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Effect of atazanavir versus other protease inhibitor-containing antiretroviral therapy on endothelial function in HIV-infected persons: Randomized controlled trial

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Short title: Atazanavir and endothelial function

Key words: HIV infection, protease inhibitors, atazanavir, endothelial function, flow-mediated vasodilation

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ABSTRACT

Objective: Impaired endothelial function was demonstrated in HIV-infected persons on protease-inhibitor (PI)-containing antiretroviral therapy, probably due to altered lipid metabolism. Atazanavir is a PI causing less atherogenic lipoprotein changes. We studied whether endothelial function improves after switching from other PI to atazanavir.

Design: Randomized, observer-blind, treatment-controlled trial.

Setting: Three university-based outpatient clinics.

Patients: 39 HIV-infected persons with suppressed viral replication on PI-containing regimens and fasting LDL-cholesterol >3mmol/L.

Intervention: Patients were randomized either to continue the current PI or change to unboosted atazanavir.

Main outcome measures: Endpoints at week 24 were endothelial function assessed by flow-mediated vasodilation (FMD) of the brachial artery, lipid profiles, high sensitive C-reactive protein, malondyaldehyde, total antioxidative capacity and oxidized LDL.

Results: Baseline characteristics and mean FMD values of the two treatment groups were comparable (3.9±1.8% on atazanavir vs. 4.0±1.5% in controls). After 24 weeks’ treatment, FMD decreased to 3.3±1.4% and 3.4±1.7%, respectively (all p=n.s.). Total cholesterol improved in both groups (p=<0.0001 and p=0.01, respectively) but changes were more pronounced on atazanavir (p=0.05, changes between groups). HDL and triglyceride levels improved on atazanavir (p=0.03 and p=0.003, respectively) but not in the control group. Serum inflammatory and oxidative stress parameters did not change; oxidized LDL improved significantly in the atazanavir group.

Conclusions: The switch from another PI to atazanavir among treatment experienced patients did not result in improvement of endothelial function despite significantly improved serum lipids. Not only atherogenic lipid profiles but also direct effects of reverse transcriptase inhibitor plus PI-containing combination on the endothelium may affect vascular function.

ClinicalTrials.gov Identifier:  NCT00447070
INTRODUCTION

Morbidity and mortality of HIV-infected persons with access to combination antiretroviral therapy (cART) have dramatically improved. However, there is a major concern that cART is associated with premature manifestation of coronary artery disease (CAD).\(^1,2\) Protease inhibitors (PI) are among the key components of cART and several studies propose a direct association between the use of PI and an increased risk of CAD,\(^3-6\) although this relation has been questioned. Combination ART, particularly PI-containing regimens, is associated with hyperlipidemia, hyperglycemia, insulin resistance, central obesity, and other metabolic factors known to promote vascular disease and premature CAD.\(^7\)

Endothelial function, a powerful surrogate marker of early atherosclerosis,\(^8\) has been found to be reduced in treated HIV-infected persons,\(^9\) even in young children,\(^10\) when compared with uninfected individuals. Recent reports have demonstrated impaired endothelial function especially in HIV-infected persons on PI, probably due to an altered lipid metabolism.\(^10,11\) In contrast, improvement of endothelial function was measured in HIV-infected persons on PI-containing cART who started lipid lowering therapy with statins although this could also be attributed to their pleiotropic effect.\(^12\) In comparison with other PI, atazanavir is a PI causing less atherogenic lipoprotein changes.\(^13\) Whether this feature of atazanavir leads to preserved endothelial function and subsequently to a lower incidence of cardiovascular complications, however, is unclear at present because long-term observations are missing.

Hence, this randomized controlled multi-centre study aimed to prospectively evaluate endothelial function, serum lipid profiles and serum inflammatory and oxidative stress surrogate parameters over a period of 24 weeks in persons on PI-containing cART and suppressed viral replication who either continued the current PI or switched to atazanavir-containing combination therapy.

METHODS

Patient characteristics, inclusion and exclusion criteria

Eligible were male and female HIV-infected persons between 18 and 65 years old on stable combination antiretroviral treatment with 2 nucleoside reverse transcription inhibitors (NRTI) and one PI (other than atazanavir) for at least 12 weeks, with two consecutive viral load assessments below 50 copies/mL within 60 days prior to study entry, CD4 lymphocyte counts above 100 cells/\(\mu\)L, a treatment history and results of prior resistance testing allowing replacement of the current PI by unboosted atazanavir, and LDL cholesterol above 3.0 mmol/L.

Exclusion criteria were known coronary artery disease, hypertension, peripheral artery disease, cerebrovascular disease, and diabetes mellitus. Furthermore, patients with serious illness requiring systemic treatment and/or hospitalization within 14 days prior to study entry, current drug or alcohol addiction or patients participating in other studies were excluded, as well as patients with previous virologic failure on PI containing regimens and previously documented protease resistance mutations. The use of non-nucleoside reverse transcriptase inhibitors,
testosterone or anabolic steroids, systemic glucocorticoids, long-acting inhaled steroids or other immunomodulators at study entry or during the study led to exclusion, as did the use of any lipid-lowering drugs within 4 weeks prior to study entry. Pregnancy was ruled out by pregnancy test.

**Study protocol**

A total of 41 patients were enrolled in this observer-blinded, randomized controlled multi-centre study using a parallel group design between 4. August 2004 and 24. October 2005. During the screening period, two assessments of endothelial function by flow mediated dilation (FMD) were performed (at least one week apart), and the mean of the 2 assessments was the baseline value. Because FMD is dependent on many factors including gender, smoking, alcohol consumption, degree and duration of hypertension or dyslipidemia, the participants were stratified by the FMD-operator after the two baseline measurements into two groups: (1) persons with mean FMD below 2.0%, and (2) persons with mean FMD equal or above 2.0%. The allocation schedule for the two treatment arms, including the two different strata with randomly permuted block sizes of 2 and 4, was generated in advance with the program RandList Version 1.0.0.107 (DatIns GmbH Tübingen, Germany), and two separate series of sealed envelopes were prepared for the two FMD strata.

Participants were then randomized by the study nurse of the HIV outpatient clinic to receive either 400 mg open-label unboosted atazanavir daily, instead of the current PI, or to continue the currently used PI. Both groups maintained the two reverse transcriptase inhibitors unaltered. Clinical visits were performed after 4, 12 and 24 weeks. At weeks 12 and 24 measurement of flow-mediated vasodilation was repeated. Patient’s compliance to treatment and procedures were assessed at each visit. Returned study medications were counted to determine whether they were consistent with the number prescribed.

Screening and study visits were performed at specialized HIV outpatient clinics of 3 tertiary care University Hospitals in Switzerland (Zurich, Bern and Lausanne). The local ethics committees of these University Hospitals approved the study protocol and all procedures were in accordance with institutional guidelines and the Declaration of Helsinki. Written informed consent was obtained from all study participants.

**Assessment of endothelial function**

Assessments were performed in dedicated cardiovascular ultrasound laboratories of the University hospitals, maintaining a standardized protocol. Physicians of these centers and the Flow-mediated dilation (FMD) operator remained blinded throughout the study and data analysis. FMD examinations were performed in a temperature controlled, quiet room in the morning. Patients were examined in the fasting state and were asked to quit smoking at least 2 hours before examination and to refrain from coffee, tea, fruits and chocolate for at least 24 hours. Flow-mediated dilation (FMD) and glycerol-trinitrate (GTN)-induced vasodilation (0.4 mg sublingual, Nitrolingual Spray, Pohl-Boskamp, Germany) of the brachial artery were assessed by a high-resolution ultrasound vessel wall tracking device with a 10-MHz linear array transducer according to guidelines. Flow-mediated reactive hyperemia reflects endogenous nitric oxide (NO) formation, resulting in endothelium-dependent vasodilation, while GTN acts as an exogenous NO donor.
directly on vascular smooth muscle cells inducing endothelium-independent vasodilatation. FMD of the brachial artery was induced by release of a wrist cuff inflated to 220mmHg pressure for 5 minutes. After release, we recorded the arterial diameter every 15 seconds for 2 minutes. After GTN application, we recorded the diameter every 30 seconds for 6 minutes.

**Serum inflammation and oxidative stress parameters**

Malondialdehyde was derivatized and measured on a HPLC system with a fluorescence detector with a limit of detection of 0.01 µmol/l and a coefficient of variation of 3% (Chromsystems, Munich, Germany). Highly sensitive C-reactive protein (hsCRP) was measured on an Immulite 2500 immunoanalyzer using commercial assays with a limit of detection of 0.1 mg/l and a coefficient of variation of 6% (DPC, Los Angeles, CA, USA). The oxidized LDL was measured by ELISA with a limit of detection of 1 mU/l and a coefficient of variation of 8% (Mercodia, Uppsala, Sweden) and the total antioxidative capacity (TAOC) was measured using Trolox as a standard with a limit of detection of 0.04 mmol/l and a coefficient of variation of 3.4% (Cayman Chemicals, Ann Arbor, MI, USA).

**Statistical analysis**

The authors had full access to the data and take responsibility for its integrity. Statisticians remained blinded throughout the predefined primary data analysis. For comparison of baseline characteristics between treatment groups, t-tests or Fisher's exact tests were used. Primary endpoints were changes of endothelial function at the end of the 24-week study period comparing the two treatment arms using t-tests and individual changes using paired t-tests. The sample size of 38 participants was calculated to detect a 20% difference in the change of FMD (power of 80%; \( \alpha = 0.05 \)). The following secondary endpoints were analyzed: changes of lipid profiles, serum inflammatory and oxidative stress parameters. Repeating the analyses with non-parametric methods yielded comparable results and therefore we only report results from two-sided parametric tests. We used Stata 9.2 (Stata Corp, College Station, Tx).

**RESULTS**

**Study participants**

Two of 41 enrolled patients dropped out before randomization. We therefore report results on 39 participants (20 in the atazanavir arm and 19 in the continued PI arm). Baseline characteristics including PI treatment at enrollment are presented in Table 1. Except for the baseline serum triglycerides, which were significantly higher in the atazanavir group, both treatment arms were equally balanced. Mean duration of HIV-seropositivity as well as duration of antiretroviral and PI therapy tended to be slightly longer in the atazanavir group (n.s.). HIV surrogate markers did not differ between the two treatment arms.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Atazanavir (n=20)</th>
<th>Continued protease inhibitor (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, no. (%)</td>
<td>15 (75 %)</td>
<td>15 (79%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean age [years] (± SD)</td>
<td>46 (±7.5)</td>
<td>47 (±12.5)</td>
<td>0.80</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>9 (45%)</td>
<td>7 (37%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Mean body-mass-index [kg/m²] (± SD)</td>
<td>23 (±3.0)</td>
<td>24 (±2.3)</td>
<td>0.37</td>
</tr>
<tr>
<td>Mean (± SD) duration of HIV-seropositivity [years]</td>
<td>10 (±5.5)</td>
<td>7.6 (±4.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean (± SD) duration of antiretroviral therapy [years]</td>
<td>7.7 (±3.7)</td>
<td>5.7 (±3.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>Protease inhibitors at time of randomization*</td>
<td></td>
<td>0.426</td>
<td></td>
</tr>
<tr>
<td>Lopinavir/Ritonavir</td>
<td>5 (25.0)</td>
<td>3 (15.8)</td>
<td></td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>13 (65.0)</td>
<td>16 (84.2)</td>
<td></td>
</tr>
<tr>
<td>Ritonavir (therapeutic dose)</td>
<td>1 (5.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Indinavir/Ritonavir</td>
<td>1 (5.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Clinical HIV stage CDC C, no. (%)</td>
<td>5 (25 %)</td>
<td>5 (26 %)</td>
<td>0.45</td>
</tr>
<tr>
<td>Mean (± SD) systolic blood pressure [mmHg]</td>
<td>125 (±13)</td>
<td>125 (±17)</td>
<td>0.96</td>
</tr>
<tr>
<td>Mean (± SD) diastolic blood pressure [mmHg]</td>
<td>77 (±8.8)</td>
<td>77 (±9.0)</td>
<td>0.91</td>
</tr>
<tr>
<td>Mean (± SD) pulse (1/min)</td>
<td>75 (±7.4)</td>
<td>74 (±9.5)</td>
<td>0.64</td>
</tr>
<tr>
<td>No. (%) with HIV-1 RNA &lt;50 copies/mL</td>
<td>20 (100%)</td>
<td>19 (100%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean (± SD) CD4 count [cells/µL]</td>
<td>539 (±250)</td>
<td>520 (±324)</td>
<td>0.84</td>
</tr>
<tr>
<td>Mean (± SD) total cholesterol [mmol/L]</td>
<td>6.5 (±0.95)</td>
<td>6.5 (±1.1)</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean (± SD) LDL [mmol/L]</td>
<td>4.0 (±0.96)</td>
<td>4.2 (±0.9)</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean (± SD) HDL [mmol/L]</td>
<td>1.2 (±0.4)</td>
<td>1.2 (±0.3)</td>
<td>0.85</td>
</tr>
<tr>
<td>Mean (± SD) triglycerides [mmol/L]</td>
<td>3.2 (±1.6)</td>
<td>2.3 (±1.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean (± SD) fasting glucose [mmol/L]</td>
<td>5.1 (±0.6)</td>
<td>5.0 (±0.5)</td>
<td>0.57</td>
</tr>
<tr>
<td>Mean (± SD) insulin [mIU/L]]</td>
<td>16.5 (±18)</td>
<td>10.5 (±8.4)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Footnote of table 1
* The reverse transcriptase inhibitor backbone in the atazanavir and continued protease inhibitor groups, respectively, were statistically not different: zidovudine plus lamivudine, 14 and 13 patients; abacavir plus lamivudine, 0 and 4 patients; stavudine plus lamivudine, 2 and 1 patients; didanosine plus lamivudine, 1 and 1 patient; lamivudine plus tenofovir, 1 and 0 patients; zidovudine plus tenofovir, 1 and 0 patients; didanosine plus stavudine, 1 and 0 patients, respectively.
Course of treatment
Of the 39 patients, 38 (97%) completed the 24 week trial. One patient in the atazanavir arm stopped the assigned treatment after 2 months. There were no serious adverse events or new AIDS-defining illnesses throughout the study. However, 4 (21%) patients in the continued PI arm experienced virological failure versus none in the atazanavir arm.

Endothelial function
Baseline brachial artery diameter matched well at baseline and did not significantly change during the 24-week study period (from 4.60±0.78 mm to 4.56±0.76 mm, p=0.90, in the atazanavir group, and from 4.68±0.61 mm to 4.74±0.61 mm, p=0.78, in the continued PI group). Flow-mediated vasodilation did not change after the switch to atazanavir or during continued PI therapy (from 4.0±1.5% to 3.4±1.7%, p=0.40, and from 3.9±1.8 to 3.3±1.4%, p=0.37, respectively; Figure 1). Atazanavir and continued PI arms combined showed FMD changes from 3.9±1.6% to 3.4±1.5% (p=0.22) during the study period. Also, nitroglycerine-mediated vasodilation and blood flow did not change during the study (all p=n.s.).

Serum lipid levels and liver enzymes
During the study period, serum lipid levels improved both in the atazanavir and continued PI arm (Table 2, Figure 2). In the atazanavir group, however, the improvement was more pronounced. Total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were significantly improved after 24 weeks of atazanavir treatment, whereas triglycerides and HDL did not change in the continued PI arm. Liver enzymes were slightly but statistically significantly higher after the 24 weeks of atazanavir treatment compared with the baseline values, whereas the liver enzymes remained stable on continued PI therapy.
### Table 2. Comparison of the two treatment groups at baseline and at 24 weeks

<table>
<thead>
<tr>
<th></th>
<th>Atazanavir arm</th>
<th>Continued protease inhibitor arm</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n=20)</td>
<td>W24 (n=19)§</td>
<td>p* Baseline (n=19)</td>
<td>W24 (n=19)</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>125 (±13)</td>
<td>121 (±13)</td>
<td>0.08</td>
<td>126 (±17)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>77 (±9)</td>
<td>77 (±9)</td>
<td>0.94</td>
<td>77 (±9)</td>
</tr>
<tr>
<td><strong>Pulse (1/min)</strong></td>
<td>75 (±7)</td>
<td>72 (±7)</td>
<td>0.19</td>
<td>74 (±10)</td>
</tr>
<tr>
<td><strong>No. (%) with HIV-1 RNA &lt;50 copies/mL</strong></td>
<td>20 (100%)</td>
<td>19 (100%)</td>
<td>0.15</td>
<td>19 (100%)</td>
</tr>
<tr>
<td><strong>HIV-1 RNA (log10 copies/mL)</strong></td>
<td>0.03 (±0.15)</td>
<td>0.17 (±0.4)</td>
<td>0.15</td>
<td>0.32 (±0.6)</td>
</tr>
<tr>
<td><strong>CD4 lymphocyte count (cells/µL)</strong></td>
<td>539 (±250)</td>
<td>543 (±247)</td>
<td>0.14</td>
<td>520 (±324)</td>
</tr>
<tr>
<td><strong>ALAT (U/L)</strong></td>
<td>31.7 (±14.6)</td>
<td>40.4 (±17.4)</td>
<td>0.002</td>
<td>28.4 (±14.7)</td>
</tr>
<tr>
<td><strong>ASAT (U/L)</strong></td>
<td>31.5 (±12.1)</td>
<td>37.4 (±20.1)</td>
<td>0.02</td>
<td>29.2 (±12.5)</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>6.5 (±0.95)</td>
<td>5.5 (±1.2)</td>
<td>&lt;0.001</td>
<td>6.5 (±1.1)</td>
</tr>
<tr>
<td><strong>LDL (mmol/L)</strong></td>
<td>4.0 (±0.96)</td>
<td>3.3 (±1.2)</td>
<td>&lt;0.001</td>
<td>4.2 (±0.9)</td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
<td>1.2 (±0.4)</td>
<td>1.3 (±0.4)</td>
<td>0.03</td>
<td>1.2 (±0.3)</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>3.2 (±1.6)</td>
<td>2.0 (±1.6)</td>
<td>0.003</td>
<td>2.3 (±1.0)</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td>5.1 (±0.6)</td>
<td>5.2 (±0.5)</td>
<td>0.28</td>
<td>5.0 (±0.45)</td>
</tr>
<tr>
<td><strong>Insulin (mIU/L)</strong></td>
<td>16.5 (±18)</td>
<td>12.3 (±13.6)</td>
<td>0.35</td>
<td>10.5 (±8.4)</td>
</tr>
<tr>
<td><strong>hsCRP (mg/L)</strong></td>
<td>2.0 (±1.8)</td>
<td>2.5 (±3.3)</td>
<td>0.58</td>
<td>1.5 (±1.3)</td>
</tr>
<tr>
<td><strong>oxLDL (U/L)</strong></td>
<td>68.9 (±15.6)</td>
<td>55.1 (±14.6)</td>
<td>&lt;0.001</td>
<td>77.7 (±16.1)</td>
</tr>
<tr>
<td><strong>MDA (µmol/L)</strong></td>
<td>0.12 (±0.04)</td>
<td>0.12 (±0.02)</td>
<td>0.85</td>
<td>0.13 (±0.05)</td>
</tr>
<tr>
<td><strong>TAOC (mM)</strong></td>
<td>2.7 (±0.65)</td>
<td>2.7 (±0.67)</td>
<td>0.82</td>
<td>2.6 (±0.64)</td>
</tr>
</tbody>
</table>

**Footnotes of table 2:**
Data are presented as mean ± SD. SBP denotes systolic blood pressure, DBP=diastolic blood pressure, ALAT=Alanine-Amino Transferase, ASAT=Aspartat-Aminotransferase, LDL=low density lipoprotein, HDL=high density lipoprotein, hsCRP=high sensitivity C-reactive protein, oxLDL=oxidised LDL, MDA=malondialdehyde, TAOC=total antioxidative capacity

* p values within groups from a paired=matched t-test
† p-values between groups from two-sided t-tests: intergroup groups at week 24;
‡ p-values between groups from two-sided t-tests: intragroup (changes from baseline to week 24)
§ no data at week 24 for one patient
|| values below limit of detection are coded as 1 copy/mL (i.e. log10(1)=0)
Serum inflammation and oxidative stress parameters
The inflammation parameter high sensitivity C-reactive protein (hsCRP) as well as the oxidative stress parameters malondialdehyde (MDA) and total antioxidative capacity (TAOC) did not change significantly during the course of the study (Table 2). Oxidized LDL changed significantly in the atazanavir group; however, the changes were in parallel with LDL, therefore no conclusion concerning oxidative stress can be reached.

DISCUSSION
In this randomized, controlled study we found that a switch from another protease inhibitor to an unboosted atazanavir-containing combination antiretroviral therapy did not result in an improvement of endothelial function after 24 weeks despite a significantly improved lipid profile.

This result is intriguing as dyslipidemia is associated with endothelial dysfunction and, vice versa, improvement of blood lipids ameliorates endothelial vascular function, as previously demonstrated in HIV-infected persons on PI who received pravastatin treatment. Therefore, an improvement of endothelial function after atazanavir therapy was expected. However, because serum lipids not only improved in the atazanavir arm, as expected, but also - less profound - in the control group (although the addition of lipid lowering drugs was not allowed during the 24 week study period), the course of lipid levels might have influenced the results of endothelial function in favour of the control group. In previous studies, treatment with PI was associated with impairment in serum lipid profiles as well as impaired endothelial function, however, such effect has been seen after initiating the drug. In contrast, our patients already were on stable PI treatment for months or years before they were enrolled in this study. The improvement of serum lipids during the study could be explained by the adherence of patients and physicians to treatment guidelines which recommend changes of life-style and behaviour in order to reduce cardiovascular risks. The fact that at the end of the study systolic blood pressure tended to be lower in both groups and diastolic blood pressure and heart rate were significantly lower in the control group after 24 weeks as compared to baseline is suggestive for such a healthier life-style. However, lower blood pressure is also associated with an ameliorated endothelial function, an effect not seen in our study.

The lacking improvement of vascular function after replacement of another PI with atazanavir may also be explained by a direct effect of the NRTI plus PI-containing antiretroviral combination treatment on the endothelium. Based on in vitro experiments as well as clinical investigations in HIV-infected patients and in persons not infected with HIV, toxic effects and other direct effects on the endothelium have been postulated, including a decrease of endothelium-dependent vasorelaxation, inhibition of the nitric oxide synthase system, increase of oxidative stress, and activation of mitogen-activated protein kinases. Direct effects on the endothelium, as for example increased oxidative stress or an inflammatory response of the vessel wall, were mainly associated with PI. Nevertheless, results of animal models indicate that also reverse transcriptase inhibitors can directly
affect the vascular endothelium.\textsuperscript{21} As a consequence, not only PI inhibitors but rather the combination therapy with reverse transcriptase inhibitors plus PI could counterbalance the expected beneficial effect of an improved lipid profile associated with, e.g., atazanavir, or other single drugs that do not influence lipid profiles. The participants of the trial presented here were indeed already on long-term antiretroviral therapy before enrolment, namely for a mean of 6.7 years on any antiretroviral drugs, and a mean of 5.6 years on combination drug regimens, which may have affected vascular endothelial function. And, although patients were randomized, the mean duration of previous antiretroviral therapy among the atazanavir group was 2 years longer than in the continuing PI group.

The results of flow-mediated dilation in our study participants indicated endothelial dysfunction as compared to in healthy persons not infected with HIV, but, we do not have any indices of increased inflammation or oxidative stress at baseline or after 24 weeks of treatment. The surrogate serum biomarkers of inflammation and oxidative stress, including hsCRP, malondyaldehyde, oxidized LDL, or total antioxidative capacity were not significantly influenced by atazanavir or continued PI, although earlier reports suggested increasing oxidative stress due to PI therapy.\textsuperscript{22}

Antiretroviral drugs can cause drug-induced liver injury.\textsuperscript{23,24} In our study, liver enzymes increased mildly but significantly after switching to atazanavir treatment, possibly indicating some toxic effect of this drug, which might also affect the endothelium. A recent study in patients with diabetes mellitus type 2 showed an inverse correlation between liver enzymes and flow-mediated dilation.\textsuperscript{25} Moreover, toxic effects to the liver might lead to an inflammatory response, not only affecting liver enzymes but also leading to complex inflammatory responses possibly affecting endothelial function. However, we did not observe hsCRP serum concentrations to increase after atazanavir treatment.

The strengths of our study include the randomization of persons with suppressed viral replication, and the stratification based on baseline flow-mediated dilation, which may control for the various cardiovascular risk factors in the different treatment arms. Possible limitations include the sample size and the probably too short duration of the study. Despite the fact that randomization balanced the two groups quite well, HIV-infected persons have heterogeneous co-morbidities, co-medication, different lifestyles including heterogeneous cardiovascular risk behaviour and a high prevalence of smoking, all factors known to influence endothelial function. Therefore, such confounding factors may have influenced our study endpoints. Furthermore, although unlikely, the higher baseline triglyceride levels might have contributed to a false low endothelial function in the atazanavir group, thus confounding our results.

In conclusion, we found marked endothelial dysfunction - a powerful surrogate for atherosclerosis - among our study participants who were on antiretroviral therapy for a mean of 6.7 years. We now demonstrate that switching PI therapy to atazanavir, a new PI not negatively altering lipid-profiles and therefore considered to be less atherogenic, has no beneficial impact on the vascular endothelium, thus probably not solving the important problem of vascular dysfunction in these patients. However, the clearly demonstrated positive effect on the lipid-profile may, in the
long term, still be beneficial in respect to cardiovascular morbidity and mortality. Therefore, further long-term observations are needed to determine whether the incidence of clinical endpoints of cardiovascular diseases will decrease among persons on atazanavir-containing antiretroviral regimens. Changing current (effective) PI therapy to atazanavir merely in the hope for reduced cardiovascular risk may not be appropriate at the present time as definitive data is lacking. It is, however, important to take other preventive measures and to strictly treat cardiovascular risk factors in this patient group.

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Conflict of Interest
Rainer Weber has received travel grants or speakers honoraria from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dome, Pfizer, Roche, TRB Chemedica and Tibotec. Bruno Ledergerber has received travel grants or honoraria from Abbott, Aventis, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Merck Sharp & Dome, Roche and Tibotec. Thomas Lüscher and Kurt Quitzau have received grants for the conduct of clinical studies from Bristol-Myers Squibb.

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Figure legends:

Figure 1.  
**Vascular function.** *Panel A:* Endothelial function measurement. Flow mediated dilation (FMD) did not change after atazanavir as well as after continued protease inhibitor-containing therapy (from 4.0±1.5% to 3.4±1.7%, p=0.4 and from 3.9±1.8 to 3.3±1.4%, p=0.37, respectively). *Panel B:* Similarly, endothelial-independent, glycerol-trinitrate (GTN) [nitroglycerine] mediated vasodilation did not change during the study (from 11.7±5.1% to 10.8±4.6%, p=0.64 and from 11.7±5.3 to 10.6±3.8, p=0.45)

Figure 2.  
**Serum lipids.** *Panel A:* Total Cholesterol (tot Chol) decreased from 6.5±0.95 to 5.5±1.2 mmol/l, p<0.0001, in the atazanavir group, and from 6.5±1.1 to 5.8±0.8 mmol/l, p=0.007, in the control group. The effect of atazanavir on total cholesterol was significantly more pronounced than the control PI (p=0.48). *Panel B:* Low density lipoprotein (LDL) decreased from 4.0±1.0 to 3.3±1.2 mmol/l, p<0.001, in the atazanavir group and from 4.2±0.9 to 3.8±0.7 mmol/l, p=0.02, in the control group. *Panel C:* High density lipoprotein (HDL) increased from 1.2±0.4 to 1.3±0.4 mmol/l, p=0.03, in the atazanavir group, whereas HDL remained unchanged in the control group (1.2±0.3 and 1.2±0.3 mmol/l). *Panel D:* Triglycerides were significantly lowered in the atazanavir group (from 3.2±1.6 to 2.0±1.6 mmol/l, p=0.003), however remained stable in the control group (from 2.3±1.0 to 2.0±1.0 mmol/l).
REFERENCES


