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Review Article

New acquisitions in the development of anti-HIV agents*

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Introduction

When developing antiviral agents for the chemotherapy of AIDS, several events in the replicative cycle of the human immunodeficiency virus (HIV) could be considered as targets for chemotherapeutic attack, i.e. virus attachment to the cells, fusion of the viral envelope with the cellular membrane, penetration of the viral capsid into the cell, uncoating of the viral capsid, transcription of the viral RNA genome to proviral DNA (which is accompanied by degradation of the viral RNA), circularization and integration of the proviral DNA into the cellular genome, replication of the proviral DNA (concomitant with the replication of cellular DNA), transcription of the proviral DNA to mRNA, translation of the latter to viral precursor proteins, which are then modified by myristylation, proteolytic cleavage and glycosylation assembly and, finally, budding of the virus particles (De Clercq, 1989a).

Most of these events require the help of specific proteins encoded by the viral genome. Virus adsorption requires a specific interaction between the viral glycoprotein gp120 and the cellular CD4 receptor. The transcription of RNA to DNA is catalyzed by the virion-associated reverse transcriptase (RT), and the remaining RNA template is degraded by ribonuclease H. The integration of proviral DNA in the cellular genome is secured by another viral enzyme, called integrase or endonuclease (*end*). Proteolytic cleavage of the viral precursor proteins is effected by the viral protease (*pro*), which is autocatalytically removed from the *pro*-RT-*end* precursor. Furthermore, virus replication is under control of a set of regula-

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tory genes which either stimulate expression of viral proteins (i.e. *tat*, encoded by the *trans*-activator gene), increase the infectivity of the virus particles (i.e. *vif*, encoded by the virion infectivity gene) or suppress expression of viral proteins (i.e. *nef*, a negative regulation factor and *vpu*, also a negative regulatory factor). Another regulatory protein (*rev*) positively regulates expression of virion proteins but negatively regulates expression of regulatory genes (including the *rev* gene itself). For a review of the processes involved in the replication and pathogenesis of HIV, see Haseltine (1988).

For a number of the target events, such as penetration and uncoating of the viral capsids, integration of the proviral DNA into the cellular genome, and virion assembly, and for several of the target proteins, such as *end, pro, tat* and *rev*, no specific inhibitors have so far been identified. For various compounds which have been claimed to inhibit HIV replication (i.e. penicillamine D, amphotericin B methyl ester, oxetanocin, hypericin, somatostatin, ribavirin, papaverine), the mode of their purported anti-HIV action is unknown or at least, uncertain. For other compounds, the mode of action is well established, i.e. phosphonoformate interferes directly with the viral RT; 2′,3′-dideoxynucleoside analogues do so after they have been phosphorylated intracellularly to their 5′-triphosphates; sulfated polysaccharides block virus attachment to the cells; suramin inhibits both viral RT and virus binding to the cells; castanospermine, deoxynojirimycin and other glycosylation inhibitors reduce infectivity of the virus particles; and anti-sense oligonucleotides may interfere with both transcription and translation of the viral genome (for previous reviews on chemotherapeutic approaches to the treatment of AIDS, see De Clercq, 1986, 1987a,b, 1988).

This review will be focused on the compounds that were synthesized and/or investigated in our own research unit. According to their chemical structure these compounds could be divided into the following four categories: I, anionic substances; II, dideoxynucleoside analogues; III, phosphonylmethoxyethylpurine derivatives; and IV, sulfated polysaccharides. Compounds II and III are assumed to interact with the reverse transcriptase step, compounds IV with virus adsorption, and compounds I with either reverse transcription or virus-cell binding, or both (although for some of the compounds which have been classified under I the precise mechanism of action has not been elucidated).

Anionic substances

The first compound found to inhibit HIV at a concentration that was not toxic to the host cells was suramin (Mitsuya et al., 1984; De Clercq, 1987c). Suramin (Fig. 1A) was studied for its activity against HIV because it had previously been shown to suppress the reverse transcriptase activity associated with murine and avian retroviruses (De Clercq, 1979). Suramin is also active in suppressing retrovirus propagation in vivo (Ruprecht et al., 1985), and its anti-HIV activity has been confirmed in several cell systems (Balzarini et al., 1986a,b; Baba et al., 1988c). Suramin is inhibitory to HIV-1 RT activity at about the same concentration (ID_{50}:

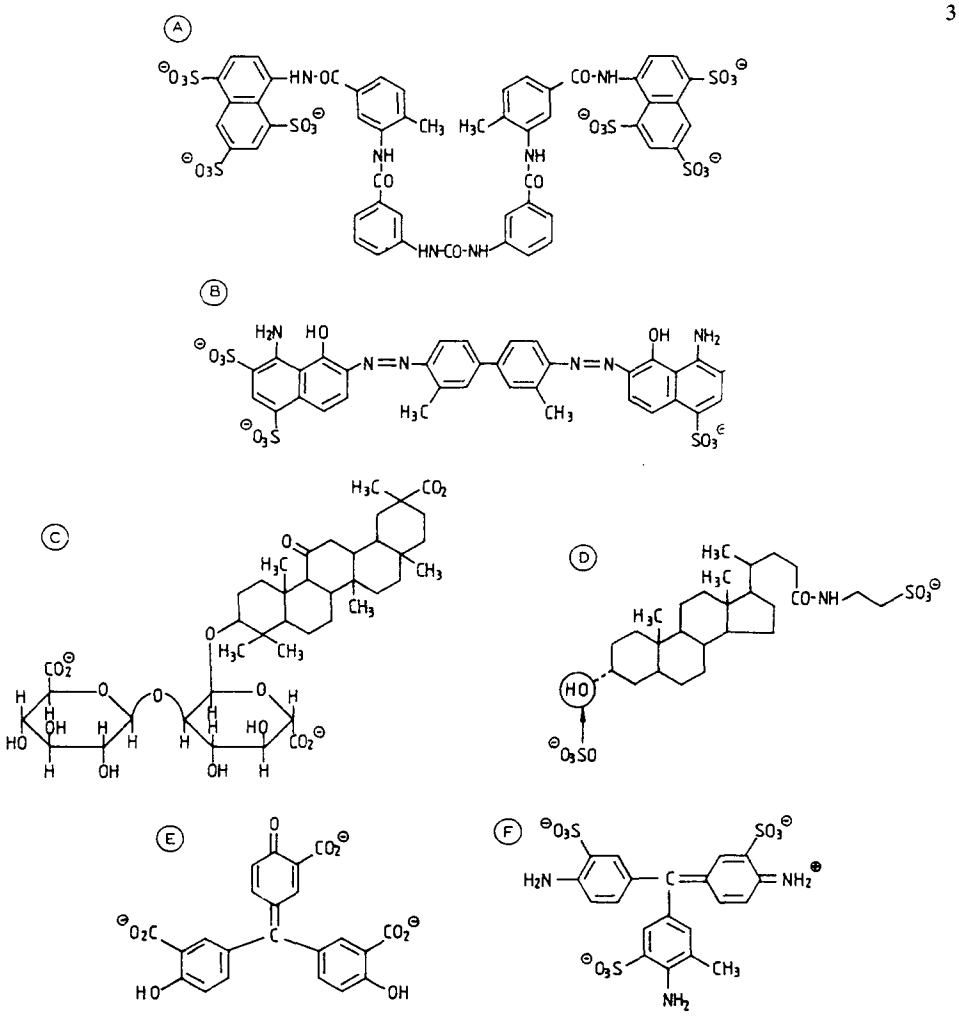

Fig. 1. Anionic compounds: sulfonates: *Suramin* (A), *Evans Blue* (B), *Fuchsin Acid* (F) and carboxylates: *Glycyrrhizin* (C), Taurolithocholic acid (3-sulphate) (D), and (E) *Aurintricarboxylic acid (ATA)*.

33 μM) as that required to inhibit HIV-1 replication in MT-4 cells (ED_{50}: 32 μM) (Baba et al., 1988c) (Table 1). Also, suramin partially blocks HIV-1 binding to the cells (Schols et al., 1989a). This means that the anti-HIV-1 activity of suramin is based on a dual mode of action, i.e. inhibition of virus adsorption and inhibition of RT activity (Fig. 5).

In addition to suramin, several other sulfonated compounds, i.e. Evans Blue (Fig. 1B), have proved effective against HIV-1 replication at non-toxic concentrations (Balzarini et al., 1986a,b). Evans Blue, like suramin may owe its anti-HIV activity partly to inhibition of RT activity and partly to inhibition of virus adsorption (Schols et al., 1989a).

Glycyrrhizin (also referred to as glycyrrhizic acid, glycyrrhizinic acid and glycyrrhetinic acid glycoside) (Fig. 1C) has been known for its antiviral activity since

TABLE 1

Anti-HIV-1 activity of selected antiviral compounds in MT-4 cell cultures

Compound	ED_{50}[a]	CD_{50}[b]	Selectivity[c] index	References
I. Anionic substances				
Suramin	32	625	20	Baba et al., 1988c
Evans Blue	10	> 100	> 10	Balzarini et al., 1986b
Glycyrrhizin	100	2400	24	Ito et al., 1987b
Taurolithocholic acid (3-sulfate)	137	1000	> 7.3	Baba et al., 1989a
Aurintricarboxylic acid	5	590	118	Baba et al., 1988c
Fuchsin acid	42	> 3125	> 74	Baba et al., 1988c
II. Deoxynucleoside analogues				
ddThd	6	> 625	> 104	Balzarini et al., 1988a
	0.2	> 625	> 3125	Baba et al., 1987c
ddCyd (DDC)	0.06	37	616	Pauwels et al., 1987
	0.3	356	1187	Pauwels et al., 1988a
ddAdo (DDA)	6.4	890	139	Baba et al., 1987a
ddGuo	7.6	486	64	Baba et al., 1987a
ddIno (DDI)	10	> 500	> 50	De Clercq et al., 1989
ddDAPR	3.6	404	112	Balzarini et al., 1987c
ddeThd (D4T)	0.01	1.2	120	Baba et al., 1987c
ddeCyd (D4C)	0.13	7.9	61	Baba et al., 1987c
AzddUrd	0.43	39	90	Balzarini et al., 1989c
	0.36	244	677	Balzarini et al., 1988a
AzddThd (AZT)	0.006	3.5	583	Pauwels et al., 1987
	0.003	4.8	1603	Balzarini et al., 1989c
	0.004	20	5000	Balzarini et al., 1988a
AzddClUrd	0.72	213	296	Balzarini et al., 1989c
AzddMeCyd	1.8	1000	555	Herdewijn et al., 1988
$AzddMe_2Cyd$	17	> 1000	> 58	Herdewijn et al., 1988
AzddGuo	1.4	190	136	Baba et al., 1987a
AzddDAPR	0.3	44	147	Balzarini et al., 1988b
FddUrd	0.06	1.1	25	Balzarini et al., 1989c
	0.04	16	400	Balzarini et al., 1988a
FddThd	0.001	0.197	197	Balzarini et al., 1988a

FddClUrd	0.38	535	1408	Balzarini et al., 1989a
FddBrUrd	0.41	24	59	Balzarini et al., 1989a
FddGuo	2.4	237	96	Balzarini et al., 1988b
FddDAPR	4.5	360	80	Balzarini et al., 1988b
III. Phosphonylmethoxyethylpurine derivatives				
PMEA	2.0	67	33	Pauwels et al., 1988b
PMEMAP	45	> 1250	> 28	Pauwels et al., 1988b
PMEDAP	1.0	18	18	Pauwels et al., 1988b
IV. Sulfated polysaccharides				
Heparin	0.58	> 2500	> 4300	Baba et al., 1988a
Dextran sulfate	0.3	> 2500	> 8300	Baba et al., 1988a
Pentosan polysulfate	0.19	> 2500	> 13150	Baba et al., 1988a
λ-carrageenan	0.54	> 625	> 1150	Baba et al., 1988a
Mannan sulfate	1.2	> 2500	> 2000	Ito et al., 1989a
Dermatan sulfate	> 625	> 2500	>< 4	Baba et al., 1988a
Supersulfated heparin	0.38	> 625	> 1650	Unpublished data, 1988
Supersulfated dermatan sulfate	0.4	> 625	> 1560	Unpublished data, 1988

[a]Fifty percent effective dose, based on the inhibition of HIV-1-induced cytopathogenicity in MT-4 cells [assessed by the MTT method (Pauwels et al., 1988a)].
[b]Fifty percent cytotoxic dose, based on the reduction of the viability of mock-infected MT-4 cells [assessed by the MTT method (Pauwels et al., 1988a)].
[c]Ratio of CD_{50} to ED_{50}.
ED_{50} and CD_{50} values of anionic substances, deoxynucleoside analogues and phosphonylmethoxyethylpurine derivatives are expressed in μM, whereas ED_{50} and CD_{50} values of sulfated polysaccharides are expressed in μg/ml.

the report of Pompei et al. (1979). It also inhibits HIV-1 replication at a concentration (ED_{50}: 0.1 mM) that is well below its cytotoxic threshold (selectivity index:24) (Ito et al., 1987b) (Table 1). Glycyrrhizin may owe its anti-HIV-1 activity, at least in part, to an inhibitory effect on virus-cell binding (Ito et al., 1989b). Glycyrrhizin also reduces protein kinase C (PKC) activity in the cells, but it is not clear to what extent this reduction in PKC activity contributes to the anti-HIV-1 activity of glycyrrhizin.

Fusidic acid, an antibiotic with a steroid-like structure somewhat similar to that of glycyrrhizin, has also been accredited with anti-HIV activity (Faber et al., 1987). However, we have been unable to confirm this activity: fusidic acid did not protect MT-4 cells against the cytopathogenicity of HIV-1 at the highest concentration that was non-toxic to the cells (25 μg/ml) (M. Baba and E. De Clercq, unpublished

data, 1987). The antiviral effects that have been accredited to fusidic acid must be attributed to toxicity to host cells, a conclusion also reached by Richman et al. (1988).

Following fusidic acid, bile acids (salts) were also accredited with anti-HIV properties (Lloyd et al., 1988): an inhibitory effect on the viability of HIV-1-infected CEM cells was noted at a concentration of 250 μg/ml, and no attempts were made to identify the active congeners in the bile acid mixture. In fact, the mixture was said to contain 60% sodium cholate and 40% deoxycholate (Lloyd et al., 1988). With neither cholate nor deoxycholate could we demonstrate a selective inhibitory effect on HIV-1 replication in MT-4 cells (Baba et al., 1989a). Of the large variety of bile acids that we evaluated for anti-HIV-1 activity (Baba et al., 1989a), only the following inhibited HIV-1 replication at subtoxic concentrations: taurolithocholic acid, lithocholic acid 3-sulfate, taurolithocholic acid 3-sulfate (Fig. 1D, Table 1) and glycolithocholic acid 3-sulfate. The anti-HIV-1 activity of these cholic acid derivatives (except for taurolithocholic acid) could be attributed in part to inhibition of virus adsorption and inhibition of RT activity.

Another anionic compound that has been identified as a selective anti-HIV agent is the triphenylmethane derivative ATA (aurintricarboxylic acid) (Fig. 1E) (Balzarini et al., 1986b). This compound has since long been recognized as a reverse transcriptase inhibitor (Givens and Manly, 1976). ATA inhibits HIV-1 RT activity at about the same concentration (ID_{50}: 5.2 μM) as that required to inhibit HIV-1 replication in MT-4 cells (ED_{50}:5.0 μM) (Baba et al., 1988c) (Table 1). The carboxylic acid groups of ATA are essential for its anti-HIV activity since the non-carboxylated parent compound aurin has no selective inhibitory effect on the replication of HIV. Of particular importance is the observation that ATA specifically interacts with CD4, and, consequently, prevents the attachment of HIV-1 particles to their cellular receptor (Schols et al., 1989b). ATA prevents binding of OKT4A/Leu-3a, but not other monoclonal antibody, to the cells. The effect was seen within 1 min at an ATA concentration of 10 μM in various $T4^+$ cells. It was dose-dependent and reversible. No other chemical has ever been reported to possess a specific affinity for CD4. What remains to be assessed is the exact binding site of ATA at the CD4 molecule, as well as the structural requirements that ATA has to fulfill to interact with CD4.

Fuchsin acid, which is structurally related to ATA (Fig. 1F), also behaves as a selective inhibitor of HIV-1 and HIV-2 replication (Baba et al., 1988c). Unlike ATA, however, fuchsin acid does not interfere with the binding of HIV to the cells. Also, fuchsin is only weakly inhibitory to RT activity. The basis for its high selectivity as an inhibitor of HIV replication (Table 1) remains therefore subject of further study.

Dideoxynucleoside analogues

Azidothymidine (3′-azido-2′,3′-dideoxythymidine, AzddThd, AZT) was the first among the 2′,3′-dideoxynucleoside (ddN) analogues shown to be a potent and selective inhibitor of HIV-1 replication in vitro (Mitsuya et al., 1985). Following AZT, various other ddN analogues, i.e. ddCyd (DDC), ddAdo (DDA) and ddIno (DDI), were reported to inhibit HIV-1 with a potency and selectivity similar to that of AZT (Mitsuya and Broder, 1986). Further search for new ddN analogues has produced a wealth of active compounds: i.e. the 2′,3′-didehydro derivatives of ddCyd and ddThd, termed D4C and D4T, respectively (Balzarini et al., 1986c, 1987b; Baba et al., 1987c; Lin et al., 1984a,b; Hamamoto et al., 1987); 3′-azido-2′,3′-dideoxyuridine (AzddUrd), 3′-azido-2′,3′-dideoxy-5-methylcytidine (AzddMeCyd), the N^4-methylated derivative of AzddMeCyd (AzddMe$_2$Cyd) and the N^4-hydroxylated derivative of AzddMeCyd (Herdewijn et al., 1988; Balzarini et al., 1988a); 3′-fluoro-2′,3′-dideoxythymidine (FddThd) (Herdewijn et al., 1987; Balzarini et al., 1988a) and the 3′-azido and 3′-fluoro derivatives of 2′,3′-dideoxy-5-chlorouridine

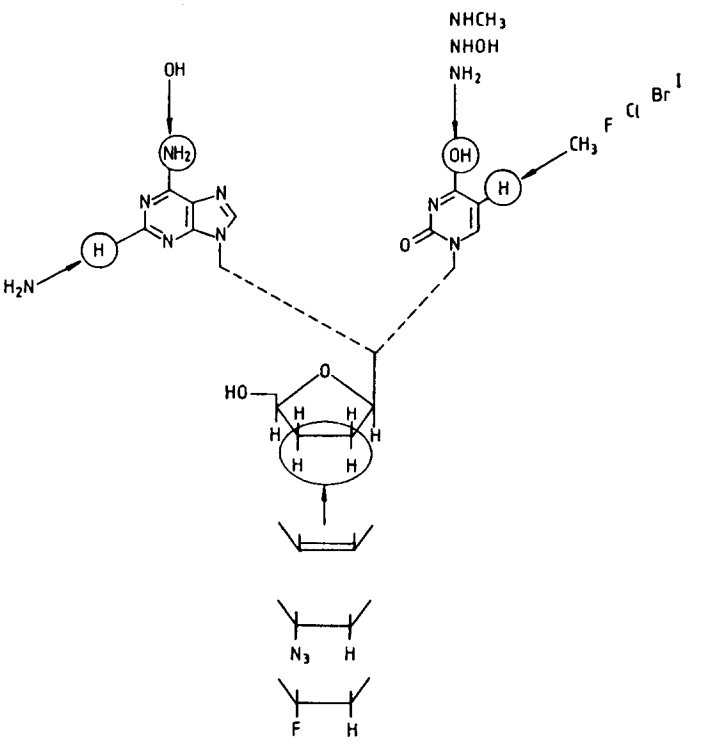

Fig. 2. Dideoxynucleoside analogues; purine base: adenine, guanine, hypoxanthine or 2,6-diaminopurine; pyrimidine base: uracil, thymine, cytosine, 5-methylcytosine, N^4,5-dimethylcytosine, N^4-hydroxyl-5-methylcytosine, 5-fluorocytosine, 5-chlorouracil, 5-bromouracil or 5-iodouracil; sugar moiety: 2,3-dideoxyribose, 2,3-didehydro-2,3-dideoxyribose, 3-azido-2,3-dideoxyribose or 3-fluoro-2,3-dideoxyribose.

(AzddClUrd, FddClUrd) (Balzarini et al., 1989c,d; Van Aerschot et al., 1989); the 2′,3′-dideoxyriboside of 2,6-diaminopurine (Balzarini et al., 1987c,d) and the 3′-azido and 3′-fluoro derivatives thereof (AzddDAPR, FddDAPR) (Balzarini et al., 1988b); and, finally, the 3′-azido and 3′-fluoro derivatives of 2′,3′-dideoxyguanosine (AzddGuo, FddGuo) (Baba et al., 1987a; Balzarini et al., 1988b). The structural formulae of these compounds are presented in Fig. 2. Their ED_{50} and CD_{50} values, as recorded from comparative studies in MT-4 cells, are listed in Table 1. From these studies it would appear that several ddN analogues, in addition to those that have already been the subject of clinical trials in AIDS patients (i.e. AZT, DDC, DDA and DDI) yield great promise as candidate anti-AIDS drugs, e.g. D4C, D4T, AzddUrd, AzddDAPR and FddClUrd. Their selective and potent anti-HIV activity has been demonstrated in several laboratories (Balzarini et al., 1986c; Baba et al., 1987c; Lin et al., 1987a,b, 1988; Hamamoto et al., 1987; Bazin et al., 1988; Mansuri et al., 1989). In fact, similar results to those obtained in MT-4 cells may be expected if the anti-HIV activity of the compounds would be determined in peripheral blood mononuclear (PBM) cells (Chu et al., 1988).

All ddN analogues may be assumed to act in a similar fashion as AZT (Furman et al., 1986), which means that they must be phosphorylated intracellularly to their 5′-triphosphate derivatives before they can interact with their target enzyme, the HIV-associated reverse transcriptase (Fig. 5). As a rule, the ddN triphosphates (ddNTPs) have a greater affinity for the HIV RT than for the cellular DNA polymerase α, and for HIV RT they also have affinities some 50-fold greater than those of the corresponding 2′-deoxynucleoside (dN) 5′-triphosphates (dNTPs) (Hao et al., 1988). At the RT level, the ddNTPs may act as either competitive inhibitors, i.e. prevent the incorporation of the natural substrates (dNTPs), or alternate substrates, and thus be incorporated (as ddNMP) in the growing DNA chain. This incorporation must lead to termination of the DNA chain, since the ddNMPs do not offer the 3′-hydroxyl groups that are necessary for chain elongation.

The differences that have been noted in the anti-HIV activity of different ddN analogues could be attributed in principle to (i) differences in the rate (or extent) by which they are phosphorylated to their 5′-triphosphates, (ii) differences in the affinity of these 5′-triphosphates for the HIV reverse transcriptase, or (iii) differences in their abilities to influence pool sizes of the substrates (dNTPs) that they ultimately compete with. Increasing experimental evidence suggests that the relative ability by which the 2′,3′-dideoxynucleosides generate 5′-triphosphates intracellularly (thus, factor i) correlates much more closely than do the other two factors (ii and iii) (Hao et al., 1988). The importance of the intracellular phosphorylation of the ddN analogues (i.e. AZT and DDC) in the anti-HIV activity they accomplish was clearly demonstrated by Balzarini et al. (1988c). The ddN analogues may differ considerably in the efficiency by which they are phosphorylated by the cells; i.e., in MT-4 cells D4T is phosphorylated to its 5′-monophosphate at a 300- to 600-fold lower extent than AZT (Balzarini et al., 1989a). The differential phosphorylation of AZT and D4T may be the principal, if not the sole, factor responsible for the differential anti-HIV activity of these compounds, since in their triphosphate form they are equipotent against HIV reverse transcriptase.

Also, combination of ddN analogues with other compounds which increase the efficiency by which the ddNs are converted to the corresponding ddNTPs may be expected to increase the anti-HIV activity of the ddNs. Thus, enhancement of the phosphorylation process may form a rational basis for the design of combination protocols. The potentiating effect of ribavirin on the anti-HIV activity of the purine 2′,3′-dideoxynucleosides (i.e. ddAdo, ddGuo) (Baba et al., 1987b) and the potentiating effect of thymidine (dThd) on the anti-HIV activity of DDC (Balzarini et al., 1987a) could readily be attributed to an enhanced phosphorylation of the ddN analogues.

The intermediary products, i.e. 5′-monophosphates, formed during the phosphorylation process of the ddNs, may themselves act as antimetabolites and thus contribute to the cytotoxicity of the compounds. Cells that have been exposed to AZT accumulate AZT 5′-monophosphate, presumably because the latter inhibits dTMP kinase that is needed for its further phosphorylation to the 5′-diphosphate. Since dTMP kinase is also required for the salvage of dThd, a blockage at the dTMP kinase level may also be expected to reduce the supply of dTDP, dTTP, and, hence suppress cellular DNA synthesis. Mutatis mutandis, those ddN analogues that do not act as inhibitors of dTMP kinase, and, thus, are not accumulated as their 5′-monophosphates, may be free of cytotoxicity which would otherwise have ensued from a reduced supply of dTTP. Examples of such ddN analogues are D4T and FddClUrd.

Phosphonylmethoxyethylpurine derivatives

Recently, a new class of antiviral agents has been identified which exhibit a broad-spectrum activity against a wide range of DNA viruses, including adeno-, herpes- and poxviruses, and retroviruses (De Clercq et al., 1987). These compounds contain an acyclic nucleoside part (as in acyclovir and ganciclovir) linked to a phosphonate group (as in phosphonoformate). The prototypes of this class of compounds are (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA] (De Clercq et al., 1986), 9-(2-phosphonylmethoxyethyl)adenine (PMEA) (Pauwels et al., 1988b) and (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine [(*S*)-HPMPC] (Snoeck et al., 1988). Also the 3-hydroxy-2-phosphonylmethoxypropyl derivative of guanine is a potent broad-spectrum anti-DNA virus agent (De Clercq et al., 1987), which has been confirmed by other investigators (Terry et al., 1988).

The mechanism of action of (*S*)-HPMPA appears to be based upon a specific inhibitory effect on viral DNA synthesis (Votruba et al., 1987). The compound is as such taken up by the cells and subsequently converted to its monophosphoryl and diphosphoryl derivatives. The latter would represent the active form of the molecule, i.e. the form under which it interacts with the target enzyme, the viral DNA polymerase. This mode of action has also been proposed for (*R,S*)-HPMPG (Terry et al., 1988) and may be applicable to the other phosphonylmethoxyalkyl derivatives as well.

Of the phosphonylmethoxyalkylpurine and -pyrimidine derivatives, only the

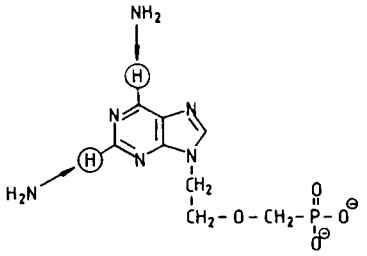

Fig. 3. Phosphonylmethoxyethylpurine derivatives: *PMEA* [9-(2-phosphonylmethoxyethyl)adenine], *PMEMAP* [9-(2-phosphonylmethoxyethyl)-2-monoaminopurine] and *PMEDAP* [9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine].

phosphonylmethoxyethylpurine derivatives, i.e. PMEA (Fig. 3) and the closely related 2-monoaminopurine (PMEMAP) and 2,6-diaminopurine (PMEDAP) analogues, have been found effective against HIV (Pauwels et al., 1988b). However, PMEA, PMEMAP and PMEDAP are less potent as inhibitors of HIV replication in vitro than are AZT and most other 2′,3′-dideoxynucleoside analogues (Table 1). Yet, in vivo PMEA suppressed tumor formation in newborn mice inoculated with Moloney murine sarcoma virus (MSV) at a dose of 1–5 mg/kg/day, that is 25-fold lower than the dose at which AZT achieved a comparable inhibitory effect on MSV-induced tumor formation (Balzarini et al., 1989b). PMEA has also been evaluated for its antiviral activity in rhesus monkeys infected with simian immunodeficiency virus (SIV) and cats infected with feline immunodeficiency virus (FIV). From these studies (done in collaboration with H. Schellekens, TNO Primate Center, Rijswijk, The Netherlands) and M.C. Horzinek (Veterinary Faculty, Utrecht University, The Netherlands), PMEA appeared to be effective in suppressing SIV and FIV replication when administered intramuscularly at a dose (10 mg/kg/day) that was well tolerated by both monkeys and cats. Initial studies also indicate that PMEA would be effective in suppressing the clinical symptoms of feline AIDS in cats.

Sulfated polysaccharides

Based on previous experience with polyanionic substances (De Somer et al., 1968a,b), virus adsorption was suggested as a possible target and polyvinyl sulfate, dextran sulfate and other polyanions were suggested as potential chemotherapeutic agents against AIDS (De Clercq, 1986). This premise was borne out when Ito et al. (1987a) and Ueno and Kuno (1987) demonstrated that dextran sulfate and heparin inhibited HIV-1 replication in MT-4 cells at a concentration which was far below the cytotoxicity threshold. Various sulfated polysaccharides (Fig. 4) are now known to inhibit HIV-1 replication in MT-4 cells within the concentration range of 0.1–1 μg/ml, while not being toxic to the host cells at concentrations up to 2.5 mg/ml, thus achieving a selectivity index of 4 orders of magnitude (Table 1). For

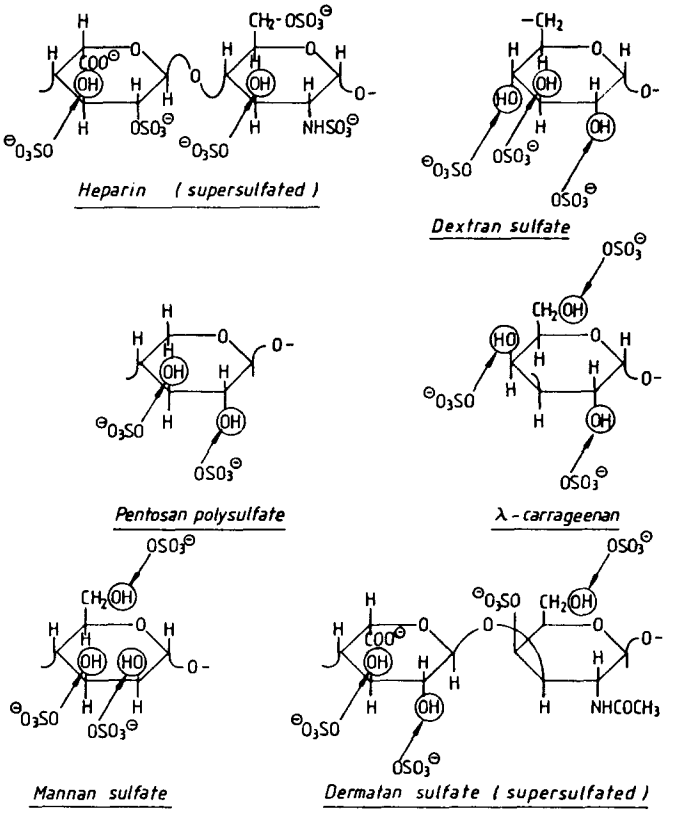

Fig. 4. Sulfated polysaccharides: repeating disaccharide or monosaccharide units (with their putative linkages). *Heparin* consists of repeating units of *L*-iduronic acid (of D-glucuronic acid)-(1 → 4)-*D*-glucosamine, with sulfamide at C-2 of D-glucosamine and sulfate groups at C-6 of *D*-glucosamine and C-2 of *L*-iduronic acid. Additional sulfate groups can be implanted at C-2 of both *D*-glucosamine and *L*-iduronic acid, which leads to the formation of 'supersulfated' heparin. *Dextran sulfate* contains a backbone of α-*D*-glucose units linked predominantly 1 → 6, with at an average two, and maximally three, sulfate groups per glucose moiety. *Pentosan polysulfate* can be considered as an oligomer of xylopyranose with at an average 1.8 sulfate groups per monomer. Carrageenans (i.e. *λ-carrageenan*) are primarily composed of α-*D*-galactose sulfate esters. *Mannan sulfate* contains a backbone of α-*D*-mannose units linked 1 → 4, with maximally three sulfate groups per mannose moiety. *Dermatan sulfate* (also called chondroitin sulfate B) consists of repeating units of *L*-iduronic acid-(1 → 3)-*D*-N-acetylgalactosamine with a sulfate group at C-4 of *D*-N-acetylgalactosamine. Additional sulfate groups can be implanted at C-6 of *D*-N-acetylgalactosamine and C-2 and C-3 of *L*-iduronic acid, which leads to the formation of 'supersulfated' dermatan sulfate.

a review on the anti-HIV activity of sulfated polysaccharides, see De Clercq (1989b).

The anti-HIV activity of the sulfated polysaccharides is critically dependent on the sulfate content: two sulfate groups per monosaccharide are required to accomplish full anti-HIV activity. Dermatan sulfate and chondroitin sulfate which contain only one sulfate group per disaccharide are virtually inactive against HIV (Baba

et al., 1988a). If, however, dermatan sulfate is 'supersulfated', so that it acquires at least two sulfate groups per monosaccharide, it becomes as active as dextran sulfate (M. Baba and E. De Clercq, unpublished data, 1988). Likewise, 'supersulfation' increases the anti-HIV-1 potency of heparin.

In addition to the degree of sulfation (or sulfate content), the molecular weight also plays an important role in the anti-HIV activity of sulfated polysaccharides: for dextran sulfate, the anti-HIV-1 activity increases with the molecular weight increasing from 1–10 kDa and then levels off (Baba et al., 1988d). With heparin fragments, no anti-HIV-1 activity is noted if the molecular weight of the fragment falls below the 5-kDa threshold (Baba et al., 1988b).

The anti-HIV activity of the sulfated polysaccharides resides primarily, if not solely, in the inhibition of virus adsorption to the outer cell membrane (Fig. 5). This has been demonstrated by several techniques based on either cell-associated radioactivity following exposure of the cells to radiolabeled HIV-1 virions (Baba et al., 1988a,b; Mitsuya et al., 1988), flow cytometric measurements of cell-associated immunofluorescence (Schols et al., 1989a) or radioimmunoassay of cell-bound virus (Nakashima et al., 1989). Sulfated polysaccharides, i.e. dextran sul-

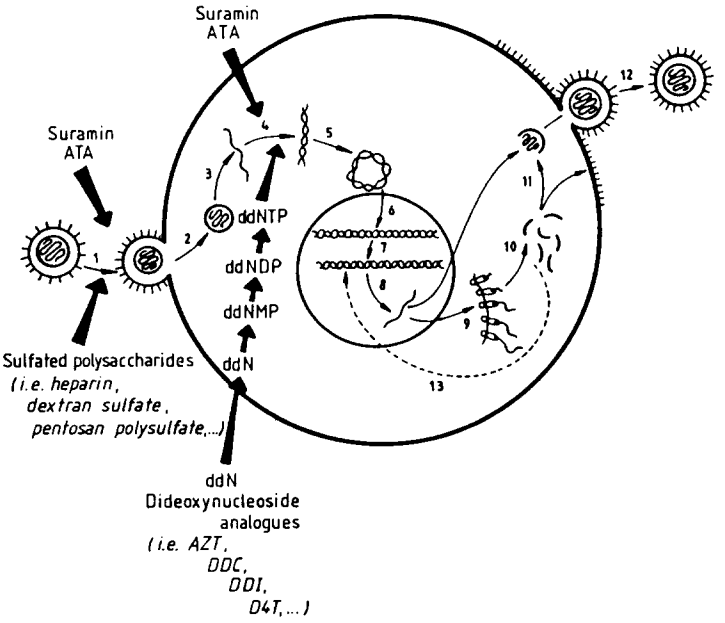

Fig. 5. Major steps in the replicative cycle of HIV: 1. adsorption; 2. penetration; 3. uncoating; 4. reverse transcription; 5. circularization; 6. integration; 7. replication; 8. transcription; 9. translation; 10. post-translational modification (i.e. proteolytic cleavage, glycosylation, myristylation); 11. assembly; 12. budding; 13. positive regulation (transactivation) or negative regulation of viral protein expression. Virus adsorption to the cell membrane serves as target for suramin, ATA and sulfated polysaccharides; reverse transcription serves as target for suramin, ATA and 2′,3′-dideoxynucleoside (ddN) analogues. To interact with the reverse transcriptase, the latter must be phosphorylated intracellularly to their 5′-triphosphates (ddNTP) *via* their 5′-monophosphates (ddNMP) and 5′-diphosphates (ddNDP).

fate, are also inhibitory to HIV-1 reverse transcriptase (Baba et al., 1988a), but this inhibitory effect is achieved only at concentrations which are about 100-fold higher than those required to inhibit HIV-1 replication or virus-cell binding. It is unlikely, therefore, that inhibition of HIV RT contributes to the inhibitory effect of the sulfated polysaccharides on HIV replication.

If the sulfated polysaccharides owe their anti-HIV activity to an inhibitory effect on virus-cell binding, they might have a differential activity against HIV-1 and HIV-2. Indeed, virus-cell binding is based on the interaction of the cellular CD4 receptor with the viral gp120 glycoprotein, and, as the HIV-1 and HIV-2 gp120 glycoproteins differ markedly from one another, their interaction with the CD4 receptor may also show differential sensitivity to the inhibitory effects of the sulfated polysaccharides. Our recent experiments have shown that, whereas AZT is equally effective against HIV-1 and HIV-2, heparin is significantly less inhibitory to HIV-2 than HIV-1, and dextran sulfate and pentosan polysulfate are significantly more inhibitory to HIV-2 than HIV-1 (Pauwels et al., 1989). In fact, pentosan polysulfate is active against HIV-2 in MT-4 cells at a concentration as low as 5 ng/ml (Pauwels et al., 1989). The HIV-2/MT-4 cell system could be used as a bioassay for the detection of pentosan polysulfate – which basically has the same structure as the polysulfated polyxylan HOE/BAY 946 recently described by Biesert et al. (1988) – in biological specimens, i.e. plasma obtained from patients treated with pentosan polysulfate. It should be pointed out, however, that Biesert et al. (1988) were unable 'to find any significant differences in the antiviral activity of HOE/BAY 946 against HIV-1 and HIV-2', which is at variance with the much higher inhibitory activity of pentosan polysulfate against HIV-2 than HIV-1 in our experiments.

The major side effect that has to be taken into account if the sulfated polysaccharides would be used in the therapy or prophylaxis of AIDS is their anticoagulant activity, which for many years has been the principal reason for the medical use of heparin. From a comparative evaluation of several sulfated polysaccharides (Baba et al., 1988a) it has appeared, however, that anti-HIV activity and anticoagulant activity are not correlated. With dextran sulfate, pentosan polysulfate and λ-carrageenan anti-HIV-1 activity is obtained at concentrations which are 200- to 400-fold below the anticoagulant threshold (1 unit) (De Clercq, 1989b). Further work along these lines has led to the development of heparin and glycosaminoglycan derivatives which have potent anti-HIV activity, yet negligible anti-thrombin activity (Baba et al., 1989b) The sulfated bacterial glycosaminoglycan (Org 31581) and chemically degraded heparin (Org 31733) inhibit HIV-1 in MT-4 cells at concentrations (0.67 and 0.52 μg/ml, respectively) which are comparable to that of standard heparin (0.89 μg/ml). Yet, Org 31581 and Org 31733 have minimal antithrombin activity (0.8 and 0.7 U/mg, respectively), as compared to heparin (141 U/mg).

A particularly interesting feature of the sulfated polysaccharides is that they are capable of blocking cell fusion (syncytium formation) between HIV-infected cells and uninfected cells. We have recently demonstrated that when chronically HIV-infected HUT-78 cells are mixed with uninfected MOLT-4 cells, the HUT-78 cells

fused with the MOLT-4 cells, and subsequently the MOLT-4 cells disappeared from the coculture. This specific destruction of the 'by-stander' $CD4^+$ MOLT-4 cells by the 'agressor' HIV-infected HUT-78 cells was completed within 24 h of coculturing the cells (Schols et al., 1989c). This phenomenon of selective killing of innocent $CD4^+$ cells by the HIV-infected 'killer' cells could be visualized by a flow cytometric method, using specific monoclonal antibodies to antigens that were expressed on either HUT-78 cells or MOLT-4 cells. Based on these observations one could easily envisage how a single HIV-infected cell, without producing any virus particles, may eliminate hundreds or thousands of innocent by-stander cells, and hence lead to the depletion of $CD4^+$ T-lymphocytes that is pathognomonic for AIDS. In this perspective, it is of utmost relevance that sulfated polysaccharides and sulfated polymers in general, but not dideoxynucleoside analogues such as AZT, are able to block fusion between HIV-infected and uninfected cells (M. Baba, D. Schols, R. Pauwels and E. De Clercq, unpublished data, 1989). This particular property of the sulfated polymers would give them a substantial therapeutic edge over AZT and other dideoxynucleosides.

Conclusion

An effective chemotherapy for AIDS is a major task requiring the combined forces of several disciplines including chemistry, biochemistry, molecular biology, pharmacology, toxicology, clinical medicine, patent law, business, economics and drug regulation. Quite schematically, the process needed to develop an effective anti-AIDS drug can be divided into ten stages: *1*, chemical synthesis of the compound; *2*, evaluation of its in vitro activity against HIV; *3*, evaluation of its in vivo activity against retrovirus infections in animal models; *4*, study of its mechanism of action; *5*, study of its pharmacokinetic profile (absorption, distribution, metabolism, elimination); *6*, toxicological examination; *7*, clinical studies in AIDS patients; *8*, patent application and granting; *9*, evaluation of market value; and *10*, approval by the drug regulatory agencies.

Except for AZT, no other compound has gone through all 10 stages. The various compounds that have been addressed in this review are in one or another stage of their development, and the prospects that at least a few of these compounds will reach the finish, that is the drug market, look bright. Considering the increasing number of compounds which have recently been found effective against HIV in cell culture systems, the crucial factor determining their ultimate success as an anti-AIDS drug will be unequivocal proof of their efficacy in patients. Such efficacy can be demonstrated only by rigorously controlled clinical trials, and, because of compliance problems with the patients in taking the prescribed medicine, proper clinical studies have proven increasingly difficult.

At present, AZT still stands as the only anti-AIDS drug that has been licensed for clinical use. Yet, several of the new compounds which have been recently described appear to be superior to AZT in one or another aspect: i.e. *D4T*, which is markedly less toxic for the bone marrow (granulocyte-monocyte progenitor cells)

than AZT (Mansuri et al., 1989); *PMEA*, which has markedly greater anti-retrovirus potency in vivo than AZT (Balzarini et al., 1989b); and *sulfated polymers* (i.e. polysaccharides) which block syncytium formation between HIV-infected and uninfected cells, whereas dideoxynucleosides such as AZT are unable to do so (M. Baba, D. Schols, R. Pauwels and E. De Clercq, unpublished data, 1989). As explained above, the fusion between infected and uninfected cells is accompanied by a selective destruction of the uninfected cells, and this phenomenon may account for the dramatic depletion of $CD4^+$ T-lymphocytes in AIDS patients. The fact that the sulfated polymers inhibit this process, whereas AZT does not, may make the sulfated polymers therapeutically advantageous.

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