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Therapeutic drug monitoring of lopinavir/ritonavir given alone or with a non-nucleoside reverse transcriptase inhibitor

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Aims
To evaluate the interindividual variability in the plasma concentrations of lopinavir in the context of routine monitoring with or without treatment with a non-nucleoside reverse transcriptase inhibitor and to assess the interaction between the coformulation of lopinavir/ritonavir and efavirenz or nevirapine.

Methods
Plasma trough and peak concentrations (C_{trough}, C_{max}) of lopinavir from 182 HIV-1-infected patients were analysed by high-performace liquid chromatography. Three lopinavir/ritonavir regimens were assessed, namely (A) 400 mg lopinavir/100 mg ritonavir twice daily given alone (n = 125), (B) 400/100 mg twice daily together with a non-nucleoside reverse transcriptase inhibitor (n = 25), and (C) 533/133 mg twice daily together with a non-nucleoside reverse transcriptase inhibitor (n = 32).

Results
Median (ng ml^{-1}) C_{trough} and C_{max} lopinavir (interquartile range, CV) were: (A) 4852 (3198–6891, 56%) and 8501 (6333–11 584, 41%), (B) 2979 (1704–5186, 74%) and 5612 (3362–11 704, 76%) and (C) 5082 (2696–7226, 74%) and 9757 (4883–12 963, 60%). Median C_{trough} of lopinavir was lower in patients taking both efavirenz [P = 0.01, 95% confidence interval (CI) for difference between medians 343, 2713] and nevirapine (P = 0.019, 95% CI for difference between medians 354, 3681) compared with those taking lopinavir/ritonavir alone. A higher interindividual variability was observed when lopinavir/ritonavir was given with a non-nucleoside reverse transcriptase inhibitor. The risk of achieving a 'suboptimal' C_{trough} of lopinavir (below a threshold of 3000 ng ml^{-1}) was statistically higher in patients treated with a non-nucleoside reverse transcriptase inhibitor (P < 0.001, 95% CI for difference between percentages 8.8, 43.1%) compared with those receiving lopinavir/ritonavir alone.

Conclusions
Our results confirmed the interaction between lopinavir and efavirenz, and also demonstrated a significant interaction between the former drug and nevirapine, resulting in lower C_{trough} of lopinavir. The wide interpatient variability in this interaction suggests that therapeutic drug monitoring may be useful in optimizing the dose of lopinavir.

Introduction

Lopinavir is a potent HIV protease inhibitor that is coformulated with ritonavir, which acts as an inhibitor of the cytochrome P450 3A4 (CYP3A4) metabolism of the former drug. Even at a low dose of ritonavir, there is a substantial increase in exposure to lopinavir [1]. The plasma trough (C_{trough}) and peak (C_{max}) concentrations (mean ± SD) of lopinavir at steady state and at standard doses of 400 mg lopinavir/100 mg ritonavir twice daily are 5500 ± 4000 ng ml^{-1} and 9600 ± 4400 ng ml^{-1}, respectively [2, 3]. These plasma drug concentrations widely exceed the inhibitory concentration (IC_{50}) for the wild-type virus corrected for protein binding (70 ng ml^{-1}). Consequently, the mean lopinavir trough concentration (C_{trough})/IC_{50} ratio or inhibitory quotient (IQ) is as high as 75 at the standard doses of the combination [4]. Based on this high IQ, lopinavir/ritonavir potentially provides a barrier to the emergence of viral resistance and activity against resistant virus.

The pharmacokinetics of protease inhibitors differ significantly between individuals, due to the variability in their absorption and metabolism. Moreover, a positive relationship between plasma concentrations of protease inhibitors and antiviral efficacy and/or toxicity has been clearly demonstrated [5–12]. Therapeutic drug monitoring during therapy with protease inhibitors is recommended in certain circumstances and in several countries such as France, although its role in routine clinical practice remains to be established [13]. Recently, a prospective study showed the potential benefit of therapeutic drug monitoring on the virological outcome at 1 year, of indinavir and nelfinavir therapy in antiretroviral naive adult patients [14, 15].

Lopinavir is metabolized almost entirely by CYP3A4. Lopinavir is also an inhibitor of this enzyme, although it is less potent than ritonavir [16]. Lopinavir is now frequently given with non-nucleoside reverse transcriptase inhibitors, such as efavirenz or nevirapine, both of which are metabolized by and induce CYP3A4. The interaction has been reported to cause a 30% decrease in the C_{trough} of lopinavir [17]. The interaction between lopinavir and nevirapine in adult patients has not been investigated. However, in a paediatric population, nevirapine significantly decreased the plasma C_{trough} of lopinavir. Thus, a higher dose of the latter should be considered when the two drugs are given together [18], although the manufacturers of both lopinavir and nevirapine do not recommend any dose adjustment except for patients with a suspected decreased response to lopinavir. Thus, the role of therapeutic drug monitoring when these drugs are given in combination needs further investigation.

In the present study, we have examined the interindividual variability in plasma lopinavir concentrations measured in samples taken for routine monitoring in adult patients receiving lopinavir/ritonavir alone or together with non-nucleoside reverse transcriptase inhibitors. We have also assessed the interaction between lopinavir and efavirenz or nevirapine to evaluate the benefit of therapeutic drug monitoring in these patients.

Methods

Patients

During routine monitoring for clinical purposes, we assessed plasma lopinavir C_{trough} and C_{max} concentrations from 182 HIV-1-infected patients followed up between January 2000 and April 2002. The study was observational, both retrospective and prospective, and carried out in eight clinical care units. Patients included in the study were treated with lopinavir/ritonavir with or without efavirenz or nevirapine with or without one or two nucleoside reverse transcriptase inhibitors for at least 1 month (allowing time to reach steady-state pharmacokinetics). The regimens assessed were lopinavir/ritonavir 400/100 mg twice daily without non-nucleoside reverse transcriptase inhibitor (group A), lopinavir/ritonavir 400/100 mg twice daily with a non-nucleoside reverse transcriptase inhibitor (group B), and lopinavir/ritonavir 533/133 mg twice daily with a non-nucleoside reverse transcriptase inhibitor (group C). Data were transferred from carers to researchers in a completely anonymized, nontraceable fashion.

Pharmacokinetic sampling and analysis

Plasma drug concentrations of lopinavir and ritonavir were measured by a sensitive and validated high-performance liquid chromatography method with ultraviolet detection [19]. The limit of quantification was 100 ng ml^{-1}. Inter- and intra-assay variability were 6.9–13.8% and 2.9–7.2 % for lopinavir and 3.3–10.5% and 1.6–9.5% for ritonavir. Blood samples were drawn at steady state, 10–12 h post-dose for the determination of C_{trough} and 3–5 h post-dose for the determination of C_{max}. The time of last lopinavir/ritonavir dose was ascertained by patient report. No other specific measure of adherence was used. None of the patients had been prescribed inhibitors or inducers of CYP3A4 activity.

Drug analysis

Interindividual variability in lopinavir concentrations was estimated using the coefficient of variation expressed as a percentage (CV%). The proportion of

patients with a lopinavir C_{trough} below the expected range was estimated for each lopinavir/ritonavir regimen, to evaluate the magnitude of any drug–drug interaction. Because the target range of lopinavir C_{trough} has not yet been defined, we first used a threshold value of 3000 ng ml^{-1} based on a previously proposed therapeutic range [13]. Therefore, a lopinavir C_{trough} below this value was defined as 'suboptimal'. We also used a threshold value of 1500 ng ml^{-1}, since the mean ± SD lopinavir C_{trough} in the population has been estimated to be 5500 ± 4000 ng ml^{-1} [3]. Thus, we took account of the interindividual variability in LPV concentrations.

Nonparametric test (Mann–Whitney *U*-test) was used to compare lopinavir and ritonavir concentrations between the different regimens. Categorical variables were compared using the χ^2 test. Statistical analysis was performed using the computer software program SPSS® PC for Windows, version 10.1 (SPSS Inc., Chicago, IL, USA). A *P*-value ≤0.05 was considered statistically significant.

Results

A total of 182 patients [135 men and 47 women with a median (range) age of 40 years (16–63)] were enrolled during the observation period. There were 125 in group A, 25 in group B (16 taking efavirenz and nine taking nevirapine), and 32 in group C (29 taking efavirenz and three taking nevirapine). Only 13% of the patients had not received a protease inhibitor previously. Five patients had impaired liver function (two in group A, two in group B and one in group C). Overall, 229 plasma C_{trough} and 57 plasma C_{max} of lopinavir and ritonavir were determined.

At a standard dose of 400 mg lopinavir/100 mg ritonavir twice daily, a 39% decrease in the median C_{trough} of lopinavir was found when the combination was given with a non-nucleoside reverse transcriptase inhibitor [$P = 0.001$, difference between medians 1718, 95% confidence interval (CI) 719, 2692], whereas no significant change was observed in C_{max} ($P = 0.35$, difference between medians 1909, 95% CI −2667, 6470). There was no statistical difference in the median C_{trough} of lopinavir between group A (400 mg lopinavir/100 mg ritomavir twice daily) and group C (533 mg lopinavir/133 mg ritomavir twice daily). At 400 mg lopinavir/100 mg ritomavir twice daily, no statistical difference was found for the C_{trough} of lopinavir {median [interquartile range (IQR)] ng ml^{-1}, *n* samples} between patients taking efavirenz [3733 (2097–4570), 24] and those taking nevirapine [2658 (1502–5199), 12] ($P = 0.61$, difference between medians 587, 95% CI −1403, 2387). The C_{trough} of lopinavir was significantly decreased both for patients taking efavirenz ($P = 0.01$, difference between medians 1549, 95% CI 343, 2713) and nevirapine ($P = 0.019$, difference between medians 2053, 95% CI 354, 3681) compared with those not taking a non-nucleoside reverse transcriptase inhibitor. Interindividual variability in both the C_{trough} and C_{max} of lopinavir was increased from 56% to 74% when a non-nucleoside reverse transcriptase inhibitor was given with lopinavir/ritonavir.

The median C_{trough} and C_{max} [(IQR), CV%] of ritonavir were 389 ng ml^{-1} [(254–624), 71%] and 709 ng ml^{-1} [(519–911), 95%] for regimen A, 311 ng ml^{-1} [(181–553), 76%] and 622 ng ml^{-1} [(226–999), 91%] for regimen B and 586 ng ml^{-1} [(195–873), 74%] and 1166 ng ml^{-1} [(570–1749), 74%] for regimen C. No statistical difference was observed in the C_{trough} and C_{max} of ritonavir at the standard dose of 400 mg liponavir/100 mg ritonavir twice daily given alone or with a non-nucleoside reverse transcriptase inhibitor. In contrast, higher C_{trough} values for ritonavir were noted in group C compared with both group A ($P = 0.033$, difference between medians 163, 95% CI 9, 322) and B ($P = 0.021$, difference between medians 235, 95% CI 35, 439). Significant interindividual variability in ritonavir concentrations was observed in all of the regimens.

On a standard dose of lopinavir/ritonavir alone, only 16% of the patients had a C_{trough} of lopinavir below the 3000-ng ml^{-1} threshold (Figure 1). The proportion of patients with C_{trough}s below this value was statistically lower in group A (16%, 95% CI 10.2, 21.8) compared with group B (42%, 95% CI 26, 58) who were also taking a non-nucleoside reverse transcriptase inhibitor ($P \leq 0.001$, 95% CI for difference between percentages 8.8, 43.1) but was comparable to group C (27%, 95 CI 13.4, 40.5) who were receiving the higher dose of lopinavir/ritonavir ($P = 0.12$, 95% CI for difference between percentages 3.7, 25.7). Patients treated with lopinavir/ritonavir 400/100 mg twice daily and a non-nucleoside reverse transcriptase inhibitor had a higher risk of having a 'suboptimal' C_{trough} of lopinavir [odds ratio (OR) 3.91, 95% CI 1.63, 9.37]. Similar results were found when using a threshold value of 1500 ng ml^{-1} (Figure 1).

Discussion

In the present work median C_{trough} and C_{max} values for lopinavir were comparable to previously reported data [2, 3] irrespective of the dosing regimen. However, we demonstrated that the median C_{trough} of lopinavir was decreased from 39% by coadministration of efavirenz or nevirapine. When the lopinavir/ritonavir dose was

Table 1
Plasma concentrations of lopinavir at two doses of lopinavir/ritonavir taken alone or together with a non-nucleoside reverse transcriptase inhibitor

	Plasma concentrations of lopinavir (ng ml^{-1})					
	(A) 400/100		(B) 400/100 + non-nucleoside reversetranscriptase inhibitor		(C) 533/133 + non-nucleoside reverse transcriptase inhibitor	
	C_{trough}	C_{max}	C_{trough}	C_{max}	C_{trough}	C_{max}
Mean ± SD	5293 ± 2959	8984 ± 3723	3555 ± 2646	7004 ± 5350	5582 ± 4123	9271 ± 5544
Median	4852	8501	2979*	5612	5082	9757
(IQR)†	(3198–6891)	(6333–11584)	(1704–5186)	(3362–11704)	(2696–7226)	(4883–12963)
CV%	56%	41%	74%	76%	74%	60%
n (samples)	152	40	36	7	41	10

**P = 0.001 (Mann–Whitney test) compared with regimen A. †Interquartile range.*

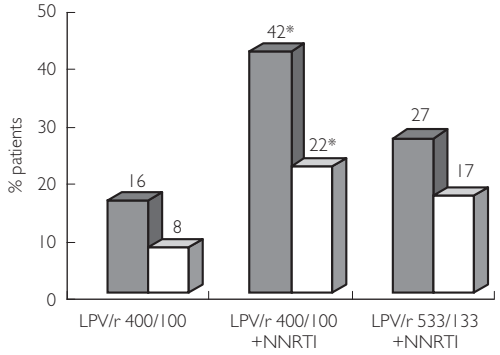

Figure 1
Proportion of patients with suboptimal C_{trough} of lopinavir with respect to lopinavir/ritonavir dose and co-treatment with a non-nucleoside reverse transcriptase inhibitor. The results are expressed as percentages
*Significant increase ($P < 0.001$) in the percentage of patients with C_{trough} of lopinavir below the threshold value between groups treated with lopinavir/ritonavir 400/100 and a non-nucleoside reverse transcriptase inhibitor and those treated with lopinavir/ritonavir 400/100 alone. LPV Ctrough <3000 ng/ml (■), LPV Ctrough <1500 ng/ml (□)

increased to 533 mg lopinavir/133 mg ritonavir twice daily, median C_{trough} values for lopinavir were comparable to those observed at the standard dose in patients not receiving a non-nucleoside reverse transcriptase inhibitor. Nevertheless, the higher dose of lopinavir/ritonavir led to elevated concentrations (above the expected values) for some patients, emphasizing the potential risk of toxicity.

In contrast, treatment with a non-nucleoside reverse transcriptase inhibitor did not affect the concentrations of ritonavir. Moreover, the C_{trough} of ritonavir was statistically higher in patients taking 533 mg lopinavir/133 mg ritonavir twice daily compared with patients from both regimen A and B taking 400/100 mg twice daily.

A lower interindividual variability in the C_{trough} of lopinavir at the standard dose of 400/100 mg twice daily (CV = 56%) was observed compared with other ritonavir-boosted regimens. For example, values of 92% and 75% have been reported for indinavir/ritonavir 800/100 mg twice daily and saquinavir/ritonavir 400/400 mg twice daily, respectively [20, 21]. The interindividual variability in the C_{trough} of lopinavir greatly increased when lopinavir/ritonavir was given with a non-nucleoside reverse transcriptase inhibitor (CV = 74%) even at the higher dose of lopinavir/ritonavir. Thus, non-nucleoside reverse transcriptase inhibitor therapy enhances the pharmacokinetic variability of lopinavir and decrease its C_{trough}, probably through induction of CYP3A4. Unpredictable lopinavir concentrations may then be obtained when lopinavir/ritonavir is given with efavirenz or nevirapine. Even if the mean C_{trough} of lopinavir remains above the proposed therapeutic threshold, concentrations in a percentage of patients may fall below this value. Indeed, we showed that the proportion of patients with a 'suboptimal' C_{trough} value was significantly higher in the group taking lopinavir/ritonavir 400/100 mg twice daily with a non-nucleoside reverse transcriptase inhibitor. However, the term 'suboptimal' should be used with caution. A C_{trough} value for lopinavir below 3000 ng ml^{-1} or 1500 ng ml^{-1} may be 'suboptimal' but, since this drug has a high inhibitory quotient, the *in vivo* minimal effective concentration may be even lower.

Because our study was observational, and partially retrospective being performed in a routine clinical setting, an accurate estimate of adherence to therapy was not possible. Therefore, prospective controlled trials assessing the relationship between plasma concentration and virological outcome and/or toxicity are required to determine the exact target range for the C_{trough} of lopinavir and to provide guidelines for using therapeutic drug monitoring in routine clinical practice.

In conclusion, therapeutic drug monitoring may provide information that can help patients achieve adequate concentrations of anti-HIV drugs. Nevertheless, it seems that for patients treated with lopinavir/ritonavir alone and not receiving other drugs affecting CYP3A4 activity, a low degree of therapeutic drug monitoring is required. On contrast, when efavirenz or nevirapine are coadministered with lopinavir/ritonavir, therapeutic drug monitoring may be useful for dosage adjustment.

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