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Severe Impairment of Endothelial Function with the HIV-1 Protease Inhibitor Indinavir is not Mediated by Insulin Resistance in Healthy Subjects

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Abstract

Endothelial dysfunction may contribute to increased cardiovascular events among HIV-1 infected patients receiving antiretroviral therapy. The HIV-1 protease inhibitor indinavir causes both vascular dysfunction and insulin resistance, but the relationship between the two disturbances is not established. Endothelium-dependent vasodilation (EDV), insulin-mediated vasodilation (IMV), and whole body and leg glucose uptake during a euglycemic hyperinsulinemic clamp (40 mU/m²/min) were measured before and after four weeks of indinavir in nine healthy men. EDV fell from 270 ± 67% above basal to 124 ± 30% (p=0.04) and IMV from 56 ± 14% above basal to 8 ± 8% (p=0.001) with indinavir. During the clamp, arteriovenous glucose difference and leg glucose uptake were not significantly different after indinavir and whole-body glucose uptake was only modestly reduced (8.0 ± 0.8 vs 7.2 ± 0.8 mg/kg/min, p=0.04). The change in EDV did not correlate with the change in whole-body glucose uptake after indinavir (r=0.21, p=0.6). Despite marked impairment of endothelial function and IMV with indinavir, only modest, inconsistent reductions in measures of insulin stimulated glucose uptake occurred. This suggests that indinavir's effects on glucose metabolism are not directly related to indinavir-associated endothelial dysfunction. Studies of the vascular effects of newer protease inhibitors are needed.

Keywords

Endothelial dysfunction; insulin sensitivity; indinavir; HIV-1 protease inhibitors

Introduction

Combination antiretroviral therapy for HIV infection is associated with increased cardiovascular events (1,2). In particular, myocardial infarction is more common with the use of HIV-1 protease inhibitors (2,3). Prospective data suggest that antiretroviral therapy-associated lipid disorders alone do not explain all of this increased risk (2). Endothelial dysfunction is a critical initial step of atherogenesis which contributes to the progression and clinical manifestations of atherosclerosis (4,5) and has been associated with protease

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inhibitor-based antiretroviral regimens (6,7). Thus, endothelial dysfunction may be responsible for increased cardiovascular events under protease inhibitor therapy that is not merely due to protease inhibitor-associated lipid changes.

Clinical states of endothelial dysfunction and insulin resistance typically co-exist (8-12), as may occur in individuals with type 2 diabetes mellitus or hypertension. Reduced skeletal muscle insulin-mediated glucose uptake, or insulin resistance, is in part due an impairment in endothelial function which can result in a restriction in the supply of glucose and insulin to the skeletal muscle (13-15). However, it is not clear whether endothelial dysfunction alone leads to insulin resistance, or if tissue insulin resistance is the initial, underlying perturbation (16-18).

Endothelial dysfunction that is seen in association with insulin resistant states is associated with impairment of nitric oxide (NO)-dependent tone (17,19). We have previously reported in a separate group of healthy, non-obese HIV-seronegative subjects that four weeks of administration of the HIV-1 protease inhibitor indinavir significantly impairs endothelial function (20). The magnitude of endothelial dysfunction due to indinavir was large and similar to the degree that occurs in patients with type 2 diabetes (21). We also reported that indinavir largely abolished NO-dependent vascular tone (20), suggesting that the marked impairment in endothelial function is most likely due to decreased NO production and/or release.

Indinavir has been reported to induce insulin resistance in healthy subjects (22,23). In the current study, we sought to determine if indinavir-induced insulin resistance was related to concomitant endothelial dysfunction. If such a relationship exists, then interventions to improve insulin sensitivity could be considered as measures to ameliorate protease inhibitor-associated endothelial dysfunction. We measured the effect of standard doses of indinavir given for four weeks on endothelium-dependent vasodilation (EDV), insulin sensitivity at the level of the whole body and skeletal muscle, and insulin effects on the vasculature in a group of healthy non-obese subjects without HIV infection.

Materials and Methods

Subjects

Demographic characteristics are shown in Table I. All were men ($n=9$, mean age 37 ± 1 years) who were HIV seronegative, healthy, non-obese (total body fat $\leq 27\%$ by dual x-ray absorptiometry scan, Lunar DPX-L; Lunar Corp., Madison, WI, system software 4.6b), normotensive by cuff measurements per JNC VI criteria (24), had normal 75 g oral glucose tolerance tests per American Diabetes Association criteria (25) and normal lipid profiles per National Cholesterol Education Program III criteria (26) and were not taking any over-the-counter or prescription medications including herbal supplements. Studies were approved by the Indiana University-Purdue University and Clarian Health Partners Institutional Review Board, and all volunteers gave written informed consent. Subjects were instructed to maintain their usual dietary and physical activity habits during study.

Study drugs

All vascular drugs were diluted in normal saline achieving concentrations of 25 $\mu\text{g/ml}$ of methacholine chloride (Clinalfa, CH-4448 Lufelfingen, Switzerland) and 8 mg/ml of N_G -Mono-Methyl-L-Arginine (L-NMMA, Calbiochem Corp., San Diego, CA). Indinavir (Crixivan, Merck, West Point, NJ) was administered as two 400mg capsules orally thrice daily (every 8 hours) on an empty stomach. Liberal water intake was encouraged to help prevent urolithiasis. Subject compliance was assessed by self-report and manual pill counts.

Procedures

Nine healthy males were studied between March and December 2003. Subjects were admitted to the Indiana University General Clinical Research Center one day before study and were fed a weight-maintaining diet. All vascular and metabolic studies were performed after an overnight fast and abstinence from smoking. The last dose of indinavir was administered at 7 AM on the last day of the study, at ~1 hour prior to methacholine infusion and ~5 hours prior to the final hour of the clamp study. Subjects had body weight, height, basal heart rates, systolic and diastolic blood pressure measurements done before and after indinavir therapy.

Vascular measurements—Hemodynamic measurements were obtained with the subjects in the supine position as previously described (27). Leg blood flow was measured using a modified thermodilution technique. Basal leg blood flow measurements were done at least 30 minutes following catheter placement, then dose-response curves for leg blood flow in response to graded intrafemoral artery infusions of 5, 10 and 15 $\mu\text{g}/\text{min}$ of the endothelium-dependent vasodilator methacholine were performed and compared to basal leg blood flow values. Subsequently, a euglycemic hyperinsulinemic clamp study was initiated and continued for a minimum of 240 minutes (described below). During the fourth hour of the insulin infusion, insulin-mediated vasodilation (IMV) was assessed by measurement of leg blood flow and compared to basal leg blood flow values. To study the effect of indinavir on the NO-dependent fraction of IMV, we then measured leg blood flow during the intrafemoral artery infusion of the endothelial NO synthase inhibitor L-NMMA at a dose of 16 mg/min for 15 minutes at the end of the clamp study.

Metabolic studies—Immediately following baseline assessments of endothelial function, subjects underwent a euglycemic hyperinsulinemic clamp study using a low-dose insulin infusion (40 mU/m²/min), the dose used by Noor et al (22) that detected reduced whole-body glucose uptake after four weeks of indinavir administration. Subjects were studied in the supine position with a catheter inserted in an antecubital vein for infusion of glucose, insulin, and potassium. The femoral arterial and venous lines described above were used for drawing blood samples. The clamp technique was a modification of the Andres method (28). Arterial plasma glucose values were determined at 5-minute intervals and the glucose infusion rate adjusted to maintain normoglycemia (target 5.0 mmol/L). Venous plasma glucose values were measured at 20-minute intervals for the first three hours of the clamp study, and at 5-minute intervals for the last hour of the clamp study. The clamp procedure was continued for at least 240 minutes after initiation of the insulin and glucose infusions, or until completion of all vascular measurements. Sampling for insulin levels was performed at 60-minute intervals during the clamp study. To prevent hypokalemia, potassium was infused during the studies at a maximum rate of 0.0038 meq/kg/min as tolerated by the volunteer.

Analytical methods

Insulin was assayed using the Abbott IMX MEIA system (Abbott Laboratories, Abbott Park, IL). Glucose was assayed by the glucokinase method at the bedside using a YSI apparatus (Yellow Springs Instruments, Yellow Springs, OH). Plasma adiponectin was measured by radioimmunoassay (Linco Research, St. Charles, MO). All plasma samples were assayed in a single run. Indinavir levels were measured by high performance liquid chromatography, with ultraviolet detection as previously described with slight modification (29). The coefficient of variation of quality control samples was less than 10%.

Calculations

Mean arterial pressure is expressed in mm Hg and leg blood flow is expressed in L/min. Changes in leg blood flow are expressed as absolute change (Δ) and percent change ($\%\Delta$) to adjust for differences at baseline. Whole body glucose uptake (M) was calculated as the mean glucose infusion rate normalized for body weight and corrected for changes in the glucose pool sizes, according to methods described by DeFronzo (28) using data from a one hour period late in the clamp (160-220 minutes) before any vasoactive agents were infused. This time period was used to avoid any effect of L-NMMA infusion on glucose uptake results. Arterio-venous glucose differences (A-VG Δ) were calculated [(arterial glucose)-(venous glucose)] every 5 minutes during the last hour of the clamp study. Leg glucose uptake was calculated as the product of leg blood flow * A-VG Δ .

Statistical analysis

Results are shown as the mean $\pm$ SE. Repeated measures ANOVA (RMANOVA) was used to compare the changes in leg blood flow in response to the graded drug infusions, before and after four weeks of indinavir administration. Paired t-test was used to compare differences in metabolic and vascular variables before and after indinavir. The Spearman Rank Order test was used to determine correlations. Statistical significance was defined as $p < 0.05$. Analyses were performed with the SAS version 9.1 (SAS Institute, Cary, NC).

Results

Metabolic effects of indinavir

Subjects had mean peak plasma indinavir levels of 4.9 ± 1.3 $\mu\text{g/mL}$. Four weeks of indinavir had no effect on BMI, fasting plasma glucose or insulin levels or HOMA-IR (Table I). Plasma adiponectin levels increased from 10.6 ± 1.3 to 13.3 ± 1.7 $\mu\text{g/mL}$ ($p=0.008$). Glucose concentrations during the clamp (Table II) were similar before and after indinavir (5.2 ± 0.1 vs 5.1 ± 0.1 mmol/L, $p=0.4$). Arterio-venous glucose differences during the final hour of the clamp (A-VG Δ) were 1.6 ± 0.2 and 1.6 ± 0.1 mmol/L before and after indinavir, respectively ($p=0.8$). In response to the NO synthase inhibitor L-NMMA, A-VG Δ increased similarly to 2.0 ± 0.2 mmol/L ($p=0.001$) before indinavir, and to 2.0 ± 0.2 mmol/L ($p<0.001$) after indinavir ($p=0.9$ for the comparison of before and after indinavir). Insulin concentrations during the final hour of the clamp were 634 ± 71 and 581 ± 52 pmol/L before and after indinavir, respectively ($p=0.4$). Rates of whole body glucose uptake (M) were only modestly reduced by 10% after indinavir (8.0 ± 0.8 vs 7.2 ± 0.8 mg/kg/min, $p=0.04$) (Table II).

Hemodynamic effects of indinavir

Resting heart rate, systolic, diastolic and mean arterial pressure did not change with indinavir (Table I). Before indinavir, leg blood flow increased in a dose-dependent fashion in response to the endothelium dependent vasodilator methacholine with a maximum increment in leg blood flow ($\Delta\%$ leg blood flow) in response to methacholine of $270 \pm 67\%$. After indinavir, leg blood flow also increased in a dose-dependent fashion. However, indinavir caused a marked blunting of the leg blood flow response to methacholine with a maximum $\Delta\%$ leg blood flow of $124 \pm 30\%$ ($p=0.04$ vs pre-indinavir). All but one subject experienced a decrease in the response to methacholine after indinavir (Figure). There was no correlation between the change in the maximum $\Delta\%$ leg blood flow in response to methacholine and the change in M (Figure; Spearman $r=0.21$, $p=0.6$).

Before indinavir, leg blood flow increased in response to insulin in the final hour of the clamp (IMV) by $56 \pm 14\%$ (Table III). After indinavir, IMV was markedly blunted, with only an $8 \pm 8\%$ increase in leg blood flow in response to insulin ($p=0.001$ vs pre-indinavir).

With administration of L-NMMA (an inhibitor of NO synthase) during the final hour of the clamp, leg blood flow decreased similarly before and after indinavir ($-36 \pm 6\%$ vs $-32 \pm 7\%$ respectively, $p=0.6$).

Before indinavir, leg glucose uptake was 0.53 ± 0.09 mmol/min during the final hour of the clamp. After indinavir, leg glucose uptake was 0.44 ± 0.04 mmol/min ($p=0.3$ compared to pre indinavir). In response to L-NMMA infusion during the final hour of the clamp, leg glucose uptake decreased similarly, to 0.42 ± 0.07 mmol/min before indinavir and to 0.40 ± 0.06 mmol/min after indinavir ($p=0.8$).

Discussion

We previously reported that four weeks of oral indinavir in healthy non-obese HIV-negative men impairs endothelial function, and nearly abolishes basal NO-dependent tone (20). The current study independently confirms our earlier finding that indinavir markedly impairs EDV and adds observations obtained during a euglycemic hyperinsulinemic clamp study. Impaired insulin sensitivity has been reported in lean males after a single dose (23) and with four weeks of indinavir (22), in addition to rat (30) and in vitro (31) findings of reversible impairments in glucose uptake after very brief exposure to indinavir. We asked if the markedly impaired endothelial function due to indinavir was related to impairments in insulin sensitivity. We tested this hypothesis in a separate group of healthy non-obese subjects, by assessing EDV, as well as insulin sensitivity at the level of the whole body, the vasculature (IMV), and skeletal muscle in a single experiment at baseline, then repeating the measurements after four weeks of indinavir.

Four weeks of daily oral indinavir in these healthy non-obese HIV-negative subjects caused marked impairment in EDV and IMV but with no impairment of insulin sensitivity at the level of skeletal muscle (leg) and only modest evidence of impairment at the level of the whole body. Shankar and colleagues recently reported that four weeks of indinavir administration (800 mg thrice daily) produced similar effects on EDV and IMV, but without affecting whole-body insulin sensitivity when using a considerably higher insulin clamp dose of $120 \text{ mU/m}^2/\text{min}$ (32). In that study, the higher-dose clamp resulted in mean insulin concentrations of approximately 2200 pmol/L (32). In the current study, the lower-dose clamp resulted in mean insulin concentrations of approximately 600 pmol/L , a level that is on the steeper, linear portion of the insulin dose-response curve for lean subjects (33,34) and thus allows greater sensitivity in discerning changes in insulin responses. Together, these findings suggest that indinavir use has major effects on the endothelium which are independent of its effects on glucose metabolism. This discrepancy between EDV and glucose uptake is consistent with a lack of acute effects on endothelial function reported with a single dose of indinavir (20), while a single dose of indinavir significantly reduced insulin sensitivity (23). It is important to note, however, that our findings only relate to indinavir-induced endothelial dysfunction/insulin resistance and should not necessarily be generalized to all clinical states of endothelial dysfunction/insulin resistance.

In our study, the reduction in the extent of whole body glucose uptake ($\sim 10\%$) was less than that reported by Noor and colleagues ($\sim 20\%$) with 4 weeks of indinavir treatment (22). This difference likely reflects a subtle difference in the timing of the final dose of indinavir in the two studies. Noor et al administered the last dose of indinavir closer to the time of determination of whole body glucose uptake and used a clamp procedure that was of shorter duration (22), presumably resulting in their glucose uptake measurements being done at higher plasma indinavir concentrations as compared to ours. A relationship between indinavir plasma concentrations and indinavir associated insulin resistance (i.e. whole body glucose uptake) effects has been previously reported in animals (30). A similar relationship

between indinavir plasma concentrations and indinavir-associated insulin resistance effects would be anticipated in humans.

It is interesting to note that while we found no impairment in insulin sensitivity at the level of the skeletal muscle (leg glucose uptake) and only modest effects at the level of the whole body, we observed a marked impairment in IMV with indinavir, as have others (32). The vasodilatory effect of insulin occurs across arterial, venous (35), and capillary (36) beds, with the latter being important in terms of indirect delivery of metabolites. IMV is largely NO-mediated (17,19), and insulin induces NO synthase in addition to releasing NO (37). Indinavir significantly impairs NO-dependent tone in healthy subjects (20). Thus, the effect of indinavir on NO release from the vasculature is likely to be the cause of the impaired IMV than is vascular tissue insulin resistance. This hypothesis is supported by our observation that there is significant impairment of both EDV (which is partially NO-mediated) and IMV (which is predominantly NO-mediated) after indinavir. Further, it is known that the metabolic and vascular effects of insulin share common signaling pathways upstream of NO synthase (38,39). Thus, the disconnect in the metabolic and vascular effects of insulin secondary to indinavir administration suggests that this drug exerts its effects at a point downstream of where these two signaling pathways diverge. Alternatively, the effect of indinavir on IMV and glucose uptake could be mediated at least in part through an altogether distinct signaling pathway or mechanism.

The arterio-venous glucose difference (AVGΔ) during hyperinsulinemia provides an estimate of skeletal muscle glucose extraction. In our study, AVGΔ did not change, suggesting that four weeks of indinavir did not directly impair Glut 4 function at the level of the skeletal muscle. Glut 4 is a member of a family of glucose transport proteins and is principally responsible for insulin-mediated glucose uptake in muscle and adipose tissue (40). In experimental models, Glut 4 function is inhibited by physiologic concentrations of indinavir (41). Leg blood flow during hyperinsulinemia, or IMV, was reduced by indinavir, potentially leading to an increase in the residence time of the glucose molecules within the capillaries and providing a greater opportunity for glucose transport across the cell membranes by Glut 4. However, leg glucose uptake, the product of leg blood flow times AVGΔ during hyperinsulinemia, another index of skeletal muscle glucose uptake, also did not change significantly. It is possible that in order for the magnitude of decrease in blood flow seen with indinavir to have a deleterious effect on glucose uptake, there needs to be an additional defect in either glucose extraction, capillary surface area, or both. Alternatively, it is possible that 1) there needs to be an even greater impairment in endothelial function/flow for there to be an effect on insulin-mediated glucose uptake, or 2) there is a compensatory capillary recruitment in response to indinavir mediated endothelial dysfunction which maintains glucose delivery and uptake, or 3) there is an upregulation of Glut4 activity compensatory to the fall in LBF that is sufficient to maintain intact insulin-mediated glucose uptake, or 4) the indinavir-induced increase in adiponectin protects against the development of insulin resistance. This in vivo increase in adiponectin levels has been reported by others in healthy subjects receiving HIV protease inhibitors (42) but contrasts with in vitro models suggesting a negative or neutral effect of protease inhibitors on adiponectin expression or secretion (43,44).

In summary, the profound impairment in EDV seen after four weeks of indinavir in healthy HIV-negative non-obese men occurs in the absence of any major impairment of insulin sensitivity. This finding indicates that, at least with the older and now seldom-used HIV protease inhibitor indinavir, insulin-mediated glucose uptake can remain largely intact despite significant impairment of EDV and IMV. These processes thus may be mediated by different mechanisms, so that the possibility of endothelial dysfunction deserves study even with protease inhibitors that have no demonstrable effects on glucose metabolism.

Therapeutic interventions to improve insulin sensitivity would appear unlikely to directly ameliorate the negative effects of protease inhibitors on endothelial function. Further studies are needed with the protease inhibitors currently in common clinical use to determine if endothelial dysfunction is a protease inhibitor drug class effect, to establish the clinical relevance of these findings, and to identify measures to improve or prevent protease inhibitor associated endothelial dysfunction.

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Non-standard abbreviations

EDV	endothelium-dependent vasodilation
IMV	insulin-mediated vasodilation
NO	nitric oxide
L-NMMA	N ^G -Mono-Methyl-L-Arginine
A-VGΔ	Arterio-venous glucose difference

Figure.

Scatterplot of the percentage change in M (glucose uptake) versus the percentage change in the maximal increase in leg blood flow during methacholine infusion, after 4 weeks of indinavir administration. Spearman $r=0.21$, $p=0.6$.

Table I

Subject characteristics (n=9) at baseline and after 4 weeks of indinavir. Results are given as mean $\pm$ SE.

	Baseline	After indinavir
Body mass index (kg/m ²)	25.0 $\pm$ 1.5	25.0 $\pm$ 1.4
Mean arterial pressure (mm Hg)	85.2 $\pm$ 1.4	85.0 $\pm$ 2.5
Fasting plasma glucose (mmol/L)	5.0 $\pm$ 0.1	5.1 $\pm$ 0.1
Fasting plasma insulin (pmol/L)	62.9 $\pm$ 10.3	68.4 $\pm$ 11.1
HOMA-IR	2.0 $\pm$ 0.3	2.2 $\pm$ 0.4
Adiponectin (μ g/mL)	10.6 $\pm$ 1.3	13.3 $\pm$ 1.7 *

HOMA-IR - Homeostasis model assessment-insulin resistance

* p=0.008 compared to baseline. All other changes were not significant.

Table II

Euglycemic hyperinsulinemic clamp measurements at baseline and after 4 weeks of indinavir in 9 subjects. Results are given as mean $\pm$ SE.

	Baseline	After indinavir	p-value
Arterial glucose concentration (mmol/L) during last hour of clamp	5.2 $\pm$ 0.04	5.1 $\pm$ 0.10	0.4
AVG Δ (mmol/L) during last hour of clamp	1.6 $\pm$ 0.2	1.6 $\pm$ 0.1	0.8
AVG Δ glucose during L-NMMA (mmol/L)	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2	0.9
Glucose uptake (M) during 160-220 min of clamp	8.0 $\pm$ 0.8	7.2 $\pm$ 0.8	0.04
Insulin concentration (pmol/L) during last hour of clamp	634 $\pm$ 71	581 $\pm$ 52	0.4

AVG Δ - Arteriovenous glucose difference; L-NMMA - NG-Mono-Methyl-L-Arginine.

Table III

Hemodynamic and vascular measurements at baseline and after 4 weeks of indinavir in 9 subjects. Results are given as mean $\pm$ SE.

	Baseline	After indinavir	p-value
Resting basal leg blood flow (L/min)	0.21 $\pm$ 0.02	0.27 $\pm$ 0.02	0.01
Maximal Δ leg blood flow with methacholine	270 $\pm$ 67	124 $\pm$ 30	0.04
Δ leg blood flow during hyperinsulinemic clamp (insulin-mediated vasodilation)	56 $\pm$ 14	8 $\pm$ 8	0.001
Δ leg blood flow with L-NMMA infusion, during last hour of clamp	-36 $\pm$ 6	-32 $\pm$ 7	0.6
Leg glucose uptake during last hour of clamp (mmol/min)	0.53 $\pm$ 0.09	0.44 $\pm$ 0.04	0.3
Leg glucose uptake with L-NMMA infusion, during last hour of clamp (mmol/min)	0.42 $\pm$ 0.07	0.40 $\pm$ 0.06	0.8

L-NMMA - NG-Mono-Methyl-L-Arginine.