Impact of reduced dosing of lopinavir/ritonavir in virologically controlled HIV-infected patients: the Kaledose trial

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Background: It is debated whether a risk of protease inhibitor mutation selection in proviral DNA exists during intermittent HIV-1 viraemia thereby impacting long-term virological control.

Methods: Virologically controlled patients treated with lopinavir/ritonavir were included in a 48 week pilot trial during which lopinavir/ritonavir dosage was reduced if lopinavir concentration was >5000 ng/mL at inclusion. Serum lipids were longitudinally assessed and proviral DNA was quantified and sequenced in patients experiencing transient viraemia.

Results: Thirty-three virologically suppressed patients while on a lopinavir/ritonavir-containing regimen were included, of whom 28 [20 males, mean age 44.6 years (SD 10.9)] completed the 48 week follow-up as scheduled. A significant decrease in lopinavir level was noted [mean Cmin, 7363 ng/mL (min, 5118; max, 12415) at baseline versus 4319 ng/mL (min, 1427; max, 8683) at week 48, P<0.03]. A significant decrease in triglycerides was also observed [1.73 mmol/L (SD 1.08) at baseline versus 1.34 mmol/L (SD 0.91) at week 48, P=0.03], whereas no significant change occurred for total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol. In the 15 patients with transient viraemia, analysis of proviral DNA for antiretroviral resistance showed that mutations had occurred when compared with baseline genotypes in three patients: I47M (n=2) and M46I (n=1). Selection of these mutations was not associated with virological failure as all three patients had a sustained virological response without treatment modification after a 3 year follow-up.

Conclusions: This pilot study evaluating the biochemical and virological impact of a reduced dosing of lopinavir/ritonavir suggests that lower exposure to lopinavir/ritonavir could be associated with a significant decrease in triglycerides during treatment, without occurrence of resistance mutations that might impact the virological response to treatment.

Keywords: resistance mutations, lipids, plasma trough concentration

Introduction

Lopinavir/ritonavir is a licensed protease inhibitor (PI) commonly used in naive and experienced patients.1 Since this treatment has a high genetic barrier to resistance in PI-naive patients, it has rarely been associated with the emergence of major PI mutations when virological failure occurs.2 A recent trial has investigated the efficacy of lopinavir/ritonavir monotherapy in exerting sufficient virological suppression, particularly as a maintenance strategy in treatment-controlled patients.3 The benefits of such strategies include conservation of certain drugs classes, tolerance (e.g. avoiding nucleoside toxicity) and cost. However, episodes of intermittent HIV viraemia (defined as blips) occurred more frequently in patients treated with lopinavir/ritonavir monotherapy.2 It is not known whether or not there is a risk of PI mutation selection in proviral DNA during intermittent HIV-1 viraemia thereby impacting long-term virological control. We conducted a pilot trial in our HIV clinic, the primary aim of which was to assess the impact on lipids of reduced-dose lopinavir/ritonavir in virologically controlled patients who were...
previously PI naïve, with a plasma trough concentration \( (C_{\text{min}}) \) of >5000 ng/mL, as well as to assess the presence of archived PI mutations in proviral DNA of patients with viral load (VL) blips and to determine their putative impact on virological control over a 72 week period following the end of study.

## Patients and methods

The study was designed as a pilot, 48 week prospective, multicentre, descriptive study. The following criteria had to be met for inclusion in the study: documented HIV-1 infection; age >18 years; first antiretroviral therapy regimen containing lopinavir/ritonavir (133.3/33.33 mg) administered as three soft-gel capsules twice daily associated with two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs); HIV-1 RNA <50 copies/mL for at least 3 months before inclusion; no change in antiretroviral treatment during the previous 3 months; \( C_{\text{min}} \) of lopinavir >5000 ng/mL 1 month prior to inclusion; and no non-nucleoside reverse transcriptase inhibitor (NNRTI) in association with lopinavir/ritonavir. The main exclusion criteria were: previous PI exposure; and association of lopinavir/ritonavir with an NNRTI plus another PI.

The lopinavir plasma concentration threshold of 5000 ng/L was chosen because we observed that >50% of the patients followed in our HIV clinic who were treated with a lopinavir/ritonavir-containing regimen presented with a \( C_{\text{min}} \) of >5000 ng/mL, although the recommended target of lopinavir \( C_{\text{min}} \) in naïve patients is between 1000 and 3000 ng/mL.\(^{1-5} \) We therefore postulated that a decrease in lopinavir posology would likely result in a \( C_{\text{min}} \) close to those recommended thresholds. The study was approved by the Ethics Committee of Saint-Antoine Hospital and informed consent was obtained from all patients at study enrolment. Socio-demographic data and history of HIV disease were collected at inclusion and included sex, age, duration of HIV infection and history of AIDS, duration of antiretroviral treatment (including any antiretroviral preceding lopinavir/ritonavir) and nucleoside backbone. At the time of study enrolment, the dose of lopinavir/ritonavir had been decreased from three soft-gel capsules (400/100 mg) twice daily to two soft-gel capsules (267/67 mg) twice daily, without modification of the NRTI backbone. Close biological and clinical monitoring was initiated and the following data were prospectively collected at baseline, week 2, week 12 and every 3 months until week 48: clinical events; \( C_{\text{min}} \); therapeutic change; association of lopinavir/ritonavir-containing regimens with a \( C_{\text{min}} \) of >5000 ng/mL; and virological blips defined as a detectable viral load in plasma with a detectable VL (50 copies/mL) and \( \leq 5000 \) copies/mL, as well as to assess the presence of archived PI mutations in proviral DNA of patients with viral load (VL) blips and to determine their putative impact on virological control over a 72 week period following the end of study.

### Results

A total of 33 patients were included of whom 28 (20 males, mean age 44.6 years (SD 10.9)) completed the 48 week follow-up as scheduled. Reasons for study non-completion were non-adherence (n=2) or patient’s decision (n=3). The mean duration of HIV infection was 5.2 years (SD 6.9) and 12 patients had a history of an AIDS-defining event. Ten patients were treatment naïve and the median number of antiretroviral regimens received by pretreated patients was 3 (IQR 2–5). At study inclusion, the combinations of NRTI agents used concomitantly with lopinavir/ritonavir were as follows: association of zidovudine and lamivudine (Combivir\(^\text{®} \) ), n=13; tenofovir and abacavir, n=5; didanosine and lamivudine, n=4; abacavir and lamivudine, n=3; didanosine and tenofovir, n=3; abacavir and lamivudine, n=2; association of zidovudine, abacavir and lamivudine (Trizivir\(^\text{®} \) ), n=1; didanosine and zidovudine, n=1; and abacavir, lamivudine and tenofovir, n=1. No NRTI treatment change was observed during the study period.

The baseline and 48 week follow-up data are presented in Table 1. After reduction of lopinavir/ritonavir dose, a significant decrease in plasma drug level was noted at week 48 compared with baseline [mean \( C_{\text{min}} \) (range), 7363 ng/mL (5118–12415) at baseline versus 4320 ng/mL (1427–8683) at week 48, P<0.0001]. The proportion of patients with a lopinavir concentration of <3000 ng/mL remained stable at ~25% of the total study population over time. A significant decrease in triglycerides was also observed [1.73 mmol/L (SD 1.08) at baseline versus 1.34 mmol/L (SD 0.91) at week 48, P=0.03], whereas no significant change occurred for total, HDL and LDL cholesterol. HIV-RNA remained undetectable in 82.1% of patients at week 2, 81.5% at week 12, 92.6% at week 24, 88.9% at week 36 and 89.3% at week 48, which corresponds to 18 episodes of VL of >50 copies/mL reported in 13 patients during the study. All episodes of detectable viraemia were followed by an undetectable value 2 weeks after and no change in lopinavir/ritonavir dose was therefore decided. Values of transient viraemia that were considered as blips varied between 53 and 1842 copies/mL [median number of blips per patient, 1.5 (min 1; max 3)]. Among the putative risk factors for blips that were tested in a univariate analysis, a borderline association was found with treatment duration before inclusion (130.7 months (SD 143) in patients with a blip versus 50.1 months (SD 62.5) in patients without a blip, P=0.06). No association was evident between the occurrence of blips and a lopinavir concentration of <3000 ng/mL.

PI mutations were found in the genotypic tests performed on plasma samples from the three patients with detectable VL at week 48 (80, 160 and 1842 copies/mL). PBMC samples at baseline and week 48 were available for all 13 patients with a VL blip during the study. The distribution of subtypes was as follows: subtype B, n=6; CRFO2-AG, n=5; subtype A, n=1; and sample...
unable to be amplified, \( n = 1 \). The genotyping analysis of proviral DNA revealed the emergence of two newly acquired PI mutations in three patients: I47M \( ( n = 2 ) \); and M46I \( ( n = 1 ) \). All viruses with PI mutations belonged to the B subtype. Long-term follow-up showed that six patients stopped lopinavir/ritonavir between week 48 and week 72 (three were included in a clinical trial, two were considered an immunological failure and one stopped treatment for personal reasons). None of the three patients with newly acquired PI mutations at week 48 presented with virological failure at week 72 without treatment modification.

**Discussion**

In this 48 week study with a follow-up extended to 72 weeks, we observed that in previously PI- and NNRTI-naive patients currently treated with reduced-dose lopinavir, transient HIV viraemia was not associated with the emergence of newly acquired PI mutations implicated in virological failure, as revealed by proviral DNA analysis. In our study, a dose reduction to lopinavir/ritonavir 267/67 mg twice daily resulted in lopinavir/ritonavir concentrations similar to those previously observed in a pharmacokinetic study using a new lopinavir/ritonavir 200/50 mg tablet formulation administered as lopinavir/ritonavir 800/200 mg once daily. As in our study, once-daily administration of the new lopinavir/ritonavir formulation was associated with a more normalized triglyceride profile than twice-daily administration. A reduction in plasma lopinavir/ritonavir \( C_{\text{min}} \) may be a risk for virological failure due to a purported sub-optimal drug exposure. Results of trials assessing the efficacy of lopinavir/ritonavir monotherapy as maintenance therapy have been recently disclosed, including the OKO4 study, which suggested that lopinavir/ritonavir monotherapy as maintenance therapy was not inferior to a combination of two nucleosides + lopinavir/ritonavir. Nevertheless, a significantly higher number of patients on monotherapy exhibited low detectable viraemia during follow-up. None harboured PI mutations, but the genotyping analysis was only performed using plasma. Episodes of transient viraemia were also observed in our study and we think that the analysis of mutations in proviral DNA would provide further valuable data on the risk of selecting protease mutations in this context. The results of our study extend those of the OKO4 trial by suggesting that the process of PI mutation selection in proviral DNA is rarely observed during intermittent viraemia in PI- and NNRTI-naive patients exposed to a lower lopinavir/ritonavir plasma drug concentration. An interesting finding is the nature of the mutations selected in PBMCs. Mutations at positions 46 and 47 have been previously reported in patients failing lopinavir/ritonavir treatment, while the mutation typically reported at codon 47 has been I47A and sometimes I47V. The long-term consequences of such mutations detected only in proviral DNA remain to be clarified, as none of the three patients included in our study who expressed PI mutations in proviral DNA developed virological failure after 3 years of treatment. However, a cohort study conducted in Spain of patients treated with various antiretroviral combinations suggested that <10% of 2720 patients who experienced blips presented with virological failure during subsequent follow-up. Besides, archived mutations have been associated with a higher risk of
subsequent virological failure only in patients who experienced a simplification strategy despite archived abacavir mutations, and in patients exhibiting minority quasispecies of NNRTI-resistant viruses.

Several limitations arise from this study. First, it is a pilot study with one treatment arm and no comparison arm with standard lopinavir dosage. However, patients acted as their own control because all were receiving lopinavir before inclusion in the Kaledose trial and lipids and lopinavir concentrations were compared with those of the pre-study inclusion. Second, the main inclusion criterion concerned the necessity of presenting a plasma lopinavir $C_{\text{min}}$ of >5000 ng/mL in order to be eligible for reduction of lopinavir posology. This means that data from this study cannot be extrapolated to all patients treated with lopinavir. Third, the number of patients is relatively low. Therefore, we cannot exclude that the non-significant association between reduced lopinavir dosing and HDL or LDL cholesterol level is not due to a lack of power.

In conclusion, we observed in this pilot study that the selection of mutations was a rare occurrence in naive patients treated with reduced-dose lopinavir and experiencing transient viraemia. Such a strategy was associated with a significant decrease in triglyceride levels, without impact of archived mutations on long-term viral suppression. Further evaluation of the potential advantages of reduced lopinavir dosing should be conducted with the current lopinavir formulation.

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Transparency declarations
None to declare.

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