Short communication

Effect of abacavir on acute changes in biomarkers associated with cardiovascular dysfunction

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Background: This study examined the effect of abacavir on acute changes in biomarkers associated with cardiovascular dysfunction.

Methods: Among the Study to Understand the Natural History of HIV/AIDS in the Era of Effective therapy (SUN) participants, we identified 25 individuals (cases) who were HLA-B5701-negative and who had ≥ 2 weeks without abacavir exposure at one visit and ≥ 2 weeks with abacavir exposure at the consecutive visit while maintaining viral suppression. We identified 43 individuals (controls) similarly unexposed and exposed to tenofovir. We assessed concentrations of prothrombin fragment F(1+2), D-dimer, high-sensitivity C-reactive protein, interleukin-8, intercellular adhesion molecule-1, vascular adhesion molecule-1, E-selectin, P-selectin, serum amyloid A and serum amyloid P. We examined the median percentage change of these biomarkers from the unexposed to exposed state among cases and controls compared with the expected assay variability using a sign test, and compared changes among cases with controls using the Wilcoxon rank-sum test.

Results: Baseline characteristics were similar between cases and controls: median age 45 versus 46 years, 80% versus 81% male, 64% versus 63% non-Hispanic White and median CD4+ T-cell count 538 versus 601 cells/mm³, respectively. Mean exposure times were 65 and 15 weeks for abacavir and tenofovir, respectively. We observed no significant changes in biomarkers from the unexposed to exposed state among cases or controls compared with the expected assay variability. We found that no biomarkers were significantly increased among cases compared with controls; however, prothrombin fragment F(1+2) was significantly lower among controls (P=0.035).

Conclusions: In virologically suppressed contemporary HIV-infected patients, abacavir exposure was not associated with increases in biomarkers associated with increased cardiovascular risk.

Introduction

In the era of combination antiretroviral therapy (cART), survival of HIV-infected individuals has improved [1–3] and mortality is increasingly attributable to non-AIDS conditions, such as cardiovascular disease [4–6]. Exposure to protease inhibitors has been associated with increased risk for cardiovascular events among HIV-infected individuals [7,8]. More recently abacavir (ABC), a nucleoside reverse transcriptase inhibitor (NRTI), has been associated with a possible increased risk of myocardial infarction (MI) [4,9–11]. The risk of MI increased shortly after initiation of ABC, attenuated with cessation of exposure, and there was no relationship between duration of therapy and MI risk [4]. These observations suggested that ABC exposure affected cardiovascular risk through an acute reversible effect [12,13]. The Strategies for Management of Anti-Retroviral Therapy (SMART) study has demonstrated both an increased risk of cardiovascular events and significantly greater levels of two proinflammatory biomarkers (high sensitivity C-reactive protein [hsCRP] and interleukin [IL]-6) among patients taking ABC compared with patients taking other NRTIs...
[14]. Several other studies examining the association between ABC exposure and proinflammatory marker concentrations, including hsCRP and IL-6, have produced conflicting results; however, only one of these studies attempted to control for the inflammatory effects of HIV replication by limiting the analysis to individuals who maintained virological suppression [15–20]. In our analysis, we examined the effect of starting or stopping ABC on acute changes in circulating biomarkers associated with coagulation, endothelial dysfunction and inflammation among SUN Study participants with undetectable HIV RNA viral loads, comparing participants exposed to ABC with participants exposed to tenofovir (TDF), an NRTI that has not been associated cardiovascular disease.

Methods

The SUN Study

The SUN Study is an ongoing prospective observational cohort study of 700 HIV-infected patients enrolled between 1 March 2004 and 30 June 2006 at seven HIV specialty clinics in four US cities: St Louis, MO, Providence, RI, Minneapolis, MN and Denver, CO. The study's design, and its data collection and management methods have been described previously [21]. Participants were generally healthy HIV-infected patients receiving routine outpatient care whose entire antiretroviral experience consisted only of cART. Patient data, including all diagnoses, all treatments and their dosages and durations, and all clinical laboratory data were abstracted from medical charts and entered into an electronic database (Clinical Practice Analyst; Cerner Corporation, Vienna, VA, USA) by trained staff. These data were then periodically reviewed for quality and analysed centrally. Additional data were collected through physical examination, non-invasive imaging and an audio computer-assisted self-interview, which collected behavioural risk data and other health related information including selected family history variables and use of tobacco, alcohol and recreational drugs.

The study protocol has been approved and has been reviewed annually by the CDC and each participating site’s institutional review board.

Nested case-control study

We included participants who were HLA-B5701-negative, maintained virological suppression (that is, <400 copies/ml) throughout observation (from April 2004 to October 2008) and who had changed antiretrovirals due to reasons other than virological failure (for example, regimen simplification, laboratory abnormalities or side effects). We chose HLA-B5701 allele-negative participants because the presence of the allele is a contraindication to treatment with ABC and can lead to a life-threatening hypersensitivity reaction; we wanted to eliminate the possibility that any inflammatory changes we noted associated with ABC were related to the presence of this allele. Cases were defined as participants who had started or stopped (that is, transitioned from an unexposed to an exposed state, or vice versa) ABC ≥2 weeks prior to the blood draw at consecutive 6-monthly study-related visits. We included as controls participants who had similarly started or stopped TDF.

Laboratory evaluation

We assessed levels of five inflammatory markers (hsCRP, IL-6, IL-8, serum amyloid A and serum amyloid P) and six markers of coagulation and endothelial dysfunction (D-dimer, prothrombin fragment F [1+2], soluble vascular adhesion molecule [sVCAM], soluble intracellular adhesion molecule [sICAM], P-selectin and E-selectin) in serum or plasma that were shipped on the day of blood draw overnight at ambient temperature to CDC and then aliquoted and stored at -70°C until time of batched assay. Testing was performed at the Core Laboratory for Clinical Studies (Washington University School of Medicine, St Louis, MO, USA) using the following assays: HLA-B5701 (peripheral blood mononuclear cells) was tested by Roche Diagnostics (Burlington, NJ, USA) PCR followed by sequence-specific oligonucleotide probes; hsCRP (serum) was tested by K-assay (Kamiya Biomedical, Seattle, WA, USA); IL-6 (plasma) was tested by Immulite 1000 IL-6 assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA); IL-8 (plasma) was tested by BD OptEIA Human IL-8 ELISA kit II (BD Biosciences, Franklin Lakes, NJ, USA); D-dimer (plasma) was tested by Hitachi 917 Tina-quant D-dimer (Roche Diagnostics); prothrombin fragment F(1+2; plasma) was tested by Enzygnost F 1+2 ELISA kit (Siemens Healthcare Diagnostics); serum amyloid A (plasma) was tested by Serum Amyloid A Bioassay ELISA kit (US Biological, Swampscott, MA, USA); serum amyloid P (plasma) was tested by an in-house Washington University developed ELISA kit (St Louis, MO, USA) [22]; sVCAM (plasma), sICAM (plasma), P-selectin (plasma) and E-selectin (serum) were tested by Quantikine Human ELISA kits (R&D Systems, Minneapolis, MN, USA). Manufacturer-specific interassay coefficients of variation for the biomarkers measured were hsCRP 1.1–4.2%, IL-6 4.0–6.0%, IL-8 4.0–5.5%, D-dimer 3.2–8.3%, prothrombin fragment F(1+2) 4.4–11.2%, serum amyloid A 7.0–7.8%, serum amyloid P 7.3–13.4%, sVCAM 5.5–7.8%, sICAM 4.4–6.8%, P-selectin 7.9–9.9% and E-selectin 7.3–8.7%.

Statistical analysis

We conducted univariate comparisons of variables between cases and controls using either the Mantel–Haenszel χ² or Fisher’s exact test for categorical variables.
Table 1. Baseline characteristics of abacavir-exposed cases and tenofovir-exposed controls at enrolment in the SUN Study, March 2004–June 2006

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>ABC-exposed (n=25)</th>
<th>TDF-exposed (n=43)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (IQR)</td>
<td>45 (40–51)</td>
<td>46 (40–49)</td>
<td>0.530</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>20 (80)</td>
<td>35 (81)</td>
<td>0.888</td>
</tr>
<tr>
<td>White non-Hispanic race/ethnicity, n (%)</td>
<td>16 (64)</td>
<td>27 (63)</td>
<td>0.921</td>
</tr>
<tr>
<td>Men who have sex with men, n (%)</td>
<td>20 (80)</td>
<td>27 (63)</td>
<td>0.139</td>
</tr>
<tr>
<td>BMI&lt;25 kg/m², n (%)</td>
<td>16 (64)</td>
<td>23 (53)</td>
<td>0.398</td>
</tr>
<tr>
<td>Median years since HIV diagnosis (IQR)</td>
<td>6.1 (2.9–9.2)</td>
<td>5.9 (3.0–7.7)</td>
<td>0.728</td>
</tr>
<tr>
<td>HIV RNA≤400 copies/ml, n (%)</td>
<td>25 (100)</td>
<td>43 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>Median CD4⁺ T-cell count, cells/μl (IQR)</td>
<td>538 (405–697)</td>
<td>601 (435–801)</td>
<td>0.487</td>
</tr>
<tr>
<td>Prior cardiovascular disease, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>12 (48)</td>
<td>22 (51)</td>
<td>0.801</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (12)</td>
<td>5 (12)</td>
<td>0.963</td>
</tr>
<tr>
<td>Current tobacco smoker, n (%)</td>
<td>6 (26)</td>
<td>19 (47)</td>
<td>0.094</td>
</tr>
<tr>
<td>Median total cholesterol, mg/dl (IQR)</td>
<td>197.5 (180–233)</td>
<td>183 (161–212)</td>
<td>0.067</td>
</tr>
<tr>
<td>Median HDL cholesterol, mg/dl (IQR)</td>
<td>39 (34–45)</td>
<td>42 (35–50)</td>
<td>0.875</td>
</tr>
<tr>
<td>Median GFR, ml/min/1.73² (IQR)</td>
<td>92 (72–103)</td>
<td>97 (79–109)</td>
<td>0.105</td>
</tr>
<tr>
<td>Median Framingham score (IQR)</td>
<td>3 (0–5)</td>
<td>3 (0–6)</td>
<td>0.600</td>
</tr>
<tr>
<td>Median CAC score (IQR)</td>
<td>0 (0–23)</td>
<td>0 (0–3.3)</td>
<td>0.856</td>
</tr>
<tr>
<td>Median cIMT, mm (IQR)</td>
<td>0.74 (0.66–0.92)</td>
<td>0.73 (0.64–0.81)</td>
<td>0.477</td>
</tr>
<tr>
<td>Prescribed lipid-lowering drugs, n (%)</td>
<td>5 (20)</td>
<td>14 (33)</td>
<td>0.266</td>
</tr>
<tr>
<td>Prescribed aspirin, n (%)</td>
<td>3 (12)</td>
<td>10 (23)</td>
<td>0.255</td>
</tr>
<tr>
<td>Hepatitis C IgG-positive, n (%)</td>
<td>3 (12)</td>
<td>6 (15)</td>
<td>1.000</td>
</tr>
<tr>
<td>Median months of cART use (IQR)</td>
<td>47 (26–67)</td>
<td>38 (13–64)</td>
<td>0.913</td>
</tr>
<tr>
<td>Median months of NRTI use (IQR)</td>
<td>47 (26–67)</td>
<td>39 (17–70)</td>
<td>1.000</td>
</tr>
<tr>
<td>Prescribed NNRTI, n (%)</td>
<td>7 (28)</td>
<td>31 (72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prescribed PI, n (%)</td>
<td>12 (48)</td>
<td>10 (23)</td>
<td>0.036</td>
</tr>
<tr>
<td>Median weeks prescribed ABC or TDF (range)</td>
<td>65 (6–248)</td>
<td>15 (4.9–61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>prior to sample collection (range)</td>
<td>14 (5–30)</td>
<td>32 (32–32)</td>
<td>0.129</td>
</tr>
</tbody>
</table>

*Cardiovascular disease includes evidence of coronary artery disease (for example, myocardial infarction, angina, coronary artery disease or heart stenting), peripheral artery disease (for example, cerebrovascular accident, transient ischemic attack, peripheral vascular disease, aneurysm or other vascular diagnoses but specifically claudication) and other cardiac structural/rhythm problems (for example cardiomyopathy, cardiomegaly, congestive heart failure, cor pulmonale, mitral valve regurgitation, prolapse and stenosis, aortic valve stenosis, aortic valve regurgitation, tricuspid valve regurgitation, cardiac arrhythmias, right ventricular heart failure, endocarditis, myocarditis and pulmonary hypertension). Lipid-lowering drugs include HMG-CoA reductase inhibitors, fibrates and niacin. Exposure to abacavir (ABC) and tenofovir (TDF) was assessed during follow-up from April 2004 to October 2008. BMI, body mass index; CAD, coronary artery calcium; cART, combination antiretroviral therapy; cIMT, carotid intima media thickness; GFR, glomerular filtration rate; HDL, high-density lipoprotein; IgG, immunoglobulin G; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

and the Wilcoxon rank-sum test for continuous variables. We compared the median percent changes in biomarker concentrations from the unexposed to exposed state among cases with the expected assay variability using a sign test; the same analysis was conducted for controls. We compared the median percentage changes of biomarker concentrations among cases with the same changes among controls using the Wilcoxon rank-sum test. All analyses were performed using SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

We identified 25 ABC-exposed cases and 43 TDF-exposed controls. Characteristics at the baseline enrolment study visit were similar between the two groups: median age 45 versus 46 years, 80% versus 81% male, 64% versus 63% non-Hispanic White and median CD4⁺ T-cell count 538 versus 601 cells/mm³, respectively (Table 1). Median time from enrolment into the SUN Study to the first specimen collection was 18 months (IQR 7–30). Risk of cardiovascular disease at enrolment was similar between cases and controls with no significant differences in median total cholesterol, high-density lipoprotein, glomerular filtration rate, Framingham risk score, coronary artery calcium scores and carotid intima media thickness (Table 1).

With regard to antiretroviral use, there was no significant difference in duration of cART or NRTI use at baseline; however, TDF-exposed controls were more likely to have been prescribed a non-nucleoside reverse
transcriptase inhibitor concomitantly, whereas ABC-exposed cases were more likely to have been prescribed a protease inhibitor concomitantly (Table 1). Median exposure times were 65 and 15 weeks for ABC and TDF, respectively (P<0.001).

Changes in biomarkers compared with assay variability
Among the 25 ABC-exposed cases and 43 TDF-exposed controls, 10 cases and 42 controls started ABC or TDF (transitioned from an unexposed to an exposed state), and 15 cases and 1 control stopped ABC or TDF (transitioned from an exposed to an unexposed state). We compared the median percentage change in the concentrations of each biomarker (except IL-6, for which 71% of participants had a result below the limit of detection) among cases and controls with the corresponding assay’s variability, first overall and then limited to individuals who transitioned from the unexposed to exposed state and vice versa. We found no significant changes in any biomarker concentrations for cases or controls in any of these three comparison groups (data not shown). Prothrombin fragment F(1+2) declined among the TDF-exposed controls (median percentage change -12.5%); however the extent of this decline was not statistically significant (P=0.080) when considering the assay variability.

Changes in biomarkers among cases compared with controls
We observed no significant differences among cases compared with controls in the concentrations of all but one biomarker: prothrombin fragment F(1+2; Figure 1). The values of median percentage change in the levels of biomarkers among cases compared with controls were prothrombin fragment F(1+2) 0.5% versus -12.5% (P=0.035), d-Dimer -8.3% versus -7.7% (P=0.794), hsCRP -19.4% versus -19.9% (P=0.512), IL-8 33.9% versus -19.1% (P=0.123), sICAM-1 1.4% versus 3.8% (P=0.160), sVCAM -2.9% versus 1.4% (P=0.546), E-selectin -8.1% versus 2.8% (P=0.234), P-selectin -2.5% versus 2.0% (P=0.615), serum amyloid A -5.1% versus -5.1% (P=0.726) and serum amyloid P -7.0% versus -3.7% (P=0.615).

Discussion
In virologically suppressed HIV-infected patients who were antiretroviral-naive or exposed only to cART, ABC exposure was not associated with increases in biomarkers known to increase risk of cardiovascular disease. We found no significant variation in biomarker levels among ABC-exposed and TDF-exposed individuals between the unexposed and exposed states with the exception of a decline in plasma prothrombin fragment F(1+2) concentrations associated with TDF exposure. Our findings are similar to those reported in other recent studies that compared levels of biomarkers among ABC-exposed with TDF-exposed individuals [16,18–20].

In our study, ABC-exposed and TDF-exposed persons had similar and generally low baseline Framingham risk scores, coronary artery calcium scores, and carotid intima media thicknesses, a distinguishing feature of this analysis. In the D:A:D study, the increased rates of MI associated with ABC exposure among participants were greatest for participants with the highest 10-year Framingham risk score (that is, >20%). Among participants with the lowest predicted 10-year Framingham risk (<10%), who were most similar to patients examined in our analysis, rates of MI were substantially lower [4]. Therefore, we hypothesize that if ABC-related cardiovascular risk is reflected in biomarker levels, the effect would be less for individuals with low baseline cardiovascular risk.

Our study had a number of limitations. Because our study population was at low risk for cardiovascular disease, our findings should not be generalized to individuals with greater risk. It is possible that we did not include relevant biomarkers; however, we attempted to create a panel that encompassed factors that have historically been associated with cardiovascular risk and across a variety of potential points of action (for example, endothelial dysfunction and coagulation). As an observational cohort study, we could not control for channelling bias and other unmeasured confounding. We cannot explain the decrease we observed in the plasma level of prothrombin F(1+2), which is a marker of thrombin activity and is increased during an acute thrombotic event, that was associated with TDF exposure compared with ABC-exposure; therefore we believe this finding should be viewed with caution. Finally, the number of participants with suitable clinical histories and specimens available for analysis was small.

Our study had two notable strengths. First, ABC-exposed participants were observed unexposed and exposed to ABC for ≥5 and ≥6 weeks, respectively, prior to collection of the blood samples we analysed. We believe this time was ample to detect changes in circulating biomarkers of interest. Median exposure times to ABC and TDF were different, which was likely due to the longer availability of ABC. However, longer exposure to ABC also ensures that ample time passed to detect a change in biomarker levels. Second, all participants included in our analysis maintained virological suppression and had changed their antiretroviral regimens for reasons other than virological failure, thereby minimizing to the extent possible any confounding proinflammatory effects related to HIV replication [23].

In summary, our findings do not support the hypothesis that ABC has proinflammatory properties that increase the risk of an acute cardiovascular event.
Figure 1. Concentrations of biomarkers associated with cardiovascular dysfunction and inflammation among ABC- and TDF-exposed virologically suppressed SUN participants

Log₁₀-transformed median percentage change (boxes) with range (bars) in the concentrations of select circulating serum and plasma biomarkers associated with cardiovascular dysfunction and inflammation among abacavir (ABC)-exposed (cases; n=25) and tenofovir (TDF)-exposed (controls; n=43) HIV-infected HLA-B5701-negative SUN Study participants with HIV RNA viral loads <50 copies/ml. hsCRP, high-sensitivity C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1.
However, ABC has been shown to induce a more atherogenic lipid profile than TDF [24]. The concern, regarding ABC’s relationship with cardiovascular disease, warrants further mechanistic studies. Given the mixed data currently available, a large randomized clinical trial comparing the cardiovascular effects of ABC and TDF may be necessary for a more definitive conclusion.

Acknowledgements

The investigation followed the guidelines of the US Department of Health and Human Services regarding protection of human subjects. The study protocol was approved and renewed annually by each participating institutions’ ethical review board. All study participants provided written, informed consent.


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Disclosure statement

TO has served as a consultant or on an advisory board for the following companies: Gilead, Bristol-Myers Squibb, GlaxoSmithKline, Tibotec, Merck and Monogram Sciences. JB receives research funding from Gilead, GSK/ViiV, American Heart Association and National Institutes of Health. KH has served as a consultant or on an advisory board for the following companies: Gilead, GSK/ViiV and Tibotec. All other authors declare no competing interests. The findings and conclusions from this review are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Additional file


References


