleosides to the macrophage reservoir of HIV infection, thereby reducing the toxicity of the antiviral nucleosides to other cells (Hostetler et al., 1990; Steim et al., 1990).

Liposomes

Liposomes are vesicles consisting of one or more concentrically ordered assemblies of phospholipid bilayers (Ranade, 1989). The antiviral activities of ddC, 2′,3′-dideoxycytidine-5′-triphosphate (ddC-TP) and liposome-encapsulated ddC-TP [L(ddC-TP)] have been compared in cultured human monocyte-macrophages (M/M) infected with HIV-1. These treatments were found to inhibit virus replication with drug at nanomolar levels. The results on L(ddC-TP) suggested that the potential of using liposomes to target drugs to macrophages could be exploited in vivo to improve the therapeutic index of dideoxynucleoside drugs (Szebeni et al., 1990).

Studying the effect of liposome encapsulation on the antiviral activity of AZT and its bone marrow toxicity in mice has shown a reduced localization in bone marrow (Phillips et al., 1991).

Membrane lectin-mediated endocytosis

Specific receptors, such as membrane lectins that mediate endocytosis upon binding of ligand to the surface of infected cells, could be applied to the delivery of antiviral drugs. New developments based on this approach have made progress with the use of neoglycoproteins, glycoproteins and glycosylated biopolymers (Roche et al., 1990).

The delivery of AZT, in its 5′-monophosphate form (AZT-MP), into human T-lymphocyte MT-4 cells in vitro through the covalent coupling to neoglycoproteins (Fig. 4) has been investigated. In vivo, this drug targeting concept may lead to an increase in the efficacy and/or the reduction of side effects of AZT. The rationale for the design of neoglycoprotein carriers is based on the concept that existence of a sugar, such as mannose, which is capable of recognizing lectins on T-lymphocytes could enhance targeting of AZT. Using a phenyl-linkage between sugar and human serum albumin (HSA), and thiophosgene-activated *p*-aminophenyl sugar derivatives, various neoglycoproteins have been synthesized (Molema et al., 1990, 1991). The recognition of the neoglycoprotein by a lectin component in the cell membrane and subsequent internalization (endocytosis) and release of the AZT from the conjugate may enhance the targeted delivery of AZT.

Human erythrocytes

ddC is known to have a rapid clearance, and it must be administered to patients every 4 h, which often attains a concentration that is toxic. 2′,3′-Dideoxycytidine-5′-phosphate (ddC-MP) has been synthesized as a prodrug and encapsulated in human erythrocytes. This prodrug has been repoted to be dephosphorylated by the endogenous pyrimidine nucleotidases in erythrocytes, and subsequently released as ddC. Encapsulated ddC-MP was found not to affect the metabolism of the erythrocyte, and was not deaminated by cytidine deaminase. The dephosphorylation reaction was also not inhibited by ATP or 2,3-bisphosphoglycerate. The efflux of ddC from the erythrocyte was discovered to be a linear function of ddC concentration and relatively insensitive to nucleoside transporter inhibitors (Magnani et al., 1989a). Thus, ddC-MP-loaded erythrocytes might be used as an endogenous bioreactor for ddC delivery (Magnani et al., 1989b).

Implantable pumps

AZT, in alkaline solution, has been administered by continuous infusion via an implantable

pump in dogs. Steady-state AZT serum concentrations of greater than 1 μM could be maintained for prolonged duration. Intraarterial delivery did not increase the CNS uptake of AZT. The morbidity associated with infusion did not seem to limit this mode of therapy (Gallo et al., 1992).

The pharmacokinetics of AZT in mice, after twice-daily subcutaneous bolus injections and during continuous subcutaneous infusion with ALZET mini-osmotic pumps, has been studied. The results suggested that moderate constant levels of AZT have achieved a greater antiretroviral efficacy than intermittent high concentrations (Sinet et al., 1992).

Ceramic capsules

Administration of AZT to treat AIDS patients by conventional methods has often produced adverse side effects, including toxic effects on bone marrow. The capability of tricalcium phosphate (TCP) and alumino-calcium-phosphorous oxide (ALCAP) ceramic capsules, an intraperitoneally implantable capsular drug delivery system, to provide a sustained release of AZT was investigated in vitro and in vivo in rats. The results indicated that ALCAP ceramic capsules can deliver AZT in rats in a controlled manner for a minimum duration of 120 days. It also suggested that ALCAP capsules probably can also be used to deliver other nucleosides in a sustained manner for long durations in mammalian systems, including humans (Benghuzzi et al., 1989, 1990).

Transdermal delivery systems

Although orally administered AZT is rapidly absorbed from the intestine, it loses considerable potency during its absorption by the hepatic first-pass metabolism (40%) and then is rapidly eliminated from the body with a half-life of only 1 h. In addition, orally administered AZT often shows strong side effects on bone marrow, which may be attributed to the attainment of an excessive plasma level of AZT immediately after administration. Development of a non-oral delivery system with zero-order drug release would be useful to decrease the high daily dose of AZT (5–10 mg/kg, every 4 h) required to maintain its anti-viral effect, to reduce the strong side effects, and to improve patient compliance (Seki et al., 1990b).

The skin has been reported to be a useful site for systemic administration to maintain a therapeutically effective plasma concentration of drugs (Chien, 1987). Percutaneous administration of AZT could be useful in improving its anti-AIDS activity and patient compliance and avoiding or minimizing the side effects. Percutaneous absorption of AZT has been examined in rats. Transdermal AZT delivery systems are compared with an AZT solution administered orally. The plasma profile of AZT in rats after oral administration is similar to that in patients with AIDS reported by Klecker et al. (1987). Since the plasma level of AZT achieved following the topical application of AZT in a simple aqueous solution was found to be too low to be detected, isopropyl myristate (IPM), *N*-methyl-2-pyrrolidone (MP) and oleic acid (OA), agents which have been permitted as additives for external preparations, were added as penetration enhancers (Barry and Bennett, 1987; Yamada and Uda, 1987; Sato et al., 1988; Sugibayashi et al., 1988; Seki et al., 1989a). Since IPM and OA have low solubility in water, emulsions were prepared with 10% of these additives. While the plasma concentration of AZT was still undetectable throughout the experimental period (24 h) when IPM or MP was used, considerable plasma concentrations of AZT were detected with the preparation containing 10% OA (Seki et al., 1989b).

The skin has been used as the site for application of transdermal rate-controlled delivery systems. In an attempt to improve the skin permeability of AZT, five aliphatic esters (acetate, butyrate, hexanoate, octanoate and decanoate) of AZT have been synthesized as the prodrugs of AZT. While the aqueous solubility of these esters is lower than that of AZT, their solubility in IPM and partition coefficient (*n*-octanol : buffer) are higher. Susceptibility to enzymatic hydrolysis in the rat skin homogenate increases as the alkyl chain length of the ester is lengthened. Among the esters, acetate and hexanoate showed a 2.4- and 4.8-fold enhancement, respectively, in human skin permeation from an apolar vehicle (IPM) over AZT itself (Fig. 5) (Seki et al., 1990a).

TABLE 3

Selected pharmacokinetic parameters for AZT, ddC, and ddI

Drug	Typical dose (mg)	Peak plasma level (μM)	Oral bioavailability (%)	Terminal plasma half-life (h)	Approximate intra-cellular triphosphate half-life (h)	CSF: plasma ratio	Chief clearance route
AZT	200	4	63	1.1	1.3	0.6	liver, kidney
ddC	2	0.1–0.2	87	1.2	2.6	0.2	kidney
ddI	250	8–10	40	0.5	12–24	0.2	kidney (probably metabolized to uric acid)

Source: Yarchoan et al. (1990a).

The in vitro permeation of AZT through the rat skin from an IPM solution was noted to be significantly enhanced by the addition of *N*-methyl-2-pyrrolidone (MP) as a penetration enhancer. To control the constant delivery of AZT, (ethylene/vinyl acetate) copolymer membrane has been used for the development of a transdermal AZT delivery system. With coadministration of MP, a considerable plasma level of AZT has been achieved and maintained for as long as 10 h from this controlled-release transdermal AZT delivery system (Seki et al., 1991).

By superposition of an electric potential gradient on the existing chemical potential gradient for diffusion, iontophoresis can enhance the flux of ionized drugs into skin (Srinivasan et al., 1990). AZT has been used as a model drug to study the effect of iontophoresis on the skin permeation of a neutral compound. The rate of in vitro permeation across hairless rat skin is low and highly variable. With iontophoresis treatment the permeation rate is 2–3-fold greater than by passive diffusion (Fig. 6). The addition of varying amounts of sodium chloride to the donor enhances the iontophoretic permeation rate another 2–3-fold possibly due to convective forces. The addition of *N*-decylmethyl sulfoxide (C_{10}MSO) to the donor increases the permeation rate by several hundred-fold over passive diffusion for hairless rat skin and approx. 75-fold for human skin (Table 4) (Wearley and Chien, 1990).

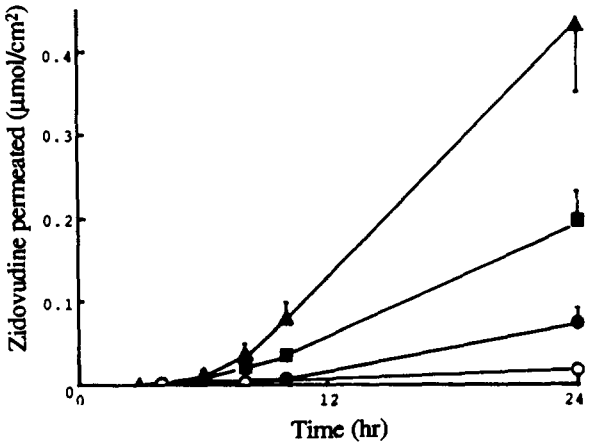

Fig. 5. Permeation profile of AZT (water, ○; IPM, ●), its acetate (IPM, ▲) and hexanoate (IPM, ■) through human skin. Data are the means ± SE ($n = 3$) (reproduced from Seki et al., 1990a).

CNS-targeted drug delivery systems

Patients with AIDS and ARC frequently develop neurological complications resulting from

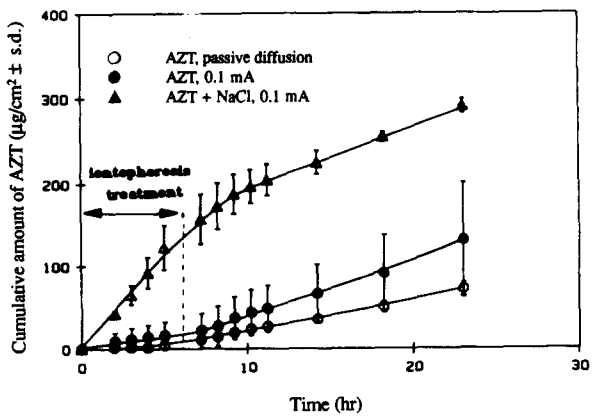

Fig. 6. Passive and iontophoresis-facilitated skin permeation profiles of AZT. Iontophoresis treatment was 0.1 mA of pulse current for 6 h (reproduced from Wearley and Chien, 1990).

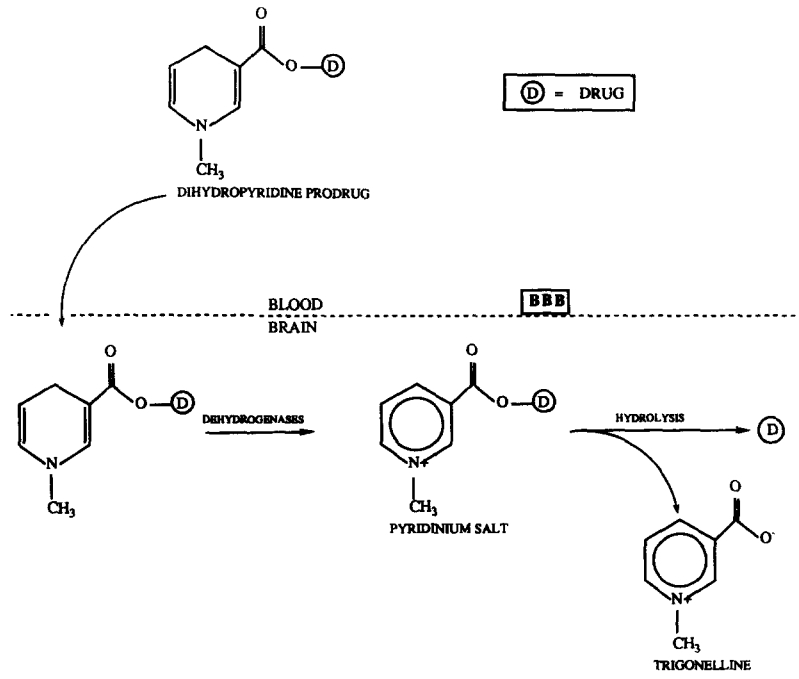

Fig. 7. Schematic illustration for the transport of a dihydropyridine prodrug of anti-HIV agents (D) through the blood-brain barrier (BBB) (modified from Palomino, 1990).

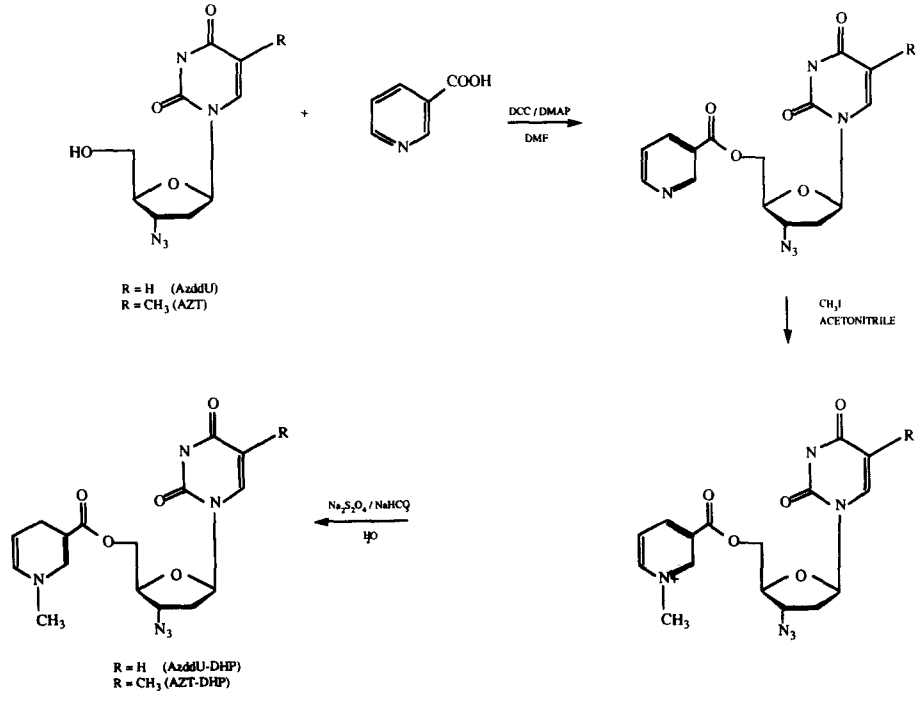

Fig. 8. Synthesis of the dihydropyridine derivatives of AzddU and AZT (modified from Chu et al., 1990b).

TABLE 4

Passive AZT permeation rate values (standard deviation) with and without C_{10}MSO treatment

Skin species	Permeation rate (mg cm^{-2} h^{-1})		Enhancement ratio
	Without C_{10}MSO	With C_{10}MSO	
Hairless rat	0.00180 (0.000550)	1.24 (0.255)	688.9
Fuzzy rat	0.00047 (0.000057)	0.42 (0.076)	893.6
Human cadaver	0.00520 (0.002500)	0.40 (0.220)	076.9

Source: Wearley and Chien (1990).

the invasion of HIV into the brain. Although the mechanism of HIV-induced dysfunction of the central nervous system (CNS) is unknown, it is believed that HIV is carried into the brain by infected macrophages/monocytes. Thus, it is essential to have anti-HIV agents permeating through the blood-brain barrier (BBB) to suppress the replication of HIV in the brain. AZT has been detected in the cerebrospinal fluid (CSF), which partially reverses the neurological complications. However, it has not been demonstrated that AZT actually permeates the BBB or maintains the concentration in CNS sufficient to effectively suppress the viral replication in the brain. Thus, it has been of interest to develop delivery systems of anti-HIV drugs which could more readily penetrate the BBB (Chu et al., 1990a).

Chemical delivery systems

It appears that AZT is highly unusual among nucleoside analogues in that it traverses the cell membrane chiefly by passive diffusion and not via a nucleoside transport system. This has been attributed to the considerable lipophilicity of this molecule gained by replacing the 3'-hydroxyl group of thymidine by an azido substituent (Palomino et al., 1989).

The chemical delivery system developed by Brewster et al. (1991) could be applied to facilitate the transport of anti-HIV drugs across the BBB using a dihydropyridine-pyridinium salt redox system (Fig. 7) (Palomino, 1990). Dihydropyridine (DHP) derivatives of AzddU and AZT

TABLE 5

Areas under the serum and brain concentration-time curves (AUC) for parent compounds, prodrugs and quaternary salts following administration of AZT-DHP and AzddU-DHP in comparison to AzddU and AZT

Compound		AUC (μg ml^{-1} h)	
Administered	Detected	Serum	Brain
AzddU-DHP		4.4	–
	AzddU-QS	1.2	3.9
	AzddU	25.8	11.4
AzddU	AzddU	25.8	2.1
AZT-DHP		1.3	–
	AZT-QS	0.6	1.2
	AZT	25.4	11.2
AZT	AZT	26.6	1.2

Source: Chu et al. (1990b).

have been synthesized (Torrence et al., 1988; Chu et al., 1990b) as shown in Fig. 8. The in vivo data generated in mouse suggested that DHP derivatives disappear rapidly from the serum and brain (Table 5). In Table 6 the enhancement in brain delivery of AzddU and AZT and their apparent brain half-lives are compared. The results indicate a significant increase in the delivery of these compounds to the brain following prodrug administration. It appears to suggest that the greater octanol/water partition coefficients of DHP derivatives (21.6 and 53.1 for AzddU-DHP and

TABLE 6

Relative brain exposure (r_e) values and apparent brain elimination half-lives ($t_{1/2}$) for AzddU and AZT

Compound		Enhanced brain delivery [a]	$t_{1/2}$ (h)
Administered	Detected		
AzddU-DHP	AzddU	5.47	4.34
AzddU		–	0.84
AZT-DHP	AZT	9.32	15.80
AZT		–	0.54

[a] Calculated from the ratio of $(AUC)_b/(AUC)_p$; As the value greater than unity indicates that a favorable brain delivery of the parent nucleosides is obtained following the administration of the prodrug. b, brain; p, plasma.
Source: Chu et al. (1990b).

AZT-DHP, respectively) relative to the parent compounds (0.45 and 1.10 for AzddU and AZT, respectively) contribute to the enhanced brain delivery, which is consistent with the notion that the lipophilic DHP derivatives penetrate the BBB more readily than do the parent compounds. Comparison of the half-lives of the parent drugs in the brain following the administration of parent drugs and prodrugs indicates an increased retention of the drugs in the brain by the DHP approach (Chu et al., 1990b).

Liposomes

Because the HIV infects the CNS, hence a successful antiviral treatment must include this organ as one of the target sites. However, many potentially useful antiviral drugs have been reported not being able to penetrate through the BBB completely when they are given by the conventional oral or intravenous route, leaving the CNS only partially treated (Terasaki and Pardridge, 1988). Even with direct administration into the CSF, bulk flow and absorption into the arachnoid granulation have resulted in a rapid clearance of antiretroviral drugs. Frequent administration is thus required. One approach to improve the CNS delivery of antiretroviral drugs is via the injection in a depot form that would slowly release the antiviral agent for prolonged period and maintain a concentration sufficient to prevent viral replication.

Use of multivesicular liposomes for administration of antiviral agents into CSF has been explored in rats. One example is that ddC was encapsulated into a multivesicular liposome prepared from the combination of dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, cholesterol and triolein. The half-lives for the release of ddC in human plasma and in saline solution were 15 and 47 h, respectively. After intraventricular injection with a stereotaxic apparatus, the ddC levels in the CNS was observed to decrease exponentially, with a half-life of 1.1 h for the unencapsulated ddC and 23 h for the liposome-encapsulated ddC (Fig. 9). The half-life of a more hydrophilic antiviral agent is expected to increase even further by liposomal encapsulation. The results of this study offer a practical intrathecal delivery system for

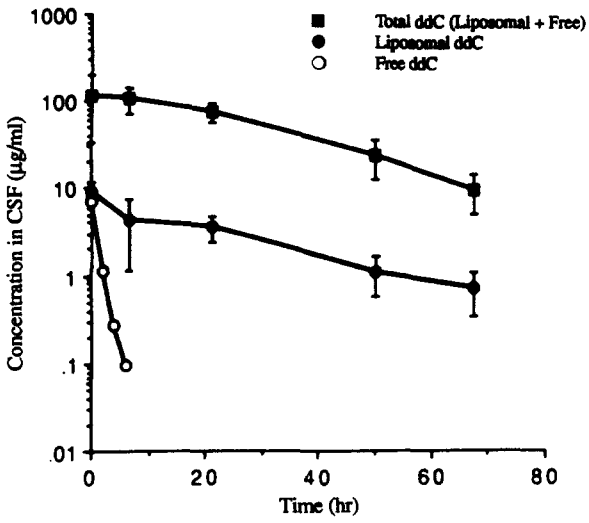

Fig. 9. Concentration profiles of free ddC (excluding ddC that remains within liposomes) in CSF (bathing brain) following the intraventricular administration of unencapsulated ddC and liposomal ddC. Top curve depicts the total concentration of ddC after the administration of liposomal ddC. Each point represents the mean value of three rats for liposomal ddC (bars = SD), from a single rat for unencapsulated ddC (reproduced from Kim et al., 1990).

drugs that do not cross the BBB (Kim et al., 1990).

Insulin coadministration

The HIV has been found to be neurotropic. The BBB is a special conformation of cellular membranes that has at times served to confound effective drug therapy for intra-CNS conditions. For these reasons, the development of an effective strategy for the delivery of antiviral drugs across the BBB is of paramount importance in the treatment of HIV infection. There are insulin receptors on the capillary endothelial cells in the BBB and it has been proposed that these may play a role in enhancing the transport of drug molecules across the BBB by coadministration with exogenous insulin. Evidence has shown that insulin may be used as a pharmacologic adjunct in the therapy of HIV infection to achieve a higher concentration of antiviral drugs in the CNS while the total dose of drugs used is reduced (Ayre et al., 1989). It has been hypothesized that this would enhance the drug's therapeutic effectiveness while obviating dose-related side-effects (Ayre, 1989).

Conclusion

The strategy for the development of antiviral therapy in HIV infection has been to use AZT as the standard drug, against which the efficacy and toxicity of all the other therapies have been evaluated.

It is still too early to predict the potential for clinical trials of the various drug delivery systems described in this article, since some of them have been evaluated only in vitro (for instance, iontophoresis for transdermal systemic drug delivery systems), while others are only hypotheses (for instance, insulin coadministration for CNS-targeted drug delivery systems). Nevertheless, it is evident that the prodrug and transdermal approaches have been extensively investigated. We believe that the delivery of anti-HIV drugs using liposomes, in combination with the prodrug approach, holds most promise since both sustained blood levels as well as CNS-targeting could be achieved.

Overall, the progress in the development of delivery systems for these drugs has been slow. It is the authors' opinion that the evaluation of the effect of drug delivery systems on the efficacy and toxicity of antiviral therapy will have a great impact on the treatment of HIV infection. A collaborative effort of scientists in various disciplines to boost research in this field is warranted and urged.

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