Antiretroviral therapy in HIV infection (2nd part)

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TAT Antagonist

HIV DNA presents at its two ends a condensed region of repeated genes - LTR (*Long Terminal Repeat*). TAT protein (*Trans-Activator of Transcription*) is a product of the viral gene tat and acts in an area of the LTR called TAR (*TAT Responsive element*). The binding to this receptor triggers a solid replication of the virus. This protein also exerts the same action on the LTR of the JC virus, which is involved in the PML (Progressive Multifocal Leukoencephalopathy); in vitro, it promotes the proliferation of cuboid endothelial cells that are characteristic of Kaposi Sarcoma and also the reduction of the multiplication of antibody-dependent T lymphocytes (this may be related to the possibility of TAT transactivating cell expression of TNF-alpha and IL6, causing a dysfunctional production of cytokines that would change the immune system).[85]

In vitro, some of these drugs also inhibit RT (reverse transcriptase), which would enable better control of viral replication.[86]

Doubts were raised relating to the possibility of other compounds (product of other genes) taking over the role of TAT, in cases where inhibition was induced.

Ro 24-7429

It comes from a set of substances derived from benzodiazepines, presenting in vitro activity against acute and chronic infection. No resistance was observed and a synergism was observed with ZDV, ddC and protease inhibitors.

Adverse effects in rats included: CNS (somnolence), GI system (stasis and diarrhoea), nephrotoxicity, color alterations in various tissues (yellow).[87]

Due to the inefficacy shown in phase II studies (ACTG 213), higher dose (200 mg/8-8h) studies were planned, to verify its potential benefit in patients with Kaposi Sarcoma, however, due to the emergence of some unexpected adverse effects (see above), investigation of this drug was suspended because of its inefficacy in combating HIV infection. A phase I trial is currently taking place in Canada, with a new compound of this group, ALX40-4C, involving 40 volunteers.

Protease inhibitors

The product of the gag (nucleocapsid and core proteins, p17, p24, p8 and p7) and pol genes (protease, RT and endonuclease – H ribonuclease and integrase) is a single long protein (polyprotein) that has to be divided into the different structural and regulatory proteins. This reaction is carried out by an enzyme – the protease, codified by the viral genome. Thus, the protease is responsible for the regulation of a cascade of proteolytic events that lead to a mature virion, capable of infecting another cell. This separation of the various compounds of the polyprotein takes place when the viral particle is already in cell membrane protrusion.[87-98]

The theoretical concept for therapeutic use of protease inhibitors is that by impeding individualization of viral, structural and regulatory proteins, it is not possible to form a mature virus capable of infecting other cells. This hypothesis was confirmed by in vitro experiments in which it was verified that the addition of those drugs to cultures of HIV-infected cells caused the emergence of immature extracellular viruses, with no core, and incapable of infecting other cells.

Nanomolar concentrations, several times lower than the concentrations that can be obtained in the plasma, are effective.

In vitro studies also revealed the good capacity of viral replication inhibition inside macrophages, which does not occur with the majority of the RT inhibitors, with poor penetration in these cells (which act as a reservoir of the virus).

Pharmacologically, protease inhibitors can be grouped into three categories: analogue peptides of the substrate, C2 symmetrical compounds, and non-

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peptides[96] Research has focused on the production of synthetic peptides with a structure similar to protein cleavage zones (therefore, the enzyme substrate).

Various difficulties have emerged in the research of these drugs, such as difficult production, poor antiviral power in cell cultures; following phase I and II clinical trials, problems with pharmacodynamics were also observed (poor absorption, interactions with other drugs, and the emergence of resistance), as well as some unexpected adverse effects (namely, hepatic toxicity).

The emergence of resistance of protease to these compounds has been reported with greater frequency, demonstrated both in vitro and in vivo; in some cases, the existence of cross-resistance between different drugs was observed (saquinavir, L524 and ABT- 538).[99-104]

The actual significance of this type of resistance is unknown, since some mutant proteases would be less efficient than that of the "wild" virus, however, in the majority of cases this resistance involved loss of viral power, with an increase in the number of circulating copies of HIV (detection of viral RNA through PCR).

Resistance patterns common to protease inhibitor groups are described; the possible mechanisms of structural alteration include: 1– mutations in the protease active centre that interfere directly in the inhibitor binding, 2– mutations outside the active centre that interfere indirectly in the inhibitor fixation, 3- mutations that lead to a more efficient enzyme and 4- mutations in the enzyme action sites that facilitate its action.[102-104]

From the study of structural alterations of protease induced by various inhibitors, certain sites were identified where the replacement of amino acids leads to the emergence of resistance (the most cited alterations are in positions 8, 48, 82 and 84). The replacement of the amino acid in position 82 (val – ala/tir/fen) was capable of conferring resistance on all the seven inhibitors tested.[102-104] These results raise doubts regarding the eventual usefulness of protease inhibitors in monotherapy after trials have already started with a combined therapy with RT inhibitors (ACTG 229)[105] and with several of these compounds; in the latter possibility, several of the resistance patterns presented have to be taken into consideration so as to not associate drugs where there is cross-resistance (this issue already existed with reverse transcriptase inhibitors, with the cross-resistance between ddl and ddC being described). (see Combined Therapy)

Analog peptides of the substrate

The objective has been to produce analogue peptides to the enzyme substrate, but which the enzyme is not able to degrade to non-functioning substrates through occupation of the active centre.

Most of these products have high vulnerability to digestive enzymes, which causes poor oral bioavailability; they are also the target of a rapid hepatobiliary metabolism (with consequent low plasma half-life).

Of the numerous compounds studied,[96] the most important (and the first to be used in human beings) is Ro 31-8959 - saquinavir.[106]

Saquinavir (Ro 31-8959)

Cytotoxicity studies have revealed that the toxic dose is 2000 times higher than the concentration with antiviral activity, which gives a very good therapeutic index. It is the protease inhibitor that is in the most advanced phase of study, being the first to enter phase III trials (currently taking place in the US and Europe with more than 6000 volunteers). The dose tested in most clinical cases (600 mg/8-8h) showed a good toxicological profile, but its antiviral capacity was modest (reduction of about 90% of viral load), raising doubts as to whether the dose being used is below the optimal level, due to the poor bioavailability of the drug (± 4%). Higher doses are undergoing clinical trial (Thomas Merigan – Stanford University), reaching 3600 and 7200 mg/d (twice and four times the dose used to date), recording an antiviral effect similar to that of L-524 (± 98%).

Perhaps the most problematic issue relating to saquinavir is the fact that it is difficult to produce, with twenty-three synthesis steps, which makes it very expensive, besides the fact that the manufacturer does not have a large amount stored (how to carry out large scale clinical trials with higher doses?).[106-109] Numerous types of resistance to this drug are described.[110-112]

In Italy and the USA, clinical trials have already been carried out in combined therapy with ZDV, reporting synergism, evidenced by higher suppression of viral load and a more marked and sustained increase of CD4+. In the USA trial (ACTG 229), the efficacy of the triple therapy (saquinavir + ZDV + ddC) was tested, being higher than the double therapy

(saquinavir + ddC or saquinavir + ZDV). Results of the association of saquinavir in triple combination with ZDV and ddI point in the same direction.[105, 106, 113]

L-735,524 (or L-524)

This is a hydroxyethylamine isoestere. In vitro, a 99% reduction of viral RNA was verified in peripheral blood, which was transitory, since after six months there was a new increase. Resistances have already been described, which might be facilitated by a plasma concentration between administrations (4-4h) that decreases to values of IC90 (concentration of drug required to inhibit 90% of viruses).[102-104, 106, 114] In a trial with 16 subjects at a dose of 400 mg/6-6h, there was a reduction to less than one tenth of the viral load, and an increase in CD4+ of between 70-100, but at the end of 24 weeks, viral load values had raised to 70%, although the increase in CD4+ was maintained and an improvement in hematologic values (PMN, platelets, haemoglobin) and weight (average increase of 8-9 kg) were recorded. In another trial with 70 volunteers (protocol 006) with Agp24 positive and CD4+ ' 500 to 600 mg/6-6h, a decrease was recorded in viral load of between 10 to 100 times (90 to 99%), which was sustained for 12 weeks, returning to basal values at 24 weeks.[106] The increase in CD4+ was of 50-100 cells, which also decreased over time. The only adverse effect was a transitory increase in bilirubin. Strains resistant to this drug also presented resistance to other compounds of this class [102-104, 114]. New trials are planned, one comparing L-525 (800 mg/8-8h) + ZDV with ZDV in monotherapy, another comparing L-524 to the association ZDV+ddI, and a third comparing higher doses of L-524 (800, 1000 and 1200 mg/8-8h) with the association of ZDV + 3TC. Its association with ddl was also initiated.

C2 symmetric compounds

The activity of these compounds is based on the fact that the protease structure is a C2 symmetric dimer. This type of drug has a portion that is symmetrical to the active centre of the enzyme to which it strongly binds, avoiding access by the natural substrate.

The compounds that have been studied the most, and for which clinical trials are underway, are C2 symmetric Diols, in a long series: A-74704, A-77003, A-76928, A-75925, all with poor oral bioavailability, a problem that has been overcome in more recent molecules, A-80164, A-80987. The most recent member of this family of compounds is A-84538 (ABT-538).

Other compounds of this class are C2 symmetrical phosphinic acid inhibitors, the dihydroxypropylamine diols, and the penicillin-derived C2 symmetric dimers.[96]

ABT-538 (A-84538)

According to the available data, this is the most potent protease inhibitor synthesized until now. Initially presented in October 1994 at the ICAAC (Interscience Conference on Antimicrobial Agents and Chemotherapy), and later in November, in Glasgow (International Congress on Drug Therapy in HIV Infection), it seems to have an impressive antiretroviral effect (reduction of viral load by 99.1% - a reduction of 113 times) and good oral bioavailability; it is unstable, requiring permanent refrigeration in its current form of oral suspension [115]. Of the phase II trials concluded in the studied doses (600 → 1200 mg/d), the adverse effects recorded were diarrhoea (75% of volunteers) and an increase in transaminase in three cases, although these had a previous history of viral hepatitis. The initial value of CD4+ lymphocytes varied between 91 and 173, with an average increase of 300%, which was more pronounced in higher doses, with increases of 300-400 CD4+ lymphocytes and 99.8% reduction in viral load (decreasing 560 times compared to the basal value) at 12 weeks.

Resistance emerged, which seemed to be caused by mutations in the same positions of protease that cause resistance to L-524, but different from the positions of saquinavir.[102-104]

Non-peptide protease inhibitors

Some of these compounds have been discovered through searches of databases that contain thousands of molecules. Small non-peptide molecules have been found, such as haloperidol, but the antiviral concentrations of the latter are 1000 times higher than its antipsychotic concentration.

New molecules under study are XM-323, which belongs to a new class of protease inhibitors called cyclic urea, and compounds U-96988 and U-103017.[96,116]

Integrase and alpha-glucosidase inhibitors

Integrase is another viral enzyme, codified by the pol gene, and which is indispensable for the insertion of the provirus into the cell genome; consequently, the

inactivation of this enzyme would make effective cell infection impossible.[117]

The structure of the enzyme and its catalytic centre have already been determined and present similarities with H RNAase and mutransposase cell enzymes.

Three classes of compounds with anti-integrase activity have been identified: DNA fixing molecules, polyhydroxylated aromatic compounds and various nucleotides (namely, the oxidized derivative of ATP, 2', 3' –dialdeid-ATP). Although this enzyme, unlike others, exists in minimal intracellular amounts, and it is therefore more easily neutralized, some obstacles remain: the integrase is only required to act during a brief period of the viral cycle, and since it is a viral enzyme, mutations can emerge and resistances created.

Alpha-glucosidases are cell enzymes that after translation from viral RNAm to proteins, proceed to glycolisation (the addition of carbon hydrate molecules to the protein structure), which is essential for the functioning of many proteins. By inhibiting its activity, it is possible to impede the formation of mature viruses.

SC-48334 (N-Butyl-Deoxynojirimycin), an alpha-glucosidase I inhibitor, currently in a phase I clinical trial, has been well tolerated, recording improvement of laboratory markers of progression (lymphocytes CD4+ and Agp24).[118]

IMMUNOMODULATING THERAPY

Interferons

These are glycoproteins discovered in 1957, which are capable of inducing non-specific resistance to viral infections. The emergence of recombinant DNA technology enabled its large scale-production.[119]

The common stimulus for cell production of interferons includes: viruses, bacteria, double-stranded RNA (double chain) and protein mediators, such as TNF, Interleukin-2 and growth factors.

First, they were classified by cell of origin, as leukocytes, fibroblasts and lymphocytes or immune cells, then the names IFN-alpha, IFN-beta and IFN-gamma emerged. The most recent classification groups the interferons according to the receptors with which they have affinity, thus, alpha and beta belong to type I (receptor codified by the gene located on chromosome 21) and gamma interferons to type II (receptor codified by the gene located on chromosome 6).

The most potent stimulus for interferon production is the introduction to the double chain RNA cell (which may or may not correspond to a virus). Generally, the stimulus is viral, but bacteria, protozoa and tumor cells are capable of the same induction. One of these stimuli activates a protein kinase that is the direct inductor through de-repression of the IFN genes.

Once the IFN is produced, it does not act intracellularly but is conducted, inside the vesicles, to the extracellular medium, where binds with the membrane receptors of the target cell. After this interaction is carried out, the IFN-R complex is internalized and conducted to the cell nucleus, where it exerts its effect on specific sites of action: transcription initiation codon, translation initiation codons (RNAt) and cleavage sites [120-122].

Briefly: IFN → R of cell surface → intracellular sign → specific proteins → proteins regulated by IFN → 1– induction of antiviral state, 2– cell growth inhibition, 3- immunomodulation and 4- regulation of oncogene expression.[120]

Induction of regulating proteins

Three of these regulating proteins induced by IFNs are described: Mx (produced by type 1 IFNs, interferes in cell exocytosis, leading to the disruption of virus production and assembly – confers resistance on the influenza virus), 2',5'-oligoadenylate synthetase (an enzyme pathway induced during infections of RNA virus, made up by three enzymes that cause viral RNA degradation; selectively protects cells from infections by picornaviruses) and Pl/elf-2 protein kinase (induced by type I IFN in the presence of single or double chain RNA virus, causes the inhibition of protein synthesis through phosphorylation of elf-2, a factor of initiation of protein synthesis – various viruses were able to cross this barrier, namely adenoviruses and HIV).[120]

Induction of antigens of the major histocompatibility complex (MHC)

These are surface glycoproteins involved in the presentation of the antigen to the T cells. The IFNs can increase the concentration of MHC I, and the IFN gamma can also increase the concentration of MHC II. Thus, the cell response against the virus can be improved through an increase in probability of viral antigen recognition by the T lymphocytes.[120]

Immunomodulating actions of IFN

IFN-gamma stimulates the cytotoxic activity of the T and NK lymphocytes, maturation of the B lymphocytes and macrophages, and the production of IL-1 by the latter. IL-1, in turn, activates the growth and differentiation of B and T lymphocytes, participates in the production of inflammation mediators, stimulates the movement of neutrophils to the peripheral compartment, as well as its activation, and induces febrile response.[120]

Interferon-alpha

IFN-alpha encompasses 22 subtypes, being produced directly in the blood circulation by various types of activated leukocytes; in response to viral and mitogenic stimuli, its production, located on chromosome 9, depends on a family of approximately 24 genes, of which apparently 9 are pseudogenes and only the other 15 are functioning. Its genetic code does not have introns, which may be important for its expression during viral infection because intron splicing mechanisms, which could be impaired in the infected cells, are not required.[119-121]

It is resistant to a pH 2 and unstable at 56ºC.

Antiviral effects of IFN-alpha (as well as on Kaposi Sarcoma) only occur in patients who have a relatively healthy immune system (CD4+ > 400) and only after long therapy with high doses.[122, 123]

In cell cultures chronically infected by HIV, the use of IFN-alpha resulted in the suppression of production (assembly and release) of complete viral particles. Electronic microscopy showed that in these cells treated with interferon, pre-formed viral particles accumulated next to the cell membrane (however, there is a rebound effect, in which, if interferon administration is interrupted, all these accumulated particles will be released to the exterior).[122-125]

In individuals with HIV infection, an increase in the production of endogenous interferon has been recorded (dependent on the increase of TNF-alpha?), which would lead to a reduction in membrane receptors for IFN-alpha in mononuclear cells of peripheral blood, partially justifying the worse response to exogenous interferon in patients with advanced HIV (described as endogenous resistance to IFN-alpha).[125-127]

The doses used varied from 1 to 15 MUI/sc/day or three times a week. The clinical effects in some trials showed a reduction of opportunistic infections in advanced stages of the disease[127] and reduction of viral load, as well as some laboratory markers of progression (AgP24 and CD4+).[128,129]

The most frequent adverse effects are flu syndrome (fever, shivers, fatigue, anorexia), which appear in almost all patients treated with the highest doses. This type of effects can be improved with administration of interferon with paracetamol or NSAIDs at bed time. Other systems affected are the gastrointestinal system (nausea, vomiting, diarrhoea, hepatitis), the central nervous system (drowsiness, confusion, anxiety and depression), hematopoietic system (cytopenia, namely neutropenia), and cardio-vascular system (rare, but potentially dangerous; previous disease and the use of cytostatics increase the risk: arrhythmia, ischemia, AMI and cardiomyopathies). In monkeys, IFN-alpha is abortive.[129]

Since it acts in the late phase of the viral replication cycle, it is a good candidate for combined therapy with RT inhibitors (normally used with ZDV).

Some authors expressed some reservations with regard to the use of low doses of IFN-alpha in association with ZDV, because besides possibly being ineffective, it could be associated with increased risk of neurotoxicity.[130]

The oral formulation of IFN-alpha was tested in clinical trials, but the lack of bioavailability impaired this form of therapy.[131]

Interferon-beta

IFN-beta encompasses about 5 subtypes (it varies depending on authors) and is produced by fibroblasts of the conjunctive tissue as a response to viral stimuli or synthetic polynucleotides; it is dependent on genes located on chromosomes 2, 5, 9 and occasionally 12. It is pharmacodynamically identical to IFN-alpha, but it presents a much lower diffusion capacity, which means it reaches high local concentrations, providing good antiviral resistance to adjacent cells.[119, 120]

Interferon-gamma

IFN-gamma does not present any subtype. It interacts with its own receptor and is produced by activated T lymphocytes T and by NK cells, in response to antigens and mitogens; generally, its production is triggered only after the action of specific components of the immune system. It has an antiviral activity 500 times lower than type I interferons, but on the other hand, it has more marked cytolytic immunomodu-

lating activity.

Like the other IFNs, it induces IL-1 and TNF syntheses, but presents synergism with TNF-alpha in the potentiation of the expression of type II MHC molecules, and with TNF-beta in cell growth inhibition. IFN-gamma also acts on TH2 lymphocytes, inducing the production of IL-4 and IL-10.[119,120]

At macrophage level, it increases its microbicide power and MHC II molecule expression.

In the B cells, it conditions not only quantitative alterations, but also qualitative ones in the class of antibodies produced.

It will be submitted to a trial on the therapy of some opportunistic infections (leishmaniasis and toxoplasmosis) and to another trial on the potential uses in the treatment of mycobacteriosis, namely infections by MAC (Mycobacterium avium-intracellulare Complex).[119,120,131]

Interleukin 2

IL-2 in vitro stimulates the proliferation of CD4+ lymphocytes and activates CD8+ suppressing cytotoxic cells, which play an important role in the control of HIV replication inside CD4+ lymphocytes.

This recombinant molecule presents a short plasma half-life and has been associated with that of polyethylene glycol (PEG), resulting in a significantly increased half-life.

In a study using this association, there was not only an improvement in laboratory markers of progression, but also an increase of the specific activity for HIV of cytotoxic T lymphocytes.

In higher doses, a marked toxicity emerged: fever, diarrhoea, skin rashes, neutropenia and thrombocytopenia and occasional cases of capillary leak syndrome; in trials with lower doses (36000 U/ twice a week) there was a marked reduction in toxicity and a significant increase in NK and activated lymphocyte activities.[133,134]

The most recent study published refers to a trial carried out at the NIAID (National Institute for Allergy and Infectious Diseases), in which IL-2 was administered in periodical cycles of continuous infusion (continuous endovenous infusion for five days, every eight weeks). The preliminary results appear to be favorable for volunteers with CD4+ > 200 cel/microl, with a significant increase in these cells (three cases described in which levels have been kept above 1000 for more than three years), which, however, was not seen in patients with more advanced infection.[135]

Doubt remains as to the role of IL-2 in HIV replication, since its capacity to potentiate it has been already shown and because it stimulates replication of CD4+ lymphocytes and activates them, increasing the number of cells that can be infected. In individuals with more than 200 CD4+ cells/microl, there was no global increase in viral load, a measurement of viral RNA by PCR, which did not occur for individuals with more advanced infection (CD4+ <200), in whom an increase in the number of HIV copies in the blood was observed. One of the possible explanations is that in the advanced stage, the infection significantly reduces the number of CD8+ cells capable of suppressing HIV replication (inside CD4+ lymphocytes) and, therefore, the administration of IL-2 would not significantly increase the number and activity of those cells, and the most pronounced effect would be the increase of viral replication; in the earlier stages, since there are still a lot of CD8+ lymphocytes, these would be stimulated and activated, and would multiply, controlling any increase in viral replication.[136]

In any case, the increase in the number of CD4+ lymphocytes has not been shown to be clinically relevant, because these new cells may by dysfunctional, since it has been verified that volunteers submitted to IL-2 treatment developed opportunistic diseases for unexpectedly high CD4+ cell values (pneumocystosis with CD4+ >400/ml).[135]

It is also not known whether the increase of CD4+ lymphocytes detected in the peripheral blood corresponds to a higher production, or merely to its movement to lymphoid organs where normally more than 95% of these cells are found.

These results were obtained with antiretroviral therapy (ZDV), and it is believed that a better response can be achieved with the use of more potent drugs. A trial is planned that associates IL-2 with the L-524 protease inhibitor, since the latter has a very strong early retroviral effect (reduction of viral load can be as high as 99%).[135]

TNF-alpha modulators

The Tumoral Necrosis Factor-alpha (TNF-alpha), besides increasing production of HIV, was also associated with: The cachexia associated with AIDS (emaciation syndrome), the hypertriglyceridemia that patients often present (inhibits hepatic LPL reducing clearance of chylomicrons and VLDL), the demye-

lination of the CNS and for stimulating astrocyte production, it may contribute to reactive glucose of the AIDS Dementia Complex (ADC).[137]

Various compounds inhibit the production of TNF-alpha. Two of the most commonly used and tested are pentoxyphiline and thalidomide.[138] These compounds have no direct effect on the HIV, and their use has not been associated with an improvement of laboratorial markers of progression (it is possible that thalidomide improves cell immunity), but rather with the symptoms of individuals in advanced stage of infection (in particular, weight increase). Other compounds of this group are L-carnitine, Beta-carotenes and BRL 61063.[18,19,139-141]

Gene therapy

In broad terms, the basic Idea of gene therapy is to introduce genes that express proteins capable of: replacing other proteins in deficit, inhibiting anomalous cell functioning or regulating the expression of other genes.[142]

Given that virus-related diseases (particularly slow virulent ones) are acquired genetic diseases, their pathogenicity could be reduced or eliminated if the proteins that produce or impede their replication could be inactivated.

Various techniques have been studied, including, among others: direct DNA injection into the cells, self bone marrow transplants with posterior injection of modified cytotoxic T lymphocytes with specific reactivity against HIV; CD4 genes or genes of transdominant proteins (they would work in the same way as CD4sr); introduction of env/rev genes through retroviral vectors in autologous fibroblasts (that then would produce large amounts of proteins of the involucrum); sensitization of cytotoxic T lymphocytes to destruct HIV infected cells (introduce genes of the extra-membrane portion of CD4, to enable the protein to be expressed by these lymphocytes, which in turn would be able to recognize surface gp120 cells and destruct them) and alterations of CD4 to intracellularly capture gp 120 [21,72,142]. RNA decoys are RNA molecules that are intracellularly expressed, with the role of binding to viral RNA and thereby impeding its expression. Two of the targets are RRE (REV responsive element) and TAR (TAT responsive element). One concern is that these elements can fasten to cell factors and then cause toxicity.[142]

Antisense RNA (or antisense oligonucleotides) the objective is to impede the gene expression. Antisense RNA (complementary RNA) is nothing more than a RNA chain complementary to a certain RNAm chain, and that can hybridize with it, there avoiding the gene expression that that RNAm chain transports (shown in vitro by the inhibition of certain cell genes after the introduction to the cells of this type of RNA).[143] Similarly, the presence of antisense RNA was verified for the transcript of herpes simplex virus, in the trigeminal ganglion with latent infection. This technique was also used in the attempt to inhibit the expression of viral genes (HIV, adenovirus, Rous Sarcoma virus and HTLV-I), but with only partial success, since total inhibition was not possible.

In fact, the larger the Antisense RNA molecule used, the higher the likelihood of cross-inhibition will be (i.e., inhibiting the viral expression of different HIV strains), but also, the larger the RNA molecule, the more difficult its entry to the cell; ideally, then, the best thing would be to produce an in loco antisense RNA, which could be achieved by using a retrovirus as a vector.[144,145]

Ribozymes are RNA molecules with catalytic functions for the RNA, being able to recognize and cleave various viral genes, therefore inactivating them.

Transdominant Mutant Proteins are altered recombinant proteins of HIV, that would bind to the same receptors, but would not activate them (inhibition through competition). The most advanced examples are transdominant proteins of REV (RevM10) and TAT of HIV-2 (R81-84A).[146]

Intracellular toxins (see also Agents That Act in the Extracellular Phase) involve combining molecules that are capable of recognizing viral products and, therefore, infected cells, with toxic compounds. Another approach is the insertion of genes codifying harmful molecules for the cell.[142]

Gene Transductor Systems are based on the use of retroviral vectors to which antiviral genes have been inserted that would block the action of viral products or could trigger the suicide of infected cells.[142]

Ex-Vivo Expansion of Lymphocytes: expansion of CD8+ cells, the separation of T lymphocytes is carried out by lymphapheresis in the early phases of the infection, stimulating them in vitro to produce cytotoxic activity against viral components and reinfusing them in a later phase; expansion of CD4+ cells, after in vitro manipulation they could become resistant to HIV infection and readministered to the patient.[147,148]

Therapeutic vaccines

Their objective is to produce an immune response in seropositive patients, in order to achieve complete eradication, or failing that, at least alter the unfavorable course of the disease.[149-158]

Sexual transmission is the main infection route, so for the vaccine to be effective, it has to ensure endovenous and mucosal protection, and enable the destruction of both free viruses and viruses inside infected cells.

Although stimulation of cell immunity is essential for the effectiveness of a vaccine, it must be taken into account that the effector T cells can be harmful, as shown by their increased activity in bronchoalveolar lavage, lymphocyte alveolitis and LCR of individuals with neurological alterations. However, the level of cytotoxicity of T lymphocytes is important because it continues to be the response presented by individuals who have had contact with the virus, but who were not infected. Neutralizing antibodies appear to be important in the reduction of vertical transmission, but cytotoxicity seems to emerge earlier. Therapeutic vaccines can stimulate the immune response of seropositive individuals, but its clinical significance is still unknown.[152,153,159] The models used to synthesize the vaccines are divided into three types:[150]

1 – in 1986, Zagury inoculated seropositive individuals with autologous cells killed in paraformaldehyde. With surface inactivated virus, there was no clinical response, but when immunomodulating therapy was associated with IFN-alpha, cell immunity returned, revealed by the restoration of the delayed hypersensitivity, proliferation of activated T cells and cytotoxic T activity. There was also stabilization of CD4+ lymphocytes. Apparently, it would be useful for patients with advanced immunodeficiency.

2 – in 1987, Salk built a vaccine from a dead virus with no involucrum, with incomplete Freund´s adjuvant. Volunteers had advanced HIV infection and the Zaire viral strain was used. There was no alteration in anti-HIV antibody titers (anti-p24, anti-RT), but there was recovery of delayed-type hypersensitivity (reversion of skin anergy) in some patients, who had reduction of incidence of opportunistic infections.[160]

3 – due to their importance in the control of other viral infections, and its scarcity in HIV infection, several authors have begun investigations to produce vaccines using proteins from the involucrum (gp160, gp120, gp41). Redfield, in 1989, started trials with recombinant gp160, obtaining evidence immunogenicity in 93% of vaccinated patients (with best responses among volunteers with CD4+ > 400/ml); later, other authors started its association with antiretroviral therapy, but with no apparent improvement of immunogenicity. The therapeutic vaccine with which most experience has been acquired is gp160, since it was well-tolerated in phase I trials (2 years of follow-up), with or without ZDV, with stabilization of CD4+ lymphocytes (for patients with low impairment of the immune system – CD4+ > 400 celmicrol).[159,161-163] There is also reference to induction of cytotoxic response restricted to MHC types I (CD8+) and II (CD4+). The association of this vaccine with another of proteins of the involucrum would induce memory cells specific to proteins of the involucrum. Not all the authors agreed with these findings.

4 – more recently, anti-idiotype vaccines have emerged, such as IOT4, derived from an antibody with affinity for the CD4 receptor; antibodies against IOT4 produced by immunization have cross-reactivity with HIV gp120, enabling neutralization in vitro. In phase II studies, there was a re-emergence of the delayed-type hypersensitivity and an increase in number of CD4+ cells of between 15% and 120%.[164,165]

5 – the most recent type of intervention in this field has been made with retroviral vectors into which HIV proteins were inserted (core – p17; rev). Some AIDS patients were auto-transfused with cells containing recombinant vectors of the vaccinia virus, containing env, pol and gag genes, leading to a reduction in the number of opportunistic infections. The use of vectors enabled the introduction of large quantities of immunogens into the host. Some potential virus vectors of note are [96,150]: viruses: poxvirus (vaccinia and avipoxvirus), adenovirus[4,7] (enable oral administration, possibility of induction of mucosal immunity), chimeras of poliovirus, retrovirus, attenuated HIV (with multiple deletions, and therefore without pathogenic power); bacteria: BCG, attenuated salmonella and E. coli; non-replicating: ag HBs and HBc of HBV; virus-like compounds: Ty particles.[160,166,167]

The efficacy of a vaccine depends not only on the type of immunogene used (recombinant proteins of involucrum or core, anti-idiotype, or recombinant virus), but also on the dose used, state of the host infection, and frequency of administration (for example, gp160 did not show immunogenicity in

three administrations, but did with six). Other important points are: the virus strain used (LAI strain is produced in laboratory only, while MN and SF2 strains are predominant in infected patients in Europe and the USA. It is not known whether there is cross-reactivity among strains; the expression system (cells of insects or mammals and fungi) and adjuvants (aluminum compounds are more potent than isolated aluminum).

COMBINED THERAPY

Converging therapy

Given that no RT inhibitor totally suppresses viral replication, and that at the end of a variable period of time there is no longer clinical efficacy, due to the emergence of resistant strains, the purpose of the association of compounds is to impede or delay this phenomenon. The use of drugs with different adverse effects enables the risk of severe toxicity to be reduced.[168-178]

Converging combined therapy presupposes the use of various drugs with action on the same point of the viral replication cycle and can be subdivided into simultaneous (two inhibitors at the same time) or alternated (alternating cycles of different inhibitors), the latter being less effective.[21,72,179,180]

The association most frequently used is that of two nucleoside analog RT inhibitors (ZDV + ddI, ZDV + ddC...). the objective is to maximize the specific characteristics of each compound: for example, ZDV better penetrates the cells with faster division (lymphocytes), while ddI better penetrates the cells with lower division potential (monocytes-macrophages). Antagonisms in these combinations are described, the most important ones being ZDV with d4T (stavudine), which compete for the same route to be activated, and ddI and ddC, which have similar adverse effects (with a higher risk of pancreatitis and neuropathy).[181-188]

The emergence of liposomic forms (ddI and ddC) enables a longer half-life, better cell penetration and lower toxicity of these drugs.[189,190]

Various clinical assays have shown that the association of two or more RT inhibitors enables a sustained reduction of laboratory markers of disease progression. The results of a trial in a sample of the MACS (Multicenter AIDS Cohort Study) showed a 34% decrease in the risk of death in patients who used combined therapy compared to those who stayed with monotherapy.[181]

Another option is the association of one of the nucleoside analogs (generally zidovudine) with a non-nucleoside (neveripine, L compounds, atevirdine, delavirdine, loviride, foscarnet...), since a reduction is observed in the emergence of resistance to the latter.[191-193]

With widespread media diffusion is the association of ZDV with lamivudine (3TC), a cytosine analogue nucleoside that leads to the rapid development of resistance through mutation in the 184 position of the pol gene, which leads to the replacement of the aminoacid in the same position of RT; this mutation is subtractive with the mutation in position 215 of the RT for the ZDV.[194-196] Faced with a mutation in position 184, a virus that had acquired a mutation that gives high resistance to ZDV, in position 215, becomes sensitive to the latter. This result was first shown in vitro, which caused some skepticism, since the same had occurred before for didanosine (ddI), in which the emergence of a mutation in position 74 provided resistance to ddl, made the virus mutant in the position 215 sensitive to ZDV, but when in vivo this "protection" was not very clear, although the association of two drugs appears to be beneficial in terms of markers of progression. In Glasgow (November 1994), the results of two European studies on the association of ZDV + 3TC in patients with and without previous exposure to ZDV were presented. In relation to viral load and increase of CD4+ lymphocytes, there was a marked advantage in the association after 48 weeks, compared with ZDV in monotherapy. However, due to the small number of volunteers in whom the viral load was analyzed (20%), the results are somewhat questionable. At the National Conference on Human Retroviruses and Related Infections, in Washington, in February 1995, new data were presented about this combination, confirming previous results in subjects with no previous ZDV treatment and less advanced infection (with 90% reduction of viral load and an increase in CD4+ of around 50 cells at 24 weeks). With regard to the results obtained for patients with previous ZDV treatment and with more advanced infection, these were not coincident, and were similar to those of the ZDV + ddC association.

With the profusion of protease inhibitors, it is expected that there will soon be a converging combined therapy with two or more drugs of this class; for this,

the pattern of protease resistance to these compounds has been studied, in order to associate drugs with different profiles of resistance (suggesting a need of more mutations in the protease, for resistance to occur). Since some of these new compounds can have a highly marked antiviral power (reducing the viral load by between 98-99%), by reducing the number of viruses in replication, they reduce the possibility of the emergence of new resistances.[102-104]

Diverging therapy

This therapy seeks to simultaneously inactivate various stages of the viral lifecycle. To achieve this, it uses drugs that act in different stages of the HIV replication cycle.[197]

Currently, the most frequently used ones use a RT inhibitor with IFN-alpha, or acyclovir [198-201]. Phase I/II clinical trials are currently underway for various other therapies, in which ZDV is associated with protease inhibitors (saquinavir), thymopentin, thymostimulin, pentoxifylline, gp160 and CD4-Pe40 (immunotoxin).[72,202-209] The knowledge of the existence of cofactors that might be important in viral replication has been important in the use of some drugs, such as acyclovir. Various organisms have been involved in the activation of HIV in a direct or indirect way, by cytokines (IL1, IL3, IL4, IL6, TNF alpha, TNF beta) or transactivators (NF kB).[18,19,210] The viruses of the herpes group (EBV, HSV, CMV), other HTLV-I retroviruses and Mycoplasma fermentans have this potential.

The role of HHV6 (Human herpes virus type 6) appears to be increasingly important, increasing the CD4 receptor membrane expression in various cells, and by infecting CD8+ lymphocytes, it leads to derepression of the CD4 receptor gene, which is then expressed at the surface of these cells These lymphocytes are then placed at risk of infection by HIV.[210]

CMV is another cofactor that has long been emphasized in the evolution of HIV infection; and several studies with hemophiliacs have shown that those who did not present antibodies against CMV at the moment of seroconversion into HIV had a longer survival time than those who had IgG to CMV during seroconversion. In vitro it was verified that the cells infected by CMV express the receptor for the Fc portion of IgG in the membrane, a doorway for the entry of HIV.

The increased survival time in patients with ZDV + acyclovir combined therapy has been the target of diverging opinions. The most recent study published, while the results of ACTG 063 and 204 are awaited, relating to the use of high doses of acyclovir (3200 mg/d) with zidovudine, suggests a reduction in mortality of between 26-34%. The data are from MACS (Multicentre AIDS Cohort Study) and the authors discuss the efficacy of acyclovir only in patients with advanced immunosuppression; almost the entire sample presented positive serology to the herpes simplex virus, and its efficacy in non-infected populations with this virus is not known.[210,211]

One well known drug that has recently been transposed to the field of antiretroviral therapy is hydroxyurea. This compound acts as a capturer of free-radicals and also inhibits the ribonucleotide diphosphate reductase enzyme, which allows the passage of ribonucleotides and deoxyribonucleotides, the units that form DNA. By diminishing the availability of deoxyribonucleotides, hydroxyurea increases the availability of dideoxyribonucleosides (ZDV, ddI, ddC and similar). In France (Centre Leon Bernard, Lyon), Malley et al [213] showed a high synergism in combined therapy of hydroxyurea and ddI (which is related to the marked reduction of intracellular dATP intracellular, the physiological nucleotide that competes with ddATP, the active form of ddI). Gallo´s team [214] demonstrated a replication suppression higher than 99% (even achieving complete inhibition, > 99.9%) in infected mononuclear cells of peripheral blood. The effective dose of drug in macrophages is lower to that required in lymphocytes. Given its good efficacy in macrophages, together with good penetration of hematoencephalic barrier, the combination of hydroxyurea + ddI is a good therapy for investigation in neurological diseases linked to HIV. A phase II trial is currently underway in France, with doses of between 500-1000 mg/12-12h + ddI 200 mg/12-12h. The immediate advantages of hydroxyurea are its clinical use for about thirty years, providing a good knowledge of its toxicity (myelotoxicity, rarely severe; it is not immunosuppressing); it has low cost, and inhibits a cell enzyme, thus the habitual resistance to viral mutation will not be a problem.

Various clinical trials are currently testing the potential benefits that the addition of different drugs (sometimes in lower doses than in monotherapy) can bring, not only in terms of efficacy, but also in toxicity reduction.[215-223]

Conclusion

Since the onset of the syndrome, the quality of life and survival of subjects infected by HIV have improved, if not by the use of retroviral compounds, then at least by the institution of prophylaxis. In this field, the emergence of new compounds (penciclovir, famciclovir and atovaquone), as well as oral formulations of ganciclovir and foscarnet, will enable a better quality of life and less physical dependence on the healthcare institutions.

I close with an extract of the address given by A. Pinching in Milan (Fourth European Conference on Clinical Aspects and Treatment of HIV Infection – March 1994), in the opening session on antiretroviral therapy: "...we have more and new compounds and potential combinations, increasingly relevant markers, and, above all, a common experience on how to move forward in the painfully slow process of discovering how to make better. The first years provided us with real progress. The relatively modest dimension of this progress should not be seen as failure, but only as a reflection the magnitude of the problem we face and the limitations of the therapeutic agents we currently have. Realism and mutual feeling on how to benefit from the acquired experience will enable a harvest of huge compensations to the individuals affected by this devastating challenge". ■

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