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Carotid artery intima–media thickness and HIV infection: traditional risk factors overshadow impact of protease inhibitor exposure

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Abstract

Context—The impact of HIV infection and exposure to antiretroviral therapy on the development of subclinical atherosclerosis is incompletely understood.

Objective—To compare intima–media thickness (IMT) of the carotid artery between HIV-infected subjects receiving protease inhibitor-containing regimens and subjects not receiving these regimens and to compare differences in the IMT of the carotid artery between HIV-infected subjects and HIV-uninfected subjects.

Methods—A prospective matched cohort study in university-based outpatient clinics. Groups of three individuals (triads) matched on the following characteristics were enrolled: age, sex, race/ethnicity, smoking status, blood pressure and menopausal status. Group 1, HIV-infected subjects with continuous use of protease inhibitor (PI) therapy for ≥ 2 years; group 2, HIV-infected subjects without prior PI use; and group 3: HIV-uninfected. Ultrasonographers at six sites sent standardized ultrasound images to a central reading site for carotid IMT measurements. Carotid IMT was compared

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within the HIV-infected groups (1 and 2) and between the HIV-infected and uninfected groups in a matched analysis.

Results—One hundred and thirty-four individuals were enrolled in 45 triads. The median IMT in groups 1, 2 and 3 was 0.690, 0.712 and 0.698 mm, respectively. There were no statistically significant differences in IMT between groups 1 and 2, or in the combined HIV groups compared with the HIV uninfected group. Significant predictors of carotid IMT in a multivariate model included high-density lipoprotein (HDL) cholesterol, the interaction of HDL cholesterol and triglycerides, age and body mass index.

Conclusions—We found no association between PI inhibitor exposure or HIV infection and carotid IMT.

Keywords

atherosclerosis; HIV infection; carotid intima; media thickness; protease inhibitors

Introduction

There is an urgent need to determine whether people with HIV infection are at an increased risk for atherosclerosis. Various lipid dyscrasias occur with protease inhibitor (PI) therapy [1–6]. In addition to lipid dyscrasias, patients with HIV infection also experience metabolic abnormalities including insulin resistance [2,7,8], visceral adiposity [4,9], and chronic immune activation [10,11]. However, whether these metabolic abnormalities increase the risk of atherosclerosis is unknown.

Several factors may contribute to an increased risk of cardiovascular disease in HIV-infected patients. These factors include chronic inflammation due to HIV infection *per se*, metabolic changes associated with PI therapy, and the intersection with traditional risk factors (smoking status, blood pressure, age, sex, race and menopausal status) with HIV-specific factors. This prospective matched cohort study was designed to identify the role of PI therapy and HIV infection on the risk of development of subclinical atherosclerosis. The objective of our study was to compare carotid intima–media thickness (IMT) between HIV-infected individuals receiving protease inhibitor-containing regimens and individuals not receiving these regimens and to compare differences in the IMT of the carotid artery between HIV-infected subjects and HIV uninfected subjects.

Methods

Study design

AIDS Clinical Trials Group Study A5078 was a limited, six center, pilot, observational cohort study including both baseline and longitudinal measurements of carotid IMT in two groups of HIV-infected subjects and one group of HIV uninfected controls. Study subjects were recruited from eight academic medical centers in the United States. A unique feature of the study design was the enrollment of study subjects at each site into a triad consisting of an individual from each group: one from group 1, one from group 2, and one from group 3 (Fig. 1).

Group 1—HIV-infected subjects with HIV-1 RNA $\leq 10\,000$ -copies/ml, who were currently receiving antiretroviral therapy including a PI. The subjects must have taken at least one PI with at least 2 years' continuous use prior to study entry. Subjects who had received two or more PIs must have a total combined duration of continuous exposure of at least 2 years to be eligible. Interruptions in therapy for at most 4 weeks for management of toxicity or to change therapy were allowed.

Group 2—HIV-infected subjects with HIV-1 RNA $\leq 10\,000$ copies/ml, who were not currently receiving PI therapy (defined as not receiving any PI-containing regimen for more than a total of 3 months at any time prior to study entry). Subjects were not required to be currently receiving antiretroviral therapy, but they must have been on non-PI containing antiretroviral treatment for at least six consecutive months sometime in the past.

Group 3—Group 3 consisted of HIV-uninfected control subjects. The triad of subjects, one subject from each group, was matched on six cardiovascular disease risk factors: age (within 5 years), race, sex, blood pressure status (normal or hypertensive), smoking status (never, current, or past), and menopausal status. The matched design was employed in order to prospectively control for important traditional risk factors for atherosclerosis while attempting to isolate the effects of HIV infection and PI therapy on carotid IMT.

Subjects were excluded from participation if they had any of the following clinical conditions: diabetes mellitus (DM) or current use of oral hypoglycemia agents and/or thiazolidinediones, family history of myocardial infarction (prior to age 55 for first-degree male relatives and prior to age 65 for first-degree female relatives), a history of coronary heart disease or stroke (including a history of angina, myocardial infarction or abnormal stress test), uncontrolled hypertension, untreated hypothyroidism or obesity [defined as a body mass index (BMI) > 30]. Subjects with the following laboratory parameters were also deemed ineligible: creatinine $> 132.6\ \mu\text{mol/l}$, alanine aminotransferase or aspartate aminotransferase $> 2.5 \times$ upper limit of normal (ULN). Finally, subjects requiring systemic chemotherapy, radiation therapy or systemic steroids were excluded.

Data collection

Visualization of the carotid artery IMT was obtained via non-invasive high-resolution B-mode carotid artery ultrasonography according to the procedure of Hodis *et al.* [12,13]. Site sonographers were uniformly trained at the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center (CIRC). Standardized ultrasound images of the right common carotid artery were acquired at baseline and at weeks 2 (for quality control purposes only), 24, 48, 72, and 96 and sent on SVHS videotape to the CIRC for review and image processing. The distal common carotid artery far wall IMT was measured by automated computerized edge detection using an in-house developed software package (Prowin, patent pending) [14,15].

Sample size

The primary hypothesis was to evaluate the pairwise differences in carotid IMT between the groups. A clinically relevant difference of 0.1 mm in carotid IMT between the groups of interest was the basis on which the power of the study was calculated. We conservatively assumed a standard deviation of 0.14 mm on this measurement. Using a standard approximation and adjusting for two comparisons (total = 0.05) and a planned non-parametric analysis, a total of 44 subjects were required in each group to detect a difference of 0.1 mm with 80% power.

Statistical methods

The primary hypothesis was to investigate the difference in carotid IMT between groups. The planned comparison of group 1 with group 2 was to assess the effect of PI therapy on carotid IMT in the HIV-infected subjects. The planned comparison of group 2 to group 3 (or group 1 and group 2 to group 3 if there was no difference between groups 1 and 2) was to assess the effect of HIV infection on carotid IMT. The analysis was designed to utilize the non-parametric Wilcoxon signed-rank test on the paired IMT data, with comparisons between group 1 and group 2, group 1 and group 3, and group 2 and group 3. The group data were paired according to triad membership. Subsequently, if there was no observable difference between groups 1

and 2, it was planned that the subjects in groups 1 and 2 were to be combined before comparing them with the subjects in group 3. In this case, there were two HIV-infected subjects matched and compared to one HIV-uninfected subject. A variation on the non-parametric Wilcoxon signed-rank test was to be used to compare the groups 1 and 2 individuals with the group 3 individuals [16].

The secondary hypothesis was to examine the association between carotid IMT and covariates via mixed effects linear regression modeling using restricted maximum likelihood estimation. IMT data was transformed using normal scores to attenuate the influence of extreme outliers. The covariates considered for all subjects were fasting lipid measurements (total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, and total cholesterol/HDL ratio), glucose, age (in years), race/ethnicity, sex, smoking status (never, current, or past), BMI, waist circumference, waist/hip ratio, and group membership. For the HIV-infected subjects, additional covariates included length of prior PI use (in weeks), baseline CD4 cell count ($\times 10^6$ cells/l), and baseline HIV-1 plasma viral load (≤ 50 versus > 50 and ≤ 500 versus > 500 copies/ml). All individuals with data were included in the linear regression models. The results of the univariate analysis were used as a guide for the multivariate analysis. Covariates considered highly correlated with cardiovascular disease were to be examined together via multivariate mixed effects linear regression models.

While the sample size was based on the ability to detect a difference of 0.1 mm between groups with a standard deviation of 0.14 mm, a two-sided α totaling 0.05, and 80% power, based on the final sample size, with an observed standard deviation of 0.11 mm, there was effectively 90% power to evaluate the primary hypothesis.

Results

Between February 2001 and May 2002, 134 subjects in 45 triads were accrued. The median age of the subjects was 42 years at entry. There were 40 male and five female triads. The racial/ethnic composition of study population included 76% whites (34 triads) 16% Hispanic (seven triads), 4% Blacks (two triads) and 4% Asian Pacific Islander (two triads). All but one subject had normal blood pressure at entry. The individual with hypertension at entry previously was normotensive during screening. Nearly half (74 of 134, or 55%) of the subjects had never smoked. Between-group differences were tested for BMI, waist circumference, and waist/hip ratio. Waist/hip ratio was significantly different between the three groups ($P = 0.001$), with waist/hip ratios tending to be higher in the group of subjects with more than 2 years of PI experience.

Baseline fasting lipid measurements are shown in Table 1. All but two of the subjects had baseline fasting glucose levels below 7 mmol/l. Forty-six percent of the subjects had total cholesterol > 5.18 mmol/l. Of the 132 subjects with an LDL-cholesterol value, 81 (61%) had an LDL-cholesterol less than 3.37 mmol/l. Eighty-four (63%) subjects had HDL-cholesterol levels of at least 1.04 mmol/l. Seventy-two (54%) subjects had triglycerides less than 1.70 mmol/l. Based on between-group comparisons, total cholesterol and triglycerides were significantly different between the three groups ($P = 0.013$ and ≤ 0.0001 , respectively). As expected, the subjects with at least 2 years of PI exposure tended to have higher levels of total cholesterol and triglycerides.

HIV-related health factors for the 89 HIV-infected subjects in the PI-treated and the PI-naïve groups are shown in Table 2. In the PI-treated and PI-naïve subjects, the median CD4 cell counts were 530 and 481×10^6 cells/l, respectively. Sixty-four (72%) subjects had HIV-1 plasma viral loads less than 50 copies/ml. The groups were distributed similarly within the CD4 and HIV-1 plasma viral load categories. Thirteen (30%) subjects in group 1 (PI use ≥ 2

years) used PIs for a total of more than 5 years. The median PI exposure in this group was more than 4 years; only one subject was exposed for less than 2.2 years. Four (9%) subjects in the PI-naïve group reported ever taking a PI and total exposure was for less than 3 months.

Antiretroviral treatment was categorized by class of drug: nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or PI. Accordingly thirty-one (70%) subjects in group 1 (PI use ≥ 2 years) were taking a combination of NRTI + PI and thirteen were receiving a PI + NNRTI or with or without an NRTI included. Overall 15 (34%) of the PI-treated subjects were using a ritonavir-boosted regimen. In group 2, thirty-four (76%) subjects were taking a combination of NRTI + NNRTI. Three subjects in this group were not taking any antiretroviral agents at study entry whereas eight (18%) were receiving nucleosides without any other agents.

Carotid IMT results

IMT values were repeatable with a high degree of precision at all sites; the median coefficient of variation of repeated baseline measures over all sites was 0.53% (range 0.09 to 0.67%).

The median IMT in group 1, group 2, and group 3 was 0.690, 0.712 and 0.698 mm, respectively. The medians of the paired differences between groups and sample sizes are shown in Fig. 2. Utilizing the Wilcoxon signed-rank test to assess the pairwise differences between the groups, we obtained a P -value of 0.21 for the comparison of group 1 to group 2 (observed difference of 0.019 mm), $P = 0.80$ for the comparison of group 1 to group 3 (observed difference of 0.01 mm), and $P = 0.34$ for the comparison of group 2 to group 3 (observed difference of 0.002 mm). Since there was no statistically significant difference between group 1 and group 2, it was concluded that there was no evidence of a PI therapy effect on carotid IMT. As such, the subjects in groups 1 and 2 were combined and compared with the subjects in group 3. A P -value of 0.45 was obtained using a modified Wilcoxon signed-rank procedure to test the difference between the carotid IMT measurements of group 1/2 and group 3 subjects.

The analysis of the secondary objectives was based on all subjects with available data at entry. The univariate mixed effects linear regression models assessed how well the baseline covariates individually predict carotid IMT on the 128 subjects with carotid IMT data. The results indicate that carotid IMT increases with age ($P = 0.0002$), BMI ($P = 0.002$), waist circumference ($P = 0.01$) and in non-Hispanic whites ($P = 0.048$); carotid IMT tended to be lower in females ($P = 0.05$) and in subjects with increased HDL ($P = 0.02$). Group membership was not predictive of carotid IMT ($P = 0.47$). Increased total cholesterol/HDL ratio and hypertension at entry were suggestive of increased carotid IMT ($P = 0.11$ and 0.08 , respectively) and were thus considered as candidate covariates for the multivariate model.

Additional covariates considered for the multivariate model were glucose, total cholesterol, LDL-cholesterol, and current smoking status. The final multivariate model contained HDL-cholesterol ($P = 0.047$), the interaction of HDL-cholesterol and triglycerides (which allowed the effect of HDL-cholesterol to be further enhanced when triglycerides > 3.39 mmol/l) ($P = 0.013$), age ($P = 0.0002$), and BMI ($P = 0.012$). Increases in IMT were predicted by older age and increases in BMI. Increases in HDL cholesterol are protective against increases in IMT; these higher HDL-cholesterol levels appear to be more protective when the triglyceride level is above 3.39 mmol/l.

When added to this multivariate model, the effect of group membership remained non-significant ($P > 0.5$).

Discussion

We conducted a prospective matched cohort study in HIV-infected adults to determine whether exposure to PI therapy or HIV infection is associated with an increased risk for subclinical atherosclerosis. Forty-five well-matched triads were successfully recruited. Prior to enrollment, subjects were matched on six risk factors associated with cardiovascular disease. As expected, the subjects in the PI-exposed group tended to have greater waist/hip ratios, total cholesterol, and triglycerides [5]. However, carotid IMT measurements on entry to the study were not significantly different between the PI-exposed members of each triad compared with the PI-naïve HIV-positive and the HIV-negative matched controls. In addition, carotid IMT was not statistically different between HIV-infected subjects and HIV-uninfected subjects. There was no evidence from the carotid IMT data of this study to support an atherogenic effect of HIV status or PI therapy, after a median duration of PI exposure of 216 weeks.

In univariate mixed effects linear regression models, where carotid IMT was the outcome of interest and triad membership was modeled as an additional source of variation, age was the strongest predictor of carotid IMT. It could be argued that if the impact of protease inhibitor therapy on subclinical atherosclerosis is mediated by changes in lipids, or by changes in body composition, that we would not expect to see an independent effect of PI therapy after control for these factors. Our results failed to show an association between PI therapy or HIV infection and carotid IMT in either the adjusted or unadjusted analyses. In the final adjusted model increases in carotid IMT were predicted by factors known to increase the risk of atherosclerosis in the general population such as older age, lower HDL cholesterol and higher BMI.

Recent data from two cohort studies suggest that the duration of combination antiretroviral therapy, and in one study, duration of PI therapy specifically, was associated with an increased risk for myocardial infarction [17,18]. These studies are limited by the absence of a control group [18] and in one study by the absence of data on smoking and other cardiovascular risk factors in the HIV population [17].

Conflicting results from studies that have investigated the relationship between HIV infection, PI exposure, lipid abnormalities and carotid IMT have been reported [19–21]. In a small case–control study with matching for smoking and age, Seminari demonstrated that carotid IMT was greater among PI-treated patients in comparison with antiretroviral naïve and an HIV uninfected group [20]. Mercie and colleagues conducted a multi-center prospective cohort study of 423 HIV-infected subjects in which extensive data on cardiovascular risk factors, HIV disease-related variables and carotid IMT were measured [19]. In this study, the median carotid IMT measurement was 0.54 mm (range, 0.50–0.60 mm). These investigators found a relationship between several factors and carotid IMT in a univariate linear regression. Carotid IMT was significantly greater with older age, male gender, greater BMI, greater waist-to-hip ratio, increased systolic blood pressure, total cholesterol, glucose, homocysteine, regular smoking, alcohol consumption, lipodystrophy, and highly active antiretroviral therapy (HAART). However, after adjustment for other cardiovascular risk factors the effects of lipodystrophy and HAART disappeared, leading them to conclude that only conventional cardiovascular risk factors are independently associated with increased IMT in HIV-infected patients. Chironi reported that lipid values but not antiretroviral therapy predicted carotid IMT in a small case–control study [21]. Finally, Hsue recently reported increased carotid IMT among a group of HIV-infected subjects compared with a control group with similar age and sex distribution but significant differences in important cardiovascular risk factors [22]. Notably, 55% of the HIV group in this study were smokers compared with 25% of controls ($P < 0.01$). In addition, rates of progression of carotid IMT were greater among the HIV-infected group compared with a subset of the control population and compared with historical

data on IMT progression; however, these results were not controlled for other cardiovascular risk factors in the historical control population.

If PIs unfavorably impact risk factors associated with CVD risk, why did our study fail to observe any difference in carotid IMT in the PI-treated patients? It is possible that the impact of antiretroviral therapy-mediated changes in metabolic parameters may take a longer time to translate into changes in carotid IMT and increased rates of atherosclerosis. Additionally, it is possible, that by excluding subjects with a family history of myocardial infarctions, uncontrolled hypertension, and diabetes, that the study population was at lower risk for observing an impact of these metabolic changes. It is important to note that 45% of the study population was former or current smokers; however only 0.7% had hypertension. One could speculate that the effect of PI-based HAART on development of atherosclerosis first becomes evident in patients who are already pre-disposed to disease on the basis of other risk factors. For example, in the recently reported DAD study, 56% were former or current smokers and 11% had a family history of coronary artery disease [18].

It is possible that other cardiovascular risk factors such as age, smoking, hypertension, all of which were matched for in our study, are stronger predictors of atherosclerosis than the impact of exposure to PI therapy and HIV infection *per se*. Our study is one of the first to carefully control for all of these factors prospectively. Finally, it is possible that the impact of PI therapy on specific intermediary risk factors for subclinical atherosclerosis are not mechanistically operative at the arterial wall level, a conclusion that is supported by this carefully controlled study. It is important to note that in this study, there was an inverse relationship between HDL-cholesterol, a common abnormality in HIV infection, and carotid IMT and perhaps with longer follow-up this will emerge as a predictor of progression of carotid IMT among the HIV-infected group.

In conclusion, in this well-matched cohort of HIV-infected and -uninfected subjects, there was no relationship detected between HIV infection or PI therapy and a static measure of carotid IMT. The longitudinal follow-up on these subjects is planned to last 3 years to determine if HIV infection or PI therapy is mechanistically important in the progression of subclinical atherosclerosis. Meanwhile, clinicians who care for HIV patients should work to reduce long-term cardiovascular risk.

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Fig. 1.
Study schema.

Fig. 2.
Distribution of pairwise differences in intima-media thickness.

Table 1

Baseline characteristics of the study population.

Characteristic	PI $\geq$ 2 years (<i>n</i> = 44)	PI naive (<i>n</i> = 45)	HIV negative (<i>n</i> = 45)	<i>P</i> -value
Median value (IQR)				
Body mass index	24.8 (23–27)	24.6 (23–27)	24.9 (23–27)	> 0.99
Waist : hip ratio	0.93 (0.89–0.97)	0.90 (0.87–0.95)	0.89 (0.83–0.92)	0.001
Cholesterol (mmol/l)	5.67 (4.01–6.32)	4.64 (4.12–5.46)	4.84 (4.33–5.41)	0.013
LDL cholesterol (mmol/l)	3.19 (2.64–4.17)	2.80 (2.41–3.55)	2.98 (2.33–3.73)	0.313
HDL cholesterol (mmol/l)	1.02 (0.91–1.30)	1.09 (0.88–1.30)	1.19 (1.01–1.35)	0.186
Triglycerides (mmol/l)	2.17 (1.20–3.22)	1.60 (1.11–2.27)	1.21 (0.93–1.67)	< 0.0001

PI, protease inhibitor; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 2

HIV disease characteristics of groups 1 and 2.

Characteristic	Group 1, PI $\geq$ 2 years (<i>n</i> = 44)	Group 2, PI naive (<i>n</i> = 45)
CD4 ⁺ T cells (cells $\times$ 10 ⁶ /l), median value (IQR)	530 (375–705)	481 (334–744)
HIV RNA % < 50 copies/ml	75%	69%
Weeks of PI use, median value (IQR)	216 (153–265)	0 (0)

PI, protease inhibitor; IQR, interquartile range.