Lopinavir-Ritonavir: Effects on Endothelial Cell Function in Healthy Subjects


To differentiate between the effects that antiretroviral drugs have on the endothelium and the secondary effects that they have on immune function, viral load, and dyslipidemia, non–human immunodeficiency virus–infected human subjects were treated with lopinavir-ritonavir for 1 month and, on the basis of forearm blood flow, the treatment’s effects on endothelial cell function were measured. Surprisingly, after exposure to lopinavir-ritonavir, absolute forearm blood-flow responses to the endothelium-dependent vasodilator, acetylcholine, increased significantly \((p = .03)\), and forearm blood flow decreased to a greater extent during specific inhibition of NO synthase by \(\text{N}^\circ\)-monomethyl-\(L\)-arginine. Thus, in this small cohort of subjects, short-term treatment with lopinavir-ritonavir does not appear to directly promote endothelial cell dysfunction.

As survival has improved for patients with HIV infection treated with highly active antiretroviral therapy (ART), studies have demonstrated that they are developing atherosclerotic complications at higher than expected frequencies [1–2]. Why patients with HIV infection develop accelerated atherosclerosis has been the subject of considerable speculation. With ART use, patients develop metabolic abnormalities such as dyslipidemia and insulin insensitivity, which could predispose them to atherosclerosis [3–5]; however, these factors by themselves do not appear to explain fully the accelerated rate of atherosclerosis [2, 6].

Direct endothelial cell damage by the protease inhibitors has been proposed as a mechanism that could contribute to accelerated atherosclerosis. Shanker et al. administered indinavir to 8 healthy volunteers for 4 weeks: endogenous NO–mediated vasodilation was impaired (there were no changes in triglyceride levels, but insulin levels increased), suggesting that indinavir induces endothelial cell dysfunction, either by insulin resistance or by a direct drug-based effect on the endothelium [7]. In vitro studies and a cross-sectional study comparing protease inhibitor–treated HIV-infected patients versus HIV-infected patients receiving other ART regimens have also supported the concept that protease inhibitors adversely influence endothelial cell function [5, 8, 9].

Many of these earlier studies have been limited by the inherent difficulty in differentiating between direct drug-based effects and potential HIV effects, secondary host immunological responses, or metabolism-mediated effects on the endothelium. Therefore, to assess the direct effects that protease inhibitors have on endothelial cell function, non–HIV-infected human subjects were treated with lopinavir-ritonavir, one of the most widely recommended and used antiretroviral agents, for 1 month, and endothelium-dependent forearm blood-flow responses were then measured [10]. These forearm blood-flow measurements have been used to correlate endothelial cell dysfunction with other risk factors in non–HIV-infected patients and have been validated as a predictor of coronary artery atherosclerosis [11–15].

Subjects and methods. Subjects were HIV-negative volunteers 18–40 years of age who, on the basis of medical history and physical examination, were considered to be healthy. Exclusion criteria included smoking, substance abuse, pregnancy/breast-feeding, abnormal results of electrocardiography, concomitant therapy with any medication (except oral contraceptives, acetaminophen, nonsteroidal anti-inflammatory medications, and loperamide), and abnormal laboratory-determined values (with respect to cholesterol-panel results; serum levels of aspartate aminotransferase, creatinine phosphokinase, and creatinine; and levels of platelets, hemoglobin, and either fasting blood sugar or blood sugar 2 h after an oral glucose-tolerance test).

Subjects underwent screening at the HIV clinic of the National Institute of Allergy and Infectious Disease (NIAID). At
screening and on day 30, an oral glucose-tolerance test and an electrocardiogram were performed, and laboratory data (including blood chemistry analyses; complete blood counts; liver-function tests; and serum levels of CD4, HIV RNA load, cholesterol, insulin, and high-sensitivity C-reactive protein) were collected. On days 1 and 30, a forearm blood-flow study was performed (as described below). Subjects began 4 weeks of treatment with lopinavir-ritonavir (3 capsules twice daily) on the evening of day 1. Adherence was assessed on the basis of pill counts at study visits on days 15 and 30.

Standard laboratory assessments were made by the Clinical Center Department of Laboratory Medicine. HIV ELISA was performed at the NIAID contract laboratory in Frederick, Maryland.

Forearm blood-flow studies were performed both before the subjects had been treated with lopinavir-ritonavir and after they had been treated with it for 4 weeks. A brachial-artery intraarterial catheter was connected to a pressure transducer, for blood-pressure measurements, and an infusion pump delivered a 0.9% saline solution at a rate of 1.0 mL/min. After a 20-min rest period, baseline forearm blood flow was measured by strain-gauge venous-occlusion plethysmography. After either acetylcholine, at sequential rates of 7.5, 15, and 30 μg/min, or sodium nitroprusside, at sequential rates of 0.8, 1.6, and 3.2 μg/min, had been infused for 3 min, forearm blood flow was assessed; there was a 20-min rest period between the 3 doses of either acetylcholine or sodium nitroprusside. After a 30-min rest period, repeat baseline forearm blood-flow measurements were made, and then N\textsuperscript{\textgamma}-monomethyl-L-arginine (L-NMMA) was infused at sequential rates of 4 and 8 μmol/min. After each of the 2 doses of L-NMMA had been infused for 5 min, forearm blood flow was measured, and then acetylcholine infused at a rate of 30 μg/min was added to the 8-μmol/min L-NMMA infusion, and coinfusion of the 2 drugs was continued for an additional 5 min.

The 2-sided Wilcoxon signed-rank test of the area under the curve (AUC), an area that was estimated by use of the trapezoidal rule, was used to compare forearm blood-flow measurements both at baseline and during infusion with each of the 3 doses of either acetylcholine or sodium nitroprusside, as well as absolute and percentage changes from baseline values, both before and after treatment with lopinavir-ritonavir. The Wilcoxon signed-rank test was used to compare forearm blood-flow measurements, as well as percentage changes from baseline values, both before and during infusion of each of the 2 doses of L-NMMA and during coinfusion with acetylcholine and L-NMMA, and was also used to compare the values of metabolic parameters before and after treatment with lopinavir-ritonavir. \( P < .05 \) was considered to be statistically significant. Analysis was performed by use of NCSS 2004 (Number Cruncher Statistical Systems) and Prism Graphpad software (version 4.03; Graphpad Software).

On the basis of power calculations using data on percentage change in leg blood flow during L-NMMA infusion before and after treatment with indinavir[7], the present study was originally planned to include 15 subjects, with an interim analysis being performed after 10 subjects had enrolled. However, in light of consistent results in the direction opposite to what had been expected, the study was stopped after an analysis of the results observed for the first 6 subjects; on the basis of the 95% confidence intervals for the results observed, the power of a 15-subject study to find that lopinavir-ritonavir has a significantly deleterious effect on endothelial cell function would be expected to be low, and it therefore was concluded that the inconvenience and risk to additional subjects was not warranted.

Results. A total of 4 women and 4 men, with a mean age of 25 years (range, 19–32 years), enrolled in the present study; 5 were white, 2 were black, and 1 was an Asian/Pacific Islander. Of the 8 subjects, 2 were withdrawn—1 because of elevated levels of total bilirubin and 1 because of elevated levels in liver-function tests. Adherence as measured by pill counts was >90%, in all 6 subjects. Data analysis was performed on the 6 subjects, all of whom completed forearm blood-flow tests both before and after treatment with lopinavir-ritonavir. Mean ± SD baseline forearm blood flows for the acetylcholine infusion were 4.3 ± 2.9 and 5.3 ± 2.0 mL/min/100 mL of forearm tissue, respectively, before and after treatment with lopinavir-ritonavir (\( P = .31 \)) (figure 1A); the corresponding values for the sodium nitroprusside infusion were 3.4 ± 1.8 and 4.5 ± 0.9 mL/min/100 mL of forearm tissue (\( P = .09 \)) (figure 1C).

Acetylcholine stimulates endothelium-dependent vasodilation via both activation of endothelial cell NO synthase and production of non-NO-dependent vasodilators, which include prostacyclin and hyperpolarizing factor. Before treatment with lopinavir-ritonavir, infusion of acetylcholine at 30 μg/min increased forearm blood flow from 4.3 ± 2.9 (the baseline value) to 28.8 ± 13.9 mL/min/100 mL of forearm tissue; the corresponding increase after treatment with lopinavir-ritonavir, from 5.3 ± 2.0 to 45.5 ± 26.2 mL/min/100 mL of forearm tissue, was nonsignificantly greater (\( P = .06 \)). Overall, the AUC of absolute forearm blood flow (\( P = .03 \)), as well as absolute forearm blood flow at 30 μg/min (\( P = .03 \)), increased after treatment with lopinavir-ritonavir (figure 1A). For each of the 3 doses of acetylcholine, neither absolute (\( P = .06 \)) nor percentage increases over baseline (\( P = .31 \)) forearm blood flows were significant (figure 1B).

To determine the sensitivity of smooth muscle–cell responsiveness to NO, sodium nitroprusside, an exogenous donor of NO, was infused. Before treatment with lopinavir-ritonavir, infusion of sodium nitroprusside at a dose of 3.2 μg/min increased absolute forearm blood flow from 3.4 ± 1.8 (the base-
Figure 1. Forearm blood flow in 6 subjects, shown in terms of absolute change (A and C) and percentage change from baseline value (B and D), during intra-arterial infusions of either acetylcholine (A and B) or sodium nitroprusside (C and D), before (Pre) and after (Post) 4 weeks of treatment with lopinavir-ritonavir. **P < .05.

To determine the effects that treatment with lopinavir-ritonavir has on baseline NO production, L-NMMA, a competitive inhibitor of NO synthase, was infused at 2 doses, 4 and 8 μmol/min. Reductions in forearm blood flow during inhibition of NO synthase correlate with the baseline contribution that endothelial cell NO makes to resting vasomotor tone. Mean ± SD percentage reductions of forearm blood flow during infusion of L-NMMA at 4 and 8 μmol/min, respectively (figure 2A). Thus, the reductions in percentage change from baseline values were greater after treatment with lopinavir-ritonavir than before treatment with it—almost significantly so (P = .06) for the infusion at 8 μmol/min. A greater decrease in forearm blood flow during infusion of L-NMMA after treatment with lopinavir-ritonavir is consistent with up-regulation of baseline NO synthase activity and/or increased sensitivity to NO.

Next, the contribution that NO synthase makes to acetylcholine-dependent forearm blood flow was determined, by coinfusion of acetylcholine and L-NMMA; with this coinfusion, the mean ± SD percentage change from the baseline value of acetylcholine-dependent forearm blood flow was nonsignificantly reduced, from 794% ± 241% to 632% ± 478% (P = .31), before treatment with lopinavir-ritonavir and was significantly reduced, from 852% ± 364% to 485% ± 206% (P = .03), after treatment (figure 2B). These data, too, were consistent with lopinavir-ritonavir having the effect of increasing the production and responsiveness of NO rather than the expected effect of reducing its bioavailability.

After treatment with lopinavir-ritonavir, serum levels of total cholesterol (P = .22), LDL (P = .56), HDL (P = .60), fasting insulin (P = .16), fasting glucose (P = .84), 2-h glucose (P = .7, L-NMMA at 4 and 8 μmol/min, respectively (figure 2A). Thus, the reductions in percentage change from baseline values were greater after treatment with lopinavir-ritonavir than before treatment with it—almost significantly so (P = .06) for the infusion at 8 μmol/min. A greater decrease in forearm blood flow during infusion of L-NMMA after treatment with lopinavir-ritonavir is consistent with up-regulation of baseline NO synthase activity and/or increased sensitivity to NO.

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Accelerated atherosclerosis is a major complication of ART. The results of the present study do not support the idea that treatment with lopinavir-ritonavir plays a direct causal role in contributing to this complication via the NO pathway; therefore, effective prevention of atherosclerotic morbidity will require further investigations into its pathophysiology.

References