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Short Communication
Initiation of an Abacavir-Containing Regimen in HIV-Infected Adults Is Associated with a Smaller Decrease in Inflammation and Endothelial Activation Markers Compared to Non-Abacavir-Containing Regimens

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Abstract

Abacavir has been associated with myocardial infarction in several studies. This may be related to inflammation and endothelial cell activation. We compared changes in inflammation and endothelial activation markers between antiretroviral-naive adults initiating zidovudine, lamivudine, abacavir, and nonnucleoside reverse transcriptase inhibitor (NNRTI) or this regimen without abacavir. Changes in soluble tumor necrosis factor receptors-I, -II (sTNFR-I, -II), high sensitivity C-reactive protein, and soluble vascular cell adhesion molecule-1 (sVCAM-1) from baseline (pre-ART) to a second time point about 24 weeks after initiating antiretroviral therapy (ART) were compared between groups using multivariable linear regression. A total of 37 met eligibility criteria; 12 received abacavir. The median (interquartile range) age was 37 years (27–45). Most were men (32/37), African-American (15/37), or white (15/37). The median nadir $CD4^+$ and baseline HIV-1 RNA were 230 cells/mm^3 (180–301) and 82,642 copies/ml (34,400–204,703). In all, 15/30 smoked, 7/37 had hypertension, 1/37 had diabetes, and 1/37 had hyperlipidemia. None had coronary or renal disease. Changes in $CD4^+$ and HIV-1 RNA level and timing of stored samples with regard to ART initiation were not different between groups. In univariable analysis, log transformed percent change in sTNFR-I ($p=0.05$) and -II ($p=0.04$) showed significant between-group differences and trended toward significance for sVCAM-1 ($p=0.08$). These markers decreased less in the abacavir group. After adjustment for confounders, significantly less decrease for sTNFR-II and sVCAM-1 was seen for those receiving the abacavir-containing regimen. When taken with an NNRTI, abacavir induced a smaller decrease in inflammation biomarkers in this cohort, suggesting a possible proinflammatory effect of this nucleoside analogue.

In 2008, the D:A:D Study Group published the results of a cohort study of HIV-infected adults in which recent use of abacavir was associated with myocardial infarction (MI) with an adjusted rate ratio of 1.90 (95% CI 1.47–2.45).[1] This result has been reproducible in some,[2–5] but not all studies[6–11] and a question of biological plausibility persists.

We performed a retrospective cohort study utilizing the Center for AIDS Research (CFAR) sample repository and clinical database. HIV-1-infected adults, antiretroviral-naive at study entry who initiated a regimen of zidovudine (AZT), lamivudine (3TC), abacavir (ABC), and a nonnucleoside reverse transcriptase inhibitor (NNRTI) or the same regimen without ABC after 1997 were included. Participants also had to have a plasma sample stored within 6 months prior to initiating antiretroviral therapy (ART) as well as 3–9 months after starting that same unchanged regimen and a signed informed consent to allow their stored samples to be used for HIV-related research. Participants were excluded if pregnant when ART was initiated, had an active opportunistic infection (OI), or had an HIV-1 RNA level >400 copies/ml when the second sample was stored. This study was approved by the University Hospitals Case Medical Center and University of North Carolina Institutional Review Boards.

Previously stored samples were tested for markers of inflammation [soluble tumor necrosis factor alpha receptors (sTNFR-I and -II) and high-sensitivity C-reactive protein

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(hs-CRP)] and endothelial activation [soluble vascular cell adhesion molecule-1 (sVCAM-1)] from before starting ART and 3–9 months later. Biomarkers with compelling data linking them to CVD risk, and especially those known to be elevated in HIV patients, were selected. Elevated hs-CRP has been associated with subclinical atherosclerosis and is independently predictive of future cardiovascular disease events in both the general population and in HIV.[12,13] The inflammatory marker tumor necrosis factor-α (TNF-α) has been implicated in MI after acute coronary syndromes and levels have been shown to be higher in patients who subsequently experience recurrent MIs or cardiac death.[14,15] The effects of TNF-α are mediated by two receptors, sTNFR-I and sTNFR-II. These soluble receptors are stable in serum, easily measured, and reflect activity of TNF-α. Likewise, sVCAM-1 arises from shedding or proteolytic cleavage from activated endothelial cells and is a useful marker for increased activation of endothelial cells in atherosclerosis.[16–18] All markers were measured using a commercially available enzyme-labeled immunosorbent sandwich assay at Aushon Biosystems, Billerica, MA. Tests were done in duplicate and averaged. For sTNFR-I, sTNFR-II, hs-CRP, and sVCAM-1, the median intraassay and interassay coefficients of variation were 8.8% and 10.8%, 8.6% and 6.0%, 6.9% and 4.5%, and 8.7% and 13.4%, respectively.

Demographic, HIV and cardiovascular characteristics, inflammatory and endothelial markers are described using median and interquartile range (IQR) for continuous variables and frequency and percent for categorical variables. Changes in the markers are reported as absolute and log-transformed percent change from baseline [$\log_e$(percent change from baseline +1)]. Differences within and between groups were tested using paired and unpaired Student's *t*-tests or Wilcoxon signed rank or rank sum tests as distributionally appropriate for continuous variables. Fisher's exact tests or exact Pearson Chi square tests were used for between-group comparisons as appropriate for categorical variables. All tests were two-sided with a type I error of 0.05. For confounding adjustment, univariable followed by multivariable linear regression was performed with log-transformed percent change in each marker as the dependent variable. Multivariable models were created by including ART treatment group, duration of time between starting ART and second sample, and baseline $CD4^+$ count, and those variables found to be statistically associated with the outcome in univariable analysis at $p \leq 0.05$. Backward elimination by least significant variable was used for model selection (ART treatment group and duration to second sample were left in the model regardless of significance). Once models were created in this way, each variable tested in univariable analysis was added in turn to see if the regression coefficient estimate for ART treatment group changed by ≥10%. Final models were tested to be sure the assumptions of linear regression were met. All statistical analyses were performed using SAS version 9.2 (The SAS Institute, Cary, NC).

Thirty-seven patients met eligibility criteria: 12 in the ABC group and 25 in the control (non-ABC) group. Overall, most were men (86%), and African-American (41%) or white (41%). Median nadir and baseline $CD4^+$ counts (cells/mm^3) were 230 (180–301) and 244 (180–316), respectively. Median baseline HIV-1 RNA level (copies/ml) was 82,642 (34,400–204,703). Median known duration of HIV prior to ART initiation (years) was 0.6 (0.2–1.2) and median year ART initiated was 2002 (2000–2003). Fifty percent (15/30) were current smokers. Out of the 37 participants, seven had hypertension, one had diabetes mellitus, one had hyperlipidemia, and none had coronary artery disease. The median body mass index (kg/m^2) was 26.3 (25.2–27.2). The median creatinine (mg/dl) was 0.9 (0.8–1.0). Three percent (1/32) used intravenous drugs. The majority (26/37) received care at Case Western Reserve.

There were no statistically significant differences between groups with regard to the change in $CD4^+$ cell count or HIV-1 RNA level over the study period ($p = 0.84$ and $p = 0.91$, respectively). By design (as indirect reflection of good adherence to ART), all participants had HIV-1 RNA ≤400 copies/ml at the time of the second biomarker measurement point. In the ABC group, 10/12 had HIV-1 RNA ≤50 copies/ml and in the control group, 13/25 had HIV-1 RNA ≤50 copies/ml. Baseline demographic and clinical characteristics were similar between groups, although age trended toward being significantly lower for the ABC group [median age (years) for controls was 38 (32–48) vs. 31 (27–35) for the ABC group ($p = 0.06$)].

Sample storage dates ranged from April 14, 1999 to November 20, 2008 and the timing of the stored samples with regard to ART initiation was not different between groups ($p = 0.43$ and $p = 0.2$ between groups for the first and second sample, respectively). The overall median times between the first sample and starting ART and duration on ART before the second sample (months) were 0.5 (0–1.3) and 5.6 (4.8–5.9), respectively.

Baseline, absolute change, and log-transformed percent change in the markers over the study period are shown in Table 1 with results of between- and within-group analyses. In the control group, all markers appeared to improve, with changes in sTNFR-I, sTNFR-II, and sVCAM-1 reaching statistical significance (all $p < 0.001$). In the ABC group, sTNFR-II significantly improved ($p = 0.05$). Between groups, differences in the change in sTNFR-I and sTNFR-II were statistically significant and change in sVCAM-1 neared significance. After adjustment, the ART treatment group remained independently associated with changes in sTNFR-II and sVCAM-1. For both of these markers, there was a greater degree of improvement in the control group when compared to the ABC group.

In this study we compared changes in several markers of inflammation and endothelial activation between ART-naive adults starting AZT/3TC/ABC + NNRTI or the same regimen without ABC. After adjustment for factors known to affect inflammation, sTNFR-II and sVCAM-1 decreased less in the group taking ABC. As such, it is possible that the ongoing heightened inflammation and endothelial activation occurring while on ABC contribute to risk of myocardial infarction observed in some studies. Although not congruent across all studies,[19–24] this notion that ABC may lead to a higher degree of inflammation is supported by other research. The SMART/INSIGHT investigators showed that hs-CRP and IL-6 levels were higher in those SMART study participants receiving ABC.[2] In ART-experienced participants switching to either an ABC-containing regimen or another NRTI, Kristoffersen *et al*. showed that matrix metallopeptidase 9, myeloperoxidase, and hs-CRP increased in the group changed to ABC.[25] Also, De Pablo *et al.* showed that ABC induces an interaction between human leukocytes and

Table 1. Baseline, Absolute Change, and Log-Transformed Percent Change in Biomarkers

	AZT + 3TC + ABC + NNRTI n = *12*	*AZT + 3TC + NNRTI* n = *25*	p[a]
Baseline			
sTNFR-I, pg/dl	555 (444–769)	761 (524–1228)	0.08
sTNFR-II, pg/dl	922 (662–1167)	1244 (826–1967)	0.13
hs-CRP, μg/dl	0.26 (0.16–3.24)	0.49 (0.3–1.03)	0.36
sVCAM-1, ng/dl	1593 (845–3319)	2532 (2150–3214)	0.05
Absolute change			
sTNFR-I, pg/dl	27 (−198–146)	−125 (−281–3)[b]	0.13
sTNFR-II, pg/dl	−294 (−599–108)[c]	−497 (−1291–349)[b]	0.04
hs-CRP, μg/dl	0.06 (−2.89–0.22)	−0.1 (−0.5–0.05)	0.64
sVCAM-1, ng/dl	−477 (−1528–176)	−1385 (−1906–594)[b]	0.07
Log-transformed percent change			
sTNFR-I	0.05 (−0.39–0.32)	−0.30 (−0.37–0.01)[b]	0.05
sTNFR-II	−0.38 (−0.76–0.08)[c]	−0.53 (−0.95–0.48)[b]	0.04
hs-CRP	0.16 (−1.31–1.51)	−0.18 (−1.18–0.30)	0.42
sVCAM-1	−0.29 (−0.79–0.23)	−0.58 (−1.12–0.42)[b]	0.08

All variables are median (interquartile range).
[a]Between groups; Wilcoxon two-sample test or Student's *t* test as distributionally appropriate.
[b]Change within-group significant at $p < 0.05$.
[c]Trend toward significant within-group change ($0.05 < p < 0.1$).
AZT, zidovudine; 3TC, lamivudine; ABC, abacavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; sTNFR-I, soluble tumor necrosis factor α receptor-I; sTNFR-II, soluble tumor necrosis factor α receptor-II; hs-CRP, high sensitivity C-reactive protein; sVCAM-1, soluble vascular cell adhesion molecule-1.

endothelial cells in a dose-dependent fashion through activation of Mac-1, which interacts with ICAM-1.[26]

Major limitations of this study include the retrospective observational study design in which ART allocation was not randomized and the small sample size. Because of this, we attempted to minimize the effect of confounding by restricted eligibility, excluding those pregnant and with active infectious/inflammatory conditions and by adjusting for factors determined or known to confound the relationship between ART group and the outcome. However, unmeasured confounding cannot be excluded as an explanation for the results that we obtained. Other limitations include having only one biomarker measurement after ART initiation and not having data on HLA-B5701 status for the participants. Having repeated measures would have better characterized changes in these markers over time. However, in choosing a time point on average 6 months after ART initiation and after participants had become virologically suppressed, we hoped to be measuring these markers at a point when the levels would be stable, i.e., after a period of expected improvement. Also, not having data on HLA-B5701 status limits our ability to exclude possible subclinical or early abacavir hypersensitivity reaction as a reason for the results that we obtained. However, we did thoroughly evaluate the data for outliers with regard to the biomarkers measured and did not find a subgroup.

In conclusion, in the setting of NNRTI treatment, there was a smaller decrease in sTNFR-II and sVCAM-1 after adjustment for important confounders in the group that included ABC. This suggests that chronic heightened inflammation may play a role in the increased risk of MI observed in some studies with ABC.

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