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Subtherapeutic Antiretroviral Plasma Concentrations in Routine Clinical Outpatient HIV Care

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Summary: The objective of this study was to evaluate plasma concentrations of nonnucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) within several dosing schemes in a cohort of HIV-infected patients in routine clinical practice and to find possible explanations for subtherapeutic plasma concentrations. Patients were included if a PI or NNRTI was part of their antiretroviral regimen, at least one plasma concentration was obtained, and a complete medication overview from community pharmacy records was available. The study period was from January 1998 to September 2001. Each plasma concentration was related to median plasma concentrations of a pharmacokinetic reference curve, yielding a concentration ratio (CR). A cutoff CR was defined for each antiretroviral drug per specific regimen, discriminating between ≥therapeutic and subtherapeutic concentrations. For the patients with subtherapeutic concentrations, it was sorted out whether drug interactions, adverse events and self-reported symptoms, or nonadherence could be the cause of the lower than expected plasma concentration. Ninety-seven HIV-infected patients fulfilled the criteria. During the defined period, 1145 plasma concentrations were available (median, 11; interquartile range, 8–14). Three hundred fourteen (27.4%) plasma concentrations were classified subtherapeutic. Drug interactions (2; 0.6%), adverse events and self-reported symptoms (67; 21.3%), and nonadherence (14; 4.5%) could only partly explain the subtherapeutic drug levels. Consequently, a large number of the subtherapeutic plasma concentrations (73.6%) remained inexplicable. A high number of subtherapeutic plasma concentrations were observed. No clear causes were found; thus, corrective measures will be difficult to employ. Therefore, therapeutic drug monitoring (TDM) must maintain its crucial place in routine clinical care to be able to identify patients who need extra attention so that therapeutic plasma concentrations are achieved. **Key Words:** Therapeutic drug monitoring—Protease inhibitors—Nonnucleoside reverse transcriptase inhibitors—Target trough concentration.

Since the introduction of highly active antiretroviral therapy (HAART), which combines antiretroviral drugs from two or more drug classes, therapeutic drug monitoring (TDM) of antiretroviral drugs has evolved from an infrequent to a nearly routinely applied clinical tool in human immunodeficiency virus (HIV) care in The Netherlands. TDM is, in general, reserved to the protease inhibitors (PIs) and the nonnucleoside reverse transcriptase inhibitors (NNRTIs) (1). For these drugs, relationships between plasma concentrations and efficacy and toxicity have been established during recent years (2–6). Variations in plasma concentrations could, therefore, have far-reaching clinical consequences. For several PIs and NNRTIs, minimal plasma concentrations have been estimated that should be targeted to maintain adequate

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viral suppression (2–4,7–12). Therefore, patients not reaching and maintaining these target trough plasma concentrations risk suboptimal treatment and the development of drug-resistant HIV strains. Development of resistance to one antiretroviral drug may also limit future treatment options since cross-resistance between drugs within a drug class exist (13,14).

Causes for subtherapeutic drug concentrations are multifactorial. Patient characteristics, including pharmacokinetic parameters such as low bioavailability and high metabolic clearance, but also drug interactions, nonadherence, and drug-related toxicity (resulting in nonadherence) contribute to low plasma concentrations (15–18).

We have investigated the occurrence of subtherapeutic drug concentrations in our HIV-infected patient population. Since we have a complete overview of their drug use (by use of community pharmacy records), our data set is very well suited to also study medication-related factors (e.g., drug interactions with coadministered drugs) that may contribute to subtherapeutic PI and NNRTI plasma concentrations. The outcome may aid in further optimization of HIV therapy. As far as we know, no such study has been described to date.

MATERIALS AND METHODS

Patients and Bioanalysis

Patients were included if a PI or NNRTI was part of their antiretroviral regimen. In addition, these patients' community pharmacy records (for which patients gave written informed consent to collect) needed to cover the period from January 1998 to September 2001. Furthermore, at least one drug plasma concentration should be available during the defined period.

All plasma samples were single random samples obtained by standardized procedures. The samples were drawn during routine clinic outpatient visits for the purpose of TDM. Time after ingestion (interval between last dose intake and plasma sampling) and dosing scheme were documented.

Steady-state concentrations of efavirenz, indinavir, nelfinavir, nevirapine, ritonavir, and saquinavir were determined by validated high-performance liquid chromatographic (HPLC) methods (19–21). Some modifications were made to quantify lopinavir within the HPLC method. Lower limits of quantification (LLQ) were 0.10 mg/L, 0.03 mg/L, 1.02 mg/L, and 0.03 mg/L for efavirenz, indinavir, lopinavir, and saquinavir, respectively. For nevirapine, nelfinavir, and ritonavir, LLQs were 0.05 mg/L.

Concentration Ratios and Trough Plasma Concentrations

For each PI and NNRTI, trough plasma concentrations that need to be reached for therapeutic success were defined based on published data. These target trough plasma concentrations are presented in Table 1. For each patient, trough plasma concentrations were compared with the target trough plasma concentration (Table 1). Since the included plasma concentrations were randomly drawn during the dosing interval, and thus trough plasma concentrations were not available at each occasion, concentration ratios (CR) were determined to correlate the measured plasma concentration with a trough value. To obtain a CR, observed PI and NNRTI plasma concentrations were related to median plasma concentrations of a pharmacokinetic curve obtained in a reference population, at the same time after ingestion. This pharmacokinetic reference curve was recorded under supervised ingestion and steady-state conditions. The assumption in this approach is that the CR determined at any time point during the dosing interval reflects the CR at the trough. The cutoff CR of each antiretroviral drug in a specific regimen was calculated by dividing the target trough plasma concentration (Table 1) by the trough plasma concentration of the pharmacokinetic reference curve. A plasma concentration with a CR below or above the cutoff CR was defined as subtherapeutic or ≥therapeutic, respectively. Table 2 presents the dosing schemes that were included in this analysis and for which pharmacokinetic reference curves were available. In addition, the cutoff CR and the trough concentration of the reference curve for each antiretroviral drug in each regimen are presented in Table 2.

Ritonavir administered in a (nontherapeutic) dose of 100 or 200 mg was not included in this analysis.

Causes of Subtherapeutic Plasma Concentrations

For each plasma concentration that was classified subtherapeutic, it was investigated if a coadministered agent

TABLE 1. *Target trough concentrations of antiretrovial drugs*

Antiretroviral drug	Target trough concentration (mg/L)	Reference
Efavirenz	1	[2]
Nevirapine	3.4	[3]
Indinavir	0.15	[7–9]
Lopinavir	5.7	[10]
Nelfinavir	1	[11]
Ritonavir	2.1	[12]
Saquinavir	0.05	[4]

TABLE 2. *Cut-off concentration ratio for each antiretroviral regimen*

Antiretroviral regimen	C_{trough} reference curve (mg/L)	Cut-off CR*
Efavirenz 600 mg qd	2.07	0.5
Nevirapine 200 mg bid	4.32	0.8
Indinavir 800 mg bid[a]	0.85	0.2
Indinavir 800 mg tid	0.2	0.75
Lopinavir 400 mg bid	5.48	1
Nelfinavir 1250 mg bid	1.3	0.8
Ritonavir 400 mg bid[b]	2.36	0.9
Ritonavir 600 mg bid	3.94	0.5
Saquinavir 1000 mg bid[a]	0.45	0.1
Saquinavir 400 mg bid[c]	0.20	0.25
Saquinavir 1200 mg bid[d]	0.12	0.4
Saquinavir 1200 mg tid	0.08	0.6

* The cut-off CR is defined as: target trough concentration divided by trough plasma concentration of the pharmacokinetic reference curve.

bid, twice daily; qd, once daily; tid, thrice daily; CR, concentration ratio; C_{trough}, trough plasma concentration; [a]combined with ritonavir 100 mg bid, [b]combined with saquinavir 400 mg bid, [c]combined with ritonavir 400 mg bid, [d]combined with nelfinavir 1250 mg bid.

could be the cause of the lower than expected plasma concentration. Drug interactions were identified according to earlier reports (22,23). The use of coadministered agents was obtained from the community pharmacy records. Furthermore, outpatient medical records were reviewed for adverse events or self-reported symptoms in the same period that subtherapeutic plasma concentrations were encountered. Nonadherence reported by the patient and noted in the outpatient medical records was documented.

RESULTS

Patients and Plasma Concentrations

Ninety-seven HIV-infected patients with a median age of 41.4 years (interquartile range [IQR], 36.2–48.5) fulfilled the inclusion criteria. Patients were predominantly men (93.8%) and white (91.8%). Patients had a median duration of antiretroviral therapy use of 13.5 months (IQR, 0.7–22.2). Twenty-four patients were antiretroviral naive (24.7%). A total of 1145 plasma concentrations were available during the defined study period, yielding a median of 11 plasma concentrations per patient (IQR, 8–14). Nevirapine and efavirenz were used by 84 and 6 patients, respectively. Saquinavir 1200 mg thrice daily, 1000 mg twice daily, 400 mg twice daily (combined with ritonavir 400 mg twice daily), and 1200 mg twice daily was used by 19, 4, 20, and 2 patients, respectively. Twice-daily regimens including nelfinavir, lopinavir, ritonavir (without other PIs), and indinavir were used by 15, 8, 17, and 20 patients, respectively. Indinavir without ritonavir boosting (800 mg thrice daily) was used by 14 patients. The observed plasma concentrations are presented in Figure 1. As can be observed, a large variability in plasma concentrations was encountered for each antiretroviral dosing scheme. Undetectable plasma concentrations were observed in 13 cases, of which 8 were saquinavir in a thrice-daily dosing scheme.

Concentration Ratio

Table 3 presents the distribution of CRs divided in subtherapeutic and ≥therapeutic CRs, and for each dosing scheme, a median CR and corresponding IQR are given. In addition, Figure 1 presents the distribution of plasma concentrations around the cutoff curve below which plasma concentrations were classified subtherapeutic. More than a quarter (27.4%) of the 1145 CRs were classified subtherapeutic by our definition. The subtherapeutic CRs involved 80 patients with a median number of subtherapeutic CRs per patient of 3 (IQR, 1–6). This indicates that more than 80% of the patients had at some moment during the studied period a subtherapeutic CR. Lopinavir showed the highest percentage of subtherapeutic CRs. Of the two dosing schemes including indinavir, the twice-daily dosing scheme appeared to result in the fewest number of subtherapeutic CRs (8.5%). Ritonavir 600 mg twice daily resulted in the fewest number of subtherapeutic CRs (11.5%) when focusing on the ritonavir regimens. Of the four dosing schemes including saquinavir, the 1000 mg twice-daily dosing scheme involved the fewest subtherapeutic CRs (4.3%), whereas the 1200 mg thrice-daily dosing scheme showed a high number of subtherapeutic CRs (53.2%).

Subtherapeutic Plasma Concentrations

Table 4 presents the possible causes for the 314 observed subtherapeutic plasma concentrations. Concomitant use of rifampicin (600 mg once daily) with indinavir (800 mg twice daily) and combined use of rifabutin (150 mg once daily) with nelfinavir (1250 mg twice daily) were found as possible causes in two cases. Sixty-seven subtherapeutic plasma concentrations could be related to adverse events or self-reported symptoms, of which diarrhea and nausea were most frequently reported. Self-reported symptoms classified as "other" included influenza and dizziness. Reports of nonadherence included ingestion problems related to diet restrictions (indinavir, nelfinavir) or dosing scheme (saquinavir thrice daily) and comprised 4.5% of subtherapeutic plasma concentrations. Overall, a quarter (26.4%) of the subtherapeutic plasma concentrations could be explained

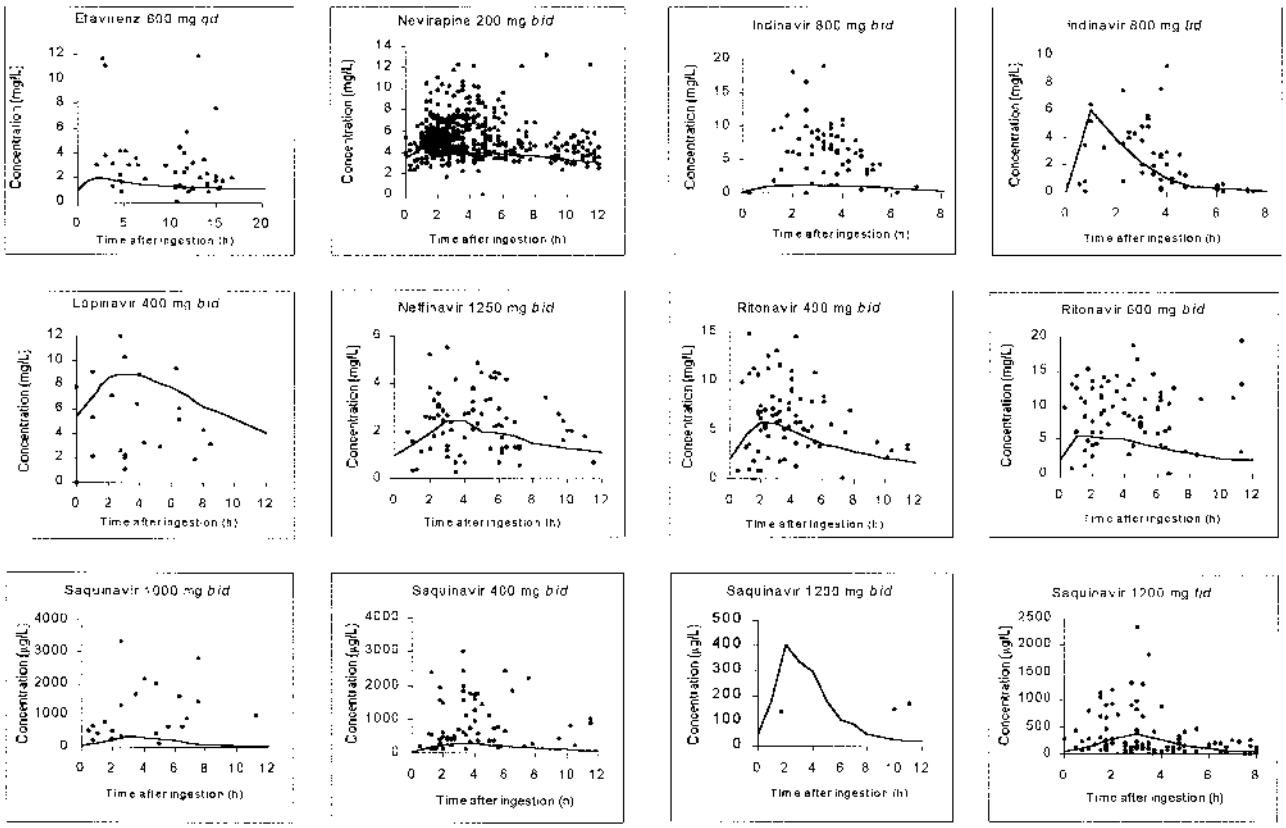

FIG. 1. Observed plasma concentrations for each dosing scheme versus time after ingestion. The solid line represents the (cutoff) curve below which plasma concentrations are classified as subtherapeutic.

by drug interactions, adverse events or self-reported symptoms, or nonadherence. Consequently, in 231 occasions, the reasons for subtherapeutic concentrations are not known.

DISCUSSION

The objective of this study was to explore the distribution of plasma concentrations of antiretroviral drugs

TABLE 3. *Distribution of calculated concentration ratios divided in subtherapeutic and ≥therapeutic concentration ratios*

	Total		Subtherapeutic		≥Therapeutic	
Antiretroviral drug	Number	Median CR [IQR]	Number (%)	Median CR [IQR]	Number (%)	Median CR [IQR]
Efavirenz	45	0.83 [0.53–1.18]	9 (20)	0.37 [0.35–0.45]	36 (80)	0.96 [0.72–1.29]
Nevirapine	515	0.97 [0.80–1.25]	126 (24.5)	0.73 [0.65–0.76]	389 (75.5)	1.06 [0.93–1.31]
Indinavir	120		25 (20.8)		95 (79.2)	
bid regimen[a]	71	1.13 [0.74–1.65]	6 (8.5)	0.05 [0.02–0.11]	65 (91.5)	1.21 [0.94–1.53]
tid regimen	49	1.02 [0.61–1.65]	19 (38.8)	0.53 [0.17–0.65]	30 (61.2)	1.59 [1.07–1.86]
Lopinavir	22	0.70 [0.30–0.89]	16 (72.7)	0.36 [0.29–0.70]	6 (27.3)	1.26 [1.18–1.34]
Nelfinavir	80	0.99 [0.58–1.33]	30 (37.5)	0.53 [0.30–0.60]	50 (62.5)	1.20 [1.01–1.57]
Ritonavir	160		36 (22.5)		124 (77.5)	
400 mg bid regimen[b]	82	1.10 [0.73–1.66]	27 (32.9)	0.43 [0.26–0.72]	55 (67.1)	1.48 [1.10–1.88]
600 mg bid regimen	78	0.95 [0.72–1.26]	9 (11.5)	0.30 [0.27–0.38]	69 (88.5)	1.03 [0.81–1.27]
Saquinavir	203		72 (35.5)		131 (64.5)	
1000 mg bid regimen[a]	23	0.47 [0.25–0.85]	1 (4.3)	0.06	22 (95.7)	0.49 [0.26–0.87]
400 mg bid regimen[c]	66	0.63 [0.34–1.47]	11 (16.7)	0.15 [0.10–0.21]	55 (83.3)	0.86 [0.45–1.55]
1200 mg bid regimen[d]	3	2.34 [1.24–2.67]	1 (33.3)	0.15	2 (66.7)	2.34; 3.00
1200 mg tid regimen	111	0.48 [0.20–1.40]	59 (53.2)	0.21 [0.15–0.34]	52 (46.8)	154 [1.08–2.29]
Total	1145		314 (27.4)		831 (72.6)	

[a] + ritonavir 100 mg bid, [b]+ saquinavir 400 mg bid, [c]ritonavir 400 mg bid, [d]+ nelfinavir 1250 mg bid.
bid, twice daily; CR, concentration ratio; IQR, interquartile range; qd, once daily; tid, thrice daily.

TABLE 4. *Possible causes for subtherapeutic plasma concentrations*

Possible cause	Number (%)
Drug interaction	2 (0.6)
Adverse events/self-reported symptoms	67 (21.3)
Diarrhea/Nausea	31 (9.9)
Fatigue	5 (1.6)
Insomnia	2 (0.6)
Psychological problems	9 (2.9)
Neuropathy	12 (3.8)
Lipodystrophy	6 (1.9)
Other	2 (0.6)
Nonadherence	14 (4.5)
Total explained	83 (26.4)
Unknown	231 (73.6)
Total subtherapeutic	314 (100)

that were determined for the purpose of routine TDM. By our definition, a high percentage (27.4%) of the observed plasma concentrations reached a trough plasma concentration (based on the calculated CR) below the target trough concentration. Only for a quarter of these subtherapeutic plasma concentrations could we find an explanation.

A complete overview of drug use (antiretroviral drugs and coadministered agents) was available, making it possible to screen all patients for drug interactions. Two subtherapeutic plasma concentrations could be explained by drug interactions with coadministered agents. Concomitant use of alternative and complementary therapies may also influence the pharmacokinetics of antiretroviral drugs (24). For example, St John's wort has been shown to significantly increase the clearance of nevirapine (25) and indinavir (26) by induction of CYP3A4 and/or P-gp, resulting in decreased plasma concentrations. However, the use of these agents was not documented, and the interacting potential for most of these agents has not been completely elucidated. The low number of drug interactions encountered as cause for subtherapeutic drug concentrations probably reflects the profound knowledge about drug interactions by the treating physicians.

Adverse events or self-reported symptoms as a cause for subtherapeutic plasma concentrations were documented 67 times. HIV- and HAART-related symptoms have been shown to be significantly associated with adherence to HAART (18,27). Therefore, a particular adverse event related to a PI or NNRTI may cause reluctance to ingest that drug. However, this may not immediately lead to clinical failure since HAART regimens usually contain at least two more antiretroviral drugs that suppress the viral replication (28). It has been shown that when patients are nonadherent to the antiretroviral regimen, however, they often show nonadherence for all the antiretroviral drugs taken at that time (29).

We also documented nonadherence as possible cause for subtherapeutic plasma concentrations. Only 14 plasma concentrations were possibly subtherapeutic because of nonadherence. This reporting of nonadherence is, however, based on spontaneous reporting of patients and thus likely underestimates the true extent of nonadherence (16).

Low PI drug levels have been described to be indicative of nonadherence (15,17,30); therefore, the unexplained subtherapeutic drug concentrations may largely involve nonadherence (including nonadherence to diet restrictions and pill schedules). Furthermore, interindividual differences in metabolic clearance will certainly have contributed. For the NNRTI nevirapine, for example, body weight has been shown to increase the apparent clearance of nevirapine significantly (31). In addition, CYP3A4, the enzyme responsible for the metabolism of all PIs and NNRTIs, shows marked interindividual variability (32,33). Moreover, its activity appears to be even more variable in HIV-positive patients than in noninfected subjects (34). Also, habits like intake of alcohol and cigarette smoking could possibly interfere with the metabolism of drugs.

Lopinavir showed the highest percentage of subtherapeutic plasma concentrations. Lopinavir is the most recently introduced PI, and data on pharmacokinetics and relationships with efficacy are still scarce. The estimated target trough concentration (5.7 mg/L) that was used in this study was extracted from a study that was performed in multiexperienced HIV-infected subjects with a high number of mutations related to PI resistance (10). Possibly, patients infected with HIV strains with fewer or no mutations could suffice with lower trough concentrations.

Noteworthy is the IQR of ≥therapeutic CRs obtained from patients on saquinavir 1000 mg twice daily. In more than 75% of the cases, CRs are lower than 1. Obviously, the median plasma concentrations from the pharmacokinetic reference curve obtained after observed ingestion are higher than the plasma concentrations reached in routine clinical practice. However, target trough plasma concentrations for saquinavir were reached (Table 3).

The use of a CR to predict whether a patient will maintain a plasma level above a certain target minimal concentration is debatable (35). For instance, the CR concept assumes that each person has the same pharmacokinetic profile, while in reality this is not true. However, CRs have previously been used as a way to reflect exposure to antiretroviral drugs and have been related to virologic failure (36,37), toxicity (38), and adherence (15,30). Possibly, Bayesian estimation for the prediction of a trough plasma concentration is a better approach

(31). The preference of this technique versus the CR concept, however, still has to be proven.

It is still not conclusively established whether the used target trough plasma concentrations will be sufficient for patients experienced with other antiretroviral regimens. When taking into account that 75% of the included patients were antiretroviral-experienced, this would suggest that the percentage of subtherapeutic plasma concentrations would be even larger than calculated.

The clinical relevance of our observations is difficult to establish, and it was not investigated whether, for example, "failures" parallel subtherapeutic drug levels. In our clinical practice, however, patients with subtherapeutic drug levels receive additional attention (e.g., are given renewed instruction how to ingest their drugs, with or without food).

In conclusion, more than a quarter of the observed plasma concentrations in an outpatient clinic population appeared to be subtherapeutic; consequently, these patients could risk treatment failure. Since many of the subtherapeutic plasma concentrations could not be explained by drug interactions, self-reported nonadherence, or toxicity, TDM must have a crucial place in routine clinical care to be able to identify patients who need extra attention in the optimization of plasma concentrations and, thus, in the optimization of antiretroviral treatment.

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