Approaches to Combat HIV : A Review

Swati Gupta & Pallavi Sharma
T.Y. B Pharm

Recently, HIV has emerged as a major world wide health problem involving a persistent viral infection with a long incubation period terminating in AIDS. It is estimated that more than 12 million people are infected with this virus. HIV wreaks havoc on the body's immune system which slowly declines. Once established, the immuno deficiency state is generally progressive and leads to death. The hope is that this progression can be handled and even reversed by antiviral chemotherapy which is indeed most challenging, requiring penetration of the blood-brain barrier and prolonged treatment with a relatively non-toxic agent.

Morphology of HIV

HIV belongs to a family of human retroviruses. Retroviruses are unique RNA viruses characterised by the transcription of their single stranded RNA into double stranded DNA of the host cell by the viral enzyme. Due to integration of viral DNA into host cellular DNA present, an unusual type of RNA viral infection results. HIV-1 virus is spherical in shape (Fig. 1). It contains an electron dense core surrounded by a lipid bilayer. The virus

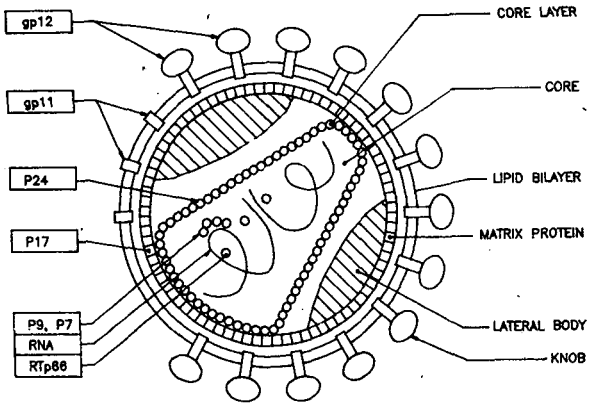


Fig.1 : HIV-Virus

core contains several core proteins, two strands of genomic RNA and the enzyme *reverse transcriptase.* Studding the viral enevelop are two viral glycoproteins gp 120 and gp 41. gp 120 projects outwards and is important for the attachment of the virus to its target cells. The proviral genome contains *gag, pol* and *env* genes that code for the core proteins, *reverse transcriptase* and enevelope proteins respectively (Fig.2), In addition, there are *vif, tat III, rev, nef, vpr* whose functions may affect its pathogenicity. Thus, it would seem that the products of these genes may be suitable targets for potenital anti-HIV drugs.

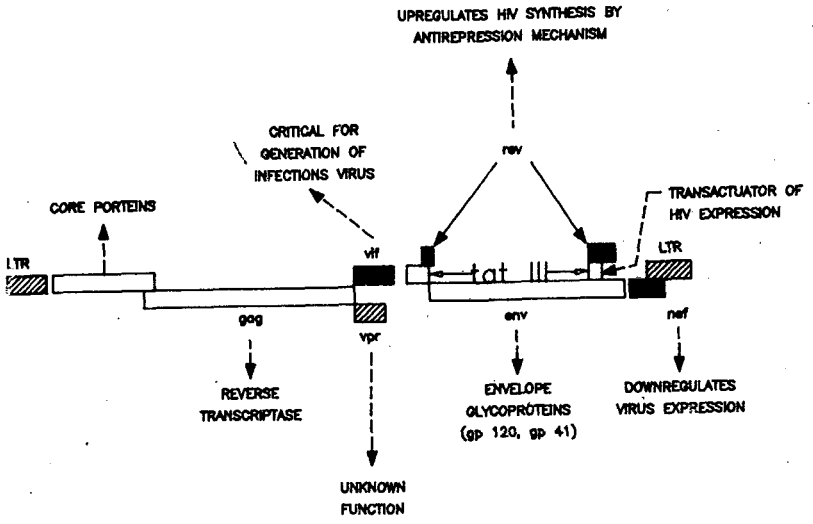


Fig.2 : Genome of HIV

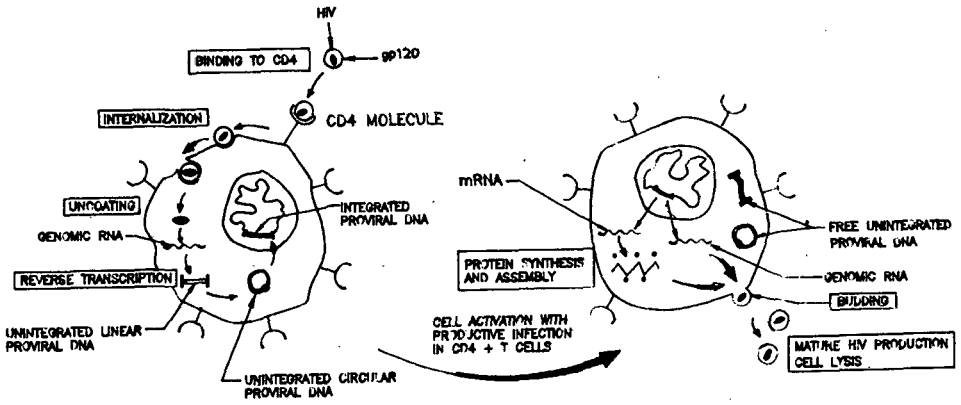


Fig. 3 : The life cycle of HIV

Strategies for Antiviral Therapy in AIDS

The life cycle of HIV is presented in Fig.3.[1] This is useful in understanding the strategies for antiviral therapy in AIDS. The retrovirus that causes AIDS has revealed enough of its life history for a variety of therapeutic strategies to be apparent. Some of these are suitable for immediate application in clinical trials or have already yielded positive results in some patients. Stages in the replicative cycle of a pathogenic human retrovirus which may be targets for therapeutic interventions are summarised in Table-1.

Table 1. The stages in the replicative cycle of a pathogenic human retrovirus which may be targets for therapeutic intervention[2,3]

	Stage	Potential Intervention
1.	Virus adsorption	Polyanionic substances, e.g.: Polyacrylic acid, dextran sulphate.
2.	Binding to target cell	Antibodies to the virus. Blocking or modification of CD4 receptor.
3.	Early entry into a target cell	Drugs that block fusion or interfere with retroviral uncoating.
4.	Transcription of RNA to DNA by	Reverse transcriptase inhibitors. reverse transcriptase e.g.: Suramin, Evans Blue, Aurintri Carboxilic acid.
5.	Degradation of viral RNA in an RNA-DNA hybrid	Inhibitors of RNase H activity.
6.	Integration of DNA in to host genome	Drugs which inhibits *pol* gene mediated integrase function
7.	Expression of viral genes	Antisense constructs inhibitors of *tat-III* Protein *arts/trs* protein
8.	Viral component production and assembly	Myristylation, glycosylation and pretease inhibitors, e.g. : invirase, crixivan or modifiers.
9.	Budding of virus	Interferons.

Establishment of Infection via Immune System

Throughout the course of HIV infection, numbers of a key immune cell- the CD4T+ slowly decline. CD4T+ are helper or inducer cells which provide help in the generation of cytotoxic T cells and antibody secreting B cells. CD8T+ are cells which dampen the immune responses.

It is suggested that the loss of T-cells by the body during the infection is compensated by bringing the overall T-cell count upto normal.. But our body fails to distinguish between two types of T-cells - CD4 and CD8 - and makes copies of both.

HIV infects and kills CD4 cells, leaving CD8 cells intact. If the body normalises the T-cell count by making both types of T-cells the result will be a skewed ratio (too many CD8 and too few CD4 cells). It is predicted that removing CD8 cells should turn the CD4 to CD8 ratio back in favour of CD4 cells. The normal CD4 to CD8 ratio is approximately 2.

Studies have already shown that large amounts of HIV are hidden away and replicating in the lymph nodes. T-cells are constantly passing in and out of the lymph nodes and come into direct contact with the virus there[4] . This substantially adds to the depletion of cells.

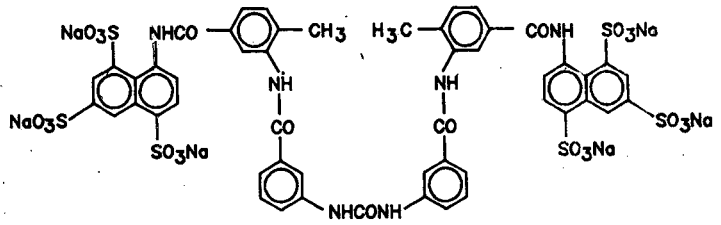
Therapeutic Approaches to AIDS

Therapeutic approaches to AIDS can be divided into conventional and nonconventional methods. These are described below.

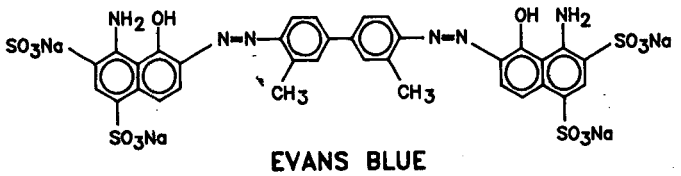
Conventional Methods

Drugs : Problems in designing Antiviral drugs:

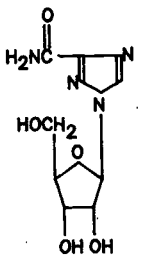
Why do researchers find it difficult to synthesise antiviral drugs rather than drugs effective against other micro-organisms ? The reason for this is that, in contrast to other micro-organisms, the viral replication is bound to affect the metabolic processes of the invaded cell. Thus, any anti-viral agent would also cause some changes in the host cell which may be lethal. Therefore, the search for drugs that would exclusively and selectively inhibit a viral process, such as attachment, uncoating, replication, or virus-directed macromolecular synthesis, etc., continues.



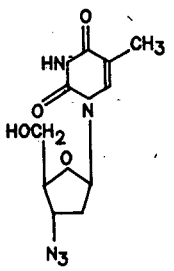
SURAMIN SODIUM



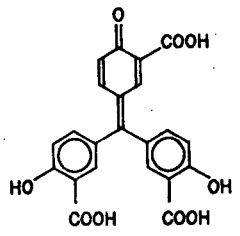
EVANS BLUE



RIBAVIRIN



ZIDOVUDINE



AURINI TRICARBOXYLIC ACID

Fig. 4

Dideoxy nucleosides: A potential antiviral target in the virus life cycle is the reverse transcription of the genomic RNA. These could be affected by inhibitors which are specific for reverse transcription. A number of compounds have been tried and the most promising appear to be derivatives of 2'-deoxyribonucleotide triphosphate. Reverse transcriptase inhibitors are nucleoside analogues which mimic the building blocks of DNA, thereby, inhibiting *reverse transcriptase* from copying HIV RNA into DNA, an essential step for initial infection of a cell. The inhibitory effect of 2', 3'- dideoxy nucleoside analogues is exerted only if they are phosphorylated. They are phosphorylated *in vivo* by cellular enzymes. Analogues, such as dideoxycytidine have severe side effects, such as peripheral neuropathy. Dideoxyinosine had been approved by FDA in 1991 for the treatment of AIDS.

AZT:- 3'-Azido-2',3'- dideoxy thymidine (Zidovudine) : This drug decreases mortality, reduces the frequency of opportunistic infections and transiently increases T4 lymphocyte counts. Less toxicity persists in low doses and it delays the onset of AIDS related complex (ARC) and AIDS in asymptomatic HIV infected patients with low T4 lymphocyte count. The drug has good oral availability and is able to cross the blood-brain barrier. This is of major importance as the virus is present in the CNS of many AIDS patients. AZT has significant toxicity for the bone marrow, producing severe anaemia in 80% patients. AZT can reduce the risk of HIV transmission from pregnant women to their babies by more than two thirds. Its side effects are reversible mild anaemia in some of the babies[5-7] (Fig.4).

Suramin, Evans Blue, Aurinitricarboxylic acid: These act in a similar manner inhibiting HIV replication *in vitro.* They inhibit cytopatho - genecity of HIV for ATH8 cells, gag p24 protein expression at a concentration well below the cytotoxicity threshold. In addition, suramin being a hexasulfonic acid derivative, may also affect virus adsorption. Safety margin (toxicity/activity ratio) of suramin is rather narrow [3] (Fig.4).

BI-RG-587: It is a member of the class of compounds called dipyridodiazepinones. It can consistently block half the activity of reverse transcriptase at a relatively low concentration. Hence, it is a potent inhibitor. It is not a nucleoside analogue. It blocks reverse transcriptase without competing with any of the nucleosides. It suggests that it binds to a different part of the enzyme. It probably damages the enzyme in such a manner that the enzyme is no longer active.

BI-RG-587 is much less damaging to bone marrow cells than Zidovudine, and is effective against some strains of HIV that are resistant to Zidovudine. The researchers have measured the effect of this drug on HIV in cultured human T cells. It reduced the amount of viral antigen produced by the cells and prevented them from clumping together to form syncytia as they normally do when infected with the virus. In addition, animal studies have shown that the drug does not have toxic effects and does not interfere with the normal enzymes in a cell[8].

Glutathione: Researchers propose that oxidative stress may play a critical role in the gradual decay of the immune system in the progression of AIDS. The damage brought about by too many dangerous oxygen molecules inside immune cells may disrupt the performance of the cells and eventually cause them to die. An important feature of AIDS is a sharp drop in the body's concentration of glutathione, a major mechanism for absorbing excess oxygen and thus protecting against oxidative harm. Thus, replenishing the body's store of glutathione may help retard the progress of AIDS. In sum, the body needs glutathione to thrive and survive[9].

ALX40-4C: This drug had completed Phase 1 clinical trials in HIV treatment in Canada in November 1993. Laboratory results have demonstrated that the drug prevents the replication of HIV by inhibiting a crucial step in the life cycle of the virus. The drug functions to inhibit transcription factors

that are involved in genetic regulations. ALX40-4C works by preventing HIV from using its genetic information to produce new virus particles. The specific target of the drug is a critical interaction between two virus components, the transactivator protein tat and the RNA structure TAR.. ALX40-4C inhibits the function of tat, thus reducing the spread of the virus. tat is small protein that is made by HIV which controls the activity of the HIV genes.[9, 11]

Heparin: Heparin binds to envelope glycoprotein gp 120 of the virus and inhibits HIV replication. The glycoprotein-binding heparin fraction was found to block HIV-1 infection of CD4 - T cells in culture. It may be possible to administer heparin at doses, below which it has significant anticoagulant effect, but which neverthless may be strongly inhibitory to HIV-1.[10]

Thalidomide: This tranquiliser, which leads to birth defects, blocks the activation of HIV-1, the virus that cause AIDS, in ceratin immune system cells. The drug could be useful in delaying the progression of HIV infection and onset of AIDS symptoms. Clinical trails are being planned to test this idea. Thalidomide blocks the biosynthesis of tumor necrosis factor-α (TNF - α.), which is known to be involved in activation of HIV-1 in latently infected immune system cells.

Ribavirin: This drug interferes with the structure and function of retroviral mRNA transcription from integrated DNA in the infected cells. But, it antagonises the effect of AZTs ability to inhibit HIV replication by suppressing the cellular enzymes that phosphorylate AZT to its active form[7].

Protease Inhibitors: HIV protease inhibitors block a viral enzyme required for the assembly of new infectious virions. Because HIV protease is only distantly related to human enzymes, selective inhibitors of the enzyme have essentially no mechanistic toxicity. In principle, this feature should allow the use of higher drug concentration that can more effectively suppress viral replication. The most useful protease inhibitors may be *invirase* and *crixivan.* Crixivan appears to help patients maintain higher levels of CD4T cells despite the fairly rapid emergence of viral resistance.

Combination Therapy : Combination of Zidovudine and Acylovir, Foscametor, Interferon, is being tested for the treatment of HIV infections when combined with interferon, AZT is required in small doses to provide greater overall efficiency than when used alone. Similarly, AZT with acyclovir has greater effect than when used alone. A protease inhibitor, *invirase* is likely to find use in combination with reverse transcriptase inhibitors and other protease inhibitors. It can be combined with AZT & DDC to give satisfactory results[5].

Therapy via Immune Systems

Vaccines (Antigen):

Vaccination with attenuated live virus or killed whole virus as well as with subunit vaccines has been a successful strategy against a number of viral diseases. The same could be used to develop a vaccine against HIV. An ideal vaccine should protect against the initial steps of the infection. This is possible by inducing a high titred long-lasting humoral and cellular immunity. Also it must increase the CD4 cells in the body which, in turn, depends upon receiving signals from increased CD8 cell levels. The adequate and sustained presence of CD4 and CD8 cells boosts the immune system and the body's capacity to fight infection. However, researchers have persistently encountered following hurdles in the development of vaccines:

(1) Strain variation of HIV

(2) An attenuated live vaccine does not seem appropriate, since it might recombine with endogenous retroviral information leading to a pathogenic virus within the infected person

BIV Vaccine: This vaccine is a non-disease producing clone of the Bovine-immuno deficiency virus. This virus was first isolated in 1972 from a cow with persistent lymphocytosis and mild CNS inflammatory lesions. BIV vaccine specifically reduces the HIV load that circulates in the infected person by improving CD4 and CD8 count. Although, the above hypothesis is not yet scientifically established, the human

chemical trials with BIV have been under way for the past six to seven months in the U.S.A.[14,15]

CEL-SCI's HGP-30: Here, a synthetic copy of a region of the p17 core protein of the AID's virus has been prepared. The vaccine was seen to elicit antidotes to HGP-30 during human trials. It also stimulated the production of HGP -30 specific T- cells[16].

Super Vaccine: It is a genetically engineered *super antigen* which has shown to raise antibodies against and neutralise a protein called nef protein which the virus produces inside the host cell. In the absence of this protein, the virus cannot multiply. Hence, the vaccine reduces the load of HIV to a level at which it cannot cause AIDS[17].

Experimental AIDS Vaccine: This involves genetically engineered versions of the HIV surface protein gpl2O,which have been designed in the U.S.A.[18]

Antibody Trials:

Antibody therapy (or passive immunity) is a concept which aims at controlling HIV by providing an infected person with antibodies that can neutralise or block the virus. Clinical trial of a monoclonal antibody against HIV (envelope protein) demonstrated that the antibody was well tolerated by AIDS patient without any side effects. Although proteins may vary from one viral isolate to another, but they all bind to T4 which is relatively constant in structure. An antibody directed against this site might bind to (neutralise) most strains of HIV and perhaps kill infected cells as they begin to expres; envelope antigens, so that spread of virus to uninfected cells can be reduced[16].

Non-Conventional Methods

Tree Bark to target AIDS:

Researchers claim that a new class of compounds derived from betulinic acid, a natural substance found in the bark of the plane tree, blocks HIV-1 replication at an early stage by inhibiting the fusion of viral envelopes to target cell membranes with efficacy in vitro[9].

Root extract against HIV: It has been confirmed that the root of the *giant knotwood,* a chinese herb, efficiently prevents the AIDS virus cell infection. In the presence of the plant root extract, the destruction of the cells by AIDS virus was suppressed. Also the enlargement of the cells rarely occurred. Although it has been successful in destroying the AIDS virus *in vitro,* further research is being undertaken in developing this herb for AIDS treatment. This herb has fewer side-effects.

Enzymes kill AIDS virus on contact:

Exact is the trade name of highly potent, purified enzymes *Myeloperoxidase* (MPO) and Eosinophil *peroxidase* (EPO) manufactured by ExOxEmis, Inc. It kills the AIDS virus without damaging surrounding tissue or helpful bacteria which occur normally in the body. *Exact* could be used prophylactically in the douches, creams, suppositories, or lozenges, etc. Other possible uses of *exact* include coating donor blood bags to prevent HIV infection and treating other sexually transmitted diseases.

A new approach pays off:

It has been found that ordinary light and a dye like the brightness used in washing powder may help to prevent the spreading of HIV virus. The work has been focused on disrupting the viral envelop. *In vitro* experiments show that a hydrophobic fluorescent dye belonging to a class of 1,8-naphthalimide photochemicals containing bromine dissolves in the fatty portion of the envelop and locks the envelop proteins together when exposed to blue light, in a photodynamic reaction.The envelop becomes rigid trapping the viral genome inside and prevents a key protein on the surface of the virus (gp 120) from binding to receptors on healthy cells. The method thus disrupts reproduction and further infection. Very low concentration of this dye is needed to neutralise HIV-1. The main drawback faced is that blue light does not penetrate human tissues. Hence, the dye has to be modified to be activated by red light, which could allow treatment by

incandescent light or even sunlight. There also could be a possibility of using Azulene, a compound that absorbs red light. Currently researchers are speculating on this idea[18].

Conclusion

At present it is not premature to speculate on the possibility of a cure for AIDS. Here it is worth stressing that the effective therapy of HIV infection may well depend on combination strategies that attack multiple steps in viral replication. Conceivably, therapeutic agents could be designed.

(1) being, capable of jamming CD26 lock because only HIV strain uses CD26 to infect cell. According to researchers at Pasteur Institute the doorway to the human cell for virus is a CD26 receptor molecule[4].

(2) to alter properties (lipid composition) of the surface of virus or target cells.

(3) to target the envelop protein itself.

(4) vaccines which combine the positive aspects of immune stimulation inherent in live attenuated vaccines with the safety of recombinant, submit type capable of developing humoral and cellular immune response[19].

Surely, the more selective the inhibitory effect on HIV, the greater the likelihood, that the compound may be efficacious in the treatment of AIDS. All the queries regarding the efficacy of the drugs currently under trial and others which are yet to be brought in the therapy of AIDS must await the results of properly controlled long term studies, monitored for as many parameters (virological, immunological, clinical) of the disease as possible.

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