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Boosting-Dose Ritonavir Does Not Alter Peripheral Insulin Sensitivity in Healthy, HIV Seronegative Volunteers

Steven A. TAYLOR, MD^{1,2}, Grace A. LEE, MD^{1,2}, Vivian Y. PAO, MD^{1,2}, Jayaranjan ANTHONYPILLAI², Francesca T. AWEEKA, Pharm.D.³, Jean-Marc SCHWARZ, PhD^{1,4,5}, Kathleen MULLIGAN, PhD^{1,4}, Morris SCHAMBELAN, MD^{1,4}, and Carl GRUNFELD, MD PhD^{1,2}

¹ Department of Medicine, University of California, San Francisco, CA, USA

² Division of Endocrinology and Metabolism, Department of Veterans Affairs Medical Center, San Francisco, CA, USA

³ Drug Research Unit, Department of Clinical Pharmacy, San Francisco General Hospital, University of California, San Francisco, CA, USA

⁴ Division of Endocrinology, San Francisco General Hospital, San Francisco, CA, USA

⁵ College of Osteopathic Medicine, Touro University, Vallejo, CA, USA

Abstract

Background—Some HIV protease inhibitors (PI), including full dose ritonavir (800 mg) and ritonavir-boosted lopinavir, acutely induce insulin resistance in the absence of HIV infection and changes in body composition. Boosting-dose ritonavir (100 to 200 mg) is the most commonly prescribed PI, yet its effects on glucose metabolism have not been described in the absence of another PI.

Methods—In this randomized, double-blind, cross-over study, a single dose of ritonavir 200 mg or placebo was given to healthy HIV-seronegative volunteers before assessment of insulin sensitivity by euglycemic hyperinsulinemic clamp.

Results—Boosting dose ritonavir had no effect on insulin-mediated glucose disposal (M/I, placebo: 8.59 ± 0.83 vs. ritonavir: 8.51 ± 0.64 mg/kg per min per $\mu\text{U/mL}$ insulin, $p=0.89$).

Conclusion—A single boosting dose of ritonavir does not alter insulin sensitivity, suggesting lopinavir is likely responsible for the induction of insulin resistance demonstrated in prior short-term studies of lopinavir/ritonavir. There is a dose-dependent effect of ritonavir on insulin sensitivity.

Keywords

HIV protease inhibitors; insulin resistance; ritonavir; lopinavir; glucose

Address correspondence and reprint requests to: Carl Grunfeld, MD, PhD, Metabolism Section VA Medical Center, 4150 Clement Street, 111F, San Francisco, CA 94121. Phone: 415-750-2005, Fax: 415-750-6927 carl.grunfeld@ucsf.edu.

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Introduction

Following the introduction of highly active antiretroviral therapy with HIV protease inhibitors (PI), an increase in abnormalities of glucose metabolism was reported [1-6]. These included induction of insulin resistance, impaired glucose tolerance, and frank diabetes. Studies have since established that individual PIs have varied effects on glucose metabolism, especially the acute induction of insulin resistance [7-10]. Previously, we showed that a single dose of ritonavir at levels used to suppress HIV ("full" dose=800 mg) acutely induces insulin resistance in healthy HIV-seronegative volunteers [8]. However, ritonavir is generally prescribed at lower "boosting" doses (100 or 200 mg) in combination with a second PI, to achieve therapeutic levels of the other PI. Boosting-dose ritonavir is the most commonly prescribed PI, yet its effects on glucose metabolism have not been described in the absence of another PI. A single standard dose of ritonavir-boosted lopinavir acutely induces insulin resistance in healthy human subjects [9]. Short term administration of ritonavir-boosted lopinavir induces more insulin resistance than ritonavir-boosted atazanavir [11]. It is unclear whether the induction of insulin resistance observed in these studies occurs as a direct effect of ritonavir, lopinavir, or both.

Here, utilizing the euglycemic hyperinsulinemic clamp technique, we compare the effects of a single boosting dose of ritonavir to placebo in a cross-over study. We studied healthy, normal volunteers to eliminate contributions of HIV infection itself, treatment-induced restoration of health, and other concomitantly-prescribed antiretroviral medications that might alter insulin sensitivity.

Methods

Ten healthy volunteers were recruited. They had no history of medical illness, a normal screening physical examination, normal hematology and chemistry laboratory results and negative HIV-1 antibody tests before study. The protocol was approved by the Committee on Human Research at the University of California, San Francisco (UCSF). Informed consent was obtained from each subject.

Exclusion criteria included a fasting glucose >5.6 mmol/L; body mass index (BMI) >30 kg/m²; fasting triglyceride level >2.13 mmol/L; LDL-cholesterol >4.87 mmol/L; blood pressure >140/90 mm Hg; taking anti-hypertensive medications; creatinine >140.8 µmol/L; liver function tests > the upper limits of normal; pregnancy; > three alcoholic drinks per day for men or two per day for women; use of amphetamines, cocaine, or heroin; or use of drugs affecting glucose or lipid metabolism within the previous thirty days.

Study design

This was a randomized, double-blind, placebo-controlled, cross-over study. Subjects were admitted to the UCSF Clinical and Translational Science Institute Clinical Research Center (CRC) at San Francisco General Hospital (SFGH) the day before the study. After an overnight (10h) fast, subjects received either a single dose of ritonavir (Abbott Laboratories, Abbott Park, Illinois, USA) 200 mg or placebo 3 hours before the start of the euglycemic hyperinsulinemic clamp. Subjects were re-admitted to the CRC within 14-28 days, and studies were repeated using the alternative treatment (active drug or placebo). All subjects were examined by a study investigator between admissions to assess for complications or intercurrent illnesses.

Ritonavir plasma concentrations are highly variable; the half-life is 3 to 5 hours when administered in the fasting state [12]. Therefore, a single dose of ritonavir 200 mg was administered three hours before the clamp to achieve plasma ritonavir levels of 0.7-1.8

μmol/L during the clamp, the range achieved in patients on boosting doses of ritonavir [9, 11, 13-15].

Euglycemic hyperinsulinemic clamp

A 3-hour euglycemic hyperinsulinemic clamp was performed as described by DeFronzo et al. [16] and employed by us previously [7-9]. A cannula was inserted into an antecubital vein for infusion. A vein in the dorsum of the contralateral hand was cannulated and warmed to 50-55°C for arterialized blood sampling. At the start of the clamp (t=0), insulin (Humulin R, Eli Lilly, Indianapolis, Indiana, USA) was administered as a primed continuous intravenous infusion for 10 minutes, followed by a constant infusion at 40 mU/m² per minute for 180-minutes. Glucose was measured every five minutes throughout the clamp. A primed 20% glucose infusion was begun and adjusted every five minutes to maintain plasma glucose at 4.5 mmol/L (coefficient of variation <5%). Blood samples were collected during the clamp for serum insulin levels. Insulin-mediated glucose disposal (M/I) was calculated using data from the last hour of the clamp (t=120 to 180 min).

Resting energy expenditure (REE)

Oxygen consumption and carbon dioxide production were measured by indirect calorimetry (DeltaTrac II metabolic monitor, Yorba Linda, California, USA) to calculate REE.

Laboratory measurements

Glucose and lactate were measured using a YSI 2300 STAT-Plus Glucose and Lactate Analyzer (YSI Inc., Yellow Springs, Ohio, USA). Serum insulin levels were measured by radioimmunoassay (Millipore Bioscience, St Charles, Missouri, USA). Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated from fasting plasma glucose and serum insulin at the start of the clamp (3 hours after administration of ritonavir or placebo) [17]. Fasting total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured by spectrophotometric methods (Thermo Scientific, Middletown, Virginia, USA, Wako Diagnostics, Richmond, Virginia, USA and Sigma, St. Louis, Missouri, USA).

Ritonavir levels were measured by liquid-chromatography, tandem mass-spectrometry by the Drug Research Unit, SFGH (lower limit of quantitation 50 ng/mL, inter- and intra-assay coefficients of variation 4.2 to 10.6% and 1.1 to 9.1%, respectively). Area under the concentration time curve (AUC) from t=0 to 180 min was estimated using the linear-linear trapezoidal rule.

Statistical analysis

The study was designed to enroll 10 patients, with 80% power to detect a 9% decrease and 90% power to detect an 10.6% decrease in M/I for active drug compared to placebo (two-sided alpha = 0.05). Data are presented as mean ± SEM, except median ± IQR are used where not normally distributed. Paired *t*-tests with two-tailed *p*-values are used.

Results

Eight men and two women, ages 38 to 65 years (54±3 years), completed the study; six were Caucasian, two were Hispanic, one African-American, and one Asian. No serious adverse clinical events occurred.

Weight, BMI, REE and fasting serum insulin, plasma glucose, HOMA-IR and lipids measured after administration of active drug or placebo but prior to the clamp did not differ between the study arms (Table 1A). Plasma ritonavir levels reached 2.5±0.5 μmol/L at the start of the clamp and the mean level remained >1.2 μmol/L until the end of the study (Fig.

1A). The 3-hour time-averaged AUC for ritonavir was 1.66 $\mu\text{mol/L}$ per hr. All study subjects reached ritonavir concentrations above 0.7 $\mu\text{mol/L}$ during the clamp.

Similar steady-state insulin and glucose levels were maintained during the final hour of the clamp (Fig. 1B). Glucose levels were 4.39 ± 0.01 and 4.40 ± 0.02 mmol/L ($p=0.42$) for ritonavir and placebo, respectively. Insulin levels were 447 ± 22 and 456 ± 23 pmol/L ($p=0.46$) for ritonavir and placebo, respectively.

M/I measured after administration of boosting dose ritonavir and placebo did not differ during the last hour of the clamp study (Fig. 1C). M/I was 8.51 ± 0.64 and 8.59 ± 0.83 mg/kg per min per $\mu\text{U/mL}$ insulin for boosting dose ritonavir and placebo, respectively ($p=0.89$).

Discussion

Boosting-dose ritonavir is the most commonly prescribed PI. Its potent inhibition of the enzyme responsible for the metabolism of most PIs (CYP3A4) enhances the antiretroviral activity of a concomitantly-dosed PI by allowing it to achieve higher plasma concentrations for more sustained periods of time. Despite its prevalent use, the effects of boosting-dose ritonavir on glucose metabolism had only been studied concomitantly with a second PI. Here, we show that boosting-dose levels of ritonavir do not acutely alter peripheral insulin sensitivity as measured by insulin-mediated glucose disposal during a euglycemic hyperinsulinemic clamp. Given that the effects of PI are greater in short-term administration [7, 9] than after four weeks [18-19], it is unlikely that there will be effects of boosting dose ritonavir with longer exposure. We studied HIV-seronegative subjects to avoid potential confounding effects on glucose metabolism introduced by HIV-related factors.

In the past, ritonavir was prescribed at full doses to suppress HIV. Previously, we showed that a single full dose of ritonavir caused a 15% reduction in insulin-mediated glucose disposal, also measured by the euglycemic hyperinsulinemic clamp [8]. Plasma ritonavir concentrations achieved during determination of M/I in that study (12.5 ± 1.5 $\mu\text{mol/L}$) were substantially higher than those observed here (1.5 ± 0.3 $\mu\text{mol/L}$). Thus, our studies demonstrate that boosting-dose ritonavir has no effect on M/I and that there is a dose-dependent effect of ritonavir on peripheral insulin sensitivity.

Ritonavir-boosted lopinavir is one of the most common PI combinations used in the treatment of HIV infection [20]. Lopinavir cannot be studied in isolation due to its low bioavailability and rapid metabolism. Previously, we showed that a single dose of lopinavir/ritonavir (533 mg/133 mg) acutely decreased M/I by 13% [9]. Noor *et al.* also demonstrated significant reductions in insulin-mediated glucose disposal measured by the clamp after short-term administration of lopinavir/ritonavir (400 mg/100 mg twice daily for 5 or 10 days) [10-11]. In all three studies, plasma lopinavir levels reached therapeutic concentrations. However, ritonavir levels achieved during determination of M/I in those three studies were one-quarter of those found here. Given that we did not detect an effect of boosting-dose ritonavir on insulin sensitivity at the higher levels achieved here, it is likely that lopinavir was responsible for the induction of insulin resistance observed in those studies.

Noor *et al.* found less effect on M/I of short-term administration of atazanavir/ritonavir (300 mg/100 mg daily for 10 days) in HIV-seronegative subjects [11]. Clamp ritonavir levels achieved in that study were lower than those found here but were more than two times greater than those reached in prior short-term studies of lopinavir/ritonavir. Thus, while previous data from our group and Noor *et al.* [9-11] suggested that boosting-dose ritonavir was unlikely to be a major contributor to the acute induction of peripheral insulin resistance observed in prior short-term studies of lopinavir/ritonavir, the data presented here directly

demonstrate the lack of an effect of boosting-dose ritonavir on insulin resistance. The major effect is likely due to lopinavir, although we cannot rule out some additive effects.

In summary, we show a single boosting dose of ritonavir does not alter insulin-mediated glucose disposal. Given our previous finding that a single full dose of ritonavir (which yielded significantly higher plasma levels than those achieved here) acutely induced insulin resistance, we also demonstrate a dose-dependent effect of ritonavir on peripheral insulin sensitivity. Further, our data suggest that lopinavir likely makes the major contribution to the induction of insulin resistance that has been observed in short-term studies of lopinavir/ritonavir. Consideration should be given to these findings when assessing the metabolic effects of antiretroviral regimens and advising patients.

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Figure 1.

(A) Plasma ritonavir levels during the euglycemic hyperinsulinemic clamp. Values presented are mean $\pm$ SEM. (B) Whole blood glucose levels (mg/dL) and rate of glucose infusion (mL/h) during the euglycemic hyperinsulinemic clamp after administration of placebo and boosting-dose ritonavir. Values presented are median $\pm$ IQR. (C) Insulin-mediated glucose disposal (mg/kg*min per $\mu\text{U/ml}$) with placebo and boosting-dose ritonavir administration. Values presented are mean $\pm$ SEM.

A: Ritonavir Levels

B: Whole blood glucose levels (mg/dL) and rate of glucose infusion (mL/h)

C: Insulin-mediated Glucose Disposal

Table 1

Fasting metabolic parameters at baseline and at the start of the euglycemic hyperinsulinemic clamp.

	Placebo	Ritonavir	P-value
Baseline *			
Weight (kg)	75.8 ± 3.3	75.8 ± 3.3	0.98
Body mass index (kg/m ²)	24.3 ± 0.6	24.3 ± 0.6	0.99
Total cholesterol (mmol/L)	4.7 ± 0.2	4.7 ± 0.2	0.85
LDL cholesterol (mmol/L)	2.0 ± 0.05	2.1 ± 0.03	0.23
HDL cholesterol (mmol/L)	1.4 ± 0.1	1.4 ± 0.1	0.49
Triglycerides (mmol/L)	0.9 ± 0.1	1.0 ± 0.1	0.81
At the start of the euglycemic hyperinsulinemic clamp			
Glucose (mmol/L)	5.3 ± 0.05	5.2 ± 0.05	0.11
Insulin (pmol/L)	60.0 ± 5.0	60.0 ± 4.6	>0.99
HOMA-IR	2.3 ± 0.2	2.3 ± 0.2	0.82
Resting energy expenditure (kcal/day)	1689 ± 65	1697 ± 60	0.64

All values are mean ± SEM

LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance index.

HOMA-IR = (insulin × glucose)/22.5

* prior to administration of medication