

Inhibition of HIV-1 Entry into Cells

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Abstract: Treatments for HIV-1 include drugs which act to inhibit specific steps in the virus life cycle such as reverse transcription and viral maturation. In 1995 breakthroughs were made in our understanding of the entry of HIV-1 into cells. HIV-1 was shown to use, in addition to the CD4 receptor, chemokine co-receptors, primarily CCR5 and CXCR4, for entry into CD4⁺ T cells and macrophages. These discoveries have provided another target for the treatment of HIV-1 infection. Drugs developed to block HIV-1 entry include CD4 receptor inhibitors, chemokine receptor inhibitors and inhibitors of attachment and membrane fusion. These drugs may add a further treatment for HIV-1 infection along with protease inhibitors and reverse transcription inhibitors. Several of the entry inhibitors are currently being used in clinical trials and demonstrate efficacy *in vivo*. In this review, the entry blocking drugs that have recently been patented, their mode of action in inhibiting HIV-1 and their efficacy in clinical trials will be discussed.

Keywords: CD4, receptor, chemokine, entry, attachment, fusion, T cell, inhibitor.

INTRODUCTION

Infection with HIV-1 is initiated upon binding of the viral envelope glycoprotein gp120 to the CD4 receptor. Following binding, a conformational change occurs allowing binding of gp120 to a chemokine co-receptor. The interaction between gp120 and the co-receptor in turn results in alterations in gp41. The two alpha helices in gp41 undergo change resulting in the insertion of a hydrophobic fusion peptide region into the cell membrane. The viral and cell membranes are then aligned to allow fusion.

In HIV infection, specific chemokines may block the entry of HIV-1 into the cell by binding to their natural receptors. Cellular entry of T-cell tropic isolates of HIV-1 may be blocked by the ligand for CXCR4, stromal cell-derived factor-1 (SDF-1), a member of the β -chemokine family. SDF-1 may block T cell tropic HIV-1 entry by binding to the CXCR4 receptor [1, 2]. The β -chemokine receptors CCR5 [3-5], CCR3 [6, 7] and CCR2b [7] were identified as co-receptors used by macrophage tropic isolates for entry into CD4⁺ T cells. The natural ligands for CCR5, macrophage inhibitory protein-1 (MIP)-1, MIP-1 and regulated on activation normal T cell expressed and secreted (RANTES), block entry of macrophage tropic HIV-1 [3, 4]. Several other co-receptors have been identified including CCR8 [8], CXCR3 [9], STRL33/Bonzo and GPR15/BOB which mediate entry of HIV-2, SIV and dual tropic strains of HIV [10-13]. The mechanisms of action of the blockage of entry of HIV-1 into cells are summarized in (Fig. 1 and Table 1).

INHIBITORS OF CD4 AND VIRAL ATTACHMENT

In studies of the inhibition of the interaction between HIV-1 gp120 and the CD4 receptor, soluble CD4 has demonstrated strong inhibition of HIV-1 infection *in vitro*

[14, 15]. However, *in vivo* administration of sCD4 required very high doses to be effective [16-19]. It has since become clear that interactions of sCD4 with gp120 result in conformational change in gp120 and increased association with CCR5, thus increasing HIV-1 replication [20-23]. The use of gp120-soluble CD4 complexes can result in a failure in exposing many important epitopes of the HIV-1 envelope. This may be due to the trimeric structure of gp120 and gp41. In a recent patent, the design of HIV envelope-CD4 complexes which are better able to interact with hidden gp41/gp120 epitopes is being studied. This would involve the production of polynucleotides for CD4 and the HIV-1 envelope individually and in combination [24]. Several recent patents concern the use of fusion proteins, antibodies or fragments of antibodies to induce a neutralizing antibody response *in vivo*, thus impeding the ability of HIV-1 to infect cells [25-27]. One patent describes using an attachment inhibitor (compounds which can bind CD4) and a fusion inhibitor (pentafuside) to synergistically improve antiviral treatment [28].

Using another strategy, synthetic peptide to the C terminus of cell surface nucleolin has been shown to inhibit HIV-1 replication at the stage of attachment. This patent describes the composition of these peptides and a method for treating viral infections, including HIV-1 [29]. Nitrogen has been reported to change the conformation of cell membrane proteins and can be used to prevent the attachment of viruses to their host cells [30]. In addition, nitrogen has been shown to inhibit HIV-1 replication [31]. One recent patent describes exposing AIDS patients to nitrogen in hyperbaric chambers to inhibit HIV-1 infection of cells *in vivo* [32]. Nitrogen treatment also improves CD4/CD8 ratios, preserves lymph node structure and helps to stabilize immune responsiveness [32].

CHEMOKINE RECEPTOR INHIBITORS

Several chemokine-derived compounds have been tested for inhibitory effects on HIV-1 replication. These include

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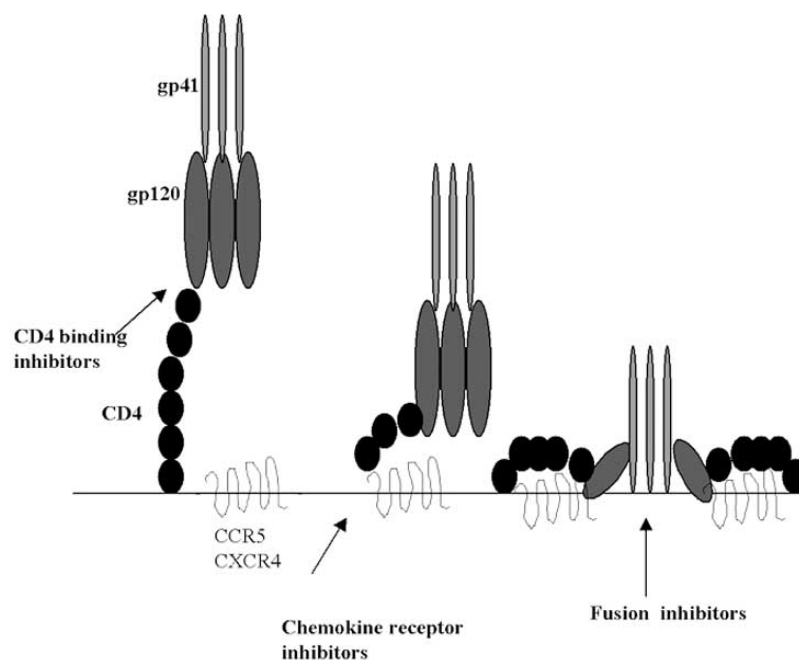


Fig. (1). The mechanisms of blockage of HIV-1 entry into cells are shown including the inhibition of CD4-gp120 interactions, blockage of viral interaction with chemokine co-receptors and inhibition of virus-cell fusion.

Table 1. HIV-1 Entry Inhibitor Classes

Viral Attachment Inhibitors:
gp120-CD4 complexes
Fusion proteins
Neutralizing antibodies, antibody fragments
Nucleolin inhibitors
Chemokine Receptor Inhibitors:
Modified chemokines
Antibodies to chemokine receptors (PRO 140)
Small molecule inhibitors
G1 phase inhibitors
Spiropiperidine compounds
Membrane Fusion Inhibitors:
T20 (enfuvirtide; DP178)
T21 (DP107)
T1249
Chemically modified peptides
Polymer bound peptides
Fusonex
ADS-J1 (phenylazo-naphthalene sulfonic acid)

modified chemokines such as aminoxyptane-RANTES, *n*-nonanoyl-RANTES and PSC-RANTES [33]. These modified forms have greater potency than unmodified RANTES in the inhibition of HIV-1. Monoclonal antibodies to chemokine receptors have also been established including PRO 140 which targets CCR5 [34]. Several small molecule antagonists of CCR5 and CXCR4 have also been identified as inhibitors of HIV-1 replication [34].

The V3 loop is a molecular mimic of the P-hairpin structures that are present in the natural ligands for CXCR4 and CCR5. Peptides from this region are expected to be useful as immunogens which would elicit broad neutralizing antibodies against HIV-1 and as well would serve as antagonists inhibiting the binding of HIV-1 to chemokine co-receptors [35]. Two recent patents describe the use of compounds which arrest the G1 phase of the cell cycle thus disrupting the response of lymphocytes to IL-2 resulting in reduced transcription of CCR5 [36]. This would also result in the accumulation of chemokines in the cell. G1 phase arresting compounds include sodium butyrate, apidicolin, hydroxyurea, olomucine, roscovitine, tocopherols, tocotrienols and rapamycin. Administration of such compounds in addition to another antiretroviral may result in synergistic enhancement of the activity of the antiviral agent.

In another approach to modulate CCR5 activity, spiropiperidine compounds and their salts and hydrates are being tested for the ability to interfere with the interaction of HIV-1 with CCR5 [37]. Novel tetramic acid-type compounds isolated from a CCR5 active complex are also being used as CCR5 antagonists to inhibit HIV entry [38, 39]. In addition, quinolyl amide derivatives are being tested as CCR5 antagonists [40, 41]. Several patents address the establishment of high throughput screening assays to identify inhibitors of HIV-1 fusion with the cell membrane. These assays involve the establishment of cell lines expressing the required receptors and reporter systems to allow the measurement of inhibition by several procedures including flow cytometry, fluorometry, immunoprecipitation, chemiluminescence, enzyme assays and radiolabeling [42-46]. Another patent describes the development of a method to identify ligands for CCR5 which have extended residency time. This could result in the identification of ligands with

more potent inhibition of HIV-1 infection and provide improved pharmacodynamics, possibly resulting in increased efficacy *in vivo*. Residency times could extend from 1-9 hours depending on the ligand [47, 48]. In addition to CCR5, antibodies or antibody fragments will be used to test inhibition of HIV-1 replication *via* the CCR2 receptor [49, 50]. Another chemokine receptor utilized by HIV-1 is the orphan receptor APJ. Cell lines expressing APJ and CD4 are under development to be used in the identification of inhibitors of HIV-1 entry *via* this chemokine receptor [51].

MEMBRANE FUSION INHIBITORS

Within the gp41 ectodomain are two heptad repeat regions which are adjacent to the N- and C-termini and peptides from these regions are strong inhibitors of HIV-1 infection. The peptides from these regions can interact resulting in a six-stranded, coiled-coil which represents the fusogenic structural core of gp41 [52]. Antiviral compounds which are targeted to the core structure of HIV-1 gp41 continue to be developed and improved upon. These include synthetic peptides comprised of amino acid sequences of the HR1 and HR2 regions of gp41. These peptides contain amino acid substitutions in the hydrophobic domain which allow the peptides to assemble into trimers in solution. A peptide based on similarity to the HR2 region may bind to the triple stranded coiled-coil formed by the HR1 region in gp41, thus preventing the formation of the 6-helix bundle required for fusion [53]. The peptides or trimers are then tested for their ability to inhibit transmission of viral fusion. Two of these fusion inhibitors and their use have been described in several recent patents, DP107 and DP178. These synthetic peptides mimic the HR2 region of gp41 and bind competitively to the hydrophobic grooves of the HR1 trimer, preventing the formation of hair pin structure of gp41 [52, 54-56]. DP107 is a 39 amino acid peptide derived from the NH₂ terminus of the gp41 HR2 region (amino acids 558-595 of gp41 of HIV-1_{LAI}) [57, 58]. DP107 was later named T21. DP178 is a 36 amino acid peptide mimetic of the carboxy terminus of the gp41 HR2 region (amino acids 127-162 of gp41 of HIV-1_{LAI}) [59]. This inhibitor was later named T20 and subsequently enfuvirtide, fuzeon or pentafuside. A second generation synthetic peptide derived from the carboxy terminus is T1249 [60]. These peptides have been demonstrated to inhibit viral fusion with the cell membrane and to reduce the infectious titer of cell-free HIV-1 [52, 61-66]. Several patents have examined DP178 and DP107 and fragments, analogs and homologues of the peptides to be used as inhibitors of human and non-human retroviral transmission to cells [67-69]. Methods are in development to identify antiviral compounds that disrupt the interaction between DP178 and DP107 or between DP178-like and DP107-like peptides [68-70]. Research is ongoing to further develop methods to examine the inhibition of complex formation by peptides derived from HR1 and HR2 regions of gp41 by inhibitory compounds [67, 70, 71]. Research is also being conducted to examine the ability of these peptides and modified peptides to inhibit intracellular events involving coiled-coil peptide structures [71]. Some patents continue to examine the use of DP107 and DP178 peptides in combination with other antiviral treatments. The studies suggest that since these peptides are effective antivirals and that they work by novel mechanisms, inclusion with other

therapies may improve or perhaps have synergistic effects in blocking HIV-1 transmission [72, 73]. Other synergistic combinations of treatments include one patent using an antibody to CCR5, an inhibitor of viral fusion that would retard binding of gp120 to CD4 and an inhibitor retarding gp41 from undergoing conformational change mediating fusion [74]. Another examines the use of an antibody to CD4 and the fusion inhibitor pentafuside [74].

In a clinical trial of enfuvirtide administered 3, 10, 30 and 100 mg twice daily by intravenous injection, a 1.5-2.0 fold reduction in viral load was observed at the highest dose by 2 weeks [57]. Two open label, randomized Phase III clinical trials (TORO 1, TORO 2) have also shown effectiveness of enfuvirtide *in vivo* [75, 76]. In these trials enfuvirtide was administered to patients receiving optimized antiretroviral therapy. Patients receiving enfuvirtide experienced increased time to virological failure and increased CD4⁺ T cell counts [77]. By 48 weeks of treatment patients receiving enfuvirtide in addition to their optimized antiretroviral treatment demonstrated a 1.48 log₁₀ copies/ml reduction in viral RNA levels as compared to 0.63 log₁₀ in patients receiving optimized antiretroviral treatment alone [77].

Resistance mutations may develop within weeks of treatment with enfuvirtide [78] indicating that it should not be administered as a monotherapy [79]. Resistance occurs in a 10 amino acid motif which is located between residues 36 and 45 in gp41 [78, 80-82]. This region forms part of the binding site for enfuvirtide which is critical for viral fusion. In addition, viral isolates of individuals treated with enfuvirtide demonstrate decreased replicative capacity [83]. Following the withdrawal of enfuvirtide, reversion to wild type can occur [84].

Modifications of identified peptides with antiviral and antifusogenic properties are also in development. The modifications include chemically reactive groups reactive with amino groups, hydroxyl groups and thiol groups on blood components to allow more stable covalent bonding [85]. The modified peptides have greater stability *in vivo* and are protected from degradation by proteases or peptidases. These improved properties may result in reduced need for frequent administration. Among the peptides under modification are DP107 and DP178. In addition, modified peptides from other nonHIV viruses whose amino acid sequences correspond to the DP107 and DP178 derived gp41 region of HIV-1 are also under development. These non-HIV viruses include respiratory syncytial virus (RSV), human parainfluenza virus (HPV), measles virus (MeV) and simian immunodeficiency virus (SIV) [85]. Within the scope of this patent is modification of peptides involved in viral transfection, including Hepatitis and Epstein Barr Virus. Other modifications include the development of conjugates containing a polymer bound to two or possibly more synthetic peptides derived from HIV-1 gp41 to inhibit HIV transmission. Synthetic peptides are operably bound to the polymer *via* a reactive functionality [86]. Another fusion inhibitor described is Fusonex, a peptide derived from amino acids 117-151 of the C peptide of E subtype HIV-1 gp41 [87]. This peptide has high stability and high valence for the inhibition of HIV-1 infection. Greater efficacy may be

achieved at lower doses of Fusonex than T20 resulting also in lower toxicity.

Synthetic peptides require frequent injection (i.e. daily) to attain effective therapeutic benefit. To overcome this, attempts are being made to chemically modify peptide-based therapeutic agents to a water soluble polymer, such as polyethylene glycol, to enhance efficacy *in vivo*. One patent describes the conjugation of peptides of the HR1 and HR2 regions of gp41 to a polymer to test the effects on half-life and biological activity *in vivo* [67]. Another patent describes reacting a novel peptide up to 51 amino acids in length with macromolecules to form covalently linked complexes which have extended lifetimes and biological activity [88].

Peptide based therapies such as T20, lack oral availability, are sensitive to proteolytic digestion and are expensive to produce. To aid in the identification of non-peptide inhibitors of HIV-1 fusion, a monoclonal antibody (NC-1) was generated which binds to the peptide complex but not to individual peptides from gp41 HR1 and HR2 regions [89]. This antibody was used to develop an ELISA for screening non-peptide fusion inhibitors [89]. The same group has also developed a high throughput ELISA for the testing of large numbers of compounds [89, 90]. One of these is ADS-J1, a phenylazo-naphthalene sulfonic acid.

CURRENT AND FUTURE DEVELOPMENTS

The use of inhibitors of HIV-1 entry into cells may have profound effects on HIV-1 replication *in vivo* in combination with drugs which target other steps in the viral lifecycle. This could provide the potential to reduce the size of the reservoir particularly when combining this treatment with an immune stimulator to increase virus production in latently infected cells in the face of a strong cytolytic response and a strong neutralizing antibody response. However, resistance mutations may also develop in response to entry blocking treatments. With respect to compounds which inhibit *via* chemokine receptors, a question arises. If there is continued blockage of a specific chemokine receptor, would HIV-1 develop mutations in the V3 region to allow another chemokine receptor to be used as a portal of entry? Blocking *via* a chemokine receptor may also have implications for normal signal transduction activity in cells. Another aspect of this research is the examination of how entry blocking compounds affect HIV-1 infection of other cell types. In addition, the use of compounds which do not have oral availability may be more difficult to administer by daily injections. However, the ongoing research on entry blocking compounds and modulations to further improve their activity *in vivo* will provide further agents to the therapeutic arsenal for HIV-1 infection.

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