Stavudine plasma concentrations and lipoatrophy

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Objectives: The objective of this study was to determine the correlation between plasma stavudine concentrations and lipoatrophy (LA), one of the major adverse events in patients on stavudine and one of the major reasons to discontinue stavudine.

Methods: Plasma drug concentrations were retrospectively analysed in patients who were on a stavudine-containing regimen for at least 12 months. We defined two groups of patients: 21 patients with LA and 15 patients without LA or other stavudine-related side effects (i.e. neuropathy).

Results: We analysed stavudine concentrations in 212 plasma samples: 87 in the control group and 125 in the LA group, with a mean of four plasma samples per person (at least two a year). Demographics were comparable in LA patients and controls, except the duration of stavudine use, which was longer in the LA group: 55 versus 42 months in the control group. Overall, LA patients had higher drug exposure to stavudine when compared with the controls, and this was seen in the geometric concentration ratios (CRs), which were 0.978 and 0.741, respectively (P = 0.04), and also a higher percentage of CR values >1.0, representing a drug concentration above the normal population curve (46% versus 23%, P = 0.02). In addition, the duration of stavudine therapy was independently associated with LA (P = 0.05). In the multivariate analysis, both duration of stavudine (P = 0.05) and CR > 1.0 (P = 0.02) were independently correlated with LA.

Conclusions: Monitoring of plasma stavudine concentrations can be useful to prevent stavudine-related LA.

Keywords: HIV infection, pharmacokinetics, nucleoside reverse transcriptase inhibitors, stavudine serum levels

Introduction

Thymidine nucleoside reverse transcriptase inhibitor (tNRTI)-containing regimens have been associated with various adverse events (AEs) such as lipoatrophy (LA), peripheral polyneuropathy and lactic acidosis. These AEs seem to be class-specific, even though the pathophysiology is not yet fully elucidated. In particular, the development of LA is one of the major concerns for patients and physicians when choosing drugs for a first-line antiretroviral regimen. Several prospective studies have shown evidence that side effects are dependent on plasma drug concentrations.9-12 There are conflicting data about the best representative of NRTI levels, intracellular or plasma.13 To assess the clinical use of plasma stavudine concentrations and their relationship with the development of toxicity, we performed a study regarding plasma stavudine levels in relation to LA. A major impediment for the success of HIV treatment is poor adherence, which is often a

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result of side effects. In this respect, LA is the most feared event by the patient. With this in mind, we wanted to investigate the relationship between stavudine plasma levels and LA appearance.

The purpose of this study was to verify whether the side effect LA, which often occurs after at least several months of therapy, could be related to elevated plasma concentrations of stavudine. In this way, drug monitoring can be helpful in the decision to change a probable toxic antiretroviral regimen to a less toxic regimen that can be continued for a prolonged time, resulting in chronic suppression of HIV infection.

Patients and methods

Study design

Plasma concentrations are routinely measured in all HIV patients on antiretroviral therapy visiting our outpatient department. We analysed the data of all patients who used or had used stavudine in the period 1996–2006. We analysed our database to find all patients on a stavudine-containing regimen. All patients had to be on their first antiretroviral therapy regimen. From our database, we selected 106 patients with a stavudine-containing regimen. Patients were divided into two groups based on the development of AEs due to stavudine. Patients who used or had used stavudine without experiencing AEs were enrolled in group 1 (32 controls). Patients who experienced LA due to stavudine were enrolled in group 2 (49 LA patients). Patients with other AEs due to stavudine were not enrolled. Because body shape changes with fat accumulation (lipohypertrophy) have been largely associated with protease inhibitors (PIs), we excluded patients who experienced only lipohypertrophy.14,15

Because LA mostly occurs after at least several months of therapy, we decided to enrol only patients who used or had used stavudine for at least 12 months.16–18 After correction for duration of therapy, 36 patients were left from the initially selected 81 patients on a stavudine-containing regimen (Figure 1). Of all the patients meeting the criteria, we retrospectively measured two samples a year with an interval of at least 4 months between the samples. LA was defined as peripheral fat loss (face, buttocks and extremities) and lipohypertrophy as central fat accumulation (abdominal, neck and breasts), both reported by the patient and confirmed by physical examination (i.e. waist-to-hip ratio measurements and lipodystrophy scoring system).19,20 Patients who were clinically suspected of severe abdominal fat accumulation had indeed increased intra-abdominal fat tissue (evaluated by DEXA or CT scan).

Concentration ratio (CR)

A CR was calculated for every plasma sample by comparing the stavudine plasma level of the patient with time-adjusted stavudine plasma concentration for the population. Based on multiple measurements over time, for every individual, the geometric mean value of the stavudine CR value was calculated. In addition, we looked at CR levels >1.0, which represent higher exposure when compared with the overall population.21 For a normal distribution, we used the geometric mean CR.

Ethics

All our HIV-infected outpatients are asked for informed consent to use their demographic data in an anonymous database. Maintenance of our database and its use for retrospective analysis of patient outcomes are approved by the Ethics Committee.

Drug monitoring

Stavudine plasma concentrations were determined by an HPLC assay, as reported previously.22 The volume of the plasma sample was 500 µL. The lower limit of quantification is 0.015 mg/L. Average accuracy ranged from 98% to 101% and the precision ranged from 1.3% to 2.2%.

Statistical analysis

Statistical evaluation was performed with SPSS for windows, version 11.0. Geometric mean values with 95% confidence intervals were calculated for all samples in each individual. For the analysis, a P value of ≤0.05 was considered significant. We performed a logistic multiple regression analysis with LA (present or not present) as the outcome variable versus demographic factors, co-medication in highly active antiretroviral therapy (except NRTI because this was lamivudine in the majority of the patients) and CRs. In addition, the variable duration of treatment was analysed as an independent factor for LA.

Results

Retrospectively, we analysed plasma samples in 36 patients using stavudine. Plasma stavudine concentrations were measured in 15 controls (10 males and 5 females) and 21 LA patients (15 males and 6 females). A total of 212 samples were analysed; 87 in the control group and 125 in the LA group. The range of samples per patient varied from 2 to 10, with a mean of four samples per individual in both groups. The mean age at the start of stavudine was 40 and 39 years in the control and LA groups, respectively. There was an equal percentage of females in the two groups (33% in controls and 29% in LA patients). In both groups, the majority of patients were Caucasian; 60% and 86% in the control and LA groups, respectively. In the control group, there were four Black patients (27%), one Asian patient (7%) and one Hispanic patient (7%). Furthermore, the LA group consisted of two Black patients (10%) and one Asian patient (5%). The mean CD4 cell count at initiation of stavudine was 210 and
205 cells/mm³ in the control group and in the LA group, respectively \((P = 0.84)\). In the control group, 10 of 15 patients were on a non-NRTI (NNRTI)-containing regimen and the other 5 used a PI versus 10 of 21 patients using a NNRTI and 11 patients using a PI in the LA group \((P = 0.33)\). The concomitant medication consisted of lamivudine in both groups (only one patient in every group was on didanosine). Except for duration of therapy, which was longer in the LA group, there were no statistically significant differences in patient characteristics between the groups. The duration of stavudine was 55 months (range 30–84) in the LA group and 42 months (range 15–86) in the control group. The dosage of stavudine was 40 mg twice daily; only one patient in each group used 30 mg twice daily due to low body weight \(<60$ kg\).

The overall drug exposure to stavudine expressed as geometric mean values was higher in the LA group when compared with the control group, 0.978 and 0.741, respectively (median values 0.683 in controls versus 0.864 in LA patients, \(P = 0.04\); Figure 2). In both groups, there was one value far above the other geometric mean values (Figure 2), which did not lead to statistical significant differences. In addition, CR values >1.0, representing a drug concentration above the normal population curve, were more often seen in the LA group than in the control group (mean 46% versus 23%, median 43% versus 13%, \(P = 0.02\); Figure 3). Table 1 provides an overview of the results.

In the multivariate regression analysis, duration of stavudine therapy \((P = 0.05)\) and CR level >1.0 \((P = 0.02)\) were both independently associated with LA.

No association between body weight and stavudine levels was found.

**Discussion**

As clinicians and patients are often confronted with side effects, we wanted to evaluate drug exposure in patients on antiretroviral therapy. Our analysis showed higher plasma stavudine levels in patients with LA when compared with those without AEs. Besides, these patients had more often a CR >1.0, representing a drug concentration above the normal population curve. Yet, no such analysis concerning NRTIs has been reported. Our results confirm earlier data reporting longer duration of stavudine use in patients experiencing LA, but we show that high exposure to stavudine is an independent predictive factor.\(^{16–18}\)

Until now, therapeutic drug monitoring (TDM) has been successfully applied to NNRTIs and PIs and to a lesser extent for NRTIs. Efficacy of NRTIs has not been correlated with plasma drug levels because these drugs must be converted into active intracellular metabolites to become active. Intracellular concentrations of NRTI-triphosphate compounds have been related to plasma HIV-RNA levels and CD4 cell counts.\(^{23}\) There is only limited experience with the use of NRTI drug monitoring. There are two studies by Fletcher \(^{13,24}\) that investigated concentration-controlled NRTI management in zidovudine and lamivudine therapy. They especially compared antiviral response in fixed-dose regimens with concentration-controlled regimens; however, the trials did not assess NRTI-associated toxicity in relation to plasma levels.\(^{13,24}\)

For dose-related AEs, patients who experience AEs are hypothesized to be those with higher plasma concentrations. Possible reasons for this phenomenon might be greater absorption, slower metabolism, change in drug transporters or reduced body weight and thereby a small volume of distribution.\(^{25–28}\)

For example, Gatti \(^{8}\) discovered a clear relationship between ritonavir plasma concentrations and side effects. In addition, Dieleman \(^{9}\) et al. found elevated indinavir plasma concentrations in patients with crystalluria. Recently, a correlation was found between neuropsychiatric AEs and efavirenz plasma levels in HIV patients receiving long-term therapy with efavirenz.\(^{12}\) Patients with higher efavirenz plasma levels were at risk of CNS toxicity. Additionally, increased bilirubin levels are associated with elevated concentrations of lopinavir, indinavir and atazanavir.\(^{10,11}\)
Our data provide interesting insights into possible use for TDM and/or lower doses of stavudine. Our data suggest that the occurrence of LA can be predicted by higher plasma drug concentrations of stavudine. In this way, tolerance of antiretrovirals can be improved by avoiding excessive plasma concentrations. By monitoring the drug levels of stavudine or starting with a lower dose of stavudine, toxicity can be found in an early phase when irreversible damage could still be prevented. A lower dose of stavudine with or without drug monitoring may contribute to the safe use of stavudine in therapy-naive and NRTI-experienced patients who need this antiretroviral drug. Eventually, stavudine plasma monitoring can be applied in stavudine-naive patients to prevent LA by adapting the stavudine dose when CR exceeds the normal level of 1.0. In the same way, stavudine can be applied in patients experiencing AEs from other NRTIs. Few studies looked at the use of a lower dose of stavudine to reduce side effects.29–31 Under strict control of viral load, it was possible to reduce stavudine dosage resulting in less side effects when compared with standard dose regimens. Recently, an interesting study investigated the role of lowering stavudine dose or switching to tenofovir, compared with standard stavudine dose in patients on long-term stavudine therapy.32 Lowering stavudine dose was associated with improvement of LA. No virological failures were reported in both groups. However, none of these studies looked at plasma stavudine concentrations. Combining these data and our results, we suggest that reduction to a twice-daily dose of 30 mg stavudine might be safer and even effective in a majority of patients. In addition, we suggest that a plasma stavudine CR < 1.0 (for example, 0.8–1.0) might be a safe level for the treatment of HIV patients to prevent LA. Although the above-mentioned studies described efficacious control of HIV, detailed studies have to be performed to prove this hypothesis. Interestingly, a recent addendum to the WHO guidelines on antiretroviral therapy for HIV infection in adults and adolescents now recommends a lower dosage for stavudine (30 mg twice daily).

Owing to genetic and environmental factors, there is a wide inter-patient variability when measuring drug exposure to a standard dose. Earlier studies have proven a relationship between drug exposure and efficacy or toxicity.9,26,33–37 This inter-individual variability makes drug monitoring useful in these specific situations to predict not only virological response but also toxicity. Because symptoms are not always present or appear only when an irreversible phase has been reached, timely identification of toxicity is essential for the optimal treatment of HIV patients.

Although it is frequently stated that plasma concentrations do not correlate with intracellular active NRTI-triphosphate metabolites, our data show that plasma NRTI concentrations give a good reflection of toxicity.23,35,38 Therefore, high plasma drug levels of stavudine are probably a useful reflection of its intracellular concentrations. Until now, less attention has been paid to the monitoring of plasma concentrations of NRTIs in contrast to PIs and NNRTIs. In the absence of easier techniques for measuring NRTI in drug monitoring, plasma NRTI levels can be well used as a replacement of intracellular measurement.

It has not clearly been elucidated which factors might additionally have played a role in the elevated plasma drug levels in the LA group. Enhanced plasma concentrations may have a multifactorial origin: absorption, elimination, cytochrome P450 system (isoenzyme CYP3A4), gender, liver or urinary clearance, drug–drug interaction or co-medication for other diseases.39–45 This study was not intended to look at these factors although we compared the latter two factors between the groups and did not find significant differences. In addition, in the controls as well as the LA patients one-third were females. Another explanation for the differences in plasma drug levels between the groups might be adherence. However, we also looked at levels of concomitantly used PIs or NNRTIs and these levels were overall within the normal range and there were no differences between the groups.

### Table 1. Patients’ characteristics

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>Controls</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Sex (%)</td>
<td>10 males (67)</td>
<td>15 males (71)</td>
</tr>
<tr>
<td></td>
<td>5 females (33)</td>
<td>6 females (29)</td>
</tr>
<tr>
<td>Race (%)</td>
<td>9 Caucasian (60)</td>
<td>18 Caucasian (86)</td>
</tr>
<tr>
<td></td>
<td>4 Black (27)</td>
<td>2 Black (10)</td>
</tr>
<tr>
<td></td>
<td>1 Asian (7)</td>
<td>1 Asian (5)</td>
</tr>
<tr>
<td></td>
<td>1 Hispanic (7)</td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>87</td>
<td>125</td>
</tr>
<tr>
<td>Mean number of samples per person (range)</td>
<td>4.3 (2–10)</td>
<td>4.6 (2–9)</td>
</tr>
<tr>
<td>Mean HIV-RNA level (log copies/mL) at start of stavudine ± SD</td>
<td>4.63 ± 0.93</td>
<td>4.63 ± 0.90</td>
</tr>
<tr>
<td>CD4 cell count at initiation of stavudine (cells/mm³) ± SD</td>
<td>210 ± 125</td>
<td>205 ± 128</td>
</tr>
<tr>
<td>Weight (kg) ± SD (range)</td>
<td>68 ± 10 (52–82)</td>
<td>70 ± 10 (48–85)</td>
</tr>
<tr>
<td>Age at start of stavudine (years), mean (range)</td>
<td>40 (23–57)</td>
<td>39 (25–58)</td>
</tr>
<tr>
<td>Duration of stavudine (months) ± SD (range)</td>
<td>42 ± 21 (15–86)</td>
<td>55 ± 18 (30–84)</td>
</tr>
<tr>
<td>Mean stavudine concentration (mg/L) ± SD</td>
<td>0.174 ± 0.102</td>
<td>0.270 ± 0.172</td>
</tr>
<tr>
<td>Mean CR ± SD</td>
<td>0.799 ± 0.309</td>
<td>1.126 ± 0.514</td>
</tr>
<tr>
<td>Median CR ± SD</td>
<td>0.833 ± 0.414</td>
<td>0.952 ± 0.288</td>
</tr>
<tr>
<td>Geometric mean ± SD</td>
<td>0.741 ± 0.295</td>
<td>0.978 ± 0.348</td>
</tr>
<tr>
<td>% CR &gt; 1.0 ± SD</td>
<td>23 ± 27</td>
<td>46 ± 29</td>
</tr>
</tbody>
</table>
Stavudine plasma concentrations and lipoatrophies

Our study was a retrospective analysis. There are only a few prospective studies that have examined the utility of plasma drug concentrations in the treatment of HIV infection.19,23 More prospective studies have to be performed in order to get more insight into the use of plasma drug monitoring for antiretroviral therapy and decision-making in the case of toxicity. It would also be helpful to investigate the use of lower-dose stavudine more closely, given the increasing number of HIV-infected patients in developing countries where stavudine is used widely, owing to low costs and inclusion in generic fixed-dose combination drugs. A decrease in stavudine dosing might result in additional cost and side-effect reduction.

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Transparency declarations

None to declare.

References