Metabolic complications of antiretroviral therapy
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Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile

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Introduction

The use of highly active anti-retroviral therapy (HAART) including protease inhibitors (PIs) for HIV-1 infection is often associated with increases in total and low density lipoprotein-cholesterol (LDL-c) plasma levels, as well as marked elevations of triglycerides (TG)[1-3], whereas high density lipoprotein-cholesterol (HDL-c) levels remain unchanged.[4] This constellation of lipid abnormalities has led to concern about patients being at increased risk of developing atherosclerosis.[5] A recent cross-sectional study indeed revealed a higher than expected prevalence of atherosclerotic lesions in the carotid arteries in PI-treated patients.[6] Numerous anecdotal reports have suggested that patients treated with PIs are at increased risk of developing vascular disease.[7-10]

Many epidemiological studies have indicated that lipoprotein metabolism and in particular plasma levels of high-density lipoprotein (HDL) are strongly and independently inversely correlated with the risk of CAD[11,12]. In fact, in many of these studies the level of HDL-c and the ratio of total cholesterol over HDL-c (TC/ HDL-c ratio) have been the most powerful predictors of future coronary events.

This inverse relationship between the level of HDL-c and the risk of developing premature CAD has been a consistent finding in a large number of prospective studies, such as the Framingham Heart Study[13,14], the PROCAM Study [15], the Helsinki Heart Study[16] and the Lipid Research Clinics Prevalence Mortality Follow-up Study[17]. Overall, it has been concluded from these studies that for every 0.025 mmol/l increase in HDL-c, the risk for CAD is reduced by 2-5%.

In contrast with what has been observed with PI-containing regimens, increases in HDL-c have been observed in HIV-1-infected patients who have received non nucleoside reverse transcriptor inhibitor (NNRTI)-
based therapy. In one study of antiretroviral-naive HIV-1 infected patients, significant increases in HDL-c were reported in those treated with nevirapine (NVP), a NNRTI, but not in those treated with the PI nelfinavir [18].

We performed an extensive assessment of plasma lipids and lipoproteins in a representative subset of patients enrolled in the Atlantic Study, in which previously untreated HIV-1-infected patients received the nucleoside analogue reverse transcriptase inhibitors (NRTI) stavudine (d4T) and didanosine (ddI), and were randomised to the addition of either the PI indinavir (IDV), the NNRTI NVP, or a third NRTI lamivudine (3TC).
Methods

Trial design

The Atlantic Study is an ongoing, open-label randomised comparative study of three triple antiretroviral regimens for the treatment of HIV-1 infection. The primary study objective is to assess the comparability of the three regimens in reducing the serum HIV-1 RNA load to undetectable levels. The three regimens being studied contain d4T and ddI in all treatment arms as the NRTI backbone, with the addition of either NVP, 3TC or IDV. The Atlantic Study enrolled a total of 298 subjects with asymptomatic HIV-1 infection (Centers for Disease Control and Prevention (CDC) stage A), who were antiretroviral drug naive, with plasma HIV-1 RNA >500 copies/ml and a CD4+ cell count >200x10^6/L. Standard dosing schedules were used, except for NVP and ddl, which were dosed once daily. The results from this main study have been presented elsewhere[19].

Patients were included in the current substudy if they had remained on randomised treatment for at least 24 weeks, and if at least two aliquots of cryopreserved, previously unthawed EDTA plasma samples were available at baseline, as well as at week 6 and 24 of treatment. All samples had been prospectively collected as part of the main trial protocol and had been shipped to a central laboratory for cryopreservation at -70°C. Fasting was not mandated for blood draws.

Lipid and lipoprotein determinations

Concentrations of plasma total cholesterol, HDL cholesterol, and triglycerides were measured in a single laboratory using enzymatic assays. LDL-cholesterol concentrations were calculated by the Friedewald formula. Lipoprotein subclass levels were measured on freshly thawed cryopreserved plasma specimens using a dedicated 400 MHz proton NMR analyser at LipoMed, Inc. (Raleigh, NC). Spectra of
each specimen (0.25 ml) were acquired in duplicate at 47°C and the lipid signal envelope at 0.8 ppm deconvoluted to give the amplitudes of the contributing signals from 16 lipoprotein subclasses[20].

Concentrations of apolipoprotein AI (apoAI), apolipoprotein AII (apoAII), apolipoprotein B (apoB), Lipoprotein (a) (Lp(a)) were measured by nephelometry. Lipoprotein AI (LpAI) concentrations and the lipoproteinAI: lipoproteinAII ratio (LpAI:AII) were measured by immuno-electrophoresis (Sebia hydragel LpAI). Cholesterol ester transfer protein (CETP) mass was measured using an enzyme-linked immunosorbent assay (ELISA) using the CETP monoclonal antibody TP2 (produced by Dr. R. Milne, University of Ottawa Heart Institute, Ottawa).

**Statistical analysis**

The subset of patients selected was compared to the remaining Atlantic Study patients to assess representativeness. Potential differences in randomisation arm, gender, mode of HIV-1 transmission and CDC classification, were examined between the two groups using a chi-square test. Baseline age, CD4+ cell count, and log_{10} HIV-1 RNA level were compared between the two groups using t-tests.

To test for possible differences in baseline values for the lipid parameters, an ANOVA procedure was carried out.

To examine the change from baseline to week 24 in the lipid parameters, both the absolute change in concentration, as well as the percentage change in concentration from baseline were calculated for each patient. Both were used as outcome variable in a linear regression model with treatment arm as the explanatory variable. The model using the absolute change in concentration as the outcome variable was adjusted for baseline concentration by including the baseline value as additional explanatory variable. A t-test statistic was calculated to test
if the percent change in concentration from baseline was statistically significantly different from zero in each of the treatment arms, while an ANOVA procedure was used to test if the percentage change in concentration differed between the arms. When the F-statistic was significant (p<0.05), a pairwise comparison between the different treatment arms was performed. These pairwise comparisons were judged to be statistically significant only if the p-value was less than 0.01.

To adjust for the possible effect of treatment induced immune restoration or decreased virus replication on lipid parameters, a multivariate linear regression model was used with the proportional change in their concentration from baseline as the outcome variable. Explanatory variables were the three treatment arms (with indinavir as the reference group) and one of the following: CD4+ cell count at baseline, log_{10} HIV-1 RNA copies at baseline, increase in CD4+ cell count or decrease in log_{10} HIV-1 RNA copies after 24 weeks.

The possibility of the occurrence of a type I error was decreased by testing on only one predefined timepoint (24 weeks). Furthermore, pairwise comparisons were only carried out when the alpha-level was below a predefined value, while during these comparisons the alpha level at which statistical significance was assumed, was adjusted downwards. The possibility of a type II error and, related to this, the power of the analyses can not be assessed due to the exploratory nature of the analysis, and the absence of information from previous research addressing the same topic.
Results

Patient selection, baseline characteristics, and treatment response (Table 1)

Of the 298 patients randomised into the Atlantic study, 114 patients (38%) fulfilled the inclusion criteria for the current study. At baseline, subgroup patients did not differ significantly between each of the three treatment arms. For the remaining 184 (62%) patients insufficient samples were available and/or treatment had been changed prior to week 24. Except for having a somewhat lower body mass index, at baseline, the subgroup of patients did not differ significantly from the remaining Atlantic Study population with respect to randomisation arm, gender, mode of HIV-1 transmission, CDC classification, baseline CD4 cell count and HIV-1 RNA levels at baseline. The HIV-1 RNA and CD4 cell responses over 24 weeks of treatment in each of the arms in the subgroup did not differ significantly from those observed over the same period in the entire study population, using an as-treated analysis (data not shown). The proportion of patients in our subgroup with an HIV-1 RNA level below 50 copies/mL at week 24 did not differ between the three arms (82.5 ± 11.8%, 87.9 ± 11.1%, 71.1 ± 14.4% in the IDV, 3TC and NVP-arm, respectively). Likewise, the mean CD4 cell count increase (± SD) after 24 weeks of treatment did not differ significantly between the three treatment (+124 ± 137 cells/mm³, +143 ± 147 cells/mm³ and +124 ± 194 cells/mm³ in the IDV, 3TC and NVP-arm, respectively). The final week 48 virological and immunological responses of the Atlantic main study have been presented elsewhere. [19].
### Table 1 Baseline characteristics of patients selected for lipid profiling compared to remaining patients from the Atlantic Study and treatment responses of the patients selected

<table>
<thead>
<tr>
<th></th>
<th>indinavir (n=41)</th>
<th>nevirapine (n=34)</th>
<th>lamivudine (n=39)</th>
<th>total selected for subsample (n=114)</th>
<th>not selected for subsample (n=184)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, %</td>
<td>83</td>
<td>88</td>
<td>79</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td>Age, years mean (SD)</td>
<td>35.6 (7.1)</td>
<td>39.7 (10.3)</td>
<td>36.1 (8.1)</td>
<td>37.0 (8.6)</td>
<td>35.4 (8.0)</td>
</tr>
<tr>
<td>BMI, kg/m² mean (SD)</td>
<td>25.4 (5.6)</td>
<td>26.2 (4.1)</td>
<td>25.7 (4.5)</td>
<td>25.7 (4.8)</td>
<td>23.8 (3.8) *</td>
</tr>
<tr>
<td>Risk factors for infection, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Homo- or bisexual</td>
<td>49</td>
<td>56</td>
<td>59</td>
<td>54</td>
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</tr>
<tr>
<td>Heterosexual</td>
<td>29</td>
<td>35</td>
<td>26</td>
<td>30</td>
<td>21</td>
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<td>Injection drug use</td>
<td>10</td>
<td>9</td>
<td>15</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Other</td>
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<td>0</td>
<td>4</td>
<td>5</td>
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<td>Unknown</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>A</td>
<td>98</td>
<td>94</td>
<td>90</td>
<td>94</td>
<td>91</td>
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<tr>
<td>B</td>
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<td>3</td>
<td>10</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CD4+ counts, cells/ml median (IQR)</td>
<td>423 (328 - 511)</td>
<td>408 (332 - 540)</td>
<td>396 (306 - 473)</td>
<td>407 (323 - 506)</td>
<td>400 (317 - 542)</td>
</tr>
<tr>
<td>HIV-1 RNA, log10 copies/ml median (IQR)</td>
<td>4.33 (3.90 - 4.65)</td>
<td>4.27 (3.93 - 4.83)</td>
<td>4.22 (3.85 - 4.71)</td>
<td>4.28 (3.88 - 4.75)</td>
<td>4.23 (3.68 - 4.76)</td>
</tr>
</tbody>
</table>

* p < 0.001 versus patients selected. CDC: Centers for Disease Control and Prevention; IQR: interquartile range.
HDL and HDL-related proteins (Table 2 and Figure 1)

None of the HDL-related parameters differed at baseline between the three treatment arms. The HDL-c level increased significantly from week 0 to 24 by 49% (+0.44 ± 0.05 mmol/l, p < 0.001) in the NVP arm, as opposed to only 16% (+0.10 ± 0.05 mmol/l, p = 0.010) in both the 3TC arm and the indinavir arm (+0.11 ± 0.05 mmol/l, p = 0.010). The HDL-c rise in the NVP arm was significantly greater than in either of the other two treatment arms (p < 0.001). ApoAI concentrations increased significantly in the NVP and 3TC arms (19% or +208.3 ± 28.7 mg/L; p<0.001, and 7% or +61.2 ± 26.8 mg/L; p = 0.007, respectively), but not in the IDV arm. The rise in apoAI however was significantly greater in the NVP arm than in the 3TC arm (p < 0.01).

Figure 1 Percentage change (from baseline) in levels of lipid subclasses, weeks 0-24. Bars represent standard errors.
LpAI concentrations increased significantly by 38% (+0.12 ± 0.02 g/L, p<0.001) in the NVP arm. LpAI also increased significantly in the 3TC and IDV arm, by 18% (+ 0.03 ± 0.02 g/l; p=0.007) and 17% (0.03 ± 0.02; p= 0.009), respectively. HDL particle size as measured by NMR increased significantly by 3% in the NVP arm (+ 0.35 ± 0.08, p < 0.001) while no significant changes were observed in the other two arms. The ratio between total cholesterol and HDL-cholesterol decreased significantly by 14% in the NVP arm (-0.99 ± 0.29, p = 0.002) and by 9% in the 3TC arm (-0.51 ± 0.27, p = 0.029), but not in the IDV arm. CETP mass did not change significantly in the NVP and 3TC arms, while a small but significant increase was observed in the IDV arm. All remaining HDL-related baseline parameters, their absolute changes adjusted for baseline and their percentage change from baseline are also shown in Table 2.
Table 2 Baseline levels and changes in high-density lipoprotein (HDL) cholesterol (C)-related lipoparameters at baseline and absolute (adjusted for baseline) and percentage change from baseline to week 24 (mean ± standard error) in the three treatment arms.
Total Cholesterol, triglycerides, LDL and VLDL-related proteins
(Table 3)

None of the parameters shown in Table 3 differed at baseline between the three treatment groups. Between 0 and 24 weeks of treatment both total and LDL-c levels increased significantly in the NVP and IDV arms, but not in the 3TC arm. Both small and large LDL-c levels increased significantly in the NVP arm, but not in the other two arms, while neither intermediate LDL-c, LDL size, nor VLDL subclass concentrations or size changed significantly in either of the three arms. Triglyceride levels were low at baseline in all three arms ranging from a mean of 1.46 to 1.59 mmol/l, and did not change significantly during treatment.

Multivariate linear regression model

The multivariate linear regression model did not reveal any statistically significant contribution from having been randomised to IDV or 3TC-containing therapy to the percent change in HDL-cholesterol and HDL-associated parameters when adjusting for CD4+ cells at baseline, CD4+ cell rise from week 0 to week 24, HIV-1 viral load at baseline or HIV-1 viral load decrease from week 0 to week 24 (data not shown).

This allowed us to use a less complex model in which only randomisation to the NVP arm was used as explanatory variable in conjunction with the variables denoting immune restoration or viral load decrease as mentioned above. As mentioned earlier, the increase in HDL cholesterol in the NVP arm was 49% in the unadjusted analysis (Table 2). Increases in HDL cholesterol remained highly significant after adjusting for baseline CD4+ cells (+32%; p<0.001), CD4+ cell increase (+36%; p<0.001), baseline HIV-1 viral load (+33%; p<0.01), and HIV-1 viral load decrease (+34%; p<0.001). A similar significant pattern was observed for the changes in LpAI, apoAI and HDL size.
Table 3 Baseline levels and changes in total cholesterol (c), low-density lipoprotein (LDL)-c and triglycerides at baseline and absolute (adjusted for baseline) and percentage change from baseline to week 24 (mean ± standard error) in the three treatment arms

<table>
<thead>
<tr>
<th></th>
<th>nevirapine (n=34)</th>
<th>indinavir (n=41)</th>
<th>lamivudine (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>Absolute change 0-24 weeks</td>
<td>Percentage change 0 - 24 weeks</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.51 ± 0.21</td>
<td>0.80 ± 0.17</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.90 ± 0.18</td>
<td>0.41 ± 0.14</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>Large LDL-c (mmol/L)</td>
<td>1.17 ± 0.15</td>
<td>0.32 ± 0.11</td>
<td>112 ± 50</td>
</tr>
<tr>
<td>Intermediate LDL-c (mmol/L)</td>
<td>0.44 ± 0.11</td>
<td>-0.15 ± 0.09</td>
<td>-11 ± 33</td>
</tr>
<tr>
<td>Small LDL-c (mmol/L)</td>
<td>1.02 ± 0.11</td>
<td>0.32 ± 0.11</td>
<td>117 ± 47</td>
</tr>
<tr>
<td>LDL size (nm)</td>
<td>20.16 ± 0.11</td>
<td>-0.03 ± 0.11</td>
<td>-0.4 ± 0.6</td>
</tr>
<tr>
<td>LDL particles (nmol/L)</td>
<td>1253 ± 66</td>
<td>215.3 ± 47.8</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>Apo B (ma/L)</td>
<td>807 ± 41</td>
<td>87.6 ± 26.9</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Lp(a) (mg/L)</td>
<td>134.5 ± 36.7</td>
<td>46.4 ± 14.4</td>
<td>44 ± 307</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.46 ± 0.13</td>
<td>-0.07 ± 0.26</td>
<td>2 ± 14</td>
</tr>
</tbody>
</table>
Discussion

We observed a striking increase in plasma HDL-c in patients randomly assigned to NVP-containing HAART, which was accompanied by significant increases in LpAI, apoAI and HDL particle size. Less pronounced increases in HDL-c, LpAI and apoAI were observed in the 3TC and IDV containing arms. The increase in LDL-c observed in the NVP arm was offset by a much more striking reduction of the total cholesterol (TC)/HDL ratio. This ratio is considered a powerful risk predictor of future CAD events[12], and the degree in which the TC/HDL ratio was reduced in patients treated with NVP would be expected to confer significant benefit if maintained over a prolonged period of time. A lesser statistically significant reduction of the TC/HDL ratio was seen in the 3TC arm, but not in the IDV arm. The effect of NVP on HDL-cholesterol and other HDL-parameters remained present even after adjusting for CD4+ cell count and HIV-1 RNA level, both prior to and during treatment.

High-density lipoprotein (HDL) is strongly protective against atherosclerosis. An important mechanism underlying this protective effect is the role of HDL in the removal of excess cholesterol from peripheral tissues (reverse cholesterol transport) as reviewed by Hill ea[21]. In addition, HDL also protects by inhibiting lipoprotein oxidation. The antioxidant properties of HDL are due in part to serum paraoxonase, an enzyme carried on HDL that can degrade biologically active oxidised phospholipids[22,23]. Genetic syndromes associated with high HDL levels are usually associated with longevity and a decreased risk of CAD[24].

Furthermore, animal studies have provided substantial proof-of-principle that intervention targeted at increasing HDL could be a viable therapeutic strategy. Repeated infusion of HDL, or transgenic overexpression of apoAI in mice and rabbits inhibits progression of atherosclerosis. Using liver-directed gene transfer, others have also
demonstrated that hepatic overexpression of the HDL proteins could increase HDL-c levels and result in a rapid and marked regression of pre-existing atherosclerotic lesions in genetically modified mice[25,26].

Intervention studies in humans such as the Helsinki Heart Study[27] and the VA-HIT[28] likewise have shown that treatment which raises HDL-c from low levels at baseline is accompanied by substantial decreases in CAD events.

The potential mechanisms underlying the changes in lipoprotein profile, which were observed in recipients of NVP-containing HAART, remain speculative. It is known that HDL-c as well as HDL particle size increases with decreasing CETP activity, as has been shown in CETP deficient patients. We measured CETP mass, which may be used[29] as a surrogate for CETP activity, but in fact observed either no significant change (NVP and 3TC), or if anything an increase (IDV) in CETP mass. Since inhibition of CETP would also lead to decreases in LDL-cholesterol, which was not observed in our study, it is very unlikely that the effect of nevirapine-containing HAART, which we observed, is the result of inhibition of the CETP enzyme.

Other possible mechanisms such as an increase of apoAI production in the liver or an increase in ATP binding cassette (ABC) A-I activity in the peripheral vasculature need to be explored. It seems highly unlikely that HAART-induced suppression of HIV replication and/or immune reconstitution would be responsible for our findings, both in view of the results from the multivariate linear regression model, and the fact that HIV-RNA and CD4 responses over 24 weeks of treatment did not differ significantly between the three treatment arms, whereas the changes in lipoprotein profile clearly did.

A limitation of this study was the fact that whether bloods were drawn fasting or not was not recorded. Although patients in the IDV-containing arm may have been more likely to attend clinics fasting as
tid indinavir preferably should be ingested in a fasting state, it is very unlikely that these results would have been influenced to any significant extent by a non-fasting state, given the finding that triglyceride concentrations were low in all three study arms at baseline (approximately 1.5 mmol/l) and did not show a significant rise over 24 weeks. Importantly however, whether bloods were drawn fasting or not should not have influenced the results regarding changes in HDL.

In conclusion, patients are surviving longer as a result of HAART but concern exists that complications of therapy, such as the dyslipidemia commonly observed with PI-containing therapy could result in premature morbidity and/or mortality from potentially accelerated atherosclerosis. The lipoprotein profile which was observed in patients receiving NVP-containing HAART, including a sharp increase in HDL-c, in itself would independent of other known CAD risk factors be expected to be associated with an approximate 50% decrease in risk of CAD, as has previously been shown in other settings [30]. Long-term follow up of trials such as Atlantic as well as observational studies are needed to demonstrate whether this will translate into a clinical benefit. Trials which prospectively look at surrogates for atherosclerosis such as the non-invasive measurement of intima-media thickness of the carotid artery, should also be considered. Furthermore, it will be important to unravel the mechanism underlying the changes in lipoprotein profile associated with NVP, and determine whether these changes are unique to NVP or may likewise occur with other drugs belonging to the class of NNRTIs, for which there is preliminary evidence[31]. Ultimately, the findings from further studies may not only effect the choice of the initial type of HAART regimen for treatment of HIV-1 infection, but may also lead to novel pharmacological interventions targeted at raising HDL-cholesterol for prevention of CAD.
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Lipid profile and nevirapine containing therapy


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