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The Acute Effects of HIV Protease Inhibitors on Insulin Suppression of Glucose Production in Healthy HIV-negative Men

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Abstract

Background—The effects of different HIV protease inhibitors (PIs) on peripheral insulin resistance have been described, but less is known about their effects on insulin suppression of endogenous glucose production (EGP).

Methods—We tested the acute effects of three PIs, indinavir, ritonavir and amprenavir, on EGP quantified by stable isotope techniques during the hyperinsulinemic, euglycemic clamp in three similar placebo-controlled protocols.

Results—EGP was higher with indinavir in the hyperinsulinemic state than with placebo (4.1 ± 1.3 vs. 2.2 ± 0.8 $\mu\text{g/kg} \cdot \text{min}$, $p=0.04$). A trend towards higher EGP was seen with ritonavir (3.6 ± 0.3 vs. 3.0 ± 0.5 $\mu\text{g/kg} \cdot \text{min}$, $p=0.08$). There was no evidence that amprenavir blunted insulin suppression of EGP compared to placebo (2.9 ± 0.04 vs. 3.2 ± 0.7 $\mu\text{g/kg} \cdot \text{min}$, $p=0.63$).

Conclusions—Some PIs can acutely blunt the ability of insulin to suppress EGP, but, as with insulin resistance, the effects of PIs on EGP are drug-specific, not class-specific.

INTRODUCTION

HIV protease inhibitors (PIs) have been associated with acute induction of peripheral insulin resistance and impaired insulin secretion^{1–3}. We previously reported that four weeks of indinavir treatment in healthy men caused a blunting of the ability of insulin to suppress endogenous glucose production (EGP) during a euglycemic hyperinsulinemic clamp, in the absence of significant changes in body composition or lipid profiles⁴. It is unknown whether other PIs also alter EGP in the hyperinsulinemic state. Therefore, we determined the rates of EGP during a euglycemic hyperinsulinemic clamp using stable isotopic tracer methods in three separate studies of identical design with indinavir, full-dose ritonavir, and amprenavir administration to healthy normal volunteers. Previously we found that a single dose of indinavir or ritonavir, but not amprenavir, acutely block insulin-mediated glucose disposal,

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which was consistent with an acute blockade of GLUT4 found *in vitro*^{1,3}. Here, we present results on the ability of PIs to acutely alter EGP in the hyperinsulinemic state.

METHODS

We report data from three separate studies of similar protocol design^{1,3}. The studies were approved by the Committee on Human research at the University of California, San Francisco. Indinavir, full-dose ritonavir, and amprenavir were each studied in randomized, double-blind, placebo-controlled, crossover trials, as described previously^{1,3}. The timing of drug administration was designed to take into account the pharmacokinetics of each PI to achieve therapeutic plasma levels during the hyperinsulinemic clamp study.

In the indinavir study, six subjects randomly received a single dose of indinavir (Merck and Co., Rahway, New Jersey, USA) 1200 mg or placebo at time 0 at the start of the clamp study¹. In the ritonavir study, nine subjects randomly received ritonavir (Abbott Laboratories Abbott Park, IL, USA) 800 mg soft gel caps or placebo two hours before the start of the clamp study³. In the amprenavir study, amprenavir (GlaxoSmithKline Research Triangle Park, NC) 1200 mg soft gel caps or placebo was given to six subjects one hour before the start of the clamp study³. Indinavir levels averaged 6–9 $\mu\text{mol/l}$, ritonavir 6–10 $\mu\text{mol/l}$, and amprenavir 2–6 $\mu\text{mol/l}$ throughout the clamp^{1,3}. A 3-h euglycemic hyperinsulinemic clamp was performed in each case. The subjects underwent the alternative treatment (drug or placebo) within 7–28 days, and the above studies were repeated. As reported previously, one subject in the ritonavir study failed to achieve therapeutic drug levels at the beginning of the clamp and was excluded from data analysis.

Tracer Protocol

During the hyperinsulinemic clamp, we used the labeled exogenous glucose infusate technique⁴. In the indinavir study, a continuous infusion of $[6,6\text{-}^2\text{H}_2]$ glucose (3.6 mg/kg*hr) was begun prior to the start of the clamp. In the ritonavir and amprenavir studies, a continuous infusion of $[^{13}\text{C}_6]$ glucose (1.2 mg/kg*hr) was used. Stable isotopes were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and Isotec (Miamisburg, OH, USA). Steady state blood sampling was performed every 10 minutes during the final 30 minutes of the study. Rate of appearance of glucose was determined using gas chromatography/mass spectrometry by traditional dilution techniques⁴.

Statistical Analyses

Paired t-tests were used for normally distributed data. Data are presented as mean $\pm$ SEM.

RESULTS

As reported previously^{1,3}, during these studies insulin-mediated glucose disposal decreased with indinavir and ritonavir by 34% and 16% respectively, compared to placebo ($p < 0.001$ and $p = 0.008$, respectively). There was no statistically significant change in insulin-mediated glucose disposal with amprenavir³.

EGP Measurements During the Euglycemic Hyperinsulinemic Clamp

With indinavir, EGP was higher in the hyperinsulinemic state than with placebo (placebo 2.2 ± 0.8 ; indinavir $4.1 \pm 1.3 \mu\text{g/kg*min}$, $p = 0.04$, Figure 1a). With ritonavir, there was a trend towards higher EGP during insulin infusion (placebo 3.0 ± 0.5 , ritonavir $3.6 \pm 0.3 \mu\text{g/kg*min}$, $p = 0.08$, Figure 1b). There was no significant difference in EGP during hyperinsulinemia with amprenavir compared to placebo (Figure 1c). There was no significant difference in EGP with placebo administration between the indinavir, ritonavir,

and amprenavir studies. When the effects of indinavir and ritonavir on insulin suppression of EGP during the clamp are taken into account, they are not of sufficient magnitude to eliminate the previously reported effects of either PI drug on the inhibition of insulin-mediated glucose disposal^{1, 3}.

DISCUSSION

In three separate studies, we found that indinavir, ritonavir, and amprenavir had different effects on EGP in the hyperinsulinemic state. Indinavir had the greatest effect on increasing EGP during the euglycemic hyperinsulinemic clamp. The effect of indinavir was approximately half the difference in EGP between type 2 diabetes and controls at this insulin concentration^{5, 6}. Ritonavir, given at the full therapeutic dose rather than boosting dose, had a more modest effect on EGP, whereas amprenavir had no significant effect. These studies were performed in subjects of very similar age, ethnicity, and family history of diabetes^{1, 3}. All subjects in the amprenavir study had participated in the ritonavir study, and one subject participated in all three studies. Although a direct comparison is not possible, these three studies of identical study design in similar populations of healthy normal men suggest these PIs have different effects on hepatic insulin sensitivity.

Both insulin-mediated glucose disposal and insulin inhibition of EGP play a role in determining the levels of glucose after a meal. EGP is also the major determinant of fasting glucose levels. Due to the single dose design and the pharmacokinetics of the drugs, we did not measure the effects of PIs on fasting EGP in these acute studies. We have previously shown that four weeks of indinavir leads to increased fasting glucose and EGP in the fasting and hyperinsulinemic state⁴. In this study, EGP in the hyperinsulinemic state was 86% higher on indinavir, whereas it was 46% higher after four weeks on indinavir⁴. We have previously found that the effects of indinavir on insulin mediated glucose disposal are also higher acutely than after four weeks^{1, 7}.

Although the mechanism by which indinavir and ritonavir alter EGP is not known, our findings suggest that it is likely to be an acute process. Indinavir and ritonavir increased EGP in the hyperinsulinemic state within several hours of administration. The degree to which the PIs altered EGP paralleled the previously reported effects of these PIs on acute induction of peripheral insulin resistance in humans^{1, 3}. PIs have been shown to decrease insulin-mediated glucose disposal through an acute blockade of GLUT4 *in vitro*⁸. Mice that have a tissue-specific knock out of GLUT4 in muscle and adipose tissue manifest an impaired ability of insulin to inhibit EGP⁹. Thus it is likely that some of the effects of PIs on EGP during insulin infusion or post-prandially may be mediated via an acute blockade of GLUT4.

In summary, we found that indinavir and full-dose ritonavir acutely increase EGP in the hyperinsulinemic state whereas amprenavir had no significant effect on EGP, indicating that the effects of PI are drug-specific, not class-specific. These findings emphasize the acute and heterogeneous nature by which PIs induce alterations in glucose metabolism.

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Figure 1.

Effect of (A) indinavir vs. placebo, (B) ritonavir vs. placebo, and (C) amprenavir vs. placebo on endogenous glucose production during the hyperinsulinemic, euglycemic clamp. Values are mean $\pm$ SEM.