ORIGINAL ARTICLE

Protease inhibitor treatment effect on aortic stiffness in normotensive patients with human immunodeficiency virus infection

Effet des inhibiteurs de protéase sur la rigidité aortique chez des patients normotendus infectés par le virus d’immunodéficience humaine (VIH)

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Summary

Objectives. — Human immunodeficiency virus (HIV) infection and protease inhibitor (PI)-based antiretroviral treatment might increase large artery (aortic) stiffness compared with healthy untreated controls. To clarify the role of PI therapy in the progression of subclinical arteriosclerosis in patients with HIV, we investigated the impact of PI treatment on arterial stiffness.

Methods and results. — In our single-centre, cross-sectional study, normotensive male HIV patients free from overt cardiovascular disease received PI treatment (n = 60) or no PI treatment (n = 42). The PI group had a significantly higher pulse wave velocity (PWV) than the PI-free group (9.0 ± 1.4 vs. 8.1 ± 1.3 m/s; P = 0.016). There was a significant positive correlation between age and PWV in the PI-free group (R² = 0.310; P < 0.0001) and, to a lesser extent, in the PI group (R² = 0.181; P < 0.0001). PI treatment was associated with a significant increase in the adjusted slope of the curve relating age to PWV as compared with no PI treatment.

Conclusions. — In normotensive HIV patients, PI treatment significantly increases both aortic stiffness and the positive correlation between PWV and age. Aortic stiffness predicts cardiovascular mortality, thus these results provide new insight on the relationship between PI treatment, mechanical arteriosclerotic and cardiovascular risk.

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MOTS CLÉS
Rigidité aortique ;
Artériosclérose ;
Virus d’immunodéficience humaine ;
Inhibiteurs de protéases

Résumé
Objectif. — L’infection par le virus d’immunodéficience humaine (VIH) et son traitement par des stratégies basées sur les inhibiteurs de protéases (IP) pourraient augmenter la rigidité de l’aorte. Afin de clarifier l’impact des traitements IP sur la progression de l’artériosclérose chez les patients VIH, nous avons étudié l’impact des traitements par IP sur la rigidité aortique.
Méthodes et résultats. — Une étude monocentrique, cross-sectionnelle a inclus des hommes normotendus infectés par le VIH et indemnes de toute pathologie cardiovasculaire avérée recevant (n = 60) ou non (n = 42) un traitement par IP. Les patients du groupe IP avaient une vitesse de l’onde de pouls (VOP) aortique significativement plus élevée que celle des patients n’ayant pas reçu de traitement par IP (9,0 ± 1,4 vs 8,1 ± 1,3 m/s ; p = 0,016). La corrélation positive et significative entre âge et VOP au sein du groupe sans IP (R² = 0,310 ; p < 0,0001) était retrouvée à un moindre degré dans le groupe IP (R² = 0,181 ; p < 0,0001). Le traitement par IP était associé à une augmentation significative de la pente ajustée de la relation âge—VOP.
Conclusions. — Chez des patients normotendus atteints par le VIH, le traitement par IP augmente significativement la rigidité aortique et renforce la relation âge—VOP. La rigidité aortique étant un prédicteur de morbi-mortalité cardiovasculaire, ces résultats apportent un nouvel éclairage sur les relations existant entre le traitement par IP, la physiopathologie de l’artériosclérose et le risque cardiovasculaire.
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Use of protease inhibitor (PI)-based highly active antiretroviral treatment (HAART) regimens is one of the preferred therapeutic approaches to human immunodeficiency virus (HIV) infection and has led to a dramatic reduction in HIV-associated morbidity and mortality rates [1]. HAART is associated with a range of adverse metabolic effects including dyslipidemia, fat redistribution, insulin resistance, glucose intolerance, metabolic syndrome and type 2 diabetes, all well-known cardiovascular (CV) risk factors in HIV-negative populations.

Lipid abnormalities have been observed in untreated patients with HIV. HIV-infected subjects have decreased low-density lipoprotein (LDL) cholesterol [2], high-density lipoprotein (HDL) [2,3] cholesterol or total cholesterol (TC) [2,3] levels (versus seronegative controls). Lipid disturbances, mainly abnormally low HDL-C concentrations, might be involved in the early atherosclerotic process in HIV-infected patients [4]. During HAART treatment, the profile of dyslipidemia changes [5]. PI-based antiretroviral therapy, in particular, is associated with an atherogenic dyslipidemic profile (elevated levels of triglyceride-rich lipoproteins and elevated LDL cholesterol levels vs PI-untreated patients with HIV) [6,7]. There is increasing evidence that PI therapy is associated with increased risk of myocardial infarction, explained in part by dyslipidemia [8]. PI therapy has also been linked to early signs of atherosclerosis such as endothelial dysfunction and increased intima-media thickness (IMT), both of which might be linked to increased risk of vascular disease [9–11].

Since the absolute CV event rate is low in this patient population, large artery stiffness is used as a simple and reproducible surrogate marker of subclinical arteriosclerotic disease [12]. Large artery stiffness has been identified as a strong predictor of total and CV mortality [12–15], coronary morbidity and mortality [14], and fatal stroke [16], in a variety of clinical settings such as essential hypertension [12,14,16], end-stage renal failure [13], diabetes mellitus [15] and chronic inflammatory diseases, such as lupus erythematosus and rheumatoid arthritis [17]. Few studies have investigated the impact of PI-based antiretroviral therapy on arterial stiffness in HIV-infected adults and the available data are inconclusive. Evidence suggests that both HIV infection and PI treatment increase large artery stiffness compared with healthy untreated controls, with longer PI exposure associated with increased aortic stiffness [18]. However, studies comparing carotid IMT (a surrogate marker of subclinical atherosclerosis) in treated and untreated HIV-infected subjects failed to show an association between PI exposure or HIV infection and carotid IMT [19,20]. A subsequent study comparing aortic stiffness in untreated HIV-infected individuals and matched non-HIV-infected controls confirmed that HIV infection was associated with an increase in aortic stiffness and atherosclerotic risk [21]. The same paper [21] reported data from a case-control comparison using the same aortic stiffness data in a comparison with data from an earlier study in a cohort of HIV-infected patients receiving stable antiretroviral therapy [18]. These post hoc findings showed that aortic stiffness tended to be higher in HIV-infected subjects receiving antiretroviral therapy versus untreated HIV-infected subjects (P = 0.07) [21]. Another cross-sectional study investigating the impact of antiretroviral therapy on arterial stiffness in HAART-treated HIV-infected patients showed that prolonged antiretroviral treatment was a significant risk factor for arterial stiffness and associated CV mortality [22].

However, these data are far from conclusive and the relationship between artery stiffness and PI-based antiretroviral treatment warrants further investigation. Our study is the first to investigate the impact of the PI-based antiretroviral therapy in disease-, age- and obesity-matched groups of normotensive HIV-infected subjects as an a priori objective. This study compares the effect of PI treatment on large artery stiffness (as determined by carotid-femoral pulse wave velocity [PWV] assessment) in PI treated HIV-infected subjects versus PI-untreated HIV-infected subjects to determine whether PI treatment has a significant independent impact on aortic stiffness as a predictor of CV disease. We
also investigated the impact of PI-based treatment on the established relationship between PWV and age.

Methods

Study design and objectives

This study was a single-centre, two-parallel groups, controlled, cross-sectional study. HIV seropositive male outpatients were divided into two groups according to PI treatment status: PI-untreated (never treated or treated for less than 1 year) and PI-treated.

The primary study objectives were to compare the impact of PI treatment on arterial stiffness as assessed by PWV in PI-treated or PI-untreated HIV-infected patients and to determine the between-group difference in the relationship between age and PWV. The protocol was approved by a Medical Ethics Committee as well as the National Committee for the Protection of Privacy and Civil Liberties.

Patients

The cohort included documented HIV-infected male patients (first diagnosis > 5 years) who attended the Department of Internal Medicine of the Avicenne Hospital, Paris. Subjects were divided into two groups:

• HIV-infected patients (documented diagnosis greater than 5 years) never treated with a PI or treated for less than 1 year;
• HIV-infected patients (documented greater than 5 years) who had been receiving PI therapy for more than 1 year.

The two groups were matched for age, systolic blood pressure (SBP), height, weight, body mass index (BMI), waist and hip circumference.

Patients excluded from the study included those with atrial fibrillation; arterial hypertension; vasodilatation and/or BP-lowering treatment; hypercholesterolemia; known diabetes or fasting glycaemia greater than 7 mmol/L (> 126 mg/dL); serum creatinine concentration greater than 176.8 μmol/L (> 2 mg/dL); clinical or laboratory evidence of coronary heart disease; and previous stroke. Hypertension was defined as a systolic/diastolic BP of greater or equal to 140/90 mmHg on three consecutive measurements or anti-hypertensive drug treatment. Hypercholesterolemia was defined as a serum cholesterol concentration of greater than 6.46 mmol/L (> 250 mg/dL) or hypolipidemic drug treatment. All participants gave their informed consent to participate in the study.

Clinical assessment

All assessments were performed within the Department of Internal Medicine. All participants abstained from smoking at least 2 hours before assessment. Their height, weight, and waist and hip circumferences were measured, and their BMI (kg/m²) was calculated.

Subjects rested in a supine position for 10 minutes in a quiet room at 22 °C before the baseline hemodynamic parameters (BP, heart rate, PWV) were measured. Clinic BP and heart rate were measured immediately before PWV measurement, and in compliance with World Health Organization guidelines using an automated digital sphygmonometer with a cuff appropriate to the subject's arm circumference.

Arterial stiffness was assessed by duplicate measurement of the automatic carotid-femoral PWV using the Complior® device (Colson, Paris), adapted to the national regulatory and custom requirements of France for PWV measurement; the technical characteristics of this device have been described and validated [23]. The inter-observer repeatability (repeatability coefficient) for the automatic PWV calculation was assessed in subjects selected at random. The basic principle of PWV assessment is that the pressure pulse generated by ventricular ejection is propagated along the arterial tree at a speed determined by the geometric and elastic properties of the arterial wall [24]. PWV was calculated from measurements of pulse transit time and the distance travelled by the pulse between two recording sites, according to the following formula: \( \text{PWV (m/s)} = \frac{\text{distance (m)}}{\text{transit time (s)}} \). Carotid-femoral PWV was calculated from the time delay between the recorded proximal (carotid) and distal (femoral) feet of the wave, and then superficially measured from the superternal notch to the femoral artery.

Statistical analysis

The sample size required to show a significant between-group difference in PWV was calculated as 47 based on data from a previous study where there was a difference of 0.8 m/s in PWV between HIV-infected subjects and seronegative controls [18].

Quantitative data were expressed as the mean ± SD (1 standard deviation) or the mean ± SE (1 standard error). Qualitative data were expressed as the percentage of subjects.

Since our aim was to explore the relationship between PI treatment and PWV and the effect of PI treatment on the PWV–age relationship, the statistical analysis involved several steps. For the analysis of effect of PI treatment on the age–PWV relationship, a test of heterogeneity of slopes was conducted. This test represents a natural extension of covariance analysis, and involves features from both ANOVA and linear regression. Firstly, the relationship between age and PWV in each group was explored by a linear regression analysis in order to find the slope value. To do so, regression coefficients were calculated as the least-squares estimates of the parameters. Thereafter, a slope comparison test (F-test) was conducted to evaluate whether or not the regression coefficients of the age–PWV relationship differed between the two patient groups. The result of the F-test (Table 2) was considered to be one of the principal outcomes of the study. The SPSS statistical package, release 10.0 (SPSS, Inc) was used for all statistical analyses. A P value of less than 0.05 was considered significant.
Table 1  Patient characteristics.
Caractéristiques des patients.

<table>
<thead>
<tr>
<th></th>
<th>Without PI (n = 42)</th>
<th>With PI (n = 60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>44 ± 12</td>
<td>47 ± 10</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24 ± 4.5</td>
<td>24.1 ± 4.5</td>
<td>0.89</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>124 ± 13</td>
<td>127 ± 14</td>
<td>0.25</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>77 ± 10</td>
<td>76 ± 8</td>
<td>0.63</td>
</tr>
<tr>
<td>PP, bpm</td>
<td>73 ± 11</td>
<td>72 ± 14</td>
<td>0.78</td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>84.0 ± 16.8</td>
<td>84.9 ± 15.9</td>
<td>0.53</td>
</tr>
<tr>
<td>Serum glucose, mmol/L</td>
<td>4.9 ± 0.6</td>
<td>4.9 ± 1.1</td>
<td>0.47</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
<td>62 ± 37</td>
<td>73 ± 56</td>
<td>0.29</td>
</tr>
<tr>
<td>LDL-C, g/L</td>
<td>1.18 ± 0.4</td>
<td>1.27 ± 0.4</td>
<td>0.29</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.6 ± 0.8</td>
<td>1.9 ± 1.4</td>
<td>0.13</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>8 ± 13</td>
<td>8 ± 10</td>
<td>0.85</td>
</tr>
<tr>
<td>CD4, n/mm³</td>
<td>390 ± 204</td>
<td>485 ± 378</td>
<td>0.17</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>69</td>
<td>55</td>
<td>0.27</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>8.1 ± 1.3</td>
<td>9.0 ± 1.4</td>
<td>0.013</td>
</tr>
</tbody>
</table>

CD4: CD4 positive T cell; CRP: C-reactive protein; PP: pulse pressure; TG: triglyceride; PI: protease inhibitor; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; LDL: low-density lipoprotein; PWV: pulse wave velocity.

Results

Patients

Sixty subjects satisfied inclusion criteria for the PI treatment group (mean ± SD age 47 ± 10 years; mean ± SD SBP/diastolic BP [DBP] 127 ± 14 /76 ± 8 mmHg) and 42 subjects were included in the PI-untreated control group. Baseline demographic and clinical characteristics in the two treatment groups were similar (Table 1).

PWV comparison by treatment group

Patients who were currently receiving PI treatment had a significantly higher PWV (9.0 ± 1.4 vs. 8.1 ± 1.3 m/s; P = 0.016) than the PI-untreated group (Fig. 1).

PWV—age analysis

In a scatter plot analysis (Fig. 2), correlation coefficients for the correlation between PWV and age were calculated for patients receiving and not receiving PI treatment. The multiple coefficient of determination (R²) showed a significant positive correlation between age and PWV in the PI-untreated group (R² = 0.310) and, to a lesser extent, in patients with PI treatment (R² = 0.181) (Table 2). PI treatment was associated with a significant increase in the adjusted slope of the PWV—age correlation curve compared with no PI treatment (P = 0.048; Table 2).

Discussion

This analysis, the first to investigate PI-related arterial stiffness in HIV-infected individuals using disease-matched controls, showed a clear positive correlation between PI treatment and aortic stiffness (PWV), an early marker of atherosclerosis and a strong predictor of CV mortality in different clinical settings. The analysis of PWV—age correlation showed that PI treatment had a significant impact on arterial stiffness independent of age after adjustment for other potential confounding factors.

Although PI use is known to be associated with early vascular structural and functional alterations [9—11], premature vascular disease is also linked to the HIV infection itself [25,26] and previous studies have been unable to show a significant impact of antiretroviral therapy on atherosclerosis risk independent of the effects of HIV infection. In an investigation of subclinical markers of atherosclerosis in HIV-infected patients, data show atherosclerotic change suggesting an increased CV risk in HIV-infected patients, both with and without the presence of clusters of metabolic risk factors, although more advanced atherosclerotic changes were seen in patients with metabolic syndrome [27]. From this, it would seem likely that both HIV

Figure 1  Mean pulse wave velocity (PWV) in patients receiving (PI+) or not receiving (PI-free) PI treatment.
Vitesse de l’onde de pouls (VOP) moyenne dans les deux groupes.
infection and antiretroviral therapy promote atherosclerosis directly and/or indirectly via metabolic risk factors [27]. This increased atherosclerotic potential may explain the observations of an increased risk of adverse CV outcomes with antiretroviral therapy in a large prospective safety study (the Data Collection on Adverse Events of Anti-HIV Drugs [DAD] study; n = 23,468) in which a 26% increase in myocardial infarction risk was seen per year of exposure to antiretroviral therapy over the first 4 to 6 years of use [28]. A subsequent analysis of data from the same study showed that antiretroviral therapy was also associated with an increased risk of all CV and cerebrovascular events [29]. In a large retrospective study, the likelihood of moderate-to-high 10-year predicted risk of coronary heart disease was significantly lower among HAART-naive individuals compared with users of PI-based regimens [30]. In this study, patients receiving non-PI antiretroviral therapy and former HAART users were also less likely than users of PI-based HAART to have moderate-to-high coronary heart disease risk (although this was not statistically significant) [30]. Conversely, in another large retrospective study in over 36,000 HIV-infected patients, no relation between the use of antiretroviral therapy and the risk of CV or cerebrovascular events was found [31].

Given the conflicting evidence for the impact of PI-based antiretroviral therapy on CV outcomes in HIV-infected individuals, the present study adds to the growing amount of evidence to show that PI treatment promotes atherosclerosis and accelerates the natural progression of arterial stiffness with age.

Long-term follow-up of large populations has shown that the rate of increase of arterial stiffness with age contributes greatly to CV risk [32,33]. The demonstration of wide inter-subject variations in the slope of the curve relating PWV or pulse pressure (another marker of vascular stiffness) to age suggests that a number of environmental or genetic factors or the combination of both might substantially influence this relationship [34,35].

**Figure 2** Scatter plot of pulse wave velocity (PWV) by age in patients receiving (PI+) or not receiving (PI−) PI treatment. *Nuage de points de la relation vitesse de l’onde de pouls (VOP)—âge chez les patients recevant un traitement par IP (PI+) et les patients sans IP (PI−).***

<table>
<thead>
<tr>
<th>No PI treatment (n = 42)</th>
<th>With PI treatment (n = 60)</th>
<th>P1</th>
</tr>
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<tbody>
<tr>
<td>R²</td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>0.310</td>
<td>0.071</td>
<td>0.017</td>
</tr>
</tbody>
</table>

β: slope, regression coefficient; R²: partial adjusted explanation of PWV by age; SE: standard error of regression coefficient; P-value: probability of regression between PWV and age; P1-value: probability of interslope comparison between the two groups (see text).
Given the strong correlation between large artery stiffness and age reported in the literature [36], the significant impact of PI treatment on the PWV—age correlation shown in our PWV—age analysis is an important finding. Our data clearly demonstrate that PI treatment has an impact on artery stiffness independent of age and HIV infection and other demographic and clinical variables.

Current guidelines on the management of CV risk in HIV-infected patients support treating CV risk in HIV-infected patients in the same manner as recommended for the general population [37]. The US guidelines for the evaluation and management of dyslipidemia in HIV-infected adults receiving antiretroviral therapy, recommend that alternative antiretroviral therapy should be considered along with lipid-lowering medication, if life-style interventions are not effective in normalizing the lipid profile [38]. Guidelines recommend metabolic and lipid assessment before beginning antiretroviral therapy, when switching and at regular intervals during stable antiretroviral therapy [38]. Although CV risks are higher in patients with HIV infection [39] and with HAART [28], the overall relative risks of CV events are still small compared with those associated with the classic CV risk factors (male, smoking, obesity, etc.) and compared with the AIDS-related risks associated with not taking effective antiretroviral therapy [40]. Although our findings support the use of antiretroviral therapy known to be less likely to cause metabolic abnormalities for HIV-infected patients with additional CV risk factors, the overriding clinical priority remains antiviral efficacy [40]. The CV risk can be managed effectively by closely controlling the modifiable risk factors with lifestyle and pharmacological interventions.

This study has several limitations:

- the number of patients was relatively small due to the numerous exclusion criteria, including the presence of traditional CV risk factors such as hypertension and hypercholesterolemia;
- data were obtained in a selected HIV population and, therefore, cannot be extrapolated to women, or the overall HIV population, and in particular, those undergoing CV treatments known to potentially affect arterial stiffness;
- PWV measurement using the Complior® device was assumed to be directly proportional to large artery stiffness, itself a surrogate marker of preclinical atherosclerosis – PWV may not be directly proportional to overall clinical CV morbidity and mortality in HIV patients;
- since the study was observational and the correlations were based on a cross-sectional analysis of data from a single time point, no definitive conclusions about causality can be made; the study may have been insufficiently powered to detect all factors affecting the PWV—age relationship.

**Conclusion**

This study shows that, in HIV-infected patients, treatment with PIs significantly promotes aortic stiffness and affects the PWV—age relationship. The impact of PI therapy does, however, appear to be moderate compared with that of age when traditional CV risk factors are controlled for, as in our study. In the general patient population however, the impact of PI therapy could be exacerbated by the concomitant presence of traditional CV risk factors, such as hypertension and diabetes, which need to be particularly tightly controlled in this population.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

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