Molecular Targets for Antiviral Agents

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ABSTRACT
There are a number of virus-specific processes within the virus replicative cycle or virus-infected cell that have proven to be attractive targets for chemotherapeutic intervention, i.e., virus adsorption and entry into the cells, reverse (RNA → DNA) transcription, viral DNA polymerization, and cellular enzymatic reactions that are associated with viral DNA and RNA synthesis and viral mRNA maturation (i.e., methylation). A variety of chemotherapeutic agents, both nucleoside (and nucleotide) and non-nucleoside entities, have been identified that specifically interact with these viral targets, that selectively inhibit virus replication, and that are either used or considered for clinical use in the treatment of virus infections in humans. Their indications encompass virtually all major human viral pathogens, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), human papilloma virus (HPV), orthomyxoviruses (influenza A and B), paramyxoviruses [e.g., respiratory syncytial virus (RSV)] and hemorrhagic fever viruses (such as Ebola virus).

For many years virus diseases have been considered as intractable to selective antiviral chemotherapy because the replicative cycle of the virus was assumed to be too closely interwoven with normal cell metabolism so that any attempt to suppress virus reproduction would be doomed to kill (or severely harm) the uninfected cell as well. With the elucidation of virus-specific events as targets for chemotherapeutic attack and the advent of a number of specific antiviral agents, it has become increasingly clear that a selective chemotherapeutic approach to virus infections can be achieved and that virus reproduction can be suppressed without deleterious effects on the host.

There are currently 30 antiviral drugs that have been officially approved for the treatment of virus infections (De Clercq, 2001a): zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and lopinavir for the treatment of human immunodeficiency virus (HIV) infections; lamivudine also for the treatment of hepatitis B virus (HBV) infections; acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, trifluridine, and brivudin for the treatment of herpes simplex virus (HSV) and/or varicella-zoster virus (VZV) infections; ganciclovir, foscarinet, cidovir, and fomiviren for the treatment of cytomegalovirus (CMV) infections; ribavirin for the treatment of respiratory syncytial virus (RSV) infections and, in combination with interferon-α, for the treatment of hepatitis C virus (HCV) infections; amantadine and rimantadine for the treatment of influenza A virus infections; and, finally, the neuraminidase inhibitors zanamivir and oseltamivir for the treatment of influenza A and B virus infections. Several other compounds, among which are adefovir dipivoxil and tenofovir disoproxil, are momentarily in advanced phase III clinical development.

ABBREVIATIONS: HIV, human immunodeficiency virus; HBV, hepatitis B virus; HSV, herpes simplex virus; VZV, varicella-zoster virus; CMV, cytomegalovirus; HPV, human papilloma virus; RSV, respiratory syncytial virus; HCV, hepatitis C virus; IMP, inosine 5'-monophosphate; SAH, S-adenosylhomocysteine; RT, reverse transcriptase; NRTI, nucleoside (type of) reverse transcriptase inhibitor; NNRTI, non-nucleoside (type of) reverse transcriptase inhibitor; NVAS, polyvinylalcohol sulfate; PVS, polyvinylsulfonate; TM, transmembrane; M-tropic, macrophage tropic; T-tropic, T-cell tropic; AIDS, acquired immune deficiency syndrome; FIV, feline immunodeficiency virus; SIV, simian immunodeficiency virus; dNTP, deoxynucleoside-5'-triphosphate; ddN, 2',3'-dideoxynucleoside; d4T, dideoxycytidine; D4P, 2,6-diaminopurine dioxolane; AZT, azidothymidine; NDP, nucleoside 5'-diphosphate; MP, monophosphate; TP, triphosphate; HEP, 1-(2-hydroxyethoxyethyl)-6-(phenylthio)thymine; TIBO, tetrahydroimidazo-[4,5,1-jk][1,4]benzodiazepin-2(1H)-one and -thione; BVDU, brivudin; TK, thymidine kinase; EICAR, 5-ethynyl-1-β-o-ribofuranosylimidazole-4-carboxamide; SAM, S-adenosylmethionine; ddI, dideoxyinosine; ddC, dideoxycytidine; ABC, abacavir; (-)-FTC, emtricitabine; (S)-DHPA, (S)-9-(2,3-dihydroxypropyl)adenine.
clinical trials for the treatment of HBV and HIV infections, respectively.

The viral replication cycle can be roughly divided into 10 steps: virus-cell adsorption (binding, attachment), virus-cell fusion (entry, penetration), uncoating (decapsidation), early transcription, early translation, replication of the viral genome, late transcription, late translation, virus assembly, and release. HIV (Fig. 1) follows this general strategy, albeit with some modifications: early transcription (step 4) is replaced by reverse transcription, early translation (step 5) is replaced by integration, and the final steps (assembly and release) occur concurrently as a process that has been dubbed “budding” and that is followed by maturation. All these steps could be envisaged as targets for chemotherapeutic intervention (De Clercq, 2000a). In addition to these virus-specific events, there are a number of host enzymes and processes that are innately involved with viral DNA, RNA, and/or (glyco)protein syntheses. Also, these processes [i.e., inosine 5'-monophosphate (IMP) dehydrogenase, S-adenosylhomocysteine (SAH) hydrolase, orotidine 5'-monophosphate decarboxylase, CTP synthetase, glycosylation pathways, etc.] may be considered as targets for antiviral agents (De Clercq, 1997).

Of all the potential targets for antiviral chemotherapy, I have selected the following eight to be further addressed in this overview (Table 1): 1) virus adsorption as the target for polyanionic substances that inhibit the replication of HIV and other enveloped viruses; 2) virus receptors and co-receptors as the target for antagonists such as the CXCR4 antagonists that block cell entry of T-tropic (X4) HIV strains; 3) HIV reverse transcriptase (RT) as the target for the nucleoside type of reverse transcriptase inhibitors (NRTIs); 4) a second (allosteric) site at HIV-1 RT as the target for the non-nucleoside type of reverse transcriptase inhibitors (NNRTIs); 5) herpesvirus DNA polymerase as the target for a series of acyclic guanosine analogs and 5-substituted 2'-deoxyuridines that are effective against HSV and VZV (following their phosphorylation by the virus-encoded thymidine kinase); 6) viral DNA polymerase (and reverse transcriptase) as the target for the acyclic nucleoside phosphonates cidofovir, adefovir, and tenofovir; 7) IMP dehydrogenase as a cellular target for the broad-spectrum antiviral activity of a number of IMP dehydrogenase inhibitors; and 8) SAH hydrolase as another cellular target for the activity of adenosine analogs against negatively stranded RNA viruses (including, among others, Ebola virus). The molecular targets, mechanisms of action, antiviral activity spectra, and clinical applications of these eight classes of antiviral compounds are schematically reviewed in Table 1, and chemical structures for representative prototype compounds are given in Fig. 2.

1. Anionic Polymers Targeted at the Viral Glycoproteins

Various polyanionic substances have been described to block HIV replication through interference with virus adsorption (binding) to the cell surface, e.g., polysulfates [such as dextran sulfate, dextrin sulfate, and polyvinylalcohol sulfate (PVAS) (Fig. 2)], polysulfonates [such as suramin (the first compound ever to be identified as an inhibitor of HIV replication) and polyvinylsulfonate (PVS) (Fig. 2)], polycarboxylates [such as those equipped with the cosalane pharmacophore (Cushman et al., 1999)], and polyoxometalates [heteropolytungstates containing a single, double, or triple Keggin or single or double Dawson type of structure (Witvrouw et al., 2000b)]. All these polyanionic substances can be assumed to exert their anti-HIV activity by shielding off the positively charged amino acid (lysine and arginine) residues on the V3 loop of the viral envelope glycoprotein gp120 (Fig. 3A), thus preventing the interaction of gp120 with its cellular receptor CD4.

Polyanionic (e.g., polysulfonate) dendrimers can inhibit HIV replication by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle (Witvrouw et al., 2000a). However, the fact that resistance selected upon passaging the virus in the presence of these compounds was associated with mutations in the envelope glycoprotein gp120 (and not the reverse transcriptase or integrase) points to the gp120 as the principal target.

<table>
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<th>Compounds</th>
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<td><strong>1. Anionic polymers</strong></td>
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<tr>
<td>Polysulfates (PVAS)</td>
<td>Virus adsorption (i.e., interaction between HIV gp120 and CD4 receptor)</td>
<td>Interaction with viral envelope glycoprotein (i.e., V3 loop of gp120)</td>
<td>HIV and other enveloped viruses (RSV, HSV, CMV, etc.)</td>
<td>As vaginal microbicides (under consideration) in the prevention of sexual HIV transmission</td>
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<td>Polysulfonates (PVS)</td>
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<td>Polycarboxylates</td>
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<td>Polyoxometalates</td>
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<td>Chicoric acid, zintevir, cosalane derivatives</td>
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<td><strong>2. Receptor antagonists</strong></td>
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<td>Bicyclams (i.e., AMD3100)</td>
<td>Virus-cell entry (fusion)</td>
<td>Interaction with CXCR4 or CCR5, the co-receptors for entry of T-tropic (X4) and M-tropic (R5) HIV strains, respectively</td>
<td>HIV</td>
<td>Systemic treatment of HIV infection (under clinical development)</td>
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<td>T-22, T-134, ALX-40-4C, CGP-64222, TAK-779</td>
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<td><strong>3. NRTIs</strong></td>
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<td>AZT, ddI, ddC, d4T, 3TC, ABC, and other ddN analogs</td>
<td>Substrate (dNTP)-binding site of RT (following intracellular phosphorylation to the 5'-triphosphate)</td>
<td>Chain termination after incorporation of the ddN 5'-monophosphate at the 3'-end of the viral DNA chain</td>
<td>HIV (and other retroviruses)</td>
<td>AZT, ddI, ddC, d4T, 3TC, ABC formally approved for the treatment of HIV infection (others are under clinical development)</td>
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<td><strong>4. NNRTIs</strong></td>
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<td>Nevirapine, delavirdine, efavirenz, emivirine (MKC-442), capravirine, thiorcarboxanilide UC-781, etc.</td>
<td>Nonsubstrate-binding site of HIV-1 RT</td>
<td>Inhibition of HIV-1 RT through interaction with allosteric pocket site</td>
<td>HIV-1</td>
<td>Nevirapine, delavirdine, and efavirenz formally approved for the treatment of HIV infection (others are under clinical development)</td>
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<td><strong>5. Acyclic guanosine analogs and 5-substituted 2'-deoxyuridines</strong></td>
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<td>Acyclovir, valaciclovir, penciclovir, famciclovir, bromovinylethoxycytidine (BVDU)</td>
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<td>First phosphorylation step by the virus-encoded TK, incorporated at 3'-end (acyclovir) or via internucleotide linkage (BVDU)</td>
<td>HSV</td>
<td>Formally approved for the systemic and/or topical treatment of HSV and/or VZV infections</td>
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<td><strong>6. Acyclic nucleoside phosphonates</strong></td>
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<tr>
<td>Cidofovir</td>
<td>DNA-viral DNA polymerase</td>
<td>Chain termination after incorporation at the 3'-end of the viral DNA chain</td>
<td>Herpes-, adeno-, pox-, papillomaviruses</td>
<td>CMV retinitis (approved in AIDS patients)</td>
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<td>Adefovir dipivoxil</td>
<td>HIV and HBV RT</td>
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<td>HIV, HBV</td>
<td>Phase III (HBV)</td>
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<tr>
<td>Tenofovir disoproxil</td>
<td>HIV and HBV RT (for all three, following intracellular phosphorylation to the diphosphate)</td>
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<td>HIV, HBV</td>
<td>Phase III (HBV)</td>
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<td><strong>7. AICAR analogs</strong></td>
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<td>(Ribavirin, EICAR, etc.)</td>
<td>IMP dehydrogenase (apoenzyme or coenzyme)</td>
<td>Inhibition of apoenzyme after intracellular phosphorylation to the 5'-monophosphate (ribavirin) or inhibition of coenzyme (mycophenolic acid), ultimately leading to a suppression in the supply of GTP and dGTP</td>
<td>Broad spectrum of (+)RNA viruses and (−)RNA viruses</td>
<td>Ribavirin approved for treatment of RSV and HCV infections (for the latter, in combination with interferon-α)</td>
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<td>Mycophenolic acid</td>
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<td><strong>8. Adenosine analogs</strong></td>
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<td>Neplanocin A, 3-deazaneplanocin A, 6'-C-methylneplanocin A, DHCeA, cDHCeA, etc.</td>
<td>SAH hydrolase</td>
<td>Inhibition of methylation reactions that depend on SAM as methyl donor, ultimately leading to inhibition of maturation of viral mRNAs</td>
<td>Broad spectrum of (−)RNA viruses</td>
<td>Under consideration for the treatment of filo (i.e., Ebola) virus infections</td>
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AICAR, 5-amino-4-imidazolecarbonamide ribonucleotide; DHCeA, 9-(trans-2', trans-3'-dihydroxycyclopent-4'-enyl)adenine; cDHCeA, 9-(trans-2', trans-3' dihydroxycyclopent-4'-enyl)-3-deazaadenine.
of action for the dendrimeric polysulfonates. In earlier studies (Esté et al., 1997), we demonstrated that development of resistance of HIV to dextran sulfate is associated with the emergence of specific mutations in the envelope gp120 glycoprotein; and following the same strategy [that is, looking for the site(s) of occurrence of mutations after repeated passages of the virus in the presence of the test compound], we found that for two purported integrase inhibitors, L-chicoric acid (Pluymers et al., 2000) and zintevir [a 17-mer oligonucleotide containing two stacked guanine quartets (Cherepanov et al., 1997; Esté et al., 1998)], the primary target of anti-HIV action was the gp120 glycoprotein and not integrase.

Of clinical relevance in the mode of action of PVAS, PVS, and their congeners is that they not only prevent the fusion between virus and cell but also between infected cells (expressing gp120) and uninfected cells, and in doing so, they may block HIV infection through both virus-to-cell and cell-to-cell contact. Furthermore, polyanionic substances are not only inhibitory to HIV but also other enveloped viruses, including HSV, CMV, RSV, etc. In addition, they are also active against sexually transmitted disease pathogens other than HIV and HSV, such as Neisseria and Chlamydia. These properties make PVAS, PVS, and their congeners particularly attractive as vaginal microbicides in the prevention of the sexual transmission of HIV, HSV, and other sexually transmitted disease pathogens.

2. Receptor Antagonists Targeted at the Co-receptors for HIV

To enter the cells following binding with the CD4 receptor, the HIV particles must interact, again through the viral envelope glycoprotein gp120, with a co-receptor, either CXCR4 [the receptor of the CXC-chemokine SDF-1 (“stromal cell derived factor”) or CCR5 [the receptor of the CC-chemokines RANTES (“regulated upon activation, normal T-cell expressed and secreted”), MIP-1α and -1β (“macrophage inflammatory proteins”). T-tropic or X4 HIV strains use the CXCR4, and M-tropic or R5 HIV strains use the CCR5 to enter the cells. At these sites, viral entry can be blocked by CXCR4 antagonists [i.e., bicyclams, e.g., AMD3100 (Fig. 2), polyphemusin T22, nonapeptide ALX40-4C, and CGP-64222] or CCR5 antagonists (e.g., TAK-779), respectively (De Clercq, 2000b).

The bicyclams are the most potent and the most specific CXCR4 antagonists that have been described to date (De Clercq, 2000c). They had been known for a number of years as highly potent and selective HIV inhibitors before their target of action was identified as the CXCR4 co-receptor (De Clercq, 2000c). The bicyclam AMD3100 inhibits the replication of X4 HIV-1 strains within the nanomolar concentration range. As it is not toxic to the host cells at concentrations up to 500 μM, its selectivity index or ratio of the 50% cytotoxic
concentration (CC_{50}) to the 50% antivirally effective concentration (EC_{50}) can be estimated at $100,000$. It has proved very difficult to engender resistance to AMD3100 in cell culture upon passaging the virus in the presence of the compound. The resistance-associated mutations appeared to be scattered over the whole gp120 glycoprotein. Resistance to AMD3100 did not lead to a switch in co-receptor use.

Akin to AMD3100, several other polycationic compounds, e.g., CGP-64222, a peptoid oligomer of nine residues, that had been previously reported as a Tat transactivation antagonist, were found to owe their anti-HIV activity primarily to a selective interaction with CXCR4 (Daemelans et al., 2000). The interaction of the bicyclam AMD3100 with CXCR4 has been investigated at the molecular level by mutated analysis (S. Hatse, K. Princen, L.-O. Gerlach, G. Bridger, G. Henson, E. De Clercq, T.W. Schwartz, and D. Schols, submitted for publication). In the interaction of AMD3100 with CXCR4 (Fig. 3B), the aspartate residues at positions 171 and 262 (located in the TM4 and TM6 segments of the seven-transmembrane receptor, respectively) play a crucial role since substitution of a neutral asparagine residue for either aspartate residue greatly reduces the antagonistic action of AMD3100 against CXCR4 (S. Hatse, K. Princen, L.-O. Gerlach, G. Bridger, G. Henson, E. De Clercq, T.W. Schwartz, and D. Schols, submitted for publication).

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mouse model of HIV infection when used at a dosage of ≥1 mg/kg/day, and following a phase I clinical trial for safety in (healthy) human volunteers (Hendrix et al., 2000), it has now entered phase II clinical trials in HIV-infected individuals. AMD3100 can be considered as a highly specific CXCR4 antagonist that through blockade of CXCR4 may prevent the switch from the less pathogenic M-tropic R5 to the more pathogenic T-tropic X4 strains of HIV, a switch that in vivo hallmarksthe progression to AIDS.

3. Nucleoside Analogs Targeted at the HIV Reverse Transcriptase

The substrate (dNTP)-binding site of HIV RT has for several years (De Clercq, 1995) been recognized as an attractive target for the design of nucleoside analogs that, in their 5'-triphosphate form, compete with the dNTPs. Thus, various 2',3'-dideoxynucleoside (ddN) analogs, such as zidovudine (AZT, azidothymidine), didanosine (ddI, dideoxyinosine), zalcitabine (ddC, dideoxycytidine), stavudine (d4T, didehydrodideoxythymidine), lamivudine (3TC, 3'-thiacytidine), abacavir (ABC), emtricitabine [(--) FTC], 2',6-diaminopurine dioxolane (DAPD), and others, have been designed and developed as specific HIV inhibitors and some of them, e.g., 3TC, (--) FTC, and DAPD, also as specific HBV inhibitors. All these ddN analogs act according to a common mechanism: as exemplified for AZT (Fig. 3C), they must be phosphorylated intracellularly consecutively by a nucleoside kinase, a nucleoside 5'-monophosphate kinase, and a nucleoside 5'-diphosphate (NDP) kinase to the 5'-triphosphate derivative, which then acts as a chain terminator when incorporated at the 3'-end of the nascent DNA chain. In fact, resistance to AZT may arise by removal of the chain-terminating residue and resumption of DNA synthesis.

The first phosphorylation step that converts the 2',3'-dideoxynucleoside analogs to their 5'-monophosphate (ddNMP) can be regarded as the bottleneck in the overall metabolic pathway of the ddN analogs to their active metabolite (ddNTP). Therefore, attempts have been made at constructing ddNMP prodrugs that, once they have been taken up by the cells, deliver within the cells the free ddNMP, which can then be converted to the corresponding ddNDP and ddNTP derivatives. Thus, phosphoramidate (Saboulard et al., 1999) and cyclosaligenyl (Balzarini et al., 2000) prodrugs of d4TMP have been designed that efficiently deliver the 5'-monophosphate d4TMP inside the cells and thus bypass the first, rate-limiting, phosphorylation step. The phosphoramidate of d4TMP, which can be considered as a triester since its phosphate moiety is linked to a phenyl group and the methyl ester of alanine (through a phosphoramidate linkage), is first converted to an alaninyl d4TMP intermediate before generating d4TMP through cleavage of the phosphoramidate linkage by a phosphoramidase (Saboulard et al., 1999).

Stavudine (d4T) (Fig. 2) was one of the first dideoxynucleoside analogs shown to be a potent and selective inhibitor of HIV replication (Baba et al., 1987). It is now widely used in the treatment of HIV infections. In addition to stavudine, five other ddN analogs, viz. zidovudine, didanosine, zalcitabine, lamivudine, and abacavir, have been formally approved for the treatment of HIV infections. As a rule, these ddN (or NRTI) analogs are used in combinations with other NRTIs, NNRTIs, or protease inhibitors. Such drug combinations have proved highly active in antiretroviral therapy (referred to as HAART).

4. Non-nucleoside Analogs Targeted at the HIV-1 Reverse Transcriptase

Whereas the ddN analogs (NRTI s), following their intracellular phosphorylation to the triphosphate form, interact with the substrate-binding site of the HIV RT, the NNRTIs block the HIV-1 RT activity through interaction with an allosterically located, nonsubstrate binding site (Fig. 3D). This NNRTI-binding site (or “pocket”) is located at a close (about 10 Å) distance from the substrate-binding site and is both spatially and functionally associated with the substrate-binding site (De Clercq, 1998). NNRTIs are notorious for rapidly eliciting virus-drug resistance resulting from mutations at amino acid residues that surround the NNRTI-binding site (i.e., L101I, K101E, K103N, V106A, E138K, V179D, Y181C, Y188H, G190A, P225H, P227L, and P236L). The most common RT mutations occurring in the clinical setting in patients treated with NNRTIs are K103N and Y181C. However, emergence of NNRTI-resistant HIV strains can be prevented if the NNRTIs are combined with NRTIs and used from the beginning at sufficiently high concentrations, as has been amply demonstrated in cell culture experiments (De Clercq, 1998).

NNRTIs are significantly more active against HIV-1 than HIV-2, SIV, FIV, or other retroviruses apparently because only HIV-1 RT offers the required allowance for interactions of the NNRTIs with their pocket, i.e., stacking interactions with the aromatic amino acids Tyr-181, Tyr-188, Trp-229, and Trp-318; electrostatic interactions with Lys-101, Lys-103, and Glu-138; van der Waals interactions with Leu-100, Val-106, Tyr-181, Gly-190, Trp-229, Leu-234, and Tyr-318; and hydrogen bonding with the main-chain peptide bonds (Jonckheere et al., 2000). While the NNRTI pocket is nonexistent in unliganded RT, it can be hypothesized that when the NNRTI is plugged into its pocket it distorts the precise geometry and/or mobility of the nearby catalytic site, so that the enzymatic function is suppressed (Jonckheere et al., 2000).

The identification of the specific binding site of NNRTIs at HIV-1 RT has prompted the search for “newer” NNRTIs with higher potency, especially against those HIV-1 strains that acquired resistance against “older” NNRTIs because of selected mutations (e.g., K103N) in the pocket site (Jonckheere et al., 2000). However, some of the pocket amino acids such as Trp-229 and Tyr-318 do not seem to mutate, or if they do, they lead to a “suicidal” loss of RT activity; such immutable amino acids should be prime targets for the rational design of new NNRTIs (Pelemans et al., 2000).

The stage for the NNRTIs was set about a decennium ago with the discovery of 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT) (Baba et al., 1989) and tetrahydromidoaz-[4,5,1-jk][1,4]benzodiazepin-2(1H)-one and -thione (TIBO) (Pauwels et al., 1990) as specific HIV-1 inhibitors. Subsequently to HEPT and TIBO, numerous other NNRTIs have been identified, and three of them, i.e., nevirapine, delavirdine, and efavirenz, have so far been formally licensed for clinical use in the treatment of HIV-1 infections. Several
others are in clinical development, including the HEPT de-
ritic emivirine (MKC-442) (Fig. 2), which is currently in
vanced (phase III) clinical trials. It is advocated in combi-
nations of NNRTIs with NRTIs, as such combinations would
lead to a cooperative interaction on the one hand and dimin-
ish the likelihood for resistance emergence on the other hand.

5. Nucleoside Analogs Targeted at
Herpesvirus DNA Polymerases

All the nucleoside analogs [i.e., acyclovir, valaciclovir, pen-
ciclovir, famciclovir, and brivudin (BVDU)] that are currently
used in the treatment of HSV and VZV infections are tar-
ged at the viral DNA polymerase (De Clercq, 2001b). The
specificity in their antiviral action is determined by a specific
virus-encoded thymidine kinase (TK), which ensures and
confines the specific phosphorylation of these nucleoside an-
logs to the virus-infected cells. While acyclovir has re-
mained the gold standard for the treatment of HSV and VZV
infections, its potency and selectivity as an anti-VZV agent is
largely superseded by brivudin (Fig. 2) (De Clercq et al.,
1979) has finally been licensed for the treatment of

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**Fig. 4.** Molecular targets and/or modes of action for A, acyclic guanosine analogs: targeted at the herpesviral DNA polymerase following three
phosphorylation steps, the first being performed by the virus-encoded thymidine kinase; B, acyclic nucleoside phosphonates (i.e., adefovir), which only
need two phosphorylation steps before their interaction (as chain terminators) with the viral DNA polymerase; C, IMP dehydrogenase inhibitors; and
D, SAH hydrolase inhibitors.
herpes zoster in both immunocompromised and -competent patients.

Both acyclovir (and other acyclic nucleoside analogs) and brivudin act according to a similar modus operandi: after phosphorylation by the HSV- or VZV-encoded TK to the monophosphate form (acyclovir) or diphosphate form (BVDU), they are further phosphorylated by cellular kinase(s) to the triphosphate form, which then interacts as a competitive inhibitor/alternate substrate with the viral DNA polymerase. If incorporated into the nascent viral DNA chain, acyclovir obligatorily leads to chain termination (Fig. 4A), whereas BVDU, which is incorporated via an internucleotide linkage, still allows further DNA elongation but then affects the normal functioning of the DNA product.

Valaciclovir (Fig. 2) and famciclovir represent the oral prodrug forms of acyclovir and penciclovir, respectively, which by themselves possess only limited oral bioavailability. Valaciclovir came from the search for amino acid esters of acyclovir that would have increased oral bioavailability over the parent compound; such amino acid esters had been shown to be equally active as acyclovir itself due to the fact that they are readily hydrolyzed to release the parent compound (Colla et al., 1983).

The treatment of HSV and VZV infections has been revolutionized by the advent of acyclovir, brivudin, and the other viral TK-dependent anti-HSV and anti-VZV compounds. These compounds are now widely used in the treatment of several manifestations of HSV and VZV infections, including primary and recurrent herpes genitalis, herpes labialis, herpetic keratitis, herpetic encephalitis, herpes zoster, and the often severe and life-threatening mucocutaneous HSV and VZV infections in immunosuppressed patients (i.e., AIDS patients, cancer patients, and organ transplant recipients receiving immunosuppressive agents).

Brivudin and (val)acyclovir are by no means the endpoints in our search for more effective and/or selective inhibitors of HSV, VZV, and other herpesviruses. Recently, two new classes of nucleoside analogs, viz. the 3- and 5-enantiomers of cyclohexenylguanine (Wang et al., 2000) and bicyclic furopyrimidine nucleosides bearing a long alkyl or an aryl side chain (McGuigan et al., 2000), were reported to offer marked activity against a wide array of DNA viruses, including picorna-, toga-, flavi-, bunya-, arena-, reo-, rhabdo-, and particularly ortho- and paramyxoviruses, and this broad-spectrum activity against a wide range of DNA viruses was (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ([S]-HPMPA); it was found active against virtually all DNA viruses (De Clercq et al., 1986). Its cytosine counterpart ([S]-HPMPC, cidofovir) (Fig. 2) was developed as an antiviral drug (De Clercq, 1993) and finally approved for clinical use, i.e., for the treatment of CMV retinitis in AIDS patients. Two congeners of cidofovir, namely adefovir (Fig. 2) and tenofovir (Fig. 2), both in their oral prodrug forms adefovir dipivoxil and tenofovir disoproxil, have progressed to advanced (phase III) clinical trials for the treatment of HBV infections (i.e., chronic hepatitis B) and HIV infections (i.e., AIDS), respectively.

Acyclic nucleoside phosphonates offer a number of attractive features not shared by any other antiviral drugs. They display a particularly long intracellular half-life (lasting for 1–3 weeks), thus allowing infrequent dosing (for cidofovir, even once every week or every other week). They do not easily lead to resistance even after prolonged treatment (longer than 1 year). They can be added onto each drug combination regimen, as shown for tenofovir in the treatment of HIV infections. They exhibit a broad antiviral activity spectrum, which in the case of cidofovir extends to virtually all DNA viruses. Moreover, acyclic nucleoside phosphonates also possess considerable antitumor potential, i.e., induction of tumor cell differentiation (as shown for adefovir) and induction of tumor cell apoptosis (as shown for cidofovir).

Cidofovir holds great potential for the treatment of a number of diseases associated with DNA viruses, i.e., viral TK-deficient herpesvirus infections that are resistant to acyclovir (or brivudin), polyomavirus infections (i.e., progressive multifocal leukoencephalopathy), adenovirus infections (i.e., keratoconjunctivitis), poxvirus infections (i.e., smallpox, monkeypox, cowpox, orf, molluscum contagiosum), and human papilloma virus (HPV) infections. Cidofovir has been able to achieve a complete and durable remission of a number of HPV-associated diseases, i.e., pharyngeal, esophageal, and laryngeal papillomatosis; ordinary, plantar, and genital warts (condylomata acuminata); and cervical intraepithelial neoplasia (CIN, type III) as well as other HPV lesions (Snodgrass et al., 2001).

6. Acyclic Nucleoside Phosphonates Targeted at the Viral DNA Polymerase

The acyclic nucleoside phosphonates can be conceived as acyclic nucleoside analogs extended by a phosphate moiety. The latter is equivalent to a phosphate group, but unlike phosphate, phosphonate can no longer be cleaved through esterases that would normally convert nucleoside monophosphates back to their nucleoside form. As a consequence, this type of molecule may be expected to show a broadened spectrum of antiviral activity, also encompassing those DNA viruses that would not encode for a specific viral TK or would have become resistant to nucleoside analogs (such as acyclovir and brivudin) through TK deficiency. In this sense, acyclic nucleoside phosphonates could be regarded as stable TK by-pass nucleotides that after their update by the cells would only require two (instead of three) phosphorylation steps to be converted to their active (diphosphorylated) form (Fig. 4B). In this form, they would essentially act as chain terminators of the DNA polymerase reaction.

The first acyclic nucleoside phosphonate recognized for its broad-spectrum activity against a wide array of DNA viruses was (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ([S]-HPMPA); it was found active against virtually all DNA viruses (De Clercq et al., 1986). Its cytosine counterpart ([S]-HPMPC, cidofovir) (Fig. 2) was developed as an antiviral drug (De Clercq, 1993) and finally approved for clinical use, i.e., for the treatment of CMV retinitis in AIDS patients. Two congeners of cidofovir, namely adefovir (Fig. 2) and tenofovir (Fig. 2), both in their oral prodrug forms adefovir dipivoxil and tenofovir disoproxil, have progressed to advanced (phase III) clinical trials for the treatment of HBV infections (i.e., chronic hepatitis B) and HIV infections (i.e., AIDS), respectively.

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spectrum antiviral activity could, at least partially, be ascribed to inhibition of IMP dehydrogenase by the 5′-monophosphate of ribavirin (Fig. 4C).

Ribavirin is a relatively weak inhibitor of virus replication; attempts to increase its potency led to the identification of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) (Fig. 2) as a 10- to 100-fold more potent antiviral agent and ditto IMP dehydrogenase inhibitor with an antiviral activity spectrum similar to that of ribavirin (De Clercq et al., 1991). EICAR may be a promising lead compound for the treatment of infections by various RNA viruses (including flav-, bunya-, arena-, reo-, rhabdo-, and paramyxoviruses) that are presently not amenable to antiviral therapy.

Mycophenolic acid (Fig. 2) is another potent inhibitor of the IMP dehydrogenase reaction that is currently used as an immunosuppressive agent in kidney transplant recipients. However, mycophenolic acid also has marked activity against some viruses, e.g., yellow fever virus, and in addition, markedly potentiates the inhibitory effects of acyclic guanosine analogs (acyclovir, penciclovir, ganciclovir) against HSV, VZV, and CMV infections both in vitro and in vivo (Neyts et al., 1998). Mycophenolic acid also potentiates the activity of the guanine derived dideoxynucleoside ddN analogs (e.g., abacavir) against HIV, and these potentiating effects could be explained by a depletion of the normal substrate (i.e., dGTP) pools relative to the competing ddGTP analogs.

From a clinical viewpoint, the potential applications of the IMP dehydrogenase inhibitors have not been fully realized. Ribavirin has been officially approved as an aerosol for the treatment of RSV infections and in combination with interferon-α for the treatment of HCV infections. Mycophenolic acid is being used as an immunosuppressant in kidney transplant recipients. It is obvious that the therapeutic potential of IMP dehydrogenase inhibitors extends to other clinical situations such as single drugs in the treatment of various (+)RNA and (−)RNA virus infections and in combination with acyclic or dideoxy guanosine analogs for the treatment of herpesvirus (HSV, VZV, and CMV) and HIV infections, respectively.

8. SAH Hydrolase Inhibitors

SAH hydrolase is a key enzyme in methylation reactions depending on S-adenosylmethionine (SAM) as the methyl donor, including those methylation reactions that are required for the maturation of viral mRNA (Fig. 4D). SAH is both a product and inhibitor of these methyltransferase reactions. However, SAH is rapidly hydrolyzed by SAH hydrolase into homocysteine and adenosine, and this prevents the accumulation of SAH that would otherwise lead to an inhibition of the SAM-dependent methylation reactions. SAH hydrolase inhibitors may be expected to lead to an accumulation of SAH and concomitantly inhibit the methylation reactions required for viral mRNA maturation (De Clercq, 1987).

SAH hydrolase has long been recognized as a suitable target for antiviral chemotherapy and broad-spectrum antiviral agents, and in fact, (S)-9-(2,3-dihydroxypropyl)adenine [(S)-DHPA], the first aliphatic nucleoside analog reported as a broad-spectrum antiviral agent (De Clercq et al., 1978), was later shown to exert its antiviral action through inhibition of SAH hydrolase. Following (S)-DHPA, various other adenosine analogs, e.g., carbocyclic adenosine, carbocyclic 3-deazaadenosine, neplanocin A, 3-deazaneplanocin A (Fig. 2), and their 5′-nor derivatives (De Clercq et al., 1989), have been identified as potent inhibitors of SAH hydrolase on the one hand and to exhibit broad-spectrum antiviral activity on the other hand.

SAH hydrolase inhibitors possess a characteristic antiviral activity spectrum, encompassing, in particular, poxviruses (i.e., vaccinia), (+)RNA viruses (reovirus and (−)RNA viruses (bunyavirus, arenavirus, rhabdovirus, filovirus, ortho- and paramyxoviruses); this includes a number of hemorrhagic fever viruses, such as Ebola). The antiviral effects of the compounds are correlated with their ability to elevate the intracellular SAH levels (as the result of their inhibitory effect on SAH hydrolase), and a close correlation has been found between the inhibitory effects of the (acyclic and carbocyclic) adenosine analogs on SAH hydrolase and their activity against those viruses that fall within their antiviral activity spectrum (De Clercq, 1987).

SAH hydrolase inhibitors have not yet reached the clinical scene. Yet these compounds offer great potential for the treatment of a number of fearful viral diseases (presently intractable to therapy), such as those caused by arenavirus, rhabdovirus, and filoviruses; i.e., they were shown to be effective in lethal mouse models for rhabdovirus (i.e., vesicular stomatitis virus) (De Clercq et al., 1989) and filovirus (i.e., Ebola virus) (Huggins et al., 1999) infections.

Conclusions

A number of virus-specific proteins or processes have been identified as targets for chemotherapeutic intervention, i.e., HIV reverse transcriptase, herpessitial DNA polymerase, virus adsorption and entry into the cells, and cellular enzymes (such as IMP dehydrogenase and SAH hydrolase) that are innately associated with virus replication. Concomitantly with their targets, a variety of antiviral drugs were discovered that are now widely used (or considered for use) in the treatment of several important viral diseases, i.e., HIV adsorption inhibitors, which are considered for use as vaginal microbicides in the prevention of AIDS; bicyclams, which are considered for use in the therapy of infections with X4 (T-tropic) HIV strains; NRTIs and NNRTIs, which are invariably part of all current treatment regimens of HIV infections; brivudin, valaciclovir, and famciclovir, which have been licensed for the treatment of herpes zoster; acyclic nucleoside phosphonates, which are indicated in the treatment of various DNA virus (cidofovir), HBV (adefovir), and HIV (tenofovir) infections; IMP dehydrogenase inhibitors, which should be pursued as such for the treatment of various RNA virus infections, and in combination with acyclic guanosine analogs for the treatment of herpesvirus infections; and SAH hydrolase inhibitors that hold great promise for the treatment of hemorrhagic fever virus infections (such as Ebola).

There are various other targets and compounds interacting therewith of great actual or potential value as chemotherapeutic approaches that have not been addressed here, i.e., compounds that inhibit HIV-cell fusion through their interaction with the viral gp41, HIV nucleocapsid p7 zinc finger-binding compounds, HIV integrase inhibitors, viral (HIV, HSV, CMV, HCV, etc.) protease inhibitors, picornaviral capsid binders (such as pleconaril), influenza A virus uncoating


References

Acknowledgment

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