Metabolic complications of antiretroviral therapy
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Citation for published version (APA):

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1 General introduction to current treatment of HIV-1 infection

For the treatment of HIV-1-infected patients three classes of antiretroviral drugs are widely available in the developed world. These consist of the nucleoside reverse transcriptase inhibitors (NRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and the protease inhibitors (PIs). In general, patients are being treated with combinations of three or more drugs from at least two of these classes. All licensed antiretroviral drugs are summarised in table 1. The mechanism by which replication of HIV-1 is inhibited by each of these three classes of antiretrovirals is summarised in the next paragraph. Subsequently, the current standard of care for the treatment of HIV-1-infected patients will be briefly discussed.

1.1 The different antiretroviral drug classes

Currently there are 7 licensed NRTIs. All of these have to be phosphorylated intracellularly into their respective active triphosphates. Because HIV-1 is a single-stranded RNA-virus reverse transcription of its RNA to double-stranded DNA by the virus-encoded reverse transcriptase has to take place prior to integration into the host cell DNA. The NRTI-triphosphates inhibit reverse transcription by being incorporated into the growing proviral DNA chain, thereby resulting in premature chain termination. [1-3]

Likewise the NNRTIs inhibit reverse transcription. However, unlike the NRTIs, NNRTIs do not need to be activated into an active moiety, but directly bind to the reverse transcriptase enzyme, thereby inhibiting its action.

The eight currently available PIs act by the inhibition of the HIV-1 specific protease enzyme. This viral enzyme is responsible for the post-translational processing of the gag-pol polyprotein that is crucial to allow the formation of infectious viral particles.
Chapter 1

This inhibition results in the formation of immature non-infectious particles, which are not able to enter and infect still uninfected host cells.

Table 1 Antiretroviral drugs currently licensed in Europe.

<table>
<thead>
<tr>
<th>Nucleoside reverse transcriptase inhibitors (NRTIs)</th>
<th>Non-nucleoside reverse transcriptase inhibitors (NNRTIs)</th>
<th>Protease inhibitors (PIs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>zidovudine (AZT, Retrovir\textsuperscript{tm})</td>
<td>nevirapine (Viramune\textsuperscript{tm})</td>
<td>indinavir \textsuperscript{\textsuperscript{1}}</td>
</tr>
<tr>
<td>lamivudine (3TC, Epivir\textsuperscript{tm})</td>
<td>efavirenz (Stocrin\textsuperscript{tm})</td>
<td>ritonavir \textsuperscript{\textsuperscript{1}}</td>
</tr>
<tr>
<td>didanosine (ddl, Videx\textsuperscript{tm})</td>
<td></td>
<td>saquinavir (Invirase\textsuperscript{tm})</td>
</tr>
<tr>
<td>zalcitabine (ddC, Hivid\textsuperscript{tm})</td>
<td></td>
<td>/Fortovase\textsuperscript{tm}</td>
</tr>
<tr>
<td>abacavir (Ziagen\textsuperscript{tm})</td>
<td></td>
<td>amprenavir (Agenerase\textsuperscript{tm})</td>
</tr>
<tr>
<td>stavudine (d4T, Zerit\textsuperscript{tm})</td>
<td></td>
<td>nelfinavir (Viracept\textsuperscript{tm})</td>
</tr>
<tr>
<td>tenofovir (Viread\textsuperscript{tm})</td>
<td></td>
<td>ritonavir-boosted lopinavir (Kaletra\textsuperscript{tm})</td>
</tr>
</tbody>
</table>

\* Recently enfuvirtide (T-20, Fuzeon\textsuperscript{tm}) from a new class of antiretroviral drugs, 'the fusion inhibitors', was registered in the United States for the treatment of HIV-1 infection in treatment-experienced patients with evidence of HIV-1 replication despite ongoing antiretroviral therapy.

1.2 Current treatment of HIV-1-infected patients

Prior to 1996 HIV-1 infected patients were generally treated with one or two NRTIs. In the great majority of patients such treatment, however, was insufficiently potent to provide sustained suppression of virus replication, resulting in the emergence of resistant viral strains, followed by progressive cellular immune-deficiency and ultimately clinical disease progression and death. [4] To avoid the emergence of drug resistance, current treatment strategies include the use of more potent combinations of three or more drugs, commonly known as highly
active antiretroviral therapy (HAART). In most cases two NRTIs are being combined with either a PI, a NNRTI or a third NRTI. Since the introduction of such potent combination antiretroviral therapy the morbidity and mortality of people living with HIV has declined dramatically in developed countries.[5,6]

2 The lipodystrophy syndrome

The use of combination antiretroviral therapy is frequently associated with side effects. One of these side effects is the lipodystrophy syndrome.[7-9] This syndrome consists of a redistribution of body fat. Typically, after the initiation of combination antiretroviral therapy, patients may complain of loss of fat from the face, buttocks and extremities (lipoatrophy). The latter is often first recognised by patients as superficial veins on the arms and legs becoming more prominently visible, as a result of subcutaneous fat loss. Lipoatrophy is often accompanied with an accumulation of visceral fat in the abdomen or less frequently an increase in the amount of fat in the neck (buffalo hump) or in the breasts. (Figure 1) In addition to these changes in fat distribution, a large proportion of patients develops insulin resistance and elevated plasma lipid concentrations. This combination of hyperlipidemia, insulin resistance and visceral fat accumulation closely resembles the abnormalities seen in ‘the Metabolic Syndrome’ [10] and has given rise to the concern that HIV-1 infected patients treated with potent combination antiretroviral therapy may be at increased risk of developing premature coronary artery disease. [11-15]
Figure 1 Patient with HIV-1-associated lipodystrophy syndrome

Pictures published after written informed consent and review of the pictures by the patient

2.1 Fat redistribution

By the end of 1997, the first patients were described with this abnormal fat distribution, resembling that seen in multiple symmetrical lipomatosis (Madelung’s or Launois-Bensau de syndrome) [16]. Although these abnormalities also resembled the abnormalities seen in Cushing syndrome, dexamethason suppression test and cortisol concentrations were normal, making Cushing syndrome an unlikely explanation.[9,17,18] Considering the fact that these abnormalities were not recognised prior to 1996, they were understandably initially attributed solely to the introduction of the PIs, which first became widely available in 1996. The increase in girth size was even designated “Crix-belly”, named after Crixivan\textsuperscript{tm} (indinavir), one of the first PIs to
be licensed. Several case-control studies were published, which all confirmed an association between the lipodystrophy syndrome and the use of HIV-1 PIs. [7,19] This did however not explain why some patients developed lipodystrophy without ever having been exposed to PIs but solely to NRTIs. [20]

There is considerable evidence from several cohort studies that exposure to both PI- and NRTI-based therapy seems independently related to the development of the changes in fat distribution. [7,20,21] The pathogenic mechanisms by which each of these two classes of antiretrovirals contribute to the development of lipodystrophy remains to be fully elucidated. Other known risk factors for lipodystrophy are increased age, longer duration of HIV-1-infection, more advanced infection and greater degree of virus suppression. The latter may in fact be the result of better adherence resulting in more exposure to antiretroviral drugs.

The objective of chapter 2 is to explore the contribution to the development of lipodystrophy of treatment with PIs alone, compared to the combination of PIs with an NRTI. In this chapter lipodystrophy was assessed in patients who as part of a clinical trial had been randomly allocated to treatment with the PIs ritonavir and saquinavir with or without the addition of the NRTI stavudine.

2.2 Hyperlipidemia

In the natural course of HIV-1 infection minor changes in the plasma lipoprotein profile have been observed. Early after infection with HIV-1 HDL-cholesterol slightly decreases.[22] This decrease is followed by a decrease in the size of LDL particles leading to a rise in more artherogenic small dense LDL particles (LDL type B).[23] Late in the infection once patients develop symptomatic HIV-1 disease triglyceride concentrations may rise.[24]
Following the introduction of PI-containing antiretroviral treatment numerous studies demonstrated that patients developed potentially more profound atherogenic changes in their lipoprotein profile, comprised of markedly elevated triglycerides, total cholesterol and LDL-cholesterol. This raised the concern of HIV-1 infected patients being at long-term increased risk of developing coronary artery disease.[12,14,15,25] This concern is supported by evidence from several cross-sectional studies comparing untreated HIV-1-infected patients with patients treated with protease inhibitors that suggest that protease inhibitor based therapy is associated with a decrease in endothelial function and an increase in carotid intima media thickness.[11,26-28] Moreover, in healthy volunteers two weeks of treatment with the PI ritonavir was shown to result in elevations of triglycerides, apolipoprotein B and VLDL-cholesterol.[29] This observation strongly suggests that PIs directly contribute to the potentially deleterious effects on lipids. The most pronounced changes are seen with ritonavir-based regimens, but all currently licensed PIs to some extent result in hyperlipidemia.

In chapter 3 we explore the effects of treatment with PI-based therapy on the lipoprotein profile and compare these effects of treatment with either a NNRTI or a NRTI-based regimen. In Chapter 4 these results are put into perspective with respects to their potential implications for the optimal management of HIV-1 infected patients.

2.3 Insulin resistance

In addition to the very stigmatising changes in body fat distribution and the induction of hyperlipidemia the use of PIs is also associated with marked disturbances in glucose metabolism. In contrast, in the natural course of HIV-1 infection no changes in glucose metabolism were observed, and if anything insulin sensitivity was demonstrated to be slightly increased.[30] Following the introduction of the PIs this picture has dramatically changed. Approximately 30 to 40% of patients treated
with protease-inhibitor containing therapy develop impaired glucose tolerance.[31] Several studies have demonstrated this to be the result of insulin resistance in HIV-1 infected patients suffering from the lipodystrophy syndrome.[7,8] Insulin resistance is frequently described only in terms of impairment of the effects of insulin on the uptake of glucose in peripheral tissues. It is important to note however that insulin influences several additional metabolic pathways. In addition to the fact that insulin increases the peripheral uptake of glucose, it decreases endogenous glucose production, which occurs mainly in the liver and kidney, and it decreases the rate of lipolysis and protein breakdown.

This raises the question which of these aspects of glucose metabolism are disturbed in the lipodystrophy syndrome. In chapter 5 we assess the different effects of insulin in patients with severe lipodystrophy while using PI-based therapy.

2.4 Disturbances in lipolysis and energy expenditure

In HIV-1-infection, like in several other chronic diseases resting energy expenditure, is increased by approximately 10% when compared to healthy controls, as was demonstrated by M. Hommes in her academic thesis.[32,33] This increase in energy expenditure does not result from an increase in plasma catecholamines, thyroid hormones or cortisol concentrations [33] and seems to occur regardless of the stage of HIV-1 infection.[34] In addition, our group has previously shown that in the natural course of an HIV-1-infection the increase in basal metabolic rate is accompanied by an increase in lipolysis, with a normal lipolytic response to an infusion of epinephrine.[35] The cause for this increase in basal metabolism and lipolysis is yet unresolved, although increased cytokine production, especially of tumor necrosis factor-α (TNF), might be involved in the pathogenesis. For instance, in untreated HIV-1-infected patients the plasma concentrations of soluble TNF receptor (sTNF) are increased, suggestive of an increased cytokine production,
when compared to healthy controls. [36] However, this increase in sTNF does not correlate with the extent of elevation in resting energy expenditure.[34]

Since lipoatrophy by definition may only result from an increased release of fat from the affected peripheral adipocytes, it seems likely that lipolysis plays a role in the development of these changes. A potential explanation for this increase in lipolysis might be an increase in the basal energy expenditure, which is likely to result in an increase in substrates for fat oxidation. In chapter 6 we explore the effect HIV-associated lipodystrophy has on lipolysis and energy expenditure. Furthermore we assess the lipolytic response to an epinephrine infusion in patients suffering from this condition. Another explanation for lipoatrophy may be decreased uptake of fatty acids into adipose tissue, which was not addressed in this thesis.

2.5 Potential pathogenesis of the syndrome

After the association between lipodystrophy syndrome and the use of PIs was first described, a hypothesis was proposed which postulated that PIs might disturb two proteins which regulate lipid metabolism. This was based on a 60% homology between the HIV-1 protease encoding gene and the host genes encoding two enzymes crucial in lipid metabolism (cytoplasmic retinoic-acid binding protein type 1 and low density lipoprotein-receptor-related protein).[37] This hypothesis assumed an accumulation of plasma lipids as a result of impaired function of these two enzymes, eventually resulting in visceral fat accumulation. Although protease inhibitors have subsequently been demonstrated to inhibit adipocyte differentiation in vitro, no other evidence supportive for this hypothesis has been presented.[38] More importantly, this hypothesis failed to explain the subsequent finding that some patients develop lipodystrophy without ever having been exposed to PIs but solely to NRTIs. [20]
In 1999 an alternative hypothesis for the development of lipodystrophy was proposed in which the loss of peripheral fat was explained by a selective inhibition by NRTIs of mitochondrial DNA replication in peripheral adipocytes, eventually resulting in a loss of mitochondrial function and apoptosis of these peripheral adipocytes.[39] Since 1991 it has been known that the NRTI interfere with the replication of mitochondrial DNA, by a selective inhibition of DNA polymerase-γ, the mitochondrial enzyme which plays a key role in the replication of mitochondrial DNA. [40] The various individual NRTIs markedly differ in their capacity to inhibit DNA polymerase-γ in vitro (zalcitabine > didanosine > stavudine > zidovudine > lamivudine = abacavir = tenofovir). [41] This so-called ‘mitochondrial toxicity’ has been implicated in the development of several NRTI associated side effects outside the scope of this thesis (peripheral neuropathy by zalcitabine, didanosine and stavudine, pancreatitis by didanosine and stavudine, and bone marrow suppression and myopathy by zidovudine). The tissue specificity of these different mitochondria-related side effects remains intriguing and may be the result of different degrees of tissue penetration and pharmacological processing of the various NRTIs.

Since there is considerable difference in the extent to which the different NRTIs inhibit DNA polymerase-γ as stated above, a difference in the incidence of lipoatrophy between the different NRTIs may be expected. Several cohort studies had demonstrated the incidence of lipoatrophy to be higher in patients using stavudine- compared to zidovudine-based therapy, which are the two commonly used NRTIs. [20,42-44]

In chapter 7 we extensively assess fat distribution, as well as mitochondrial DNA, in patients who in the past had been randomly allocated to first initiate treatment with either a stavudine or zidovudine-based regimen.
2.6 The role of protease inhibitor replacement

As outlined above, it initially seemed likely that PIs played a key role in the development of the lipodystrophy syndrome. We therefore designed a clinical trial to investigate the reversibility of the various aspects of the lipodystrophy syndrome, when replacing the PI component of patients' antiretroviral regimens by the NRTI abacavir. In chapter 8 we characterise the effects on the disturbances in glucose metabolism and lipolysis in subjects with severe lipodystrophy, 96 weeks after replacement of PI by abacavir. We postulated that such a trial would also improve our understanding of the contribution of PIs to the derangements in glucose and lipid metabolism. Assuming that any potential beneficial changes observed might occur as a result of an improvement in fat distribution, we also assessed body composition, focussing on fat distribution in the participants of this study.
3 Brief outline of thesis

In Chapter 2 we explore the development of lipodystrophy in patients randomly allocated to protease inhibitor with or without the addition of a NRTI.

In Chapter 3 the effects of treatment with protease inhibitor based therapy on the lipoprotein profile is compared to that of treatment with either a non-nucleoside or a nucleoside reverse transcriptase inhibitor-based regimen in patients randomly assigned to either of such three treatment regimens.

In Chapter 4 the results observed in the study described in chapter 3 are put into perspective with respect to their potential clinical implications, including for the individualized treatment of HIV-1 infected patients.

In Chapter 5 we characterize glucose metabolism in patients with severe lipodystrophy. Lipodystrophy was defined as an evident loss of peripheral fat with or without visceral fat accumulation while using protease inhibitor based therapy. These results were compared to those obtained in age, gender and body mass index-matched healthy volunteers.

In Chapter 6 we explore the effect HIV-1-associated lipodystrophy has on the rate of lipolysis and on resting energy expenditure, and compare this to data obtained in asymptomatic HIV-1 infected patients. Furthermore, we compare the lipolytic response to an epinephrine infusion in these two groups of patients.

In Chapter 7 we objectively assess the presence of lipoatrophy, and quantify mitochondrial DNA content in peripheral blood mononuclear cells as well as mitochondrial DNA content in fat biopsies in patients.
who in the past had been randomised to initiate either stavudine- or zidovudine-based therapy.

In Chapter 8 the effect is described on glucose metabolism, lipolysis and fat distribution in patients with lipodystrophy, 96 weeks after withdrawal of protease inhibitors from their treatment regimen.
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