Clinical and pharmacological aspects of induction-maintenance therapy in HIV-1 positive patients: the ADAM study
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Chapter 5

The effect of plasma drug concentrations on HIV-1 clearance rate during quadruple drug therapy.

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Chapter 5

Abstract

Objective

To investigate the relationship between exposure to antiretroviral drugs and the initial decline of plasma HIV-1 RNA.

Design

Open-label study in antiretroviral naive HIV-1 infected patients using a quadruple drug regimen (nelfinavir (NFV), saquinavir (SQV), stavudine, and lamivudine).

Methods

The elimination rate constant (k) for HIV-1 clearance was calculated during the first 2 weeks of treatment in 29 patients. Exposure to NFV and SQV was quantified on each study visit. Observed NFV and SQV concentrations were related to those expected in a reference population and a concentration ratio was calculated. The median concentration ratios for NFV and SQV, the baseline CD4+ lymphocyte count and baseline log_{10} HIV-1 RNA were correlated with k.

Results

A significant positive correlation was observed between k and the median NFV (p=0.001) or SQV concentration ratio (p=0.016) in a univariate analysis. In multivariate analyses, the median NFV concentration ratio remained significantly correlated with k.

Conclusions

The variation in the rate of decline of plasma HIV-1 RNA between patients after the initiation of a quadruple drug regimen could be explained by differences in exposure to NFV or SQV. Determination of k could be used to optimize further antiretroviral drug therapy and may be a first tool to assess antiretroviral activities of new or increasing doses of drugs administered in combination regimens. Furthermore, our data suggest that exposure to antiretroviral drugs should be incorporated in mathematical models to describe HIV-1 dynamics in more detail.
Drug exposure and HIV-1 clearance rate

Introduction

The finding that replication of HIV-1 in vivo occurs continuously at high rates and the increased availability of antiretroviral drugs has led to fundamental changes in treatment strategies.\(^1\),\(^2\) They have given rise to the widespread opinion that the minimum goal of antiretroviral drug therapy should be to suppress HIV replication as much as possible for as long as possible.\(^3\) Several mathematical models have been used to describe the dynamics of HIV-1 replication in vivo.\(^1\),\(^2\),\(^4\),\(^5\) These models assume full (or at least constant) inhibition of viral replication by antiretroviral drugs in each patient. However, interindividual variability in exposure to antiretroviral drugs is large.\(^6\) This could result in different degrees of inhibition of viral replication between patients treated with the same drug regimen. We have investigated the relationship of exposure to antiretroviral drugs and HIV-1 clearance rates in the Amsterdam Duration of Antiretroviral Medication (ADAM) study.
Material and methods

ADAM is an ongoing, multicenter, open-label, randomised study to investigate the feasibility of long-term suppression of viral replication by a quadruple induction therapy followed by a double drug maintenance therapy after 6 or 12 months in antiretroviral-naive HIV-1-infected patients. Induction therapy consists of a regimen containing 750 mg thrice daily nelfinavir (NFV), 600 mg thrice daily saquinavir (SQV), 40 mg twice daily stavudine (d4T), and 150 mg twice daily lamivudine (3TC). Eligible patients have CD4+ lymphocyte counts $\geq 200 \times 10^6$ cells/L and $\geq 1,000$ copies HIV-1 RNA/ml.

Patients

Patients included in the ADAM study with at least two evaluable NFV and SQV concentrations during the first 8 weeks of therapy, and with viral load data available until 15 November 1997, were included in this substudy. Patients were instructed to ingest NFV and SQV simultaneously during a meal. The study protocol was approved by local reviewing boards and all patients gave written informed consent.

HIV-1 RNA dynamics

HIV-1 RNA levels in plasma were measured using commercially available assays (NASBA HIV-1 RNA QT and NucliSens, Organon Teknika, Boxtel, the Netherlands) according to the instructions of the manufacturer. Initially the NASBA HIV-1 RNA QT assay was used. When HIV-1 RNA levels declined to $< 1,000$ copies/ml (the lower limit of detection of the assay), levels were quantified using the NucliSens assay. Subsequently, when levels declined to $< 400$ copies/ml, an ultrasensitive protocol was used. Briefly, the RNA purified according to the NucliSens protocol was eluted in a two-step procedure from the silica particles. Subsequently, RNA was concentrated by precipitation with ethanol and sodium acetate in the presence of a pellet dye (pellet paint co-precipitate; Novagen, Madison, Wisconsin, USA). The complete RNA pellet was then used in the NucliSens RNA amplification and detection procedure according to the instruction of the manufacturer. In case of the ultrasensitive protocol, the lower limit of detection of the assay was 50 copies HIV-1 RNA/ml.

HIV-1 RNA levels in plasma were determined at baseline, and at days 7, 14, 28, and 56. For calculating the HIV-1 RNA clearance rate in plasma an exponential function was used to describe the rate of HIV-1 RNA decline during the first two
Drug exposure and HIV-1 clearance rate

weeks for each patient. The following function was used to describe the decline of viral load (first-order elimination):

\[ V(t) = V(0) \times e^{kt} \]

where \( V(0) \) represents plasma HIV-1 RNA in copies/ml at time \( t \), \( V(0) \) represents the baseline viral load, \( k \) is the elimination rate constant (day\(^{-1}\)), and \( t \) is the time after start of treatment (days). All HIV-1 RNA measurements \( \geq 50 \) copies/ml were used for each patient from the start of treatment until a value below 50 copies/ml was reached (including this measurement) or until day 14. For each patient the value for \( k \) was estimated using least squares regression analysis (\( \ln V(t) \) versus \( t \) plot). The half-life of clearance of HIV-1 RNA in plasma was calculated with the equation \( t_{1/2} = \ln 2/k \).

NFV and SQV exposure

On each study visit a blood sample was drawn for the quantitation of plasma concentrations of NFV and SQV using a validated and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) assay. The time between drug ingestion and the drawing of the sample was noted. NFV and SQV plasma concentrations were divided by the expected concentrations of NFV and SQV, respectively, at the same time after drug administration in a reference population of 18 patients in whom full (8-h) pharmacokinetic profiles of both drugs were assessed. By this means, concentration ratios for NFV and SQV were calculated at days 7, 14, 28, and 56 to estimate exposure to both protease inhibitors adequately. For each patient, at least two NFV and SQV concentration ratios had to be evaluable (e.g. the time interval between drug ingestion and drawing of the sample was known). Individual median values for NFV and SQV concentration ratios were used as a measure of exposure to these drugs in each patient.

Statistical analysis

Univariate and multivariate linear regression analysis was performed with \( k \) as the dependent variable. Median NFV and SQV concentration ratios, baseline CD4+ lymphocyte count, and baseline log\(_{10}\) HIV-1 RNA were used as independent variables. Statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 6.1 (SPSS Inc., Chicago, Illinois, USA). A significance level of \( P=0.05 \) was used throughout.
Chapter 5

Results

HIV-1 RNA measurements in plasma were available from 34 patients in the ADAM study during the first 56 days after start of treatment at the time of analysis. All patients had HIV-1 RNA levels < 50 copies/mL at day 56. For 5 patients, less than two evaluable NFV and SQV concentration ratios were available. Thus, it was possible to include a total of 29 patients in this substudy.

The median values and interquartile ranges for $k$, baseline viral load, baseline CD4+ lymphocyte count, and median NFV and SQV concentration ratios in this population of 29 patients are presented in Table 1. In our reference population of 18 patients the average values for area under the plasma concentration versus time curve (during 8 hours), maximum plasma concentration, and plasma elimination half-life were 18.1 h*mg/l, 5.2 mg/l, and 2.0 h for NFV, and 2.37 h*mg/l, 0.57 mg/l, and 2.3 h for SQV, respectively.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of baseline characteristics, HIV-1 RNA clearance rates, and exposure to protease inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline characteristics</td>
<td>median</td>
</tr>
<tr>
<td>HIV-1 RNA (copies/ml)</td>
<td>58,000</td>
</tr>
<tr>
<td>$\log_{10}$ HIV-1 RNA (copies/ml)</td>
<td>4.76</td>
</tr>
<tr>
<td>CD4+ lymphocytes (x 10^6 cells/l)</td>
<td>410</td>
</tr>
<tr>
<td>HIV clearance rates</td>
<td></td>
</tr>
<tr>
<td>$k$ (day$^{-1}$)</td>
<td>0.29</td>
</tr>
<tr>
<td>$t_{1/2}$ (days)</td>
<td>2.42</td>
</tr>
<tr>
<td>median NFV concentration ratio</td>
<td>0.83</td>
</tr>
<tr>
<td>median SQV concentration ratio</td>
<td>0.93</td>
</tr>
</tbody>
</table>

$k$ = elimination rate constant for HIV-1 RNA in plasma, NFV = nelfinavir, SQV = saquinavir

Univariate linear regression analysis was performed using $k$ as the dependent variable, and median NFV and SQV concentration ratios, baseline CD4+ lymphocyte count and baseline $\log_{10}$ HIV-1 RNA as independent variables. Results of the univariate linear regression analysis are shown in Table 2. NFV and SQV concentration ratios were significantly and positively correlated with $k$ (Figure 1).
Figure 1  Relationship between (A) saquinavir concentration ratio and (B) nelfinavir concentration ratio and the elimination rate constant k for the clearance of HIV-1 RNA from plasma.
Median concentration ratios of NFV and SQV were significantly correlated (p=0.030). If NFV and SQV concentration ratios were simultaneously analysed in a multivariate linear regression model, the median NFV concentration ratio remained significantly related with k in contrast to the median SQV concentration ratio (Table 2). If baseline CD4+ lymphocyte count was added to this model, the NFV concentration ratio was still significantly correlated with k.

Table 2  Univariate and multivariate linear regression analysis using k as the dependent variable.

<table>
<thead>
<tr>
<th>independent variable</th>
<th>Univariate linear regression analysis</th>
<th>Multivariate linear regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>coefficients</td>
<td>p</td>
</tr>
<tr>
<td>median NFV concentration ratio</td>
<td>0.108 (0.031)</td>
<td>0.001</td>
</tr>
<tr>
<td>median SQV concentration ratio</td>
<td>0.055 (0.022)</td>
<td>0.016</td>
</tr>
<tr>
<td>baseline CD4+ lymphocyte count*</td>
<td>- (0.0084)</td>
<td>-0.064</td>
</tr>
<tr>
<td>baseline log_{10} HIV-1 RNA</td>
<td>0.016</td>
<td>-</td>
</tr>
</tbody>
</table>

k = elimination rate constant for HIV-1 RNA in plasma, NFV = nelfinavir, SQV = saquinavir, *per 100 CD4 cells.
Figures are regression coefficients (with SE=standard error), and p-values.
Discussion

In this study an approximately four-fold variation in the elimination rate constant for HIV-1 RNA clearance in plasma was observed (range: 0.13 - 0.49). We have made an attempt to explain this variation, at least in part, by the differences in exposure to antiretroviral drugs between patients.

The patients in the current study used two protease inhibitors (NFV and SQV) and two nucleoside reverse transcriptase inhibitors (d4T and 3TC). Since the nucleoside analogues require intracellular phosphorylation to their active triphosphates, and plasma concentrations do not necessarily reflect the intracellular amount of pharmacological active triphosphates, these drugs were not taken into account. Protease inhibitors, on the other hand, do not require an activation step, and relationships between plasma concentrations and antiretroviral effect have been reported. Thus, we decided to investigate the relationship between exposure to both protease inhibitors and HIV-1 RNA clearance rates.

Univariate linear regression analysis revealed that baseline CD4+ lymphocyte count and baseline log<sub>10</sub> HIV-1 RNA did not correlate with k. However, significant positive relationships were found between the median NFV or SQV concentration ratio and k (Fig. 1). Since median SQV and NFV concentration ratios were positively correlated, and baseline CD4+ lymphocyte count almost reached significance in the univariate analysis, these parameters were analysed in multivariate linear regression analyses. The baseline CD4+ lymphocyte count was not correlated with k in a multivariate analysis. The NFV concentration ratio remained significantly related with k in contrast to the SQV concentration ratio. This observation can be interpreted in several ways. It could mean that NFV has a stronger effect than SQV on the initial elimination rate of HIV-1 RNA in plasma when used in this drug regimen. On the other hand, the effect of SQV on viral replication could be maximal at observed plasma concentrations (as opposed to NFV), and a relationship between exposure and k could therefore not be found at currently observed concentrations. Furthermore, SQV and NFV concentrations are significantly correlated due to the fact that NFV increases SQV concentrations in plasma. This correlation makes it difficult to separate the effects of both protease inhibitors in this study.

A more rapid decline of HIV-1 RNA in plasma after start of treatment may be important to prevent the emergence of drug-resistant virus. Recently, the results of AIDS Clinical Trials Group 343 pointed out that the time to HIV-1 RNA load below 200 copies/mL was a risk factor for treatment failure. This observation justifies that a
fast decline of HIV-1 RNA after start of therapy should be investigated as a potential objective of antiretroviral therapy.

The decrease of HIV-1 RNA in plasma was described during the first two weeks of therapy by measuring the HIV-1 RNA load on three occasions. A more frequent sampling strategy would have resulted in a more accurate estimate of k. The currently observed values for k are somewhat lower than reported by Ho et al.1 and Perelson et al.5 This can be explained by the less frequent sampling schedule in our study and the gradual decrease of the HIV-1 RNA decline after start of treatment. The possibility of an underestimation of k does not affect the primary outcome of this study (a relationship exists between drug exposure and the elimination rate constant). In this study we have used single timepoint measurements to estimate the exposure to drugs in the patients. Determination of the area under the concentration versus time curve might have been more accurate to estimate exposure of these drugs, but is also less practical.

The main outcome of this study is that higher exposure to NFV or SQV resulted in a higher clearance rate of HIV-1 RNA in this population. This observation could be used for further improvement of the initial response to antiretroviral drug therapy in individual patients by optimising plasma drug concentrations (through dose adjustment). Assessing the value of k could also be used as a first tool to measure antiretroviral activity of new or increasing doses of drugs in combination therapy regimens. Furthermore, up to now, mathematical models to describe HIV-1 dynamics in vivo have assumed full (or at least constant) inhibition of viral replication in all patients treated with the same drug regimen.5 The currently reported results justify, however, that exposure to antiretroviral drugs should be taken into account in these models to describe HIV-1 dynamics in vivo more accurately.

**Acknowledgement**

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Drugs exposure and HIV-1 clearance rate

References


