Atherosclerosis in the HIV and non-HIV setting: detecting and modifying cardiovascular risk
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Pharmacokinetics and Pharmacodynamics of Combined Use of Lopinavir/ritonavir and Rosuvastatin in HIV-infected Patients

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Abstract

Background: Lopinavir/ritonavir-containing antiretroviral therapy can cause hyperlipidaemia in HIV-infected patients. However, most statins are contraindicated due to drug-drug interactions. Rosuvastatin undergoes minimal metabolism by CYP450, so no CYP450-based interaction with lopinavir/ritonavir is expected. This pilot study explored the lipid-lowering effect of rosuvastatin and assessed the effect of lopinavir/ritonavir on the pharmacokinetics of rosuvastatin and vice versa.

Methods: HIV-infected patients on lopinavir/ritonavir (viral load<400 copies/mL) with total cholesterol (TC)>6.2mmol/L were treated with rosuvastatin for 12 weeks, starting on 10 mg QD. If fasting target values (TC<5.0mmol/L; HDL-c>1.0mmol/L; LDL-c<2.6mmol/L; TG<2.0mmol/L) were not reached, rosuvastatin was escalated to 20mg or 40mg at week 4 and 8. Plasma lopinavir/ritonavir trough levels (C_{min}) were drawn at week 0, 4, 8, 12; rosuvastatin C_{min} at week 4, 8, 12.

Results: 22 patients completed the study. Mean reductions in TC and LDL-c from baseline to week 4 (on rosuvastatin 10mg QD) were 27.6% and 31.8%. Lopinavir/ritonavir concentrations were not influenced by rosuvastatin (p=0.44 and 0.26, repeated-measures analysis). Median (IQR) rosuvastatin C_{min} for 10mg, 20mg and 40mg QD were 0.97 (0.70–1.5), 2.5 (1.3–3.3) and 5.5 (3.3–8.8) ng/mL. Rosuvastatin was well tolerated; three patients experienced transient muscle pain.

Conclusions: Rosuvastatin appeared to be an effective statin in hyperlipidaemic HIV-infected patients. Lopinavir/ritonavir levels were not affected by rosuvastatin, whilst rosuvastatin levels unexpectedly appeared to be increased 1.6-fold as compared to data from healthy volunteers. So, probably another mechanism other than CYP450 is involved in this interaction. Until safety and efficacy have been confirmed in larger studies, the combination of rosuvastatin and lopinavir/ritonavir should be used with caution.
Introduction

Lopinavir/ritonavir is one of the most widely-used HIV-protease inhibitors (PIs). One of the drawbacks when using lopinavir/ritonavir is the frequent development of hyperlipidaemia [1]. A strategy to manage lopinavir/ritonavir-induced hyperlipidaemia may be treatment with lipid-lowering drugs such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) and/or fibrates [2;3]. The concomitant use of such drugs with PIs has been hampered by the occurrence of drug-drug interactions [4]. Most statins are metabolized by cytochrome P450 (CYP450), while PIs, including lopinavir/ritonavir, are strong inhibitors of this enzyme.

Hence, there is a clear need for statins which have both potent lipid-lowering effects and are not subject to such drug-drug interactions. The newly introduced statin rosuvastatin may fulfil these requirements. CYP450-based metabolism does not play an important role in the clearance of rosuvastatin [5;6]. Rosuvastatin is only minimally metabolized by CYP3A4 [7]. Furthermore, rosuvastatin has been demonstrated to be an effective statin in HIV-uninfected patients [8]. However, the pharmacokinetics of rosuvastatin when used concomitantly with PIs has not yet been investigated.

The primary objective of this pilot study was to explore the effect of rosuvastatin on plasma lipids in HIV-infected patients with hyperlipidaemia who were on stable lopinavir/ritonavir containing antiretroviral therapy (ART). The bidirectional pharmacokinetics of rosuvastatin and lopinavir/ritonavir and the safety of the combination were also assessed.

Methods

This pilot study was conducted from May 2004 until July 2005 at Radboud University Nijmegen Medical Centre, Academic Medical Center Amsterdam, and University Medical Centre Leiden, all in the Netherlands, and at University of Bonn and Cologne in Germany.

Study population

This study was conducted in HIV-1-infected dyslipidaemic patients, aged 18 to 65 years, stable on lopinavir/ritonavir 400/100mg BID-containing ART for at least 3 months and had signed informed consent. Hyperlipidaemia was defined as fasting total cholesterol >6.2mmol/L (239 mg/dL). Plasma HIV-1 RNA had to be below 400 copies/mL. The main exclusion criteria were: sensitivity/idiosyncrasy to rosuvastatin or chemically related compounds, a relevant history or current condition that might interfere with pharmacokinetics and pregnant or breast-feeding females. Japanese/Chinese patients were excluded because Asian patients are more likely to experience side effects of rosuvastatin such as rhabdomyolysis [9]. Other exclusion criteria
were: creatinine clearance <60ml/min (calculated from serum creatinine), fasting triglyceride level >8.0mmol/L (700mg/dL), abnormal creatinekinase levels (>10 times upper limit of normal), history of statin-related rhabdomyolysis or family history of inheritable muscle disease. The use of any statin or fibrate in the 6 weeks prior to the first dose, previous use of rosuvastatin and concomitant medication, known to interfere with the pharmacokinetics of rosuvastatin or lopinavir/ritonavir, were not allowed. Subjects were advised not to change their diet during the study. At screening (within 3 weeks prior to the first dose) patients’ eligibility for inclusion was established.

**Study design**
All subjects started with an oral dose of 10mg rosuvastatin once-daily (QD) in addition to their regular dose of lopinavir/ritonavir (400/100mg twice-daily [BID] as soft gelatine capsules) and other components of their current ART-regimen. Patients used this combination until week 4 at which time fasting lipids were determined. The dose of rosuvastatin was escalated to 20mg QD for the following 4 weeks if patients had not reached each of the four following targets derived from the Guidelines of the HIV Medical Association of the Infectious Disease Society of America (IDSA) and the Adult AIDS Clinical Trials Group [10]: total cholesterol <5.0mmol/L (192mg/dL); LDL-cholesterol <2.6mmol/L (100mg/dL); HDL-cholesterol >1.0mmol/L (40mg/dL); triglycerides <2.0mmol/L (175mg/dL). If after an additional 4 weeks of 20mg of rosuvastatin QD, the above-mentioned targets were still not reached at week 8, rosuvastatin was escalated once more to 40mg QD for the subsequent four weeks (up to week 12, end of study). If patients had reached all targets at week 4 or 8, they continued their current dose of rosuvastatin for the remainder of the study. Patients continued using lopinavir/ritonavir 400/100mg BID during the whole study period.
Relatively short periods, i.e. 4 weeks, were chosen after which the dose of rosuvastatin could be escalated if the abovementioned targets were not reached, because the maximum lipid-lowering effect of rosuvastatin has mostly been shown to be achieved after a 4-week dosing period [9], and because we wanted to limit the overall duration of the trial. The study was approved by the local ethics committees of each of the participating sites.

**Biochemistry, safety assessments and pharmacokinetic sampling**
Lopinavir/ritonavir trough levels, drawn just before the next dose (within 9–15 hours after intake), were determined at week 0, 4, 8, and 12. Rosuvastatin trough levels, 24 hours after intake, at week 4, 8, and 12. Serum biochemistry, including total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, hepatic and muscular enzymes were performed at week 0, 4, 8, and 12 at the local sites. Adverse events were assessed at each of these time points.
Total cholesterol, HDL-cholesterol and triglycerides were measured using enzymatic colorimetric tests. In Nijmegen, Bonn, Cologne, and Amsterdam LDL-cholesterol was calculated from the Friedewald formula [11]. In Leiden LDL-cholesterol was directly measured with a
colorimetric test; in Cologne this assay was used if TG > 4.5 mmol/L. If in Bonn patients had TG > 4.5 mmol/L, LDL-cholesterol could not be calculated; in Nijmegen and Amsterdam none of the patients experienced high TG levels.

Bioanalysis of lopinavir/ritonavir and rosuvastatin concentrations in plasma
Plasma samples of lopinavir/ritonavir were analyzed at the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre. This department has established an HPLC assay for lopinavir/ritonavir, derived from an HPLC method which has been published previously [12].
Rosuvastatin plasma samples were measured at Covance Laboratories, Inc. Madison, Wisconsin, United States. The quantification of rosuvastatin in plasma was performed by automated solid-phase extraction using tandem mass spectrometric detection, as published previously [13].

Sample size and statistical analysis
No formal sample size calculation was performed as this was a descriptive pilot study. The changes in lipids were assessed using a Paired Samples T-test, because these parameters were normally distributed. For the comparison of lopinavir/ritonavir concentrations General Linear Model Repeated Measures analyses were performed using the logarithmic transformed trough concentrations.
Statistical evaluations were carried out using SPSS® for Windows, version 12.0.1 [SPSS Inc, Chicago, IL, USA].

Results
Baseline characteristics
Twenty-two HIV-1-infected patients with undetectable viral load and without hepatitis coinfection (20 males) were included. Median age was 48 (IQR: 40-56) years. Median CD4 at baseline was 399 (IQR: 265-482) *10^6/L. Viral load and CD4 count did not change during the trial between baseline and week 12 (p=0.794 and p=0.783, respectively; Paired Samples T-test).

Pharmacodynamics
At baseline, all patients started using 10 mg of rosuvastatin QD. At week 4, three patients continued using 10 mg QD, but nineteen patients were dose escalated to 20 mg of rosuvastatin QD. From week 8–12, one patient was still using 10 mg; seven had been escalated to 20 mg QD; fourteen to 40 mg QD. One patient had developed an elevated creatine kinase level at week 4 (493 U/L, which is about 4-fold higher than the upper limit of normal) and was therefore not dose escalated although predefined lipid targets were not met.
Table 1 shows the effect of rosuvastatin on fasted total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels. Triglyceride levels of three patients were not included in the data analysis. In spite of the fact that their triglyceride levels at screening were >8.0mmol/L, these patients met all other inclusion criteria and were nevertheless included to investigate the effect of rosuvastatin on total cholesterol, LDL-cholesterol and HDL-cholesterol, because the local investigator was determined to start lipid-lowering statin-therapy in them anyway. Mean percent reduction (standard deviation [sd]) in total cholesterol, LDL-cholesterol, and triglycerides resulting from 10mg of rosuvastatin QD from week 0 to 4 was 27.6% (7.6%), 31.8% (16.7%), and 20.7% (38.5%), respectively (p<0.001, p<0.001, and p=0.022 vs. baseline; Paired Samples T-test). Mean increase in HDL-cholesterol between week 0 and 4 was 3.1% and not statistically significant (sd: 12.5%; p=0.460). The overall mean reduction in lipid levels (sd), comparing week 12 to baseline, combining data from all dose groups, was 33.8% (9.7%), 38.9% (25.8%) and 37.0% (26.1%) for total cholesterol, LDL-cholesterol and triglycerides, respectively (p<0.001, p<0.001, and p=0.001). There was a mean increase in HDL-cholesterol of 16.9% which did not reach statistical significance (sd: 39.5%; p=0.080 vs. baseline).

At week 12, 32% (7/22) of the patients met all lipid targets; when looking at each lipid parameter individually, 68% (15/22) of the patients reached the target for total cholesterol, 68% (13/19) for LDL-cholesterol, 68% (15/22) for HDL-cholesterol and 53% (10/19) for triglycerides.

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol Mean (sd) (mmol/L) N=22</th>
<th>LDL-cholesterol Mean (sd) (mmol/L) N=20</th>
<th>HDL-cholesterol Mean (sd) (mmol/L) N=22</th>
<th>Triglycerides Mean (sd) (mmol/L) N=19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>7.1 (0.95)a</td>
<td>4.2 (0.99)c</td>
<td>1.2 (0.43)c</td>
<td>3.6 (1.8)a</td>
</tr>
<tr>
<td>Week 4</td>
<td>5.2 (0.81)b</td>
<td>2.8 (0.61)b</td>
<td>1.2 (0.41)b</td>
<td>2.6 (1.3)b</td>
</tr>
<tr>
<td>Week 8</td>
<td>4.9 (1.2)</td>
<td>2.7 (0.77)</td>
<td>1.2 (0.51)</td>
<td>2.2 (1.0)</td>
</tr>
<tr>
<td>Week 12</td>
<td>4.7 (0.79)</td>
<td>2.5 (0.61)d</td>
<td>1.4 (0.81)</td>
<td>2.3 (1.4)</td>
</tr>
</tbody>
</table>

* Two patients were excluded for data-analysis because of non-fasting; * One patient was excluded because of non-fasting; * Two patients were excluded because of non-fasting and one level could not be determined because of high triglyceride levels; * One level could not be determined because of high triglyceride levels

**Pharmacokinetics**

Lopinavir levels of twenty patients were included in our data analysis; levels of two patients were excluded, because these samples were not drawn within the prespecified time window (9–15 hours after intake). Median (IQR) lopinavir Cmin was 5.2 (3.7–6.5), 5.4 (4.1–7.6), 5.6 (4.2–7.8) and 5.2 (3.6–6.3) mg/L at week 0, 4, 8 and 12, respectively. A repeated-measures analysis showed no difference between logarithmic transformed lopinavir Cmin at weeks 0, 4, 8, and 12 (p=0.44).
For the analysis of the ritonavir trough levels, the same samples as for lopinavir data analysis were used. Median (IQR) ritonavir $C_{\text{min}}$ (9–15 hours after intake) was 0.22 (0.17–0.28), 0.25 (0.14–0.36), 0.26 (0.14–0.33) and 0.20 (0.17–0.34) mg/L at weeks 0, 4, 8 and 12, respectively (repeated-measures analysis: $p=0.26$).

For rosuvastatin, trough levels were available for 12, 13, and 9 patients on 10 mg, 20 mg, and 40 mg of rosuvastatin QD, respectively. Median trough levels were not provided for all patients, because in some cases, blood samples were drawn about 12 hours instead of 24 hours after intake. For nine patients rosuvastatin plasma levels were available for doses of 10mg, 20mg, as well as 40mg QD. Table 2 shows the median trough levels for the different dosages compared to those obtained from historical HIV-uninfected controls without hyperlipidaemia (measured with the same method and at the same laboratories as the samples from our trial) (data on file AstraZeneca; [14-16]). Rosuvastatin plasma trough levels appeared to be 1.6-fold higher compared to those in these historical healthy controls.

**Adverse events and safety assessments**

None of the included patients dropped out or temporarily stopped taking rosuvastatin during this 12-week trial. The reported adverse events were mild: diarrhea (N=2), headache (N=2), and a cold (N=2). Three patients experienced transient muscle pain/cramps: one on 10mg of rosuvastatin QD during week 3, one on 20mg QD during week 8–12, and one on 40mg QD during week 8–12, respectively. The second patient also had an elevated creatinekinase level of 436 U/L at week 12; rosuvastatin was stopped after the trial. After stopping, the symptoms disappeared and the creatinekinase level returned to normal. The other two patients had normal creatinekinase levels (range: 64–100 U/L) and continued to use rosuvastatin.

In addition, three patients had clinically asymptomatic elevations of creatinekinase above 250 U/L, ranging from 363 to 676 U/L. One of these patients already had an elevated level at baseline.

Median liver enzymes, comparing baseline to week 12, were as follows: median (IQR) ALAT (N=20; in two patients ALAT was not determined) and ASAT (N=22) were 25.5 (16.0-37.8) and 28.0 (20.0-35.3) at week 0 and 26.5 (22.0-47.5) and 27.5 (21.0-35.0) at week 12, respectively.
Table 2  Median rosuvastatin trough levels for 10, 20 and 40 mg compared to historical healthy controls

<table>
<thead>
<tr>
<th>Rosuvastatin dosage</th>
<th>Rosuvastatin C_{min} (ng/mL)</th>
<th>Ratio C_{min} rosuvastatin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Our trial Median (IQR)</td>
<td>Historical healthy controls* [14] Mean (range)</td>
</tr>
<tr>
<td>10 mg (N=12)</td>
<td>1.0 (0.69–1.5)</td>
<td>0.63 (0.27–1.2)</td>
</tr>
<tr>
<td>20 mg (N=14)</td>
<td>2.5 (1.3–3.3)</td>
<td>1.6 (0.54–4.1)</td>
</tr>
<tr>
<td>40 mg (N=9)</td>
<td>4.5 (3.3–7.5)</td>
<td>2.9 (1.7–3.6)</td>
</tr>
</tbody>
</table>

* Data on file AstraZeneca

Discussion

In a recently published study, performed in hyperlipidaemic HIV-1-infected patients on various ART-regimens using 10mg of rosuvastatin QD, similar reductions in lipid levels were observed following 24 weeks of treatment [17]. In HIV-negative patients with hyperlipidaemia, rosuvastatin in a dose range of 10-40mg, reduced LDL-cholesterol by 43%-63% (reviewed by Cheng [18]). For 10mg of rosuvastatin, we found in our trial, with a relatively small sample size, a somewhat lower effect on LDL-cholesterol. We cannot compare the results at week 8 and 12 of our study with other studies in HIV-negative patients because our trial was designed to titrate the patient to our predefined goals and only the poor responders were eligible for dose escalation.

The somewhat lower effect of rosuvastatin we found in our trial despite the increased rosuvastatin levels, could be explained by the fact that when plasma levels are increased, the intake of rosuvastatin in the hepatocytes, where it conducts its effect, is decreased. So, a decreased concentration of rosuvastatin in the hepatocytes could cause a decreased lipid-lowering effect.

Previous studies reported similar lopinavir trough concentrations [19;20], as we found. The same was true for ritonavir trough levels [21;22]. Our observations are in accordance with in vitro observations indicating an absence of inhibitory or inducing effects of rosuvastatin on CYP_{450}-enzymes [7;23].

Only a few trials investigated rosuvastatin pharmacokinetics in patients with hyperlipidaemia. In contrast, pharmacokinetic studies have been performed extensively in healthy volunteers, but trough levels have only been reported in a minority of these studies [14-16]. For comparison, we used data from a study in healthy subjects on file at AstraZeneca, the manufacturer of rosuvastatin. In our trial, rosuvastatin trough levels were 1.6-fold higher as compared to the levels found in that study in healthy, HIV-uninfected subjects without hyperlipidaemia for all dosages of rosuvastatin. These higher rosuvastatin levels could not have been caused by
A CYP$_{450}$-based interaction since 90% of rosuvastatin is excreted unchanged in faeces [9] and CYP-based metabolism was anticipated not to play an important role in the clearance of rosuvastatin [7]. Furthermore, studies in healthy volunteers showed no relevant drug-drug interactions with rosuvastatin involving CYP2C9 [24], CYP2C19 [24], and CYP3A4 [25-27]. An alternative explanation for our observation might be that lopinavir/ritonavir affects a membrane transporter of rosuvastatin. In a study by Simonson [28] a significant increase in rosuvastatin exposure was observed in heart transplant recipients using cyclosporine. The mechanism was believed to be cyclosporine-mediated inhibition of the human liver transporter organic anion transporting polypeptide C (also known as OATP-1B1). OATP-C (1B1) is a transporter protein [29], likely to be involved in the hepatic uptake of rosuvastatin [30-32]. To our knowledge, no studies have been performed investigating the effect of lopinavir/ritonavir on OATP. However, ritonavir, saquinavir and indinavir are known to inhibit OATP1B1 [31;32].

A recently performed pharmacokinetic drug-drug interaction study with lopinavir/ritonavir in 15 healthy volunteers also showed an increase in rosuvastatin exposure: 2.1-fold increase in AUC and 4.7-fold increase in $C_{\text{max}}$ [33]. This effect on rosuvastatin AUC and $C_{\text{max}}$ is in agreement with the results of our trial. However, we cannot explain why Hoody did not find an effect on rosuvastatin $C_{\text{min}}$ while observing an increased AUC and $C_{\text{max}}$.

Whether the increased rosuvastatin exposure, observed in our trial and by Hoody [33], is clinically relevant, is questionable while the orders of magnitude are less than what has been reported in previous interaction studies between several different PIs and statins other than rosuvastatin: the combination of PIs with atorvastatin (20mg QD) and simvastatin (40mg QD and 20mg QD) resulted in an increase in statin AUC by 590% [34], 3059% [35] and 505% [36], respectively.

A favourable tolerability profile of rosuvastatin including the absence of any cases of rhabdomyolysis was observed both in our study and by Calza [17]. One needs to realize however that rhabdomyolysis is a relatively rare complication of statin use, thus larger studies are needed to confirm safety of using rosuvastatin in HIV-infected patients on ART.

Our study has a number of limitations. Because of the relatively small sample size, no centralized plasma lipid measurements and the strict lipid target criteria, the results regarding safety and pharmacodynamic effects on lipids should be interpreted with caution. In addition, although patients were instructed not to change their diet during the study, formal data on diet were not collected during the trial and changes in dietary pattern may have influenced the results. Another limitation is that we did not include a randomized control group. Rosuvastatin trough levels were compared to those reported in healthy uninfected historical controls, and lopinavir/ritonavir plasma levels for each patient were compared to baseline before adding rosuvastatin. Future drug-drug interaction studies between rosuvastatin and PIs may consider including a randomized control group of HIV-infected patients using rosuvastatin in the ab-
sence of ART-regimens. Finally, in this pilot study we have chosen to only determine rosuvastatin trough levels in order not to burden patients with recording 24-hour pharmacokinetic curves. Nonetheless, determination of rosuvastatin AUCs would provide more information concerning any interaction between rosuvastatin and lopinavir/ritonavir, and for this reason we do suggest that in future trials pharmacokinetic curves are obtained.

In conclusion, in our pilot study rosuvastatin appeared to be an effective statin in hyperlipidaemic HIV-1-infected patients treated with lopinavir/ritonavir. Although plasma lopinavir/ritonavir levels were not affected by the concomitant use of rosuvastatin, rosuvastatin levels were 1.6-fold higher compared to those in HIV-uninfected healthy volunteers without hyperlipidaemia. Our findings support further research, especially a placebo-controlled randomized trial, in larger numbers of patients to more fully elucidate pharmacokinetics, efficacy, and safety of the combined use of rosuvastatin and lopinavir/ritonavir in HIV-infected patients. Until such time, the combination of rosuvastatin and lopinavir/ritonavir should continue to be used with caution.

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