Tangible effects of antiretroviral therapy in HIV-1 infected patients
Foudraine, N.A.

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Chapter 1

"Introduction and outline of this thesis"
1. A short history of antiretroviral therapy

Zidovudine (3'-azido-2', 3' dideoxythymidine, ZDV), the first approved drug against HIV-1 infection, was discovered in 1964 (1) in the search for anti-cancer agents. It was shown to inhibit HIV-1 reverse transcriptase \textit{in vitro} in 1986 (2), soon after the identification of HIV as the etiologic agent of the acquired immunodeficiency syndrome (AIDS). Shortly thereafter its antiviral effect \textit{in vivo} was evaluated and confirmed in a phase I clinical trial (3).

Following the introduction of ZDV, an increasing number of other nucleoside analogue reverse transcriptase inhibitors (NRTI's) has become available. Five NRTI's, ZDV, didanosine (ddl), zalcitabine (ddC), lamivudine (3TC) and stavudine (d4T) have now been approved for the treatment of HIV-1 infection in the Netherlands, and a sixth, abacavir, is expected to be licensed in the near future. All nucleoside analogues reduce HIV replication, both by direct inhibition of the virally encoded reverse transcriptase (RT) and by competing with natural nucleotides for incorporation into the growing HIV-DNA chain, resulting in termination of proviral DNA chain elongation (4).

Monotherapy with ZDV did not result in sustained suppression of virus replication or sustained rises of CD4$^+$ lymphocyte counts, and provided only transient clinical benefit (3,5-19). These disappointing results with antiretroviral monotherapy and the increasing recognition of the emergence of viral drug resistance with various agents used as monotherapy, led to evaluating dual combinations of NRTI's which in general were found to be superior to monotherapy (11,14,16,20-28). After the results of two pivotal clinical endpoint trials, the Delta study and the AIDS Clinical Trials Group (ACTG) 175 study, were released in 1995 dual NRTI therapy became the standard of care for the treatment of HIV-1 infection (24,25). Both trials demonstrated significant reductions in HIV-1 disease progression and mortality both for the combination of ZDV plus ddl and ZDV plus ddC as compared with ZDV monotherapy, particularly in previously untreated (antiretroviral naive) patients, with less pronounced effects in those who had received prior ZDV. In that period a second class of antiretroviral agents, non-nucleoside RT inhibitors (NNRTI's), became available. They potently inhibit the viral RT by altering the position of critical amino acids within the enzyme's catalytic site (29). Introduction to clinical practice of these agents was initially hampered by the rapid development of resistance (30-32), but when used as a part of combination regimens these intrinsically potent drugs have shown to remain
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efficacious in suppressing viral replication (33,34) and they are now commonly used in combination regimens. In 1996, a third class of potent antiretroviral drugs, protease inhibitors (PI’s), was introduced in daily practice. Protease inhibitors target the HIV-1 encoded protease. This enzyme is involved in the cleavage of several viral protein precursors into smaller structural proteins, allowing the production of new infectious virions. Currently, four PI’s have been licensed: indinavir, saquinavir, ritonavir and nelfinavir, and a few other PI’s are expected to be licensed in the near future.

The introduction of new classes of potent antiviral drugs in combination with the possibility to measure plasma HIV-1 RNA levels has provided important insight into the dynamics of HIV-1 infection, revealing that even during the clinically latent stage of infection, continued massive viral replication occurs. The number of viruses produced daily amounts to ± 1x10^8 - 1x10^10 (35-37). It has been calculated that free virions have a half-life of less than six hours and infected CD4^+ cells of two days (35,36). This high level of viral replication drives a continuous direct and indirect destructive effect on the immune system. Given these findings, it is understandable that guidelines concerning the time to start antiretroviral therapy (which were previously based on the CD4^+ cell count, or on clinical symptomatology) have shifted towards starting treatment earlier, at a time when the immune system is still relatively preserved. In addition, the results from various clinical trials have demonstrated that the expected clinical benefit of antiretroviral therapy is associated with the degree of treatment-induced reduction in plasma HIV-1 RNA levels (38,39). As a result, the measurement of plasma HIV-1 RNA has become the primary guide both for initiating and monitoring the effect of antiretroviral therapy.

The current primary aim of antiretroviral therapy is to suppress plasma HIV-1 RNA levels as much as possible and for as long as possible. As assays for measuring HIV-1 RNA became increasingly sensitive, it was demonstrated that the nadir of plasma HIV-1-RNA achieved following the start of antiretroviral therapy was strongly correlated with the duration of viral suppression (40,41) and with the improvement in clinical outcome (33,38,42-47).

The studies described in this thesis were designed during the transitional period shortly before PI’s became available on a larger scale and became the standard of care for the treatment of HIV infection.
2. Background of the studies described in this thesis

2.1 Issues concerning the optimal NRTI backbone in antiretroviral combination regimens

2.1.1 A comparative study of ZDV/3TC versus d4T/3TC

The combination of ZDV and lamivudine (3TC) in particular became a first choice NRTI combination treatment, as it was found to be potent and very well tolerated (21,23,27,48-50). Moreover, 3TC was discovered to have the ability to partially prevent and suppress pre-existing resistance to ZDV (51,52). Although adding 3TC to ZDV in ZDV-experienced patients may result in reversal of ZDV resistance (53), there is no scientific evidence supporting this particular NRTI combination as the first choice for treatment of antiretroviral naive individuals. Furthermore, the toxicity and tolerability profile of ZDV may not allow for its use in every HIV-1 infected patient (54-56). Therefore, the investigation of potential alternative NRTI combination regimens not involving ZDV was a logical and useful approach. Stavudine (d4T), another NRTI, was found to be an attractive candidate to serve as an alternative for ZDV because of its ease of administration and its well demonstrated efficacy in clinical trials (57-63). In addition the combination of d4T plus 3TC was increasingly being used in routine clinical practice in the absence of clinical trial data. Like ZDV d4T is a thymidine analogue (mainly phosphorylated in activated cells) and it has a favourable resistance and toxicity profile. The combination of ZDV plus d4T is antagonistic due to competition at the level of the intracellular phosphorylation to the active triphosphate moieties of each component (64,65).

In Chapter 2 we describe the findings of the first randomised comparison in HIV-1 infected patients who previously had never been treated with antiretroviral drugs. At the time this trial began, PI’s were just becoming available for use in clinical practice. This was taken into account by allowing the addition of the protease inhibitor indinavir to both double nucleoside combinations after the initial 12 weeks of treatment, if the plasma HIV-1 RNA level was found to be insufficiently suppressed by the double NRTI regimen.
2.1.2 The effect of antiretroviral therapy on HIV-1 RNA in the cerebrospinal fluid (CSF)

HIV-1 rapidly enters the CNS during primary infection (66,67) and, if left untreated, may result in AIDS dementia complex (ADC) in as many as 5-15% of patients (68-71).

As peripheral blood only harbours 2% of the total virus load in the body, it is not self-evident that accomplishment of adequate suppression in the blood by definition coincides with a similar reduction of virus replication in other compartments of the body such as the central nervous system (CNS) and the lymphoid tissues. The CNS, which is naturally protected by the blood-brain, brain-CSF and the blood-CSF barriers (72), is considered an important sanctuary site where HIV-1 may persist, in spite of successful control of virus replication in blood.

The pathogenic interplay between HIV-1, the immune system and the brain in leading to CNS pathology remains enigmatic. Since brain specimens are seldom available during life most information stems from post-mortem studies (73-75). It has been demonstrated that the level of viral load in the brain per se does not fully explain the syndrome of ADC (74); multiple interactions between immune system cells, microglial cells and neurones may contribute to the pathogenesis of ADC. Although in patients with ADC a correlation has been found between the concentration of HIV-1 RNA in the CSF and the severity of their symptoms (76-81), high concentrations of HIV-1 RNA in the CSF are not found in all demented patients (79). Furthermore, CSF abnormalities like elevated HIV-1 RNA levels and increased cellular and protein content can also be found in neurologically asymptomatic HIV-1 infected patients (82-85), with uncertain clinical significance. Although the pathogenesis of ADC is unclear, therapy with ZDV has been shown to prevent ADC in a significant number of patients (86,87). It was surmised that the high level of penetration of this lipophilic agent into the CSF could explain this beneficial effect (88-90). This was considered an important property of ZDV, and resulted in the therapeutic guideline to always include ZDV as a component of antiretroviral drug regimens (91). However, intolerance or resistance to AZT make it necessary to investigate alternative drug regimens which may also have a prophylactic or therapeutic effect on ADC.

For this reason, as part of the comparative trial described in Chapter 2, we investigated the penetration into the CSF not only of ZDV, but also of 3TC and d4T,
and studied the effect of these both study drug regimens in reducing CSF HIV-1 RNA levels. The results of these investigations are described in Chapter 3.

2.1.3 A long-term follow-up of a comparative study of ZDV/3TC versus d4T/3TC: 72 week results

First generation HIV-1 RNA PCR-based assays had lower detection limits of around 1000-3000 copies (c)/mL, and were followed by second generation assays which are generally able to reliably measure HIV-1 RNA concentrations as low as 200-500 c/mL. Recently, so-called ultrasensitive assays have become available with lower limits of detection of 5-50 c/mL. It has been suggested that only suppression of plasma HIV-1 RNA to levels below the detection limit of ultrasensitive assays will result in a sustained suppression of HIV-1 replication (41).

In Chapter 4 we describe the magnitude and duration of plasma and CSF HIV-1 RNA suppression as measured by an ultrasensitive assay, using stored samples from participants of the trial comparing ZDV/3TC versus d4T/3TC (see Chapter 2 and 3), after 72 weeks of follow-up. Nowadays, the sequential addition of indinavir to the two NRTI’s would be considered a suboptimal approach. Therefore, it was particularly important to measure the effects of this strategy by the use of an ultrasensitive HIV-1 RNA assay, in order to be able to demonstrate whether this approach indeed had resulted in lesser degrees of plasma HIV-1 RNA suppression over the longer-term, than the reduction of plasma HIV-1 RNA achieved in other trials in which patients had been treated with triple drug regimens from the outset.

Moreover, as part of the neurologic substudy a lumbar puncture had also been performed at week 48. HIV-1 RNA levels in CSF from week 12 and 48 samples were reanalysed by using an ultrasensitive HIV-1 RNA assay and indinavir concentrations in the CSF were measured at week 48.

2.2 Antiretroviral therapy and opportunistic infections

2.2.1 Clinically relevant beneficial effects of antiretroviral therapy-associated immune recovery

By dysregulating cells that are involved in immunologic defences, (predominantly CD4+ lymphocytes), infection with HIV-1 leads to a progressive deterioration of most
immune functions. Infected CD4\(^+\) cells in blood are reduced in number as a result of various mechanisms which may include decreased renewal (92), trapping into the lymph nodes (93) and increased cell death (94-96). CD4\(^+\) lymphocytes may die because of direct HIV-1 related cytopathic effects and cytotoxic responses of CD8\(^+\) cells but also by the induction of apoptosis (96,97).

Within the CD4\(^+\) and CD8\(^+\) T-lymphocyte population in HIV-uninfected persons, subsets of memory and naive cells can be found in equal proportions. Memory cells represent the part of the T-cell repertoire that has been activated by exposure to a recall antigen. T-cell defence against previously encountered opportunistic pathogens therefore is believed to reside primarily within this subset of lymphocytes. Naive T-cells bear the potential to generate immune responses to newly encountered antigens and may evolve to memory T-cells thereafter. Apart from the quantitative loss of immunoreactive T-cells, qualitative T-cell responses to new or recall antigens have also been found to be decreased already in the early stages of HIV-1 infection (98-101). Beside the decreased number and function of CD4\(^+\) lymphocytes, impaired functions of other cell types involved in the immune system like those of antigen-presenting cells and natural killer cells, can also be found (102). It is clear that multiple mechanisms contribute to failure of the immune response in progression to AIDS. It is hypothesised that a decrease of lymphocytes with specific reactivity against certain antigens may lead to a gap in the immune repertoire (103,104), and loss of host defence against some frequently encountered opportunistic pathogens like Mycobacterium avium, Pneumocystis carinii and Toxoplasma gondii. In general, most opportunistic diseases are related to a specific stage of HIV induced immunodeficiency related to the number of CD4\(^+\) cells in the peripheral blood (105,106). Diarrhoea is frequently observed when CD4\(^+\) cell counts have dropped to below 50 cells/\(\mu\)L. If the diarrhoea is caused by infection with Enterocytozoon bieneusi or Cryptosporidium parvum effective therapy is not available.

Potent antiretroviral combination therapy has been demonstrated to result early on in strong rises of CD4\(^+\) lymphocytes, mainly of the memory phenotype, followed by a lesser and more protracted rise in cells of the naive phenotype (107). For this reason we investigated the effect of treatment with the protease inhibitor indinavir on chronic intractable diarrhoea of unknown aetiology or caused by infection with cryptosporidium or the microsporidian E. bieneusi. The results of this study are presented in Chapter 5.
2.2.2 Antiretroviral therapy-associated immunopathologic effects of immune reconstitution

Following the introduction of highly active antiretroviral therapy (HAART), we began to observe unusual clinical presentations of what turned out to be mycobacterial infection occurring within weeks to a few months after patients started HAART. Unlike what was expected, these clinical syndromes often coincided with impressive rises of CD4⁺ cell counts. Similar anecdotal cases were described subsequently for presentation of CMV-vitraxis, viral hepatitis and mycobacterial lymphadenitis (108-113).

Functional improvement of immunity, due to the recovery of the inflammatory response, will result in immunopathology in cases where the antigenic load of the antigen has become relatively high during the immunocompromised, pre-HAART, period.

To assess whether specific antimycobacterial cytotoxicity had increased with HAART, *in vitro* T-cell proliferation studies were performed using stored peripheral blood mononuclear cells samples from patients who had presented with unusual signs and symptoms of a mycobacterial infection shortly after commencing HAART. The clinical case histories of these patients and the results of the *in vitro* experiments are described in Chapter 6.


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Introduction


