Antiretroviral therapy in HIV infection, (1st part)

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Abstract

This study briefly describes the immunopathogenesis of HIV infection. It addresses drugs currently undergoing clinical investigation, and some that may be of future interest. The compounds currently available for clinical use are highlighted, seeking to address the most important aspects in clinical practice. Clinical trials are reported in relation to the efficacy of these drugs in monotherapy or in combination.

Key words: antiretroviral therapy, resistance, combination therapy.

Introduction

Since the first cases of pneumocystosis were described in 1981, until today, infection by the Human Immunodeficiency Virus (HIV) has gone from being a scourge of the four Hs (Homosexuals, Heroin Addicts, Hemophiliacs and Haitians) to a pandemic of dimensions that are difficult to evaluate, and with unpredictable consequences.

The political negligence of the majority of governments meant that it was only when it came to light that the heterosexual community was also at risk that large-scale preventative campaigns were begun, and serious support given to its investigation; many years of research and prevention were thus misdirected, and several thousand more deaths added to the statistics.

In one decade, various compounds showed antiviral activity against HIV, both in vitro and in vivo. Hundreds of compounds are being tested in laboratories, and a few dozen are being investigated in clinical trials. However, we are still far from finding an ideal drug that will eradicate the infection, with minimal side effects. Even if a compound with these qualities were discovered, it certainly would not be available in one of the places where it is most needed; Africa.

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Immunophysiopathology and therapeutic targets of HIV infection

Two types of virus have been described to date: HIV-1 and HIV2 (*Fig. 1*), each with different epidemiological patterns, but with similar clinical evolution.

In a person infected by HIV rather than a single type of virus, we find a heterogenous group of viruses resulting from minor mutations that emerge as the infection progresses. Although cell tropism is a dominant characteristic, not all HIV infected cells express the CD4 receptor, as is the case with human chondrocytes, fibroblasts of the foreskin, the synovial cells and five human hepatoma cell lines which, despite the fact that they do not express this receptor, are susceptible to infection by the virus.[1,4] Four cell receptors are currently described that permit host infection by HIV: The CD4 receptor, Gal-C (galactosyl ceramide, which exists in the cells of the CNS and intestine), the receptor of the Fc portion of immunoglobulins (Igs) and the complement receptor.[5]

Interaction between the protein of the viral involucrum gp120 and the cell receptor leads to fusion of the membranes and passage of the viral content (RNA and the various enzymes required for its integration in the reverse transcriptase cell genome, ribonucleases and integrases) to the interior of the cell.

Once inside the cell, the RNA of the HIV is transcribed into DNA through the action of reverse transcriptase, forming a hybrid double chain of RNA-DNA; ribonuclease leads to RNA splicing, resulting in a single DNA chain, which duplicates itself through the DNA polymerase. The double chain DNA migrates to the nucleus, where through the action of integrase, it is inserted into the DNA of the host cell, becoming an integral part of its genome.

When the viral sequence is transcribed, the viral

RNA and the RNAm that encodes the viral proteins (of the core, involucrum and enzymes) are formed, gathering in the cytoplasm and amalgamating with the viral RNA to form the new viruses which, when released, will infect new cells.

In several of these stages, therapeutic intervention is possible in an attempt to eliminate, or at least reduce, the production of infecting viral particles (*Fig 2*).

The lymphocyte CD4+ has been the main target of interest in the evolution of this infection. According to classical theory, direct infection of these cells by HIV leads to their destruction and the breakdown of the immune system. Recently, new concepts have emerged concerning the way in which the destruction of these cells occurs, and how the immunity collapses. Contrary to what was initially believed, after primary infection, HIV quickly leaves the circulation and enters the lymphoid system, particularly the lymphatic ganglia (as confirmed by PCR in situ for DNA, calculating that 30% of the CD4+ of the lymphatic tissue is infected, although only 1 in every 400 with active viral replication, which is detected by positivity for PCR in situ for the viral RNA).[6-9] In these ganglions, where the vast majority of T lymphocytes are located, there are other kinds of cells (follicular dendritic cells in the germinal center, and interdigitating dendritic cells in the paracortex) which are indispensable for the presentation of antigens to the CD4+ and B lymphocytes, and for the formation of memory cells, as well the viability of existing ones. Although HIV does not infect these cells, the deposition of its proteins on their surface could lead to dysfunction and subsequent death of those cells. Meanwhile, the disappearance of this kind of cell could trigger programmed cell death (PCD) of the CD4+ and CD8+[9] cells. PCD (which in vitro is morphologically translated by apoptosis) is a physiological mechanism that plays an essential role in embryogenesis; in adults it performs important functions in the immune system, blocking the proliferation of potentially harmful (autoimmune) cells, or those with no maturation capacity (i.e. useless). This programmed death can be induced by a series of cytokines (and expression of some genes), or by the absence of others.[10]

Thus, by infecting or deregulating various types of accessory cells (but which are indispensable for the good functioning of immune system), HIV leads to

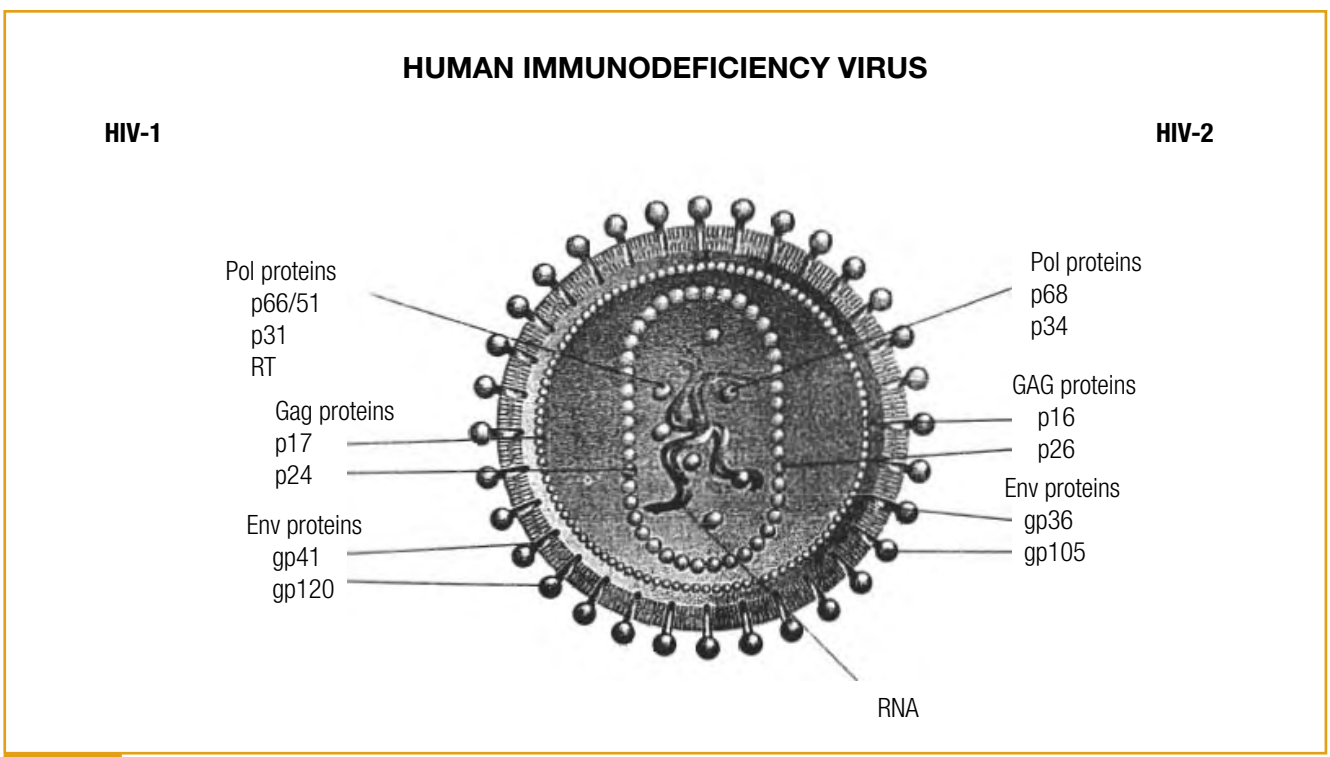


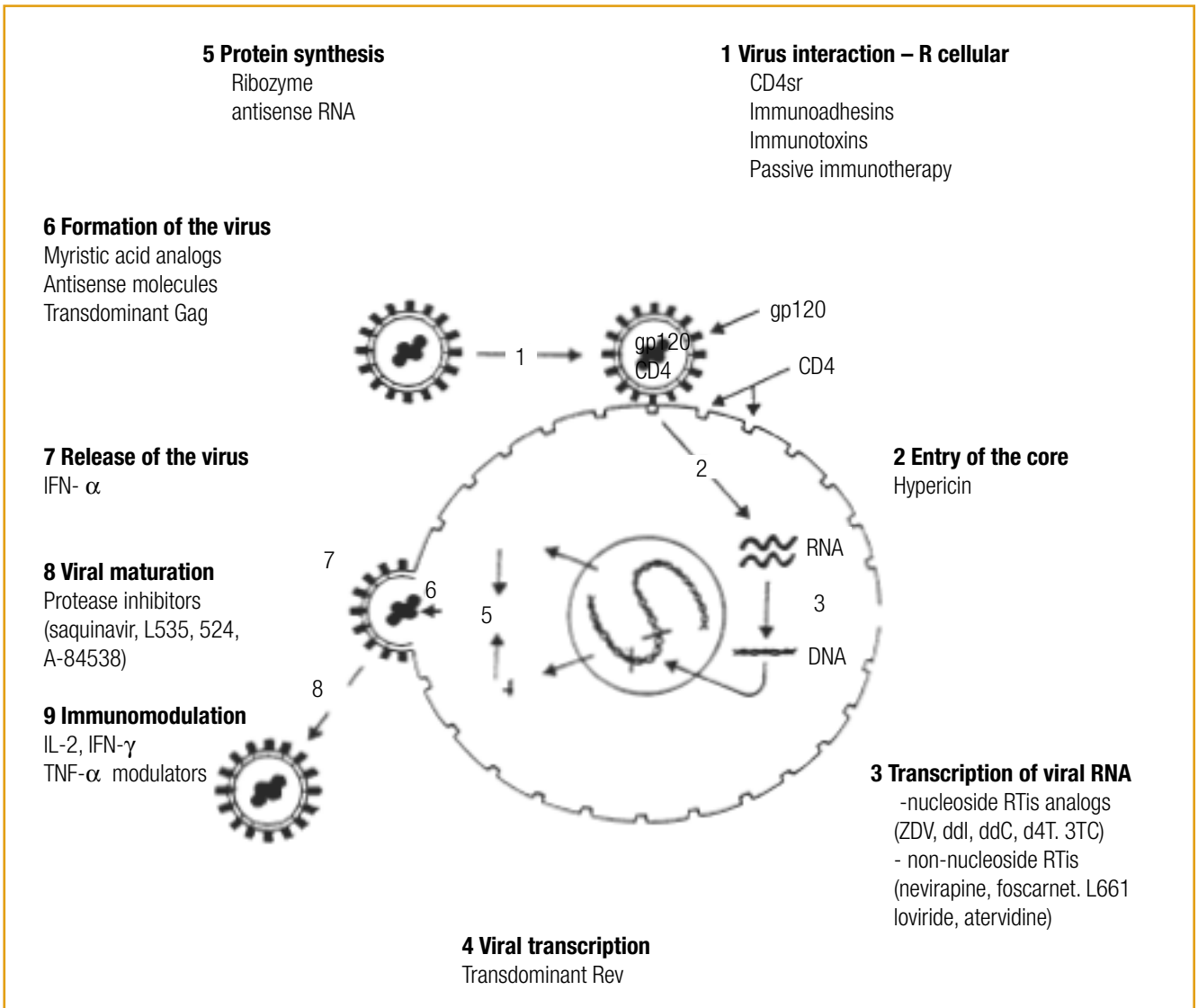
FIG. 1

depletion of the CD4+ and CD8+ cells. Great importance has been attached to the role of the latter, with a CD8+CD28+ subgroup that produces a cytokine (still unnamed) capable of suppressing viral replication within the CD4+ without destroying them; as the infection progresses, this capacity gradually disappears, leading to an increase in viral replication and the emergence of more virulent strains (such as syncytium inducers).[11]

PCD has been involved, besides depletion of the CD4+ lymphocytes, as an important mechanism in the development of muscular atrophy and multifocal progressive leukoencephalopathy associated with HIV infection.

Other authors demonstrate that some proteins of the involucrum (gp120) may cause dysfunction of the CD4+ cells simply by depositing themselves on the surface of those lymphocytes.[12]

The CD4+ cells can be subdivided into two groups, according to the cytokines they produce in TH1 and TH2. Thus, TH1 produces IL-2 and IFN-γ and stimulates cell immunity, while TH2 produces IL-4 and IL-10 and stimulates humoral immunity. Latent infection inside the monocytes, by deregula-



Schemes of potential therapeutic targets in HIV infection.

FIG. 2

tion of cytokine production (increase in production of TNF-α and reduction of IL-1),[13,14] contributes, on one hand, to the PDC of the CD4+ and CD8+[15] cells, and on the other, to the transition of the prevalence of TH1 to TH2, with the resulting weakening of cell immunity.[16-20]

Therapeutic targets

Agents that act in the extracellular phase of the infection

These are a heterogeneous group of products aimed at impeding HIV-cell receptor interaction.

They do not appear to be as efficient as single agents, but may play an important role as part of a strategy of combination therapy, and have already demonstrated synergism in vitro among some of these compounds to ZDV and IFN-α.[21]

Soluble recombinant CD4 (CD4sr)

Blocks fixation of the virus to its receptor, although the hypothesis that it may interfere in the introduction of the "core" into the cell is not rejected. However, its efficacy in bonding to gp120 of VIH-2 is significantly lower than that of HIV-1. This type of molecule does not interfere in the interactions between T lymphocytes (communication within the immune system).

Administering this compound did not cause any improvement in the laboratory markers of progression of the disease. It is assumed that the main problem of this therapy is its short half life, which would involve continuous endovenous administration.[22,23]

Immunoadesins

These emerge from the specificity of CD4sr binding with the effector fraction of an immunoglobulin.

The Fc portion of IgG1[24-26] was used, which was chosen due to its long half life and because it has binding sites for the complement. Their half life was 200 times higher than that of CD4sr, but they did not bind to the C1q of the complement cascade. They seem to prevent cell death caused by HIV, but without affecting the capacity for cell proliferation; they also facilitate the destruction of infected cells by phagocytosis or antibody-dependent cell-mediated cytotoxicity (ADCC).[27]

There were no indications that the infection of cells that express receptors with high affinity to Fc was facilitated. They cross the placenta barrier, which could provide protection to fetuses in seropositive women.

Other molecules were produced, such as the pentameric form of chimera CD4-IgM, which, in the syncytium formation tests, appears to be very effective.

In clinical trials, they did not cause any adverse effects, and stability of the laboratory markers of progression was recorded.

Immunotoxins

Immunotoxins consist of the junction of a compound with the capacity to bind a cell (antibodies, hormones or growth factors) to a toxin (vegetal or bacterial) or its sub-unit.

Ricin is a plant-derived toxin made up of two chains: A, which has the capacity to inactivate ribosomes and B, which serves as a cell ligand; after purification of the A chain (initially hepatotoxic) binding to CD4sr occurred. The theoretical concept is that by binding to the gp120 on the cell surface, this immunotoxin-gp120 group is endocytosed and, once liberated into the cytoplasm of the infected cell, the ricin A chain blocks the ribosomes and protein synthesis [28].

High toxicity was observed in vitro for H9 cells infected by HIV. This toxin was also conjugated with the light κ chain of an IgG specific for gp41, a highly preserved protein that is expressed only by infected cells. The addition of chloroquine to the medium increased the cytotoxicity of this immunotoxin 100 times for H9 and U327 cells infected by HIV.

Another toxin that has been used is Pseudomonas exotoxin A (PEA), which has the capacity to influence protein synthesis. Recombinant forms CD4-PE40 were synthesized, which demonstrated high cytotoxicity against cells infected by HIV. However, inhibition of viral replication was incomplete.[24,29]

One of the limitations for therapeutic use of these compounds is the fact that infected T lymphocytes, as well as monocytes, cannot express viral protein to the surface beyond what these proteins are able to deposit on the surface of non-infected cells.

Passive immunotherapy

The principle of using passive immunotherapy in HIV infections is based on the successful use of specific human immunoglobulins (Igs) in prophylaxis of di-

seases caused by agents such as CMV, HBV, HAV, etc., after possible exposure. Techniques to produce monoclonal antibodies and DNA recombinant production enabled the use of large quantities of highly specific Igs, affording a high level of protection (preferably using associations of specific Igs specific for different epitopes of the same agent).[21]

Anti-gp120 antibodies – all the protective (neutralizing) antibodies detected in this infection are specific for gp120, notably for two domains of this protein; anti-ansa V3 and anti-local of binding to the CD4. These antibodies, although present in infected individuals, do not reach sufficient concentrations to be efficient.[30]

Results of clinical trials involving this form of immunotherapy (but using inactivated plasma from infected individuals) were inconclusive, with reports of a decrease in Agp24, reduction in the number of opportunistic infections, and improvement in the laboratory parameters and the scores of the Karnofsky scale.[31] Inconclusive results can be attributed to the use of seropositive plasma, but with low antibody titers, many of which are non-neutralizing.

The use of different neutralizing antibodies may decrease the frequency of emergence of mutants (and resistances) by impeding the emergence of strains with mutations in the proteins of the involucrum.

Anti-p24 antibodies — some authors[32] used seropositive plasma rich in this antibody, observing not only an improvement in laboratory markers of progression, but also a reduction of the pathologies that define AIDS.

Anti-CD4 antibodies – the receptor CD4 has various regions that are susceptible to monoclonal antibodies; thus, the monoclonal antibody against domain 1 would prevent interaction with gp120. However, antibodies against domains 2 and 3 would block the infection after the binding of the virus to the cell receptor. Given that these antibodies are not directed against the portions of the CD4 receptor involved in the CD4-MHC (Major Histocompatibility Complex) interaction, theoretically there would be no risk of dysfunction in the phenomena of communication between the cells of the immune system.[21]

PASSHIV-1 – some authors use porcine antibodies targeted against proteins of the involucrum (gp160, gp120, gp41), core (p24, p55) and polymerase (p66, p53). This hyperimmune serum was produced through administration in pigs of an HIV lysate (containing the aforementioned proteins). In a study carried out with individuals infected with HIV (stage CDC B or C), the adverse effects were minimal (urticariform reaction in one patient). In the sixth month, improvements were reported in the laboratory markers of the disease (Agp24 and CD4+) and also in the clinical markers, with regression of fever, oral candidiasis, bronchitis, and peripheral polyneuropathy. Most patients showed improved appetite, and weight gain was observed in the group as a whole, to a maximum of 8.6 Kg.[33]

Agents that act in the intracellular phase of infection

Reverse transcriptase inhibitors (RTis)

As HIV is a retrovirus, in order to infect a cell, its RNA must be converted into DNA, which can then be integrated into the cell genome. For this, it transports an enzyme, reverse transcriptase (RT), the role of which is to perform this conversion. RT is a heterodimer made up of two units formed by the cleavage (mediated by protease) of polyprotein encoded by gag-pol genes.

One disadvantage of this type of inhibitor is that to be effective, there has to be active replication inside the cells (i.e., they are effective only in avoiding the infection of new cells). Several authors have demonstrated, through PCR in situ for viral RNA, that the number of cells under these conditions in the asymptomatic seropositive patient is minimal, placing in doubt the reliability of the use of these compounds in this situation.[6,9]

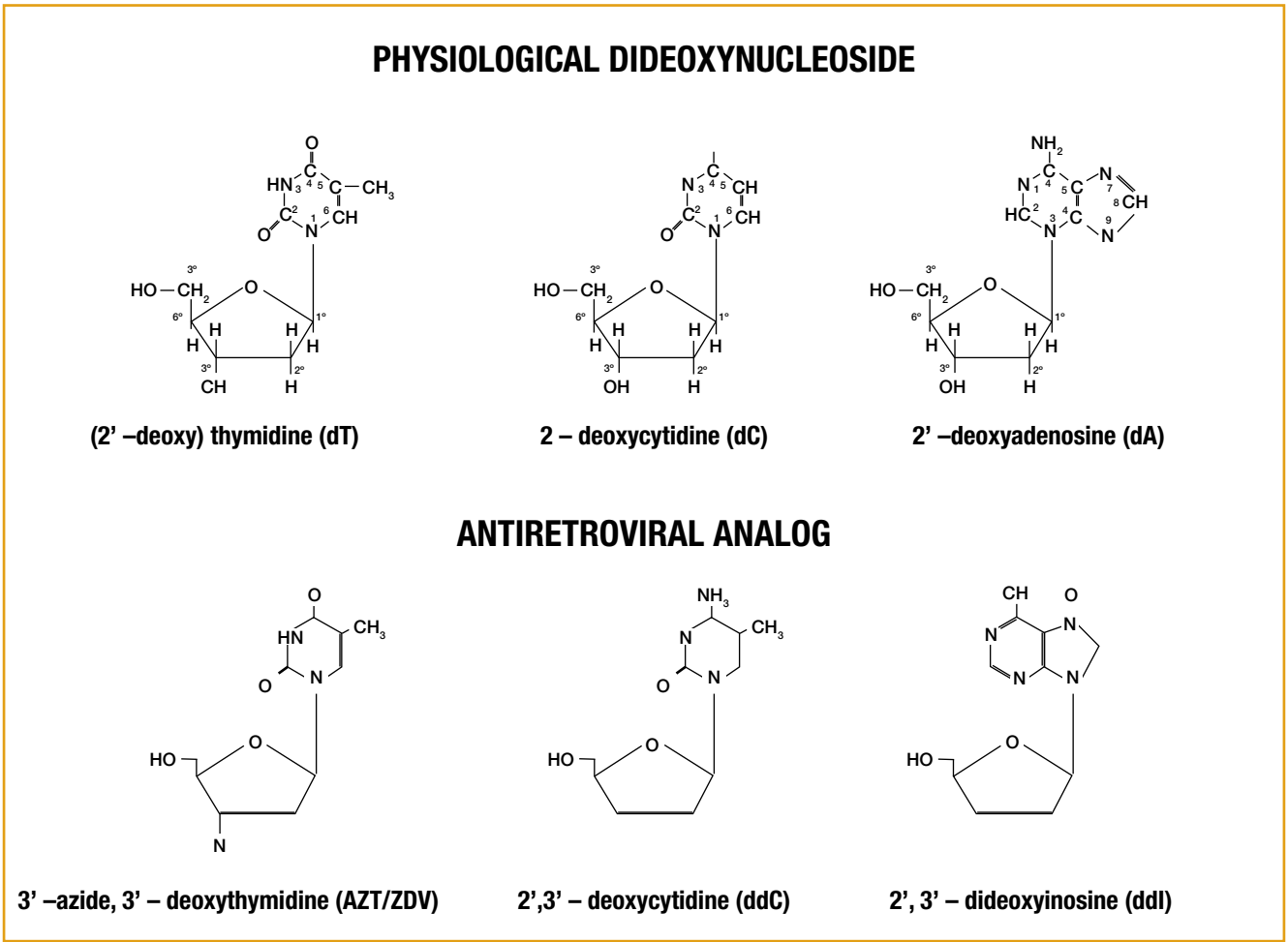
This group of drugs — reverse transcriptase inhibitors (RTis) — is divided into two families: nucleoside analogs (which have a structure similar to the basic units of nucleic acids – nucleosides) and non-nucleosides (all with different structures, and no analogy to the structure of nucleosides).

Nucleoside analog RTis

Many pyrimidine and purine analogs have shown antiviral activity against retroviruses (*Fig. 3*).

It is this group that is part of the first drug that showed clinical utility for the treatment of HIV infection — Zidovudine (ZDV).

Nucleoside analogs are gradually phosphorylated by cytoplasmic enzymes to the form 5' –triphosphate (active form), which will compete with the natural



Comparison of the chemical structure of the nucleoside analogs and physiological nucleosides.

FIG. 3

substrate to bind to the viral RT (and to the DNA polymerases of the host).

It has a double action mechanism: on one hand there are direct RT inhibitors (binding to it and blocking its activity) in their monophosphate form; on the other hand, in its triphosphate form, they are integrated into the DNA being formed, through the RT, functioning as termination codons, as the lack of a radical 3' –OH required to form the3' -5' –phosphodiester bond, which is indispensable for the addition of another nucleoside is no longer available, resulting in incomplete, non-functional DNA chains of HIV.

Its efficacy depends on its capacity to penetrate various types of infected cells, and on the possibility of these cells metabolizing the compounds to their active form (triphosphate). This explains the decreased efficacy of some dideoxynucleosides in the monocyte-macrophage cell line.

Toxicity of these compounds results from their capacity to inhibit RT function without affecting DNA polymerases of the host, essential for cell multiplication.

Thymidine analogs

Zidovudine

This is a deoxythymidine analog in which the hydroxyl radical (OH) has been replaced by an azote atom, hence the name azidothymidine (AZT).

Besides its activity on the RT of the VIH, ZDV-TP also inhibits, albeit in a weaker form, the DNA polymerase of the HBV (without clinical utility), the

same occurring with EBV, HTLV I and various viruses that cause immunodeficiency in animals. It is also active against gram negative bacteria (particularly Enterobacteriaceae) but with very rapid development of resistance.

The oral bioavailability is 60%, due to the effect of the first hepatic passage with glucuronidation in an inactive metabolite excreted by the kidneys. In patients with renal insufficiency, the half life of ZDV increased only slightly as it is the inactive form that is excreted by the kidneys.[34] In patients with hepatic pathology, particularly cirrhosis, as the metabolization capacity can be compromised, the half life can increase significantly, requiring dose adjustment.[35]

It is found in the sperm in concentrations 10-20 times higher than in the blood, but this gives does not give protection with regard to transmission, and there is not even a decrease in the amount of virus in this fluid.[36]

The increase of CD4+ lymphocytes, weight, and the feeling of well-being, as well as the decrease in Agp24 and the improvement in neurological functions can all occur in the first two weeks of therapy. A reversion of cutaneous anergy was also recorded. In various clinical studies, despite the improvement in laboratory markers of progression, the number of opportunist infections between the ZDV group and the placebo only differ significantly after eight weeks of therapy, which shows that the recovery of some immune capacity may be independent and relatively delayed in relation to the above-mentioned markers of progression.[36,37]

ZDV partially reverts and delays the progression of the cognitive dysfunctions (demonstrated by neuropsychometric tests such as memory and attention tests) and significantly decreased the incidence of the dementia complex associated with AIDS (ADC). Zidovudine significantly reduces the occurrence of productive infection in the brain, and is responsible for the decrease of cases of encephalitis, ADC and progressive multifocal leukoencephalopathy (PML).[38] The penetration of ZDV in the LCR appears to be dose-independent, which may explain its effectiveness, even in low doses, in the prevention and treatment of neurological disturbances associated with AIDS.[39]

Another effect that has been described is the improvement in thrombocytopenia associated with HIV infection; the mechanism of action is not known, however, a possible anti-inflammatory action has been described, and even an immunosuppressant action of zidovudine which, in vitro, decreases the response of the mononuclear cells of the peripheral blood to the mitogens and inhibits cell growth. This antiproliferative effect is similar to that of the corticoids and immunosuppressant agents[40] and could explain its beneficial action in situations associated with HIV infection, such as immune thrombocytopenia, leukoencephalopathy and psoriasis, although the etiology of these pathologies cannot always be considered autoimmune.[41]

The vertical transmission of HIV is related to gestation time, the risk being higher in the third term of pregnancy, if the maternal infection occurs in the puerperal period, in the normal labor, and with advanced maternal immunossuppression.[42,43]This risk ranges between 20% and 30%. However, in a state in which ZDV is administered to the mother in the antenatal period, the risk of transmission decreased by 50%.[44,45]

At the end of 6 months of therapy with ZDV, in some cases, resistance occurs, associated with the inability of the RT to incorporate triphosphates nucleosides with radicals 3' –azide, though it does so with the others (hence they are sensitive to the ddI or ddC).

The adverse effect most frequently attributed to the ZDV has been myelosuppression, with anemia heading the list. This effect is dose-dependant, emerging in the first trials in 25% of people (against 4% in the placebo group) generally observing macrocytosis, normal or increased levels of serum erythropoietin and medullary hypoplasia (which justifies the direct medullary toxic effect). Neutropenia occurred in 15% of people (compared with 2% in the placebo group). The association with other factors could promote this effect; the most well-known is with Ganciclovir. In the case of some severe opportunist infection in which it is necessary to use myelotoxic drugs (phase of ganciclovir induction, for example) ZDV should be suspended.[36]

A myositis-like syndrome has been described, characterized by paraesthesias, myalgias, edema, muscular atrophy and increase in LDH and CPK in patients undergoing prolonged ZDV therapy. This situation appears to be more frequent in patients with more advanced infection, as HIV itself can cause this pathology. This is why the two can have additive

effects on the muscle. This myopathy of ZDV apparently involves depletion of the mitochondrial DNA and may be related to inhibition of the polymerase DNA γ by the drug.

Other adverse effects are nausea, insomnia, intense headaches and hepatotoxicity (but the majority of people had concomitant infections with CMV or microbacteria). There were sporadic reports of convulsions. Black coloration of the fingernails is the adverse effect most frequently observed among the Black population.

The myelotoxic adverse effects can be controlled either by decreasing the dose (500 to 300 mg/d)[46] or with haematopoietic growth factors (EPO if endogenous EPO < 500 mUI; G-CSF/GM-CSF of severe neutropenia). Sometimes it is necessary to suspend administration of the drug. If this occurs on more than three occasions, definitive suspension should be considered, switching to another RTi.

Van Luzen et al. affirm the blastogenic capacity of the T lymphocytes in the presence of ZDV and conclude that in individuals who are asymptomatic to that drug, blastogenesis decreased significantly, compared with symptomatic patients in whom the main effect is a decrease in viral load[47]. The use of ZDV in primary infection (for example after being pricked by an infected needle) has been problematic. Its use in this situation could prevent the initial immune response against HIV (decreased initial CD8+ lymphocytosis), increased risk of progression[48] and eventually even a risk of collecting strains resistant to ZDV could occur.

Since 1989, based on the results of the ACTG 016 and ACTG 019 trials that show a decrease in infection for symptomatic disease, which advised the administration of ZDV to all the infected individuals with less than 500 CD4+ lymphocytes, even if asymptomatic. In April 1993, preliminary conclusions of the Concorde trial[49] (England/France/Ireland, the largest such trial to date, and the one with the longest ever follow-up), reported that ZDV was not effective in delaying the progression of the asymptomatic infection to the symptomatic phase (CRS, AIDS, or death).

Likewise, it was concluded that the antiviral effect of ZDV appears to be transitory, acting over a period of one or two years; the confirmation and publication of the trial occurred in April 1994.[50] These results were contested by some authors due to the fact that there had been alterations in the branch of the treatment (placebo) administered in which after 1989 up to 92.27% of the participants take ZDV. However the authors of the Concord and various others[51] affirm that the quantity of ZDV taken by the branches of the study is so different that the conclusions are untenable. In March 1994 the European-Australian collaborate group published a trial (017) in which ZDV 500 mg/d was administered over a period of 104 weeks, to 329 individuals infected by HIV in asymptomatic state, with CD4+ between 200-400 x 10^3/ml. At the end of one year of follow-up, the progression to advanced CDA or AIDS was significantly higher in the placebo group (P=0.01). However, at the end of two years, this difference was not statistically significant (P=0.26).[52]

Another conclusion of the Concord trial is the inadequacy of the CD4+ lymphocytes as a marker to monitor the progression of the disease and of the clinical trials, since despite the improvement in the overall value of the CD4+ lymphocytes that the ZDV induces, in clinical terms, infected people progress in the disease. As it is not possible to define a value from which the ZDV should be started in asymptomatic seropositive cases, these authors report that if such a value existed, it would be for CD4+ lymphocytes lower than 300 x 10^3 cells/ml.[50]

A study recently emerged which seeks to evaluate the quality of life of asymptomatic people under ZDV therapy[53] and another in which the cost/benefit of the ZDV therapy is evaluated in the asymptomatic cases[54]. In the first, the population consisted of individuals who took part in the ACTG 019, and compared a battery of severe adverse effects in three groups of asymptomatic people: placebo, ZDV 500 mg/d and ZDV 1500 mg/d for 18 months. In this analysis the authors report that the patient's quality of life following an adverse effect is not the same as during the period without symptoms of diseases in toxicity, but that this varies with the patient and the adverse effect manifested, and conclude that for asymptomatic infected patients, the reduction in quality of life due to the severe adverse effects of the therapy is the same as the increase in quality of life associated with the delay in the progression of the disease to HIV.

This polemic debate continues, as in a recent study, Cooper et al. analyze the effects of ZDV in seropositive patients with the immune system little affected (CD4+ between 500 and 749 ce/µl), concluding that there is a decrease in progression to symptomatic di-

sease.[55] Other authors do not confirm this hypothesis in asymptomatic seropositive patients with CD4+ < 500 cel/µl.[56-58]

Fluorothymidine

FLT, fluorothymidine or 3'–fluoro-ddT is a thymidine derivative, by substitution of the azotade radical of azidothymidine by fluoride. It is stronger than zidovudine in vitro and is active in strains that are resistant to that drug. Myelotoxicity continues to be the limiting adverse effect and according to some authors, the marked decrease in the number of reticulocytes should be predictive of future anemia.[3,21] Recently, clinical trials with this drug were suspended due to a possible contribution to two cases of acute hepatic insufficiency.

Stavudine

A 2', 3'–didehydro-2'. 3'–dideoxythymidine (d4T) is also a thymidine analog but with a different activation kinetic (triphosphate) from zidovudine. Apparently just as potent, but less toxic than ZDV, it acts on the viruses that are resistant to that drug.

It has good oral bioavailability (±85%); renal and non-renal excretion (50% of the drug is recovered unaltered in the urine) which distinguishes it from ZDV and ddl, with predominantly non-renal secretion, and ddC which is excreted mainly renal excretion.

The plasma peaks reach concentrations well above those necessary for the antiviral effect, while the troughs (in tripartide administration) are lower, which gives some concern due to the hypothetical emergence of resistences.[59]

There was an improvement in the laboratory markers of progression at 24 weeks: decrease in Agp24 and sustained increase in CD4+ cells. In clinical terms, there was an improvement in weight, fever, nocturnal sweating and fatigue, and also in some cases, the diarrhea disappeared.[60]

d4T is more potent than ZDV in the inhibition of polymerases β (RT, therefore higher antiviral power) and γ (higher risk of neurotoxicity as it is a mitochondrial polymerase).

The recommended dose is 2 mg/Kg/d divided into three or four administrations. With this quantity, the secondary effects may be reduced. However peripheral neuropathy and hepatotoxicity have been reported. At higher doses (4-8 mg/Kg/d), anemia has been reported.[61]

The appearance of neuropathy appears to depend not only on the dose but also on the way it is divided up. The increase in daily dose from 1 mg/Kg doubled the risk, while two administrations instead of three increased the risk fourfold. This is probably due to the good oral bioavailability of the drug: a greater interval with a higher dose can expose the tissues to much higher and more toxic peaks.

The cumulative dose of d4T and the appearance of neuropathy was only associated with the form of dosage (for the same daily dose the risk, when taken in two parts, is four times that of the dose taken in three parts).[60,62-64] According to data from the manufacturing company, in the United States up until October 1993, in the widened access program, 9165 patients had had access to the drug. The most frequent toxicity was peripheral neuropathy (63 cases), followed by nausea and vomiting (15), hot flushes (13) and diarrhea (12). Apparently, according to another study by the company, Stavudine can be administered with ddl without increased toxicity (both cause peripheral neuropathy).

ZDV inhibits the phosphorylation of d4T, which counterindicates the use of ZDV + d4T as combined therapy, as the drug would inhibit the passage to the active form of the second. In August 1994 it was approved by the FDA for use in the USA.

Adenosine analogs

Didanosine

This is Didesoxinosine (ddI) which is converted into dideoxyadenosine (ddAMP), in turn phosphorylated to dideoxyadenosine triphosphate (ddATP) which is incorporated, by the RT in the DNA chain in formation, leading to its early termination.[36]

Didanosine is extremely vulnerable to acid medium, therefore to be absorbed via the oral route, the gastric content must be alkaline.

The oral bioavailability varies between 20% and 40%. It has a short plasma half life (0.6-1.4h) but its active metabolite, ddATP, has an intracellular half life of 12 hours, which enables its administration only twice a day.

Given that 30% to 65% of the drug is excreted unaltered in the urine, in patients with renal insufficiency (Ccreat < 5 ml/m) the dose should be reduced by 2-3 times and the drug should only be administered

at the end of the haemodialysis. Penetration in the LCR is poor with the oral administration, and does not appear to have any benefit in improving the neurological symptoms like those observed with ZDV.

The current recommended dose varies according to weight: > 75 Kg 300 mg/12-12h, 50-74 Kg 200 mg/12-12h e < 50 Kg 125 mg/12-12h; (at the dose reported and for the tablets; the chewable tablets have 20% to 25% higher absorption than the sachets. However, at least two tablets are always needed, to reach the sufficient amount of buffer to neutralize the gastric pH).

Simultaneous administration with food decreases its absorption significantly (therefore it must be taken in fasting, 1.5 hours before or 2 hours after meals) and simultaneous use of ranitidine does not increase absorption. Drugs that require acid media for their absorption (dapsone, ketoconazole) should be administered with intervals of two hours between each dose. Formulae containing magnesium or aluminum decrease the absorption of quinolones and tetracyclines, therefore they should not be taken simultaneously. [29,65]

Didanosine increases (in transitory form, like ZDV and zalcitabine) the number of CD4+ cells, decreases the Agp24 and β2microglobulin and improves the hematological values (Hb, GB, lymphocytes and neutrophils). In clinical terms, improvements are described in the patients' feeling of well-being, the constitutional symptoms (fever, weight loss and nocturnal sweating), oral tricho-leukoplasty, cognitive functions and recovery of retarded hypersensitivity in anergic individuals.[66-68] The effectiveness appears to be lower in individuals with AIDS.

The manifestations of toxicity that most frequently lead to a reduction in dose are peripheral neuropathy and pancreatitis, although these are infrequent for doses lower than 13 mg/Kg/d. Diarrhea has been frequently referred to but appears to be a complication related to the buffers used to neutralize the gastric pH.

Toxicity for the bone marrow cells emerges at concentrations of around 100 times higher than those of ZDV and 10 times higher than those of ddC.

In the phase I trials, 34% of the individuals developed peripheral neuropathy at doses equal to or lower than those currently recommended. It appears that the necessary cumulative dose for the development of the neuropathy is between 1.5 and 2 g/Kg. The cause appears to be inhibition of replication of the mitochondrial DNA, and this complication appears to revert 1-12 weeks after suspension of the drug. Pancreatitis occurs in 9% of individuals on doses equal to or lower than those recommended (0.39% fatal cases) and the risk appears to correlate with a previous history of pancreatitis, advanced HIV disease, poor clinical condition and concomitant pancreatic toxic therapies (pentamidine and cotrimoxazole). The use of didanosine should be avoided in patients with risk factors for pancreatic disease (in addition to those already described, alcoholics, and those with dyslipidaemias) and frequently monitor amylase, lipase and triglycerides (substantial increases in these are described before clinical pancreatitis). Less frequently, the following were described: myalgias (myositis), headaches, xerostomia, akathisia, insomnia and abdominal pain. In very rare cases: Cardiac insufficiency (major inflow of sodium by the buffers used), hepatitis (depletion of the glycogen deposits), increase in QT, optic neuritis, rash and hypocalcemia.[29,50,68,69]

As for ZDV, at the end of some time of therapy, resistances emerge.[71,72] The combination with other antiviral agents is synergic in the majority of cases. Its addition to ZDV appears to prolong the period of effectiveness, perhaps because it delays the development of resistances. A curious result was obtained for the association with ribavirin, which decreases the antiviral activity of ZDV and, because it is an IMP dehydrogenase inhibitor, leads to an increase in ddATP (the active metabolite of didanosine) with a consequent increase in the effectiveness of the didanosine; on the other hand, the GM-CSF that potentiates the ZDV decreases the effectiveness of didanosine and zalcitabine. The G-CSF and erythropoietin did not show any interference.[29]

Cytosine analogs

Zalcitabine

2' -3' dideoxycytidine is a pyrimidine analog, and like the others members of this family, needs to be phosphorylated in order to become active (active metabolite – 2',3' – dideoxycytidine triphosphate; ddCTP).

Of the various human polymerases, the DNA polymerase α (involved in synthesis) is relatively resistant, while the DNA polymerase β (repair and RT) and γ (mitochondrial) are more susceptible to

inhibition ddCTP. This explains the adverse effects of zalcitabine.

The relationship between deoxycytidine triphosphate and zalcitabine triphosphate (natural substrate/active drug) is low among monocytes ad macrophages, which enables better antiviral power within that cell line (it is precisely the cells where the ZDV and ddI has greater difficulty acting).

It has good oral bioavailability (88%), which decreases significantly when taken with food. Most of the drug is eliminated unaltered in the urine (without hepatic metabolism), increasing its excretion time in renal insufficiency. The current recommended varies from 0.375 to 0.750 mg/8-8h.

Zalcitabine did not significant improve the cutaneous anergy in any of the clinical trials. Many of the patients did not end their participation in the clinical trials due to the adverse effects (neuropathy) or due to the appearance of clinical elements of progression of the disease.[73]

In vitro, zalcitabine inhibits the progenitor cells of the bone marrow at concentrations that are significantly lower than those of the antiviral activity. However in the clinical trials, no significant myelotoxicity has been demonstrated.

The most common adverse effects are peripheral neuropathy, cutaneous rash, aphthous stomatitis, fever, neutropenia, thrombocytopenia, arthritis, arthralgia and in rare cases, pancreatitis and myocardiopathy. Neuropathy is the most frequent complication, manifesting in the feet and hands around eight weeks after the start of therapy; it is dose-dependant. It begins with paraesthesias (burning and tingling sensation) in the feet, and 50% of cases develop symptoms in the hands. In higher doses, the neuropathy did not decrease one year after suspending the drug. In other patients, there was intensification of the symptoms soon after suspension and after a gradual disappearance. Rash is also characteristic, appearing on the trunk and limbs; it is dose-dependant, and appears at the end of two weeks of therapy, being associated with oral ulcers and sometimes fever. Hemocytopenias are more frequent in regimes which alternate zalcitabine with zidovudine than in continuous therapy with zalcitabine, but can occur in the latter situation.[21,36,74]

Resistances to zalcitabine also develop, but it may be that its appearance is slower than that of ZDV and ddI.

Synergism in vitro was demonstrated between zalcitabine and IFN-α, soluble CD4, dextran sulphate, dipyridamole and zidovudine; on the other hand, there was antagonism in the association with ribavirin and GM-CSF.

Lamivudine

2' –deoxy-3' –thiacytidine, or 3TC, is another analog of this pyrimidine. It demonstrated good antiviral strength in vitro, with activity in ZDV resistant strains, and a good cytotoxicity profile.

Apparently highly innocuous, Lamivudine (300 mg/12-12h) caused only urticaria and headache. It is well-tolerated in monotherapy or in association with ZDV.[75]

Its oral bioavailability is 82%, with mainly renal excretion (70% of the dose is recovered unaltered in urine). The triphosphate derivative (active form) has an intracellular half life of between 11 and 14h.

It has low potential for γ polymerase and therefore minimal neurotoxicity.[76] Clinical trials are now in phase II.

Other deoxynucleoside analogs

Some companies created libraries of nucleoside analogs that have been produced for antiviral and antineoplasic therapy. Thus, hundreds of compounds have been synthesized, dozens of which have shown antiretroviral activity in vitro and possibly some of which will be the object of clinical trials; These include: dideoxyguanosine (ddG), derivatives 3' –azide guanosine (AZG), various pyrimidines, such as azidodeoxyuridine, and fluorated derivatives (3' –fluor) of ddG[77]. In Europe (Denmark, France, Germany, Italy, the Netherlands, Spain and the United Kingdom), Burroughs Wellcome has begun phase II trials for a new RTi FCU (935U83) nucleoside analog, which has a strength nine times lower than ZDV, but is 1300 times less toxic. In vitro it is active against ZDV and ddI resistant strains.

Non-nucleoside RTis

Unlike dideoxynucleosides, their structure is not similar to that of basic units of nucleic acids, therefore they are not "mistakenly" used by the RT and are incorporated into the DNA being formed. These compounds bind directly to the enzyme, blocking its action, so there is no inhibition by competition (they do not bind to the active center). They have the advantage that they do not require metabolization to

becoming active (as in the case of dideoxynucleosides, which need to be phosphorized to triphosphate compounds).

As a group, they are structurally different; they have a good therapeutic score, which reflects their high specificity. They are specific for HIV -1, not exerting any activity on HIV-2 or SIV.

Several classes of compounds represent this group: benzodiazepines (dibenze, monopyrido and dipyridodiazepines), anthraquinones, phosphonoformic acid, HPA-23, suramin, flavonoids, catechin derivatives, etc.

Resistances develop quickly, in some cases one week after use. Some mutations (particularly those in positions 103 and 181 of the RT) confer a high level of resistance not only to the drug, but also to all other non-nucleoside RTis (cross resistance), which supports the idea that they act in the same site of the enzyme.

When used in conjunction with ZDV, these resistances do not appear, and when administered for viruses that are resistant to ZDV, after a while they become sensitive to zidovudine again.

Nevirapine

It has good oral bioavailability and a plasma half life of 24h (at a dose of 400 mg/d). Low toxicity and strong antiviral activity in vitro[78].

Due to the easy emergence of resistance to non-nucleoside RTis, association with other compounds has been sought, namely ZDV, IFN-α and immunoadhesins, with no record of the emergence of new toxicities or combination of toxicities. At doses higher than 400 mg/d, despite the adverse effects (rashes), resistance would take longer to appear.[21]

L- L697,661 (L661) compounds

This is a family of compounds derived from pyridones. Like other non-nucleoside RTis, it is effective in small concentrations (nanomolar, IC50 – 100 nmol/l).

It has low oral bioavailability (2-2.5%), which it can be increased by food (it increases tenfold if taken with food). It suffers the effect of the first passage in the liver. There are no data related to the passage of the haematoencephalic barrier. The dose is 600 mg/d.

Like other non-nucleosides, resistances developed in short-term monotherapy (sometimes six weeks), in particular, the mutation of position 108 (tyrosine → cytosine) of RT, which causes high resistance. It appears that these resistances do not emerge in combination therapy, and trials are currently underway L661 + ZDV (ACTG 0191 – USA and in Frankfurt).[21,80]

Loviride

A compound belonging to the α-APA group, the most recent drug of which is R 89 439, loviride.

Phase I clinical trials revealed diarrhea (13%), fatigue (12%) and alteration in appearance of the urine (12%) as the most frequent adverse effects.

Laboratory markers of disease progression revealed an improvement (increase of CD4+). At the end of three months, cultures of the virus were made (18 cultures in 65 participants), and no mutation in position 181 was observed; other less significant mutations were investigated (positions 98 and 179), but likewise were not found. The results are awaited of the phase II of trial now taking place in Belgium.[81,82]

Foscarnet

This trisodium salt of the phosphonoformic acid has an antiviral activity that covers almost all the elements of the herpes virus family, inhibiting DNA polymerase of 5 herpesviruses (HSV I and II, VZV, EBV and CMV).

Foscarnet also inhibits reverse transcriptase in a way that is non-competitive with triphosphate nucleosides, just as it does with DNA polymerases of the herpes virus. Oral bioavailability is poor (17%), not reaching through this route the minimal concentrations for antiviral effect. Penetration in the LCR is variable. It is not metabolized; it is eliminated unaltered in the urine (83% in 36 hours), part of it is sequestered in the bone (by replacement of phosphate in the bone matrix) and it is eliminated much more slowly.

The most frequent adverse effects are: nephrotoxic, hematological and metabolic. Continuous infusion, as well as previous renal pathology and nephrotoxic drugs (acyclovir, pentamidine, cotrimoxazole and cetoconazole) predispose it to higher renal toxicity. Splitting the dose (180 mg/Kg/d) into three infusions of two hours each, with previous hydration (2.5L of physiological serum/d) and adjusting the drug to Ccreat, reduces the toxicity.

The most frequent metabolic alterations are hypocalcaemia (which can be dangerously aggravated by pentamidine), because the foscarnet chelates the ionized calcium (symptoms of hypocalcaemia appear, with normal calcium values) and hyperphosphatemia,

which occurs because foscarnet replaces phosphate Recently, there have been reports of ulcers in the penis (in the glans and subprepuce) that appear to be caused by the high concentration of foscarnet in the urine, accumulating under the foreskin.

This capacity to inhibit RT appears to be associated with the advantage of an increase in life expectancy with foscarnet compared with ganciclovir in the treatment of CMV retinitis in patients with AIDS (16 weeks).

Replication of HIV is synergistically inhibited by the foscarnet-IFN-α and foscarnet-zidovudine associations.[83,84]

The second part of this article will address the TAT antagonists, the various protease inhibitors, interferons, IL-2, TNF-α modulators, combination therapy, genetic therapy and therapeutic vaccines. ■

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