Metabolic Effects of Ritonavir-boosted Atazanavir and Darunavir in HIV-negative Adults: A Randomised Comparison

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Abstract

**Objectives:** The increased cardiovascular risk historically associated with protease inhibitor (PI) use in HIV-infected individuals is only partly attributable to fasting dyslipidaemia, and may not apply to the currently recommended PI drugs - atazanavir and darunavir. In this prospective pilot study, we aimed to describe the isolated metabolic differences between atazanavir/ritonavir and darunavir/ritonavir on circulating fasting and post-prandial lipids (primary objective), and arterial stiffness, glycaemic and inflammatory markers (secondary objectives) by administering them as monotherapy to healthy, HIV-uninfected adults.

**Methods:** Participants were randomised 1:1 to receive open-label atazanavir (300 mg/day) or darunavir (800 mg/day), both boosted with ritonavir (100 mg/day), for 4 weeks. A standardised high-energy meal was consumed at weeks 0 and 4, with hourly post-prandial blood samples and arterial stiffness assessed for 4 hours. Mean fasting and post-prandial measurements were compared, the latter as the change in mean post-prandial incremental area under the curve.

**Results:** Twenty participants (10 atazanavir/ritonavir, 10 darunavir/ritonavir; 50% male, mean age 38 years, mean blood pressure 113/72 mmHg, mean total cholesterol 4.4 mmol/L, mean triglycerides 0.9 mmol/L) completed the study. After four weeks, fasting total cholesterol rose more with darunavir/ritonavir than atazanavir/ritonavir (+0.8 mmol/L vs. +0.3 mmol/L, respectively; p=0.041), as did low-density lipoprotein cholesterol (+0.1 mmol/L vs. +0.7 mmol/L, respectively; p=0.017). Between-group differences in post-prandial lipid changes were non-significant. Post-prandial decline in arterial stiffness was greater with atazanavir/ritonavir than darunavir/ritonavir (-27.6 h% vs. +0.1 h%, respectively; p=0.041). Bilirubin rose significantly with atazanavir/ritonavir but not darunavir/ritonavir (+29 µmol/L vs. +0.3 µmol/L, respectively; p=0.017). Between-group differences in post-prandial lipid changes were non-significant. Post-prandial lipid effects were similar, however. Further study of treatment-emergent cardiovascular event incidence with darunavir/ritonavir therapy is required to determine the clinical relevance of this between-group difference.

**Conclusion:** Atazanavir/ritonavir has modestly, but significantly, favourable metabolic and haemodynamic profiles over darunavir/ritonavir, evident even after short-term monotherapy. Post-prandial lipid effects were similar, however.

**Keywords:** Protease inhibitors; Atazanavir; Darunavir; Ritonavir; Dyslipidaemia; Post-prandial lipids; Arterial stiffness; Tonometry; Bilirubin; Cardiovascular risk

Introduction

Protease inhibitor (PI)-based combination therapies for human immunodeficiency virus (HIV) infection possess potent anti-HIV activity, are widely available, and present the highest genetic barrier to resistance [1]. However, PIs are subject to a wide range of drug interactions, which may complicate dosing requirements and adherence. They have also been associated, in multiple studies, with fasting dyslipidaemia and/or an increase in overall cardiovascular risk [2]. This effect is independent of other known risk factors, including HIV infection itself.

The results of the D:A:D study indicate that only half of the cardiovascular risk specifically associated with PI-based combination therapy is attributable to fasting dyslipidaemia. Postulated additional, PI-induced effects include endothelial dysfunction [3], impaired cholesterol efflux from macrophages [4], and vascular wall effects leading to greater arterial stiffness [5]. Arterial stiffness relates measurement of change in arterial diameter and pressure at the same site, and is positively correlated with premature coronary artery disease, and is a validated, independent predictor of cardiovascular mortality in HIV-uninfected populations [6]. It is also associated with the metabolic syndrome, which is of emerging importance as non-acquired immune deficiency (AIDS) events become the dominant source of morbidity and mortality in the HIV-infected population [7].

Post-prandial (non-fasting) changes in circulating lipids, particularly triglycerides, have been prospectively associated with cardiovascular risk in the general population [8-10], but have not been widely assessed in the context of antiretroviral therapy. We have previously demonstrated that 4 weeks of low-dose ritonavir (100 mg/day) in healthy, HIV-uninfected adults led to significant increases in post-prandial low-density lipoprotein (LDL) cholesterol, but not triglycerides, when compared to the HIV integrase strand transfer inhibitor raltegravir [11]. We have also demonstrated that mean arterial stiffness, as measured non-invasively via radial artery tonometry, is...
significantly higher in treatment-experienced than treatment-naïve HIV-infected individuals [12], corroborating the findings of an earlier study by Schillaci et al. [13].

The association with secondary dyslipidaemias and higher incidence of cardiovascular events has led to concerns about PI use in general, despite evidence that individual PI drugs do differ. In current United States Department of Health and Human Services antiretroviral guidelines, two PI drugs – atazanavir and darunavir (both boosted with ritonavir) – are listed as preferred options for initial therapy [14]. No association has been found between atazanavir and myocardial infarction or stroke [15]. Presently there is insufficient data to perform a similar analysis for darunavir. It would therefore be of interest to compare the metabolic and haemodynamic effects of darunavir and atazanavir; any relative differences may provide insights into mechanisms of cardiovascular risk.

While it is possible to estimate relative lipid effects of atazanavir and darunavir from separate clinical trials, such indirect comparisons are confounded by presence of active HIV infection – itself a cause of dyslipidaemia – and concomitant antiretroviral drugs [16-18]. This study simulates the isolated metabolic and vascular effects of ritonavir-boosted atazanavir and darunavir by administering them to healthy, HIV-uninfected adult volunteers.

Methods

This was a prospective open-label pilot study, conducted at a single centre in Sydney, Australia. Eligible volunteers were randomised 1:1 to receive ritonavir 100 mg/day with either atazanavir (300 mg/day) or darunavir (800 mg/day) for four weeks. All doses were to be taken with food. Randomisation was conducted according to a standard procedure, by an individual not involved in the study design or conduct.

All participants were healthy adult volunteers, capable of providing consent. They were eligible if they had a stable diet and weight, a body mass index of 20-30 kg/m², family history of hypercholesterolaemia 20%, fasting triglycerides <2.0 mmol/L, fasting total cholesterol <6.0 mmol/L and negative HIV serology at screening. Volunteers were ineligible if pregnant, breast-feeding, diabetic, or receiving anti-hypertensive or hypolipidaemic therapy, had any contraindication to atazanavir, darunavir or ritonavir, or a known allergy to any component of the study meal.

Presently, there are no data for medication-induced changes in post-prandial lipids. Consequently, our sample size was based on previously reported studies of fasting lipids, particularly our earlier study [11]. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines use LDL cholesterol levels as a recommended treatment target [20], and a prospective meta-analysis of 90,056 individuals across 14 randomised trials of statins reported that even a 0.2 mmol/L change in LDL cholesterol amongst our cohort of healthy participants.

This study was approved by the local Human Research Ethics Committee, and conducted in accordance with the Helsinki II declaration and the International Committee on Harmonisation’s Good Clinical Practice Guideline. The protocol was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000917033). Each participant provided signed, informed consent before enrolment.

At weeks 0 and 4, participants consumed a standardised morning meal (1,386 calories: 38% fat, 16% protein, 45% carbohydrate), having fasted for 12 hours. They were required to abstain from any exercise or alcohol intake for the 48 hours prior. Fasting blood samples and arterial stiffness measurements were then taken hourly post-meal. Systemic arterial stiffness was examined as the augmentation index, defined as the difference between the second and first systolic peaks, as a percentage of the pulse pressure [22]. It was recorded via applanation tonometry at the radial artery, performed by an accredited operator. A validated proprietary transfer algorithm was then used to correct for heart rate (SphygmoCor® SCOR-PVx tonometer, AtCor Medical, Sydney, Australia), giving the final, adjusted, value for the augmentation index.

The primary objective was to describe relative changes in the circulating fasting and post-prandial lipid parameters – total, LDL and high-density lipoprotein (HDL) cholesterol fractions and triglycerides – after four weeks. Secondary outcomes of interest were the fasting and post-prandial measurements of arterial stiffness, glucose and inflammatory markers (C-reactive protein, D-dimer). All changes in post-prandial parameters were assessed as the mean incremental area under the curve, using previously described methods [11]. Between-group post-prandial comparisons were made by analysis of variance, fasting parameters were compared with the Mann-Whitney U-test, and p values were assigned a significance level of 0.05.

Safety was assessed by recording clinical adverse events (of any grade/severity) and serum biochemistry (hepatic transaminases, alkaline phosphatase, gamma glutamyl transferase, albumin, total protein and bilirubin). Adherence was determined by pill counting. All concomitant medications were recorded.

Results

Participant disposition and baseline characteristics

Twenty participants (male 50%, mean age 38 years, mean body mass index 24 kg/m², family history of hypercholesterolaemia 20%) completed the study (Figure 1). The mean age of the darunavir/ritonavir group was higher (44 years vs. 33 years, respectively). Other baseline characteristics are listed in Table 1. No participants commenced cigarette smoking during the study. Mean adherence to study medication was similar in both groups (atazanavir/ritonavir 82% vs. darunavir/ritonavir 81%).

Lipid parameters

Mean fasting baseline LDL cholesterol in both groups was 2.7 mmol/L. After four weeks, fasting cholesterol fractions rose in both groups (Table 2), with significant differences for total cholesterol (+0.3 mmol/L vs. +0.8 mmol/L, respectively; p=0.041) and LDL cholesterol (+0.1 mmol/L vs. +0.7 mmol/L, respectively; p=0.017). Mean changes in all post-prandial lipid fractions were similar between the groups (p=0.05).

Non-lipid parameters

A between-group difference was identified for arterial stiffness: with atazanavir/ritonavir, there was a significant fall in post-prandial arterial stiffness compared to darunavir, which remained unchanged (-27.6 h% vs. +0.1 h%, respectively; p=0.041). This difference was restricted to the post-prandial state, as the change in fasting arterial stiffness after four weeks was similar between the groups (+2.2% vs. +0.2%, respectively; p=0.670). Spearman’s correlation was used to describe the relationship between bilirubin rise at week four and the post-prandial change in arterial stiffness. Although a moderate, negative correlation
35 individuals screened for eligibility
23 randomly assigned to study groups
12 assigned to receive atazanavir/ritonavir (300/100 mg) once-daily
11 assigned to receive darunavir/ritonavir (800/100 mg) once-daily
1 did not start drug (withdrew consent)
11 commenced assigned study drug
1 discontinuation (rash)
12 ineligible
9 entry criteria (5 elevated cholesterol, 3 high BMI, 1 high ALT)
2 withdrew consent before randomisation
1 investigator discretion (deemed unable to complete study procedures)
1 investigator discretion (deemed unable to complete study procedures)
10 completed study procedures
10 completed study procedures
BMI - Body mass index; ALT - alanine transaminase.  
Figure 1: Study profile and participant disposition.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Atazanavir/Ritonavir (n=10)</th>
<th>Darunavir/Ritonavir (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>4 (40)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Mean age, years (SE)</td>
<td>33 (4.6)</td>
<td>44 (5.7)</td>
</tr>
<tr>
<td>No previous smoking, n (%)</td>
<td>2 (20)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Family history, n (%) cardiovascular disease</td>
<td>3 (30)</td>
<td>4 (40)</td>
</tr>
<tr>
<td></td>
<td>hypercholesterolaemia</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

**Clinical measures**

<table>
<thead>
<tr>
<th>Mean blood pressure, mmHg (SE)</th>
<th>Atazanavir/Ritonavir (n=10)</th>
<th>Darunavir/Ritonavir (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>systolic</td>
<td>109 (3)</td>
<td>114 (4)</td>
</tr>
<tr>
<td>diastolic</td>
<td>69 (2)</td>
<td>72 (3)</td>
</tr>
<tr>
<td>Heart rate, beats/minute (SE)</td>
<td>70 (3)</td>
<td>69 (3)</td>
</tr>
<tr>
<td>Height, m (SE)</td>
<td>1.71 (2.5)</td>
<td>1.78 (2.2)</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SE)</td>
<td>23 (0.5)</td>
<td>24 (1.1)</td>
</tr>
</tbody>
</table>

**Fasting blood analytes**

<table>
<thead>
<tr>
<th>Triglycerides, mmol/L (SE)</th>
<th>Atazanavir/Ritonavir (n=10)</th>
<th>Darunavir/Ritonavir (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 (0.1)</td>
<td>1.0 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (SE)</td>
<td>4.3 (0.2)</td>
<td>4.4 (0.3)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L (SE)</td>
<td>1.4 (0.1)</td>
<td>1.2 (0.1)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L (SE)</td>
<td>2.7 (0.2)</td>
<td>2.7 (0.3)</td>
</tr>
<tr>
<td>Total:HDL cholesterol (SE)</td>
<td>3.2 (0.2)</td>
<td>3.8 (0.4)</td>
</tr>
<tr>
<td>Bilirubin, µmol/L (SE)</td>
<td>10 (0.8)</td>
<td>9 (1.3)</td>
</tr>
<tr>
<td>Glucose, mmol/L (SE)</td>
<td>4.5 (0.1)</td>
<td>5.0 (0.3)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L (SE)</td>
<td>1.6 (0.9)</td>
<td>1.7 (0.8)</td>
</tr>
<tr>
<td>D-dimer, mg/L (SE)</td>
<td>0.4 (0.2)</td>
<td>0.3 (0.1)</td>
</tr>
</tbody>
</table>

SE: standard error of the mean; HDL: high-density lipoprotein; LDL: low-density lipoprotein.  
Table 1: Baseline characteristics and parameters of study participants.
between these two parameters emerged ($r=0.3$), it was non-significant ($p=0.194$).

Between-group differences in glucose, C-reactive protein and D-dimer were similar for both the fasting and post-prandial states. Likewise, anthropometric measurements (body mass index, waist circumference, weight) were similar compared to baseline for both study groups.

**Safety**

Of the 22 participants who received at least one dose of a study drug, one in each group withdrew prematurely for a generalised maculopapular rash of mild severity. Both resolved completely upon drug cessation.

In total, 39 clinical adverse events (irrespective of cause) were recorded, with most participants (68%) reporting at least one event. No serious adverse events were documented. Gastrointestinal events (nausea/vomiting, reflux, diarrhoea, constipation) were most common (50%), with bilirubin rising significantly with atazanavir/ritonavir but not darunavir/ritonavir (+29 µmol/L vs. -0.6 µmol/L, respectively; $p=0.001$). No participants withdrew due to hyperbilirubinaemia.

**Discussion**

To our knowledge, this is the first prospective study to examine the fasting and post-prandial metabolic and haemodynamic effects of boosted PI drugs in isolation. The two currently-preferred PI agents for initial therapy — atazanavir and darunavir — appeared similar in their post-prandial effects on measured lipid fractions, and glycemic and inflammatory indices. However, higher fasting lipid levels were noted in both groups, with the change in total and LDL cholesterol being significantly higher with darunavir/ritonavir than atazanavir/ritonavir. Finally, we report that arterial stiffness falls post-prandially with atazanavir/ritonavir compared to darunavir/ritonavir.

In separate randomised studies of initial antiretroviral therapy, darunavir/ritonavir has had similar effects on fasting cholesterol fractions to lopinavir/ritonavir, which in turn had higher cholesterol levels than with atazanavir/ritonavir, both after 48 weeks of treatment in HIV-infected adults [23,24]. These findings are supported by a recent head-to-head comparison of atazanavir/ritonavir and darunavir/ritonavir [25]. However, these studies do not take into account the effect of HIV infection, nor of concomitant antiretroviral drugs. Our fasting lipid results are consistent with the above comparisons, but suggest it to be an independent effect of PI exposure, not confounded either by HIV viraemia or by other antiretroviral drugs. Also, that this effect is evident after only four weeks suggests that PI-associated dyslipidaemia occurs early in treatment. The modest, but significant difference in fasting LDL cholesterol also indicates that atazanavir/ritonavir may be less atherogenic than darunavir/ritonavir. This may be clinically relevant, as by study completion the mean LDL cholesterol level in participants receiving darunavir/ritonavir rose sufficiently from baseline to require consideration of hypolipidaemic therapy under the NCEP ATP III guidelines [20].

Based upon the fasting lipid results, it would have been reasonable to anticipate that atazanavir/ritonavir would also have a lesser effect upon the post-prandial lipid levels than darunavir/ritonavir. However, the two drugs were similar in their post-prandial lipid effects. Considered in light of recent D:A:D Study results indicating that atazanavir/ritonavir is not associated with an increased incidence of vascular events [15], it suggests that post-prandial lipid effects are unlikely to be a mechanism for vascular pathology with either atazanavir/ritonavir or darunavir/ritonavir.

Our finding that post-prandial arterial stiffness falls with atazanavir/ritonavir but not darunavir/ritonavir is a novel result. Haemodynamic parameters are highly sensitive to feeding states, and acute falls in arterial stiffness are a well-described physiological response to food intake [26,27]. In high cardiovascular risk conditions such as obesity and diabetes mellitus, this magnitude of this fall is attenuated. PI-based treatment of HIV infection has formerly been associated with higher indices of aortic stiffness when compared to an HIV-uninfected cohort [28], but our study is the first to compare and describe differences between specific PI drugs.

While cigarette smoking can accelerate arterial stiffness [29], the proportion of participants with a smoking history was similar between the two groups and no participants changed their smoking status throughout the study. This effect was also limited to the post-prandial state, and there were no outliers in the arterial tonometry data, making smoking unlikely to account for the between-group difference, and also making a regression to the mean effect unlikely.

The lower post-prandial arterial stiffness seen with atazanavir/ritonavir is also noteworthy given the 90% incidence of unconjugated hyperbilirubinaemia in that group. A link between hyperbilirubinaemia and cardiovascular risk has been explored in non-HIV conditions. Individuals with Gilbert's syndrome, a common congenital cause of benign unconjugated hyperbilirubinaemia, have a prevalence of

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### Table 2: Mean post-prandial changes between baseline and week 4, measured as incremental area under the curve.

<table>
<thead>
<tr>
<th></th>
<th>Atazanavir/Ritonavir (SE)</th>
<th>Darunavir/Ritonavir (SE)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>+0.4 mmol/L (0.1)</td>
<td>+0.2 mmol/L (0.2)</td>
<td>0.545</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>+0.3 mmol/L (0.1)</td>
<td>+0.8 mmol/L (0.2)</td>
<td>0.041</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td>+0.1 mmol/L (0.1)</td>
<td>+0.7 mmol/L (0.2)</td>
<td>0.017</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol</td>
<td>0.0 mmol/L (0.0)</td>
<td>+0.1 mmol/L (0.0)</td>
<td>0.495</td>
</tr>
<tr>
<td>Total Low-density lipoprotein</td>
<td>+0.1 (0.2)</td>
<td>+0.4 (0.2)</td>
<td>0.174</td>
</tr>
<tr>
<td>Glucose</td>
<td>+0.2 mmol/L (0.1)</td>
<td>-0.2 mmol/L (0.2)</td>
<td>0.120</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>-0.2 mg/L (1.1)</td>
<td>-0.9 mg/L (0.6)</td>
<td>0.805</td>
</tr>
<tr>
<td>D-dimer</td>
<td>+0.1 mg/L (0.1)</td>
<td>+0.1 mg/L (0.1)</td>
<td>0.190</td>
</tr>
<tr>
<td>Arterial stiffness</td>
<td>+2.2 % (4.2)</td>
<td>+0.2 % (2.4)</td>
<td>0.670</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.041</td>
</tr>
</tbody>
</table>

SE: standard error of the mean.
ischaemic heart disease up to six times lower than the general population. This effect is independently associated with hyperbilirubinaemia, and may be due to the potent anti-oxidant activity of bilirubin [30]. Lower mean arterial stiffness correlating with bilirubin levels has also been described in Gilbert’s syndrome [31], and atazanavir given to HIV-uninfected diabetics improved endothelial function [32]. The degree of hyperbilirubinaemia seen with atazanavir is similar to that in Gilbert’s syndrome [33], making it attractive as a biologically plausible candidate to at least partly explain the favourable cardiovascular profile of atazanavir/ritonavir. However, studies in HIV-infected cohorts have failed to identify a significant association between bilirubin, atazanavir and decreased vascular resistance [34-36]. Our study was not powered to examine this relationship, and no significant correlation was identified between bilirubin rise and post-prandial arterial stiffness. But it should be noted that these other studies used flow-mediated vasodilation as a marker of endothelial function, not applanation tonometry, and made no assessments relative to feeding state, which our study did.

The recruitment of healthy, HIV-uninfected volunteers meant that the effect of active HIV replication, itself a source of inflammation, was excluded as a confounding factor. Our study also used low-dose ritonavir as a pharmacokinetic booster – which reflects PI prescription in actual clinical use.

The principal limitations of our study were the small participant numbers and its short duration. It is possible that a longer treatment period with a larger cohort and a control group might have shown significant post-prandial lipid effects, or glycaemic and anthropometric changes consistent with nascent metabolic syndrome. It nevertheless provides a basis for further refinement of sample size and duration of follow-up for similar future studies. The small numbers also meant that despite randomisation, mean age of the darunavir/ritonavir group was 11 years higher than the atazanavir/ritonavir group. As arterial stiffness is known to increase with age, this may confound our between-group comparison of post-prandial arterial stiffness. It should also be noted that the augmentation index, although reproducible, is one of several methods of marking the systemic arterial stiffness non-invasively. It is a complex surrogate marker, influenced by multiple factors (height, blood pressure, heart rate, gender) that remain incompletely defined [6].

By our objective measurements atazanavir has favourable metabolic and vascular characteristics over darunavir, albeit modest in magnitude. A prospective examination of treatment-emergent cardiovascular events for darunavir/ritonavir, like the one completed for atazanavir/ritonavir vs. raltegravir in HIV-infected adults. AIDS 24: 1727-1731.


AIDS Info (2013) Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services, Rockville, Maryland, USA.


