Eur J Med Res (2007) 12: 391-396

The Role of Receptors in the HIV-1 Entry Process

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Abstract
The advent of highly active antiretroviral therapy (HAART) has revolutionised the management of HIV. A wide selection of antiretroviral agents with various mechanisms of action is now available, allowing patients and physicians a greater choice of effective therapy.

This article details the development of the HIV entry inhibitors, describing the physiology and pharmacology involved in their design, and their use in individuals at all stages of HIV infection. We focus on the CCR5 antagonists, a novel class of HIV entry inhibitor and detail the findings of the recent MERIT and MOTIVATE trials, designed to investigate CCR5 antagonist use in the antiretroviral naïve and highly treatment experienced populations, respectively.

Drug resistance and toxicities have emerged as major treatment challenges in the HAART era and the development of novel antiretroviral agents remains paramount. This article discusses how the entry inhibitors may meet many of these challenges and preserve the reduced morbidity and mortality we have come to expect from HAART use.

Key words: HIV; Highly active antiretroviral therapy; HIV entry inhibitors

Introduction

In the early 1980s, following an outbreak in the USA of Pneumocystis Carinii Pneumonia (now Pneumocystis jirovecci) and Kaposi's sarcoma in men who have sex with men (MSM), the Human Immunodeficiency Virus (HIV) was isolated [1, 2, 3]. It is now known that HIV preferentially infects activated CD4 cells and antigen presenting cells (APC) leading to a decline in CD4 cells due to direct infection and destruction, often secondary to 'by-stander' apoptosis. Following HIV infection, there is a subsequent aberrant activation of the immune system which evokes much of the immunopathology associated with HIV, rather than an isolated immunodeficiency attributable to CD4 cell loss.

Highly active antiretroviral therapy (HAART) has revolutionised the treatment of HIV in the Western world. However, no treatment is able to completely eradicate the virus, active drug pressure merely suppresses viral replication. Therapy may also fail. Resistant virus, patient non-adherence, inadequate drug levels and toxicity are the main culprits fuelling virological failure. The demand for novel antiretroviral agents has grown in the recent past, and considerable interest has been focussed on the development of the viral entry inhibitors.

Replication of HIV

HIV replication has been extensively investigated, forming the main target of antiretroviral therapy. Following fusion with the host cell, the virus is uncoated. As HIV is a retrovirus, reverse transcription must occur prior to incorporation into the host genome. Reverse transcription is facilitated by the specific reverse transcriptase enzyme, unique to HIV. *Vpr*, a splice gene product contained within the virion, chaperones the pre-integration complex to the nucleus where a second unique enzyme, integrase, facilitates viral incorporation. The growing mRNA chain is stabilised by *tat*, a splice gene product produced early in the replication cycle. The mRNA chain leaves the nucleus and forms a large polyprotein, which is split into active constituent proteins following cleavage by an aspartate protease. Once the constituents of the virus are assembled, the complete viral particles bud off, extracting a segment of the cellular membrane. The three unique enzymes, reverse transcriptase, integrase and protease, essential for the replication of HIV, have become obvious targets for HIV therapy. As the specific assembly process for the virus is clarified, drugs are being developed to prevent replication at each point of the assembly cycle.

The Process of Viral Entry

Shortly after the discovery of HIV, it was recognized that the virus gains access to the cell via the CD4 receptor. We now know that a second receptor is also necessary for viral entry. The 'secondary' receptors are of two broad groups, determined by the position of a cysteine molecule, and are named either CCR *or* CXCR receptors. It is now recognized that in the majority of patients, the second receptor facilitating HIV cellular entry is the CCR5 receptor. However, a minority of HIV viruses are said to be 'dual tropic', capable of using the CCR5 receptor or the CXCR4 receptor. Others use the CXCR4 receptor in isolation- so called X4 viruses. A more rapid fall in CD4 count and the development of AIDS has been observed in individu-

als with viruses which were able to form syncitia in tissue culture compared with those which were not [4]. This in vitro phenomenon is associated in most, but not all, cases of change in the tropism of the virus from R5 to X4.The phenotypic change of R5 to X4 is usually associated with genotypic changes in the codons responsible for the V3 loop of the virus. Like non-syncitium inducing and synctium inducing variants (NSI and SI), this genotypic change is not uniformly associated with the change in tropism.

Early Process of Fusion

The first stage of fusion occurs when the gp-120 envelope of the virus makes contact with the CD4 receptor. This induces a conformational change, allowing the V3 loop to link with the chemokine receptor, inducing further conformational change, bringing the surfaces of the virus and the cell into close proximity.

Blocking CD4 Interaction

It has been suggested that soluble CD4 molecules could be administered to saturate the virus and inhibit replication. This has had little success in vivo [5]. Bristol Myers Squibb developed a trial of small molecules designed to interfere with the mechanism of gp-120/CD4 interaction. A Phase 1 proof of concept study has shown that such drugs produce pronounced viral load decline [6]. It remains to be seen as to whether these agents are safe and pharmacologically available in as an oral preparation.

A series of monoclonal antibodies targeting the site of gp-120/CD4 interaction, have also been shown to produce considerable reduction in plasma viral load. TNX-355, is an anti-CD4 monoclonal antibody developed from an immunoglobulin G4 anti-CD4 molecule. In a phase II trial in 82 HIV-infected adults, all three class experienced, individuals were randomised to receive TNX-355 or placebo in conjunction with an optimised background regimen (OBR). At 48 weeks, the intention to treat analysis (ITT) revealed that individuals randomised to receive 10 mg/kg of TNX 355 had a mean viral load reduction of 0.96 log 10 copies/ml and individuals receiving 15mg/kg had a drop of 0.71 $\log_{10}$ copies/ml, contrasting with the 0.14 log 10 copies/ml drop seen in the placebo arm ($p<0.01$) [7, 8]. A second immunoglobulin, PRO 542, made by Progenics, is also being investigated [9]. This agent binds to gp120 blocking attachment and entry of virus into the host cell.

Blocking the Fusion Process

Enfuvirtide (T-20) already has a role in the management of HIV, predominantly at a later stage of disease. Binding to gp41, it is used optimally in co-administration with agents which have sufficient antiviral activity to facilitate complete cessation of viral replication. Its major limiting factor lies in the fact that it must be administered by subcutaneous injection. It is also fragile, without potent drugs in combination, resistance rapidly emerges. A second fusion inhibitor, T1249 is being developed and thought to be more potent than T20.

CCR5 and CXCR4 Inhibitors

CCR5 and CXCR4 have not been crystallized. Molecular models of their structure are all based on rhodopsin, and the impact on drug development is debatable. Blocking CCR5 receptor activity is not thought to have a deleterious effect on normal physiological functioning of the body as there is considerable redundancy within the immune system and a functional CCR5 receptor is not essential for life. Deletion of the CCR5 receptor is a common balanced polymorphism in the Caucasian population, occurring in 1% and appears to have no deleterious effects. It has been suggested that resistance to a number of diseases, including childhood asthma, multiple sclerosis and myocardial infarction, results from being heterozygous for this deletion. Interestingly, the presence of the delta 32 mutation is also thought to have an effect on HIV progression [10].

Three pharmaceutical companies were originally involved in the development of CCR5 directed antagonists. These agents were all available for oral administration, and had little evidence of toxicity in animal experiments or in initial studies, however, one of these products, Aplaviroc, in Phase 2 studies was associated with hepatotoxicity and withdrawn. A second, Vicriviroc, has been associated with abnormal QT interval on ECG.

Vicriviroc has been trialled in antiretroviral experienced individuals out to 48 weeks in ACTG 5211 [11]. In this study, 118 individuals on a failing antiretroviral regimen, a third of whom were enfuvirtide experienced, were randomised to add 5, 10 or 15mg of Vicriviroc or placebo to their regimen. At baseline, the median viral load was 4.56 $\log_{10}$ copies/ml and median CD4 was 146 cells/mm^3. Following two weeks of exposure, background regimens were changed, according to baseline resistance test findings. In the treatment arms, vicriviroc was continued, with ritonavir used as a boosting agent. Of note, individuals receiving placebo were permitted to switch to the treatments arms if no appreciable increase in viral load was seen at or after week 16. The 5mg Vicriviroc arm was closed early, following the diagnosis of 5 cases of malignancy in individuals randomised to this arm. At 48 weeks, data was available on 88 study participants, 30 of whom had received 10mg Vicriviroc, 30 received 15mg and 28 received placebo. The majority, 23 of 28 (82%) of subjects in the placebo arm ceased treatment early, in comparison with 30% of individuals in the 10mg arm and 37% in the 15mg.

At week 48, the median viral load drop was between -1.92 $\log_{10}$ copies/ml in the 10mg arm, -1.44 $\log_{10}$ copies/ml in the 15mg arm, and the median CD4 rise was 130 cells/mm^3 and 96 cells/mm^3 respectively. There is no data for placebo as only five individuals remain in this arm. Of the participants in the treatment arms, 57% of those exposed to 10mg of Vicriviroc achieved a viral load of < 400 copies/ml and 37% reached a viral load of < 50 copies/ml. In the 15mg arm, 43% achieved a viral load < 400 copies/ml, with 27% reaching a viral load < 50 copies/ml. Regardless

of previous exposure, study participants without enfuvirtide resistance were offered this agent as part of their optimised regimen. Individuals naïve to enfuvirtide were seen to have the greatest reduction in viral load.

The major concern evoked by this trial was the incidence of malignancy in the treatment arms. At 24 weeks, six malignancies were reported increasing to eight at week 48. Whether this is a reflection of the population enrolled in the trial or a drug association is unclear.

The most advanced of the CCR5 antagonists is Maraviroc, produced by Pfizer. This agent has been trialled at varying doses, from 300mg BD to 1200 mg OD [12]. The dose limiting side effect appears to be hypotension. The most common adverse effects are head-ache, dizziness and nausea. Given that Maraviroc may be used initially in more late stage disease when polypharmacy is common, the pharmacokinetics of this agent have been studied in depth. When Maraviroc is given with Ritonavir, its dose should be reduced by half. When used with Efavirenz, the Maraviroc dose should be doubled [13]. Two large, double blind, randomised controlled trials have investigated the use of Maraviroc- MOTIVATE-1 and 2 [14, 15]. In each study, three class experienced individuals with HIV viral loads greater than 5000 copies/ml known to have CCR5 tropic virus at baseline were randomised to receive OBR plus Maraviroc OD or BD vs placebo. The OBR consisted of 3-6 agents. Both studies had similar

Table 1. Baseline characteristics of individuals in MOTIVATE-1 (14).

	Maraviroc OD	Maraviroc BD	Placebo
VL (copies/ml)	4.85	4.86	4.8
CD4 (cells/mm^3)	168	150	163

Table 2. Baseline characteristics of individuals in MOTIVATE-2 (15).

	Maraviroc OD	Maraviroc BD	Placebo
VL (copies/ml)	4.87	4.84	4.89
CD4 (cells/mm^3)	174	182	174

baseline patient characteristics (see Tables 1 and 2).

In MOTIVATE-1, 601 individuals entered the trial, of whom, 585 received at least one dose of study drug. In MOTIVATE-2, 475 were randomised and 464 received at least one dose of drug. The primary end-point in each study was a reduction in plasma RNA at 24 weeks. Secondary end-points included the change in CD4 count from baseline and proportion of patients achieving a viral load of <400 copies/ml and <50 copies/ml.

In MOTIVATE-1, the reduction in viral load at 24 weeks was statistically greater in the Maraviroc OD and BD arms than in the placebo arm (see Table 3). There was a greater chance of reaching viral load undetectability in the Maraviroc arms than in the placebo arm. A greater increase in CD4 count was also seen in the treatment arms. The same was true for MOTIVATE-2 (see Table 4).

Table 3. Outcomes of MOTIVATE-1 (14).

	Maraviroc OD	Maraviroc BD	Placebo
VL reduction ($\log_{10}$ copies/ml)	1.82	1.95	1.03
Percentage of participants with VL<50 copies/ml (%)	42	49	25
Increase in CD4 (cells/mm^3)	107	111	52

Table 4. Outcomes of MOTIVATE-2 (15)

	MVC OD	MVC BD	Placebo
VL reduction ($\log_{10}$ copies/ml)	1.95	1.97	0.93
Percentage of participants with VL<50 copies/ml (%)	41	46	21
CD4 increase (cells/mm^3)	112	102	64

The combined analysis of MOTIVATE-1 and 2 illustrated that of all patients screened, 56% had a CCR5 tropic virus. Of all individuals found to be R5 tropic at screening, 8% were found to have mixed tropic virus at baseline. Those individuals with dual tropic virus had a poorer virological outcome than individuals with monotropic R5 virus.

In both MOTIVATE-1 and 2, there was no statistically significant difference in adverse events in any of the arms.

Maraviroc has also been trialled in the antiretroviral naïve population in the MERIT study [16]. All individuals included harboured R5 tropic virus at baseline, had a viral load above 2000 copies/ml and no evidence of baseline resistance to Efavirenz, Zidovudine or Lamivudine. Participants were randomised to receive either Maraviroc 300mg OD, 300mg BD or Efavirenz 600mg OD with a Combivir backbone (Zidovudine and Lamivudine in combination). The OD arm was closed early in the trial due to virological failure.

Baseline characteristics in each arm were similar, with a median CD4 count of 241 in the Maraviroc arm and 254 in the Efavirenz arm. Baseline viral loads were approximately 70 000copies/ml in both arms.

The primary end-points were the proportion of in-

dividuals achieving viral loads < 400 copies/ml and < 50 copies/ml. A complex, non-inferiority measure was applied to the study. At 48 weeks, Maraviroc was shown to be non-inferior to Efavirenz by the < 400 copies/ml end-point, with 70.6% of individuals in the Maraviroc arm achieving this end point, compared with 73.1% in the Efavirenz arm. However, Maraviroc was not found to be non-inferior (by protraction, therefore, inferior) to Efavirenz at the < 50 copies/ml measure, with 65.3% in the Maraviroc arm compared with 69.3% in the Efavirenz arm achieving this end point.

Interestingly, when the study participants were analysed by geographical region, it was seen that individuals in the Northern hemisphere were equally likely to achieve viral loads <50 copies/ml (68% and 67.8%) irrespective of exposure to Maraviroc or Efavirenz. This may reflect differing HIV clades, stage of HIV infection or potential tropism screening problems.

The main reasons for drug discontinuation were different in each arm of the trial. Those taking Maraviroc had a higher rate of respiratory tract infections. Given the data on Vicriviroc, it is interesting to note that there were fewer malignancies reported in the Maraviroc arm.

CXCR4 Inhibitors

One drug, AMD 3100, has shown significant viral load reductions in a small number of patients harbouring X4 tropic virus in a proof of concept study [17]. This drug is not being developed further because of toxicity, but other compounds in the same series are being evaluated, AMD 070 [18] and AMD887 [19] both of which exhibit activity against multiple drug resistant virus in vitro.

The Future Role of CCR5/CXCR4 Inhibitors

Measuring viral tropism is an important precursor to starting therapy with a CCR5 inhibitor. At present, these tests are limited by their thresholds for detection of quasispecies, failure of virus amplification, cost and availability of results.

The majority of individuals with a CD4 count between 250-350 cells/mm harbour predominantly R5 virus (80%), with the remaining 10%-20% having dual tropic virus. A significant proportion of patients with a CD4 count of less than 50 cells/mm^3 who may have failed antiretroviral therapy in the past or are late presenters, may have dual tropic virus, with a small percentage harbouring X4 tropic virus only.

Phase III trials investigating the benefit of R5 inhibitors in individuals with dual tropic virus are on-going. The main concern with this approach lays in the theory that blockade of the R5 receptor may allow the more virulent X4 virus to replicate more quickly. Also, if X4 virus is present in the viral population prior to starting treatment could CCR5 inhibitor use preferentially select for the up-regulation of X4 virus or fuel mutations within the genome, facilitating a tropism change? Both mechanisms have been described and as yet, there is too little data to support either theory.

Currently, viral tropism is tested both on screening and at baseline when an individual enters a R5/X4 clinical trial. Some variability has been found with the initial screening tests showing R5 tropism only, while the baseline tests showed dual tropism. This may have been a fault of the assay, or dual tropic viruses may be a relatively common finding within the viral population. In a published report [11], a patient with dual tropic virus was inadvertently treated with Maraviroc, resulting in the transient emergence of R4 tropic virus during the period of the study, reverting to R5 tropism subsequently.

In a second case, R4 tropism emerged during the study with the same drug and persisted after cessation, without apparent deleterious effect on the immune system [11]. The role of CCR5 inhibitors in individuals with dual tropic virus is being addressed in a number of experienced studies.

MOTIVATE 1 and 2 have shown the CCR5 inhibitors to be useful in antiretroviral experienced patients. This market, may, however, be growing smaller and a number of new drugs have recently been introduced, targeting this cohort of individuals specifically. Further trials, investigating the use of these agents in first line therapy, Post-exposure Prophylaxis and Pre-exposure Prophylaxis (potentially in the form of vaginal pessaries) would be welcome.

Conclusion

The entry inhibitors have heralded a new era of treatment for individuals living with HIV infection. For the first time, we have novel agents targeting areas other than the reverse transcriptase and protease enzymes. Individuals with inherited or acquired multi-drug resistance or those intolerant of established antiretrovirals have treatment options available and can look forward to achieving viral load suppression, CD4 recovery and an associated improvement in quality of life.

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Received: August 10, 2007 / Accepted: August 18, 2007

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