Endothelial function in HIV-infected patients receiving protease inhibitor therapy: does immune competence affect cardiovascular risk?

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Summary

Background: The use of HIV protease inhibitors (PIs) as a component of combination antiretroviral therapy in HIV-infected patients has been associated with dyslipidaemia, but its significance as a risk factor for cardiovascular disease is unclear. Endothelial dysfunction is an early phase of atherogenesis that may be assessed non-invasively with ultrasonography in vivo.

Aim: To evaluate vascular function and investigate potential determinants of endothelial dysfunction of the peripheral circulation in PI-treated, HIV-infected men with dyslipidaemia.

Design: Observational, case-control study.

Methods: We studied 24 HIV-infected, PI-treated men with dyslipidaemia and 24 normolipaemic, healthy male controls matched for age and body mass index. Brachial artery endothelial function was studied using high-resolution ultrasound and computerized edge-detection software. This non-invasive technique measured post-ischaemic flow-mediated dilatation (FMD), and the endothelium-independent vasodilatory response to glyceryl trinitrate (GTN).

Results: Within the HIV patient group, FMD was significantly associated with percentage of ‘naïve’ CD4+ 45RA+ T cells (p = 0.03), while plasma lipid/lipoprotein and insulin levels, body mass, and smoking status did not correlate with endothelial function. FMD was not significantly different between the study group and the controls.

Conclusions: The atherogenic potential of PI-associated dyslipidaemia may be attenuated in HIV-infected patients with decreased immune competence, reflecting a possible contribution of cell-mediated immune responses to the pathogenesis of atherosclerosis.

Introduction

Highly active anti-retroviral therapy (HAART) regimens that combine nucleoside analogue reverse transcriptase inhibitors (NRTIs) and HIV-1 protease inhibitors (PIs) have significantly improved the prognosis of HIV-infected patients. The use of PI therapy is, however, associated with a cluster of metabolic abnormalities characterized by elevated levels of triglycerides and triglyceride-enriched...
lipoproteins, associated with reduced HDL-cholesterol and insulin resistance. In the general population, this represents an atherogenic metabolic profile, in which increased risk of cardiovascular disease is predicted by this mixed dyslipidaemia phenotype, rather than by increased LDL-cholesterol levels. In HIV-infected patients receiving HAART, however, the risk of premature atherosclerosis attributable to PI-related dyslipidaemia remains to be established.

In the present study, we assessed the determinants of endothelial function in a group of dyslipidaemic HIV-infected men receiving long-term PI therapy, as well as in age- and body mass index (BMI)-matched controls. Endothelial dysfunction is an early phase of atherogenesis in which the vasodilatory properties of the endothelium are diminished, preceding the development of atherosclerotic plaque in the arterial wall, and may be measured non-invasively by assessing the degree of flow-mediated dilatation (FMD) of the brachial artery following an ischaemic stimulus. Endothelial function at the brachial artery provides a surrogate measure of the coronary circulation and a correlate of the severity of coronary artery disease. Accordingly, abnormal brachial artery endothelial function has been associated with a wide spectrum of cardiovascular risk factors, including dyslipidaemia, smoking, diabetes and hypertension.

Experimental evidence indicates that cardiovascular risk factors such as dyslipidaemia may evoke, or interact with, a chronic cell-mediated immune response at the endothelium. Hence, immunological factors were also assessed in the HIV-infected patients studied to investigate possible effects of HIV-associated immune deficiency, and the reconstitution of immune function with effective antiretroviral therapy, on vascular function.

Methods

Patient selection

Subjects were 24 HIV-infected men who were referred to a lipid disorders clinic for assessment and management of dyslipidaemia, representing all male patients who fulfilled the study criteria from a cohort of over 350 active patients (described in reference 23). Inclusion in the study required the use of HIV protease inhibitors for at least 9 months, and a diagnosis of dyslipidaemia, which was defined as a fasting total cholesterol > 6.0 mmol/l, and/or triglyceride > 2.0 mmol/l with HDL-cholesterol ≤ 0.9 mmol/l. Patients were excluded from the study if they had active opportunistic infections, diabetes mellitus, renal failure, hypothyroidism, major systemic illness, or current use of hormones, lipid-lowering drugs, fish oils or angiotensin-converting-enzyme inhibitors. A reference group of 24 healthy, normolipidaemic men matched for age and BMI was also studied. The Royal Perth Hospital Ethics Committee approved the study, and subjects gave informed consent.

Clinical and laboratory measurements

Details were taken of age, smoking habits, history of coexistent disease and medication. Blood pressure was measured using a Dinamap (Critikon). Height was measured without shoes, and weight in light clothes. Venous blood was obtained with the subject resting in the semi-recumbent position and after a 10–12 h fast. Total serum cholesterol, HDL-cholesterol and triglyceride levels were measured by an enzymic, colorimetric method. Serum LDL-cholesterol was a calculated value based on the modified Friedewald formula: LDL-cholesterol = total cholesterol − (0.46 × triglyceride) − HDL-cholesterol, where all values are in mmol/l. When serum triglyceride exceeded 5.0 mmol/l, direct measurement of LDL-cholesterol was undertaken retrospectively, using an enzymatic colorimetric assay. Lipoprotein(a) and apolipoprotein B were assayed by immunonephelometric methods. Serum glucose was assayed by the hexokinase method, and serum insulin levels were measured by a solid-phase two-site chemiluminescent enzyme-labelled immunometric assay (Immulite, DPC, Los Angeles, CA). Insulin resistance was calculated by the HOMA model: insulin resistance = fasting insulin (mU/ml) × fasting glucose(mmol/l)/22.5.

Other than C-reactive protein, which was measured using a sensitive Behring BN II nephelometric assay, virological and immunological assessments were carried out in the HIV group only. These included measurement of plasma HIV viral load (Roche Amplicor HIV Monitor, version 1.5, Ultra-sensitive, <50 copies/ml limit of detection), and T-cell subset analysis by flow cytometry, including absolute and percentage CD4 and CD8 expression, and CD45RA and CD45RO expression within the CD4 + T cell population.

In the PI-treated group, retrospective data relating to immunological and metabolic status prior to commencement of PI therapy were collected, according to the methods described above. These parameters are routinely measured in participants in the Western Australian HIV cohort, and data are stored in a centralized database.
Vascular measurements

Ultrasonography was carried out (and scans were analysed) by trained operators using high-resolution B-mode ultrasound equipped with a 12 MHz linear array transducer (Acuson 128) and computerized edge-detection software. Subjects were studied after a 4–6 h fast, being allowed a light low-fat breakfast and any medication. During the ultrasound procedure, a high-resolution transducer was placed 5–10 cm proximal to the ante-cubetal crease of the left arm, and fixed in position by a stereotactic clamp. A pneumatic cuff was placed around the subjects left forearm, and after scanning the baseline artery diameter for 2 min, the cuff was inflated to 200 mmHg for 5 min. Release of the cuff induced reactive hyperaemia, and scanning was continued for a further 4–5 min. A second resting scan was obtained at least 10 min after cuff deflation, to ensure that the brachial artery diameter returned to the basal level prior to sublingual administration of glyceryl trinitrate (GTN 400 μg) via spray. The artery was subsequently scanned for a further 5–6 min. Images were recorded on super-VHS videotape (Sony MQSE 180) for retrospective analysis.

Analyses of post-ischaemic flow-mediated dilatation of the brachial artery (FMD) and the response to glyceryl trinitrate (GTN) used semi-automated edge-detection software developed and validated by the University Department of Medicine, Royal Perth Hospital, University of Western Australia. An observer visually determined the section of the artery providing the most consistent image during the period of flow-mediated dilatation, and baseline and measurements were confined to this area. This technique was repeated to allow separate analysis of the endothelium-independent response to GTN. All diameter measurements were R-wave-gated and performed in a blinded fashion. R-wave diameters were continuously recorded throughout the entire study allowing objective determination of peak flow-mediated dilatation. Data are presented as percentage changes in brachial artery diameter from baseline in response to ischaemia (FMD) and glyceryl trinitrate (GTN).

Statistical methods

Data analysis used SPSS 9.0. For continuous variables, the normality of data distribution was assessed using the Shapiro-Wilk test. Paired t-tests were used to compare lipid and immunological data pre-commencement of protease inhibitors and while on treatment. Unpaired t-tests and the non-parametric Mann-Whitney test were used to compare demographic, metabolic and physiological variables between patient and control groups. The Pearson product moment coefficient of correlation (r) (two-tailed) was computed to identify relationships between variables. Linear regression analyses were used to establish the predictive power of variables associated with FMD and GTN.

Results

Group comparisons of demographic, immunological, metabolic status, and vascular function are presented in Table 1.

Demographics of study groups

Patients and controls in the study were male, and were matched for age (42.6 ± 9.5 vs. 43.7 ± 9.8 years, p = 0.71) and BMI (24.1 ± 2.7 vs. 24.4 ± 2.6 kg/m², p = 0.69). Ten of the HIV-infected subjects and six controls were current smokers. Among the HIV-infected patients, PI therapy consisted of indinavir (n = 13), nelfinavir (n = 6, in 1 case in combination with ritonavir), and ritonavir/saquinavir (n = 5). Patients received PI therapy for a period of 30.5 ± 4.9 months.

Immunological and virological status

In the HIV-infected study group, average pretreatment (nadir) CD4+ T cell count was 134 ± 157 × 10⁶/l (7 ± 6%), with advanced-stage HIV disease (defined as CD4+ T cells < 200 × 10⁶/l) in 21/24.

At the time of the study, 18/24 patients had undetectable plasma viral load (<50 copies HIV RNA/ml), with mean plasma HIV RNA in the group of 3.97 ± 1.1 copies/ml. Absolute CD4+ T cell counts on treatment (469 ± 240 × 10⁶/l, range 160–1140 × 10⁶/l) were consistent with mild-to-moderate immune deficiency, with evidence of significantly improved CD4+ T cell counts under PI-containing HAART (CD4+ T cell increment 335 ± 137 × 10⁶/l (12 ± 4.6%), p < 0.001). Despite this improvement, the percentage of lymphocytes expressing the CD4+ ‘naive’ T cell marker CD45RA remained relatively low at the time of study (9 ± 5%, range 2–23%). These %CD4+ CD45RA+ values were comparable to those obtained from a reference group of 30 patients who had pre-therapy CD4+ T cell counts of <50 × 10⁶/l and who had experienced immune restoration disease following HAART (11 ± 9.9%, p = 0.30); and were significantly lower than results obtained from 29 healthy controls (19 ± 7.1%, p < 0.001).

C-reactive protein levels were similar among PI-treated patients and controls (3.0 ± 2.6 mg/l vs.
In comparison with the age- and BMI-matched control group, the HIV-infected group prior to treatment with PIs had significantly reduced plasma LDL-cholesterol (2.4 ± 0.9 vs. 2.9 ± 0.7 mmol/l, \( p = 0.04 \)) and HDL-cholesterol (0.5 ± 0.2 vs. 1.3 ± 0.2 mmol/l, \( p < 0.001 \)), while triglyceride levels were significantly elevated (3.4 ± 0.9 vs. 5.5 ± 3.4 mmol/l, \( p < 0.001 \)). Plasma concentrations of total cholesterol (4.4 ± 0.8 vs. 4.7 ± 0.8 mmol/l, \( p = 0.19 \)) and apolipoprotein B (1.0 ± 0.2 vs. 0.9 ± 0.2 mmol/l, \( p = 0.14 \)) were similar in HIV-infected subjects pre-therapy and in the control group.

At the time of the study, the PI-treated study group had significantly elevated plasma levels of total cholesterol (6.9 ± 1.3 mmol/l), triglyceride (5.2 ± 2.9 mmol/l) and apolipoprotein B (1.4 ± 0.2 mmol/l) compared with control values \( (p < 0.001) \) and pre-therapy values \( (p < 0.01) \). HDL-cholesterol levels on treatment (0.9 ± 0.2 mmol/l) were significantly decreased compared with the control group \( (p < 0.001) \), but were significantly higher than pre-therapy values \( (p < 0.001) \). Sixteen of the 24 PI-treated patients had lipoprotein (a) levels < 0.1 g/l, and levels of lipoprotein (a) were similar in cases and controls \( (p = 0.69) \).

Plasma insulin and glucose levels were increased in PI-treated patients compared with controls \( (17.2 ± 21.5 \text{ vs. } 6.3 ± 2.2 \text{ mU/l}) \ (p = 0.001) \); and
5.2 ± 0.7 vs. 4.4 ± 0.9 mmol/l (p = 0.002), respectively. Accordingly, calculated insulin resistance using the homeostasis (HOMA) model was higher in PI-treated patients than controls (4.3 ± 6.0 vs. 1.2 ± 0.6, p < 0.001).

**Vascular function**

There were no significant differences between HIV-infected patients and controls in baseline brachial artery diameter (3.87 ± 0.5 vs. 3.84 ± 0.4 mm, p = 0.80), post-ischaemic FMD (6.7 ± 3.6% vs. 7.6 ± 3.7%, p = 0.40), and response to glyceryl trinitrate (15.4 ± 5.3% vs. 18.2 ± 5.3%, p = 0.08) (Table 1).

In the HIV-infected group, FMD was correlated with baseline brachial artery diameter (r = 0.46, p = 0.02), as expected, as well as with pulse pressure (r = 0.46, p = 0.02), and %CD4+45RA+ T cells (r = 0.44, p = 0.03). There was a trend towards an association between %CD4+ count and FMD (r = −0.38, p = 0.07). Among other immunological variables, no correlation could be found between FMD and, %CD4+45RO+ T cells, %CD8+ T cells or C-reactive protein levels. There were no significant correlations between FMD and age, body mass index, plasma triglyceride, LDL-, HDL- or total cholesterol, lipoprotein (a), apolipoprotein B, or smoking status in the 24 patients. There was a trend towards a positive correlation between FMD and HOMA score (r = 0.63, p = 0.10 for log HOMA) among PI-treated subjects. Response to GTN was not associated with any variable other than pulse pressure (r = −0.45, p = 0.03).

Multivariate linear regression analyses were undertaken to assess the most comprehensive explanatory models of FMD variability (Tables 2a and 2b). In the patient group, a model incorporating baseline artery diameter, pulse pressure, %CD4 T cell count, and age explained 54% of the variability in FMD (p < 0.001, adjusted R² = 0.54). Smoking status, body mass index, lipid and lipoprotein levels, fasting insulin, HOMA assessment of insulin resistance, duration of PI therapy, and C-reactive protein levels were also assessed in these analyses, and were not found to contribute to the models. A model incorporating known cardiovascular risk factors (smoking status, total cholesterol, HOMA score, body mass index, mean arterial pressure, and age) is shown in Table 2b.

Among controls, FMD correlated with baseline artery diameter (r = 0.43, p = 0.03), while no other variable tested was significant at the 10% level.

**Table 2a** Multivariate linear regression model of FMD

<table>
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<th>Variables</th>
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<th>Beta</th>
<th>t</th>
<th>p</th>
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<td>BAD</td>
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<td>0.031</td>
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<td>CD4%</td>
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<td>Age</td>
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<td>-2.788</td>
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</table>

R² = 0.622; adjusted R² = 0.542. BAD, baseline artery diameter.

**Table 2b** Multivariate linear regression model of FMD, incorporating standard cardiovascular risk factors

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<td>0.18</td>
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<tr>
<td>Mean arterial BP</td>
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<td>-0.09</td>
<td>-0.43</td>
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<tr>
<td>Total cholesterol</td>
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<td>0.63</td>
<td>0.07</td>
<td>0.32</td>
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<tr>
<td>HOMA score</td>
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<td>-0.08</td>
<td>-0.41</td>
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</table>

R² = 0.380; adjusted R² = 0.109. BAD, baseline artery diameter; BP, blood pressure.
regression revealed that no factor other than baseline artery diameter contributed significantly to the model, which explained 15% of variability in FMD.

Discussion

Brachial artery endothelial function in dyslipidaemic PI-treated patients

In this study, a group of 24 PI-treated HIV-infected patients who met current criteria for management of dyslipidaemia demonstrated comparable endothelial function of the brachial artery to age-matched normolipidaemic controls. Within the HIV group, however, the naïve T-cell percentage was a significant predictor of endothelial function of the brachial artery. Hence, despite the presence of dyslipidaemia as well as other cardiovascular risk factors such as smoking, brachial artery flow-mediated dilatation was preserved among PI-treated individuals with evidence of immune suppression. This discordance represents the most striking finding of the study, and suggests that immunological status may be a factor influencing endothelial function in these patients. Further prospective studies are required to investigate associations between metabolic and immunological status and vascular outcomes among patients who are antiretroviral-therapy-naïve progressing on to HIV protease inhibitor-containing treatment regimens.

These findings may be contrasted with those of a study of endothelial function in 22 PI-treated and 15 PI-naïve HIV-infected adults, presented by Stein and colleagues. In this study, PI therapy was associated with elevated triglycerides and triglyceriderich lipoproteins, and these metabolic parameters (chylomicrons, IDL-cholesterol and VLDL-cholesterol) were in turn predictive of impaired endothelial function. Overall, FMD in the PI-treated group was significantly reduced compared with the PI-naïve group (2.8 ± 4.6% vs. 6.1 ± 6.7%, p = 0.005). These data provide evidence for the atherogenic potential of the PI-associated metabolic phenotype, and identify associations between endothelial dysfunction and specific lipoprotein fractions that have previously been shown to determine cardiovascular risk in HIV-negative populations with triglyceriderich dyslipidaemia.

In the present study, the metabolic outcomes associated with PI therapy are consistent with published data, and are similar to those observed by Stein and colleagues, with significant mixed dyslipidaemia, elevated apolipoprotein B, and insulin resistance. Similarly, clinical parameters including age and duration of PI therapy (30.5 ± 4.9 months in this study, versus 30.8 ± 9.6 months) are also comparable, as are the methods used for measurement of flow-mediated dilatation of the brachial artery. Hence, the apparently conflicting results relating to associations between PI-associated dyslipidaemia and endothelial function cannot be readily attributed to methodological differences. These differences might be reconciled by considering the advanced stage of HIV infection at which PI treatment was initiated in the present study, and the influence of this treatment strategy on immunological as well as metabolic variables that may influence endothelial function.

Metabolic and immunological status associated with advanced-stage HIV infection

The metabolic status of HIV-infected patients in this study prior to commencing PI therapy is consistent with advanced-stage HIV infection, with markedly reduced HDL-cholesterol, reduced total- and LDL-cholesterol, and elevated triglyceride levels (Table 1). This HIV-associated lipoprotein profile has been characterized by Grunfeld and colleagues, who have also noted increased insulin sensitivity associated with this metabolic phenotype. In this context, it is notable that plasma apolipoprotein B, which provides a measure of the number of atherogenic particles in the VLDL-, IDL- and LDL-cholesterol fractions, is not elevated in pre-therapy samples compared with healthy controls. These data would suggest that ‘baseline’ metabolic status in these patients with advanced-stage HIV disease, although significantly altered compared to HIV-seronegative controls, is unlikely to be atherogenic.

Pre-therapy immunological parameters are also consistent with advanced stage disease in the study group, with an average CD4+ cell count of 134 ± 106/l (Table 1). The capacity for restoration of immunological function and of the CD4+ T cell repertoire in response to HAART, and in particular the reconstitution of naïve CD4+ T cells, appears to be diminished in individuals treated at advanced stages of HIV infection. This is apparent in the present study, as the percentage of CD4+ CD45RA+ T cells at the time of endothelial function assessment remained low (9 ± 5.4%), consistent with ongoing depletion of the naïve T-cell repertoire, despite evidence of significantly increased CD4+ T-cell counts on HAART (CD4+ cell count increment, 335 ± 137 × 106/l, p < 0.001).

In this study, the percentage of CD4+ T cells expressing the CD45RA+ ‘naïve’ T-cell marker was a predictor of post-ischaemic flow-mediated
dilatation of the brachial artery. While it is acknowledged that CD45RA expression is an incomplete marker of immunological naïvete, these findings are consistent with previous work by Stemme and colleagues, who have proposed that naive CD4+ T cells are critically involved in the inflammatory component of atherogenesis, being recruited to human atherosclerotic plaque and subsequently activated in situ to become CD4+CD45RO+ plaque T cells. Hence, these data raise the possibility that the development of endothelial dysfunction in response to PI-associated dyslipidaemia in HIV-infected patients may be contingent on the reconstitution of immune function, and that the naive CD4+ T-cell population provides a marker of the ‘atherogenic immune response’ in these individuals.

**Study limitations**

The cross-sectional design of this study does not allow for an adequate assessment of the causal relationships between immune competence, cardiovascular risk factors, and endothelial function in HIV-infected patients, and it is acknowledged that the small sample size provided limited statistical power to detect a difference in vascular function between the patient and control groups. It is notable, however, that this study is in these respects comparable to that reported by Stein and colleagues, although the primary findings are divergent.

In addition, the study was not designed to comprehensively investigate the immunological aspects of endothelial dysfunction and atherogenesis in HIV-infected patients, so that potential immunological correlates of endothelial dysfunction—for example, the balance of Th1/Th2 cytokines that has been implicated in the modulation of nitric-oxide-dependent endothelial function—remain poorly defined. Similarly, due to ethical concerns, the assessment of immunological status (other than measurement of C-reactive protein) was not undertaken in the control group in this study.

**Conclusions**

Investigating the potential for metabolic abnormalities associated with PI therapy to affect cardiovascular risk has been a high priority area of research in the HAART era. Initial case reports of premature coronary artery disease have now made way for large-scale population-based studies, which continue to produce evidence both for and against increased cardiovascular risk associated with long-term PI therapy. In this study of dyslipidaemic PI-treated individuals, in which the measurement of brachial artery flow-mediated dilation provides an ‘early’ marker of the atherogenic process, an unexpected preservation of endothelial function was found in patients with more advanced HIV-associated immune deficiency. This finding is in contrast to a published study of similar size and methodology that identified associations between endothelial dysfunction and dyslipidaemia. We propose that the apparently conflicting results of these studies may be reconciled by an appreciation of the immunological and metabolic effects of advanced-stage HIV infection, and the diminished immunological response to treatment of advanced disease, that are demonstrated in this study. These factors may modulate the capacity for endothelial dysfunction and atherogenesis to develop in response to PI-induced dyslipidaemia. The relationship between endothelial function and immune competence, as represented by the percentage of CD4+CD45RA+ T cells, warrants investigation in future studies as a factor that may explain some of the apparent discordance that continues to be observed in this field of research.

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**References**


