Postprandial lipid effects of low-dose ritonavir vs. raltegravir in HIV-uninfected adults

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Objective: Protease inhibitor therapy is associated with an increased risk of myocardial infarction. Half this risk appears attributable to fasting dyslipidemia, but half remains unexplained. We compared the fasting and postprandial effects of low-dose ritonavir and raltegravir on cardiovascular and metabolic risk factors.

Design: Randomized (1 : 1), open-label study.

Methods: Twenty HIV-uninfected volunteers (14 women, mean age 32 years) received low-dose ritonavir (100 mg daily) or raltegravir (400 mg twice daily) for 4 weeks. We administered a standardized meal (3.6 MJ, 76% fat, 10% carbohydrates) at baseline and at week 4, with hourly assessments for 6 h after each meal. The primary outcome measure was incremental area under the curve (iAUC) change in postprandial lipids.

Results: Ritonavir induced significantly higher postprandial iAUC excursions in low-density lipoprotein (LDL) cholesterol than raltegravir, mostly in the first 3 h after food ($P < 0.05$). The ritonavir-related postprandial increases in LDL cholesterol at 1, 2, and 3 h were 30–65% greater than the ritonavir-related increase in fasting LDL cholesterol (0.34–0.43 vs. 0.26 mmol/l, $P < 0.05$ for each comparison). The postprandial iAUC and fasting LDL cholesterol changes at week 4 were significantly correlated ($r = 0.64$; $P = 0.003$). There was no between-group difference for other postprandial parameters.

Conclusion: In HIV-uninfected adults, postprandial LDL cholesterol excursions with low-dose ritonavir were significantly greater than those with raltegravir. This postprandial effect of ritonavir increased by about 50% the previously observed adverse effect of ritonavir on fasting LDL cholesterol, and so may explain some of the hitherto unexplained association of protease inhibitor-based therapy with cardiovascular disease.

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Introduction

Antiretroviral therapy (ART) including a protease inhibitor increases the risk of myocardial infarction and cardiovascular disease (CVD) [1,2]. The protease inhibitors most associated with CVD are ritonavir-boosted lopinavir and indinavir [3]. Lipid effects with ritonavir-boosted fosamprenavir and saquinavir appear similar. Even low-dose ritonavir (100 mg once daily), which is used to boost atazanavir and darunavir, is associated with dyslipidemia [4,5]. Thus, any ritonavir-boosted drug may exacerbate CVD risk. In contrast, the integrase inhibitor raltegravir causes less dyslipidemia than efavirenz or ritonavir/lopinavir [6,7].

About 50% of the increase in CVD risk related to protease inhibitor therapy is attributable to protease inhibitor-related dyslipidemia, but the other 50% remains unexplained [1]. However, almost all the lipid data relating protease inhibitor therapy to CVD risk are derived from solely fasting blood samples.

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Plasma lipid levels change following a meal. In general, triglyceride levels increase, atherogenic cholesterol is stable, and high-density lipoprotein (HDL) cholesterol levels may fall. Postprandial dyslipidemia, particularly the increase in triglyceride levels, is associated with an increased risk of CVD and has been suggested to be a greater CVD predictor than fasting lipids [8]. Four studies [9–12] have examined postprandial lipids in adults receiving ART. Most of these studies were cross-sectional and none was randomized or evaluated low-dose ritonavir or a boosted protease inhibitor.

We hypothesized that raltegravir would induce less postprandial lipid disturbance than low-dose ritonavir. We compared the effects of 4 weeks of ritonavir 100 mg once daily to those of raltegravir 400 mg twice daily on postprandial lipid and other metabolic parameters in healthy HIV-uninfected adults.

Methods

Participants

Volunteers were recruited by advertisements in a tertiary referral hospital, universities and a Sydney general practice and enrolled at a single site (Garvan Institute of Medical Research) between July 2008 and April 2009.

Inclusion criteria were as follows: age greater than 18 years; fasting triglycerides less than 2.0 mmol/l (177 mg/dl); body mass index 20–30 kg/m^2; stable weight and diet; and provision of written, informed consent. Exclusion criteria were as follows: HIV infection (by antibody, western blot, and proviral DNA); use of lipid-lowering or antihypertensive therapy; use of any drug contraindicated with ritonavir or raltegravir; diabetes mellitus; serum hepatic transaminases exceeding three times the upper limit of normal; and pregnancy (known or planned) or breast feeding.

Study protocol

In this open-label, randomized trial, eligible volunteers were allocated to ritonavir 100 mg daily (one capsule with breakfast) or raltegravir 400 mg twice daily for 4 weeks. The study protocol was approved by the St Vincent's Hospital Human Research Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (number ACTRN12607000622404).

Block randomization was used to balance participants: blocks of four contained two envelopes for each of raltegravir and ritonavir, randomly ordered. Allocation was by staff not involved with study conduct (Centre for Applied Medical Research).

Before each visit (baseline, week 2, and week 4), participants refrained from vigorous exercise and alcohol for 48 h and fasted overnight for at least 12 h.

Meal study

At baseline and week 4, an intravenous cannula was inserted into an antecubital vein for sampling. Participants consumed a standardized, high-fat, cafeteria-style meal (3.68 MJ, 88 g fat, 104 g carbohydrates, 37 g protein) over 20–30 min; participants remained semi-recumbent for the study. The last dose of study medication was taken with the week 4 meal.

Assessments

Clinical assessments

At each visit, concomitant medications were recorded and physical examination performed. Safety was assessed by recording clinical adverse events and liver function tests. Weight and height were recorded with each participant in a hospital gown and BMI derived [weight/height squared (kg/m^2)]. Waist circumference was measured in triplicate and was defined as mean widest circumference between the lower edge of the ribs and the anterior superior iliac spines. Adherence was determined by patient diary and tablet count.

Metabolic parameters

After a 15-min rest, fasting samples were collected at baseline and week 4 for total, low-density lipoprotein (LDL) and HDL cholesterol, triglycerides, apolipoprotein (apo) A, apoB, glucose, insulin, and C-reactive protein (CRP); lipids were repeated immediately prior to meal commencement. After meal completion (time 0), blood was collected over 6 h: hourly for lipids, insulin, and glucose; and CRP two-hourly.

Total cholesterol, HDL cholesterol, and triglycerides were measured spectrophotometrically at 490 nm using enzymatic colorimetry (Roche, Basel, Switzerland), LDL cholesterol by the Friedewald equation, glucose by the oxidase method (NOVA14; Nova Biomedical, Waltham, Massachusetts, USA), apoA1 and apoB by immunoturbidimetry (Roche reagents, Integra analyzer, Roche Diagnostics, Sydney, Australia), high-sensitivity CRP by immunoturbidimetry (Olympus reagents, Olympus AU2700 analyzer; Integrated Sciences, Sydney, Australia), and insulin by radioimmunoassay (Linco Research, St Charles, Missouri, USA).

Energy expenditure

Resting energy expenditure was measured when patients were fasting by indirect calorimetry (Deltatrac Metabolic Monitor; Datex Instrumentarium Corp., Helsinki, Finland) for 30 min. The respiratory quotient, defined as the ratio of carbon dioxide production to oxygen consumption, was measured hourly for 6 h.

Arterial stiffness

Arterial stiffness, as measured by the arterial augmentation index (AIx), is associated with angiographically proven coronary artery disease and predicts cardiovascular mortality independent of cardiovascular risk factors [13].

Arterial stiffness was measured by applanation tonometry of the radial arterial pulse wave (in duplicate by a single trained operator) using a validated transfer function to generate AIx (SphygmoCor SCOR-PVx tonometer; AtCor Medical, Sydney, Australia). Heart rate-adjusted AIx was measured before each meal and then hourly for 6 h.

Statistical analysis

Postprandial lipid studies evaluating interventions are typically performed with 8–10 adults per intervention, but no such studies of adverse lipid effects in healthy adults have been performed [14]. Fasting lipid differences have been observed with ritonavir 100 mg daily in HIV-positive adults, and within 2 weeks in HIV-uninfected adults [4,5]. On the basis of these data, it was anticipated 10 participants per group would suffice to detect a difference in postprandial triglycerides of 0.5 mmol/l between groups.

Postprandial incremental area under the curve (iAUC) for each parameter was calculated using the trapezoidal method by subtracting baseline values extrapolated over 390 min (30-min basal period and 360-min postprandial period) from the total postprandial area. Insulin resistance was calculated using the homeostasis model assessment (fasting insulin × fasting glucose/22.5).

Data were analyzed by on-treatment analysis. Analysis of variance and Mann–Whitney *U*-tests were used to compare between-group iAUC changes. *P* values less than 0.05 were deemed statistically significant. Comparison of individual postprandial timepoints was only performed if iAUC differences were significant.

Results

Participants

Twenty-six potential participants were assessed for eligibility: four were ineligible (two for elevated lipids and one each for elevated BMI or liver transaminases) and one withdrew consent after screening, but before randomization. Of 21 randomized participants, one participant randomized to raltegravir was permanently lost to follow-up after completing the baseline assessment and was excluded from all analyses. At baseline, participants completing 4 weeks of ritonavir were similar to participants completing 4 weeks of raltegravir (Table 1). Mean adherence to study medication was 94% for raltegravir and 99% for ritonavir. Weight did not change significantly from baseline in the ritonavir group and the between-group change in weight at week 4 was also not significant (data not shown).

Lipid effects

Ritonavir induced a significantly higher mean postprandial increase in iAUC for LDL cholesterol than raltegravir (1.50 ± 0.88 vs. -0.36 ± 0.41, respectively; $P = 0.05$; Table 2). The changes with ritonavir and raltegravir at 1 h (0.35 ± 0.14 vs. -0.08 ± 0.14, respectively; $P = 0.01$), 2 h (0.28 ± 0.14 vs. -0.09 ± 0.09, respectively; $P = 0.023$), and at 3 h (0.28 ± 0.15 vs. -0.06 ± 0.06, respectively; $P = 0.028$) were significantly different, with nonsignificant trends for higher postmeal LDL cholesterol excursions with ritonavir at 4, 5, and 6 h ($P = 0.06$–0.08).

Table 1. Baseline characteristics.

	Raltegravir	Ritonavir
n	10	10
Age (years)	35 ± 4.0	29 ± 1.9
Weight (kg)	67.5 ± 4.6	66.9 ± 4.3
BMI (kg/m^2)	23.9 ± 1.0	26.5 ± 2.6
Waist (cm)	80 ± 3	80 ± 3
Total cholesterol (mmol/l)	4.4 ± 0.2	4.4 ± 0.3
High-density lipoprotein cholesterol (mmol/l)	1.4 ± 0.1	1.6 ± 0.2
Low-density lipoprotein cholesterol (mmol/l)	2.6 ± 0.2	2.4 ± 0.2
Apolipoprotein A1 (mmol/l)	1.33 ± 0.12	1.53 ± 0.12
Apolipoprotein B (mmol/l)	0.56 ± 0.07	0.59 ± 0.08
Triglycerides (mmol/l)	0.9 ± 0.1	0.9 ± 0.2
Resting energy expenditure (MJ/day)	5.61 ± 0.50	5.71 ± 0.29
Respiratory quotient (%)	0.85 ± 0.01	0.88 ± 0.02
Glucose (mmol/l)	4.8 ± 0.1	4.8 ± 0.1
Insulin (mU/l)	6.1 ± 0.9	5.4 ± 1.1
Insulin resistance (mUmmol/l^2)	1.32 ± 0.23	1.18 ± 0.28
C-reactive protein (mg/l)	1.3 ± 0.4	2.3 ± 1.2
Arterial augmentation index (%)	6.1 ± 2.3	5.4 ± 3.9

All data are mean (SEM).

The ritonavir-induced postprandial increases in LDL cholesterol at 1, 2, and 3 h were significantly greater than the ritonavir-induced increase in fasting LDL cholesterol (0.34–0.43 vs. 0.26 mmol/l; $P < 0.05$ for each). There was a significant correlation between the change in postprandial iAUC for LDL cholesterol at week 4 and the change in fasting LDL cholesterol at week 4 ($r = 0.64$; $P = 0.003$; Fig. 1).

There were also trends for greater postprandial iAUC changes in total cholesterol, HDL cholesterol, and triglyceride rises with ritonavir than with raltegravir. In contrast, there was no significant between-group difference observed for other postprandial or fasting metabolic parameters, postprandial or fasting augmentation index, or for postprandial fat oxidation, as measured by iAUC respiratory quotient.

Safety

No patient experienced a serious or grade 3–4 adverse event. One raltegravir recipient developed fatigue and nausea at day 10 with grade 2 serum alanine aminotransferase at week 2, possibly related to study drug. Serology for viral hepatitis was negative. Serum alanine

Table 2. Postprandial changes at week 4.

	Raltegravir	Ritonavir	*P*
Low-density lipoprotein cholesterol (mmol/l)			
Fasting (premeal)	0.01 ± 0.09	0.25 ± 0.13	0.10
1 h postmeal	−0.08 ± 0.14	0.35 ± 0.14	0.01
2 h postmeal	−0.09 ± 0.09	0.28 ± 0.14	0.02
3 h postmeal	−0.06 ± 0.06	0.28 ± 0.15	0.03
4 h postmeal	−0.07 ± 0.09	0.29 ± 0.16	0.06
5 h postmeal	−0.02 ± 0.08	0.32 ± 0.17	0.07
6 h postmeal	−0.07 ± 0.09	0.23 ± 0.14	0.08
iAUC (0–6 h)	−0.36 ± 0.41	1.50 ± 0.88	0.05
iAUC for other parameters			
Total cholesterol (mmolh/l)	0.2 ± 0.1	1.5 ± 0.9	0.12
High-density lipoprotein cholesterol (mmolh/l)	−0.2 ± 0.3	0 ± 0.3	0.76
Apoliprotein A1 (mmolh/l)	5.63 ± 5.11	0.37 ± 0.48	0.28
Apolipoprotein B (mmolh/l)	0.07 ± 0.14	0.14 ± 0.13	0.30
Triglycerides (mmolh/l)	1.0 ± 2.2	4.6 ± 1.1	0.14
Respiratory quotient	−0.03 ± 0.02	0.02 ± 0.02	0.15
Glucose (mmolh/l)	0.1 ± 0.6	0.6 ± 0.9	0.56
Insulin (mU/l)	0.19 ± 11.0	2.12 ± 17.6	0.91
C-reactive protein (mg/l)	−1.6 ± 1.0	−3.5 ± 5.0	0.71
Augmentation index (h%)	17.8 ± 9.9	−1.25 ± 9.7	0.19

All data are mean (SEM). iAUC, incremental area under the curve.

aminotransferase improved at day 17 and normalized at week 4 despite continuation of raltegravir.

Discussion

Four weeks of ritonavir 100 mg once daily in HIV-seronegative adults induced significantly higher postprandial LDL cholesterol excursions than raltegravir 400 mg twice daily. These postprandial LDL cholesterol changes were also significantly higher than the ritonavir-induced changes in fasting LDL cholesterol. There was a strong correlation between the change in postprandial iAUC for LDL cholesterol and the change in fasting LDL cholesterol.

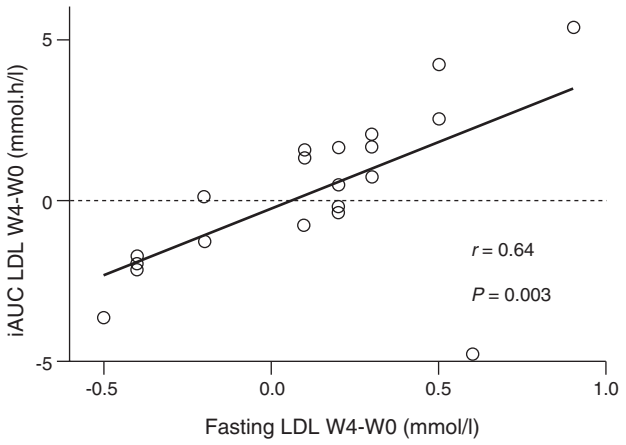

Fig. 1. Change in fasting low-density lipoprotein cholesterol vs. change in postprandial incremental area under the curve low-density lipoprotein cholesterol at week 4. iAUC, incremental area under the curve; LDL, low-density lipoprotein.

Our data suggest that the adverse effect of ritonavir on LDL cholesterol is about 50% greater than the changes previously observed in fasting patients. The average adult consumes three meals and several snacks per day, which would translate into a postprandial effect of ritonavir on LDL cholesterol of perhaps 12 h per day. The significant correlation between fasting and postprandial changes suggests that the postprandial effects will be greatest in those with the largest fasting change in LDL cholesterol. This postprandial effect might explain some of the hitherto unexplained association of protease inhibitor therapy with CVD [1], and also why the Framingham equation seems to underestimate cardiovascular risk in HIV-uninfected adults [15].

There was no significant change in fat oxidation measured by respiratory quotient. The significant LDL cholesterol increase with low-dose ritonavir without a significant change in fat oxidation suggests that ritonavir increases hepatic LDL synthesis or secretion, as has been shown *in vitro* [16].

The LDL cholesterol effect of ritonavir did not translate into a significant effect on arterial function, but the study may have been too short and small to adequately address this outcome.

We did not observe significant changes in other lipid parameters, but there were nonsignificant trends in fasting LDL cholesterol and postprandial iAUC for total cholesterol that also suggested adverse lipid effects of ritonavir and are very consistent with the significant effects of ritonavir on fasting lipids seen in larger and longer studies in HIV-infected adults. These nonsignificant trends may have arisen because of the relatively small number of participants studied. Our

data now provide a basis for sample size for future studies.

Our study has additional limitations. We used raltegravir as a control, rather than a placebo, so it is possible that some of the differences observed are due to an inhibitory effect of raltegravir on LDL cholesterol metabolism, though such an effect has not been observed in previous studies of raltegravir and no biological basis has been proposed for such an effect.

The brief duration of drug exposure, the inclusion of patients only with normal lipids, and the use of ritonavir alone rather a ritonavir-boosted protease inhibitor may collectively have resulted in an underestimation of the postprandial lipid effects of protease inhibitor therapy in HIV-infected adults receiving long-term ART. Conversely, it is possible that consumption of ritonavir with a nonfatty meal or in the fasted state may yield different results from those observed in the present study. The postprandial effects of ritonavir-boosted protease inhibitor therapy should be studied in HIV-infected adults over longer periods.

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All authors contributed to study design, participant enrollment, and follow-up, data collection, and analysis and writing of the article.

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