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Inflammation investigated as a source of pharmacokinetic variability of atazanavir in AIDS Clinical Trials Group protocol A5224s

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Abstract

Background—Inflammation is associated with the downregulation of drug metabolizing enzymes and transporters. Thus, we investigated the chronic inflammatory state associated with HIV-infection as a source of pharmacokinetic variability of atazanavir. We also explored the association of total bilirubin concentrations with markers of inflammation and endothelial activation.

Methods—Apparent oral clearance (CL/F) of atazanavir was estimated from plasma samples collected from participants in AIDS Clinical Trials Group Study A5202. Several inflammatory and endothelial activation biomarkers were measured at baseline and weeks 24 and 96 as part of metabolic sub-study A5224s: high-sensitivity C-reactive protein (hsCRP), interleukin-6, tumor necrosis factor alpha and its soluble receptors, soluble vascular cellular and intracellular adhesion molecules, and total bilirubin. Statistical analysis was performed by a matrix of correlation coefficients between atazanavir CL/F and biomarker concentrations measured at week 24. The correlation between atazanavir clearance and percentage change in bilirubin from baseline to weeks 24 and 96, and between biomarkers and bilirubin concentrations at each week were also evaluated.

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Results—Among 107 participants, there were no significant correlations observed between atazanavir CL/F and inflammatory and endothelial activation biomarkers measured at week 24 ($P \geq 0.24$). As expected, bilirubin increased with increasing exposure to atazanavir (rho = −0.25, P = 0.01). Bilirubin concentrations were inversely correlated ($P < 0.01$) with each of the biomarkers except hsCRP.

Conclusions—Atazanavir CL/F did not correlate with the inflammatory biomarkers changes. Inflammatory-mediated inhibition of cytochrome P450 3A may have been attenuated due to atazanavir-associated increases of bilirubin, which has known anti-inflammatory properties.

BACKGROUND

Despite antiretroviral therapy (ART), persons with HIV-1 have persistent, low-grade inflammation and immune activation that are associated with an increased risk of AIDS and non-AIDS events [1,2]. As part of the inflammatory response to HIV infection, endothelial activation and release of vascular adhesion molecules occurs, and several different markers reflecting ongoing inflammation and endothelial activation increase in HIV infected persons even after long-term combination ART [3]. Although multiple studies have demonstrated improvements in inflammatory cytokine levels after undetectable virus levels are achieved with ART, some cytokine levels do not completely normalize compared to HIV uninfected individuals [4–6]. The physiological changes accompanying inflammation may alter the pharmacokinetics of certain medications. These changes include an increase in gastric pH, increasing and decreasing concentrations of plasma proteins, and reduction in the expression and function of drug metabolizing enzymes and membrane transporters [7,8]. For instance, inflammatory cytokines and other agents associated with inflammatory responses, such as interleukin-1 (IL-1), IL-6, interferon-γ, IL-1β, and bacterial lipopolysaccharaide are associated with the downregulation of cytochrome P450 (CYP) 3A mRNA and protein expression [9]. Similarly, IL-6 and perhaps tumor necrosis factor-alpha (TNF-α) suppress P-glycoprotein (P-gp) expression in hepatic, intestinal, and brain tissues [10].

Clinical effects resulting from these inflammatory-induced alterations are observed in various acute and chronic inflammatory conditions [11–14]. In lung and breast cancer patients, clearance of erythromycin (a CYP3A substrate and moderate inhibitor) was inversely correlated with plasma levels of the inflammatory acute-phase protein, C-reactive protein (CRP) and IL-6 [12]. In patients receiving bone marrow transplantations, elevated levels of IL-6 and TNF-α were correlated with reduced drug metabolism of cyclosporine (a CYP3A and P-gp substrate and inhibitor) [14]. Also, plasma trough concentrations of voriconazole (a CYP2C19, 3A4, and 2C9 substrate) are estimated to increase on average by 0.014 mg/liter for every 1 mg/liter increase in CRP concentration due to reduction in CYP-mediated metabolism [15]. Given the chronic inflammatory state associated with HIV-infection, inflammatory-mediated cytokines may be a source of pharmacokinetic variability of antiretrovirals, especially those that are substrates or inhibitors of CYP3A or P-gp [16,17]. In a recent clinical study, HIV-infected ART-naïve participants had decreased intestinal expression of *CYP3A4*, measured from intestinal biopsies, compared to healthy uninfected controls. Interestingly, however, HIV-infected individuals receiving ART had

increased gene and protein expression of *ABCB1*/P-gp compared to the HIV-infected ART-naïve group [18].

Atazanavir is a potent once-daily protease inhibitor that undergoes extensive hepatic metabolism via CYP3A4/5, with subsequent uridine diphosphate glucuronsyltransferase (UGT)-mediated glucuronidation [19]. It is also a substrate for the drug efflux transporter, P-gp. In the present study, we analyzed plasma atazanavir exposure and markers of residual inflammation and vascular endothelial activation, as well as total bilirubin levels measured in HIV-infected individuals to determine their association with one another. The primary objective was to explore the relationship between atazanavir exposure and plasma concentrations of inflammatory and endothelial activation markers, with the expectation that with 24 weeks or more of ARV therapy, atazanavir exposure would be higher in those with increased inflammation. We also assessed the relationship between total bilirubin levels and plasma concentrations of inflammatory and endothelial activation markers because of the potential anti-inflammatory effects of bilirubin [20–22].

METHODS

Study Design

Data for this exploratory analysis came from AIDS Clinical Trials Group A5224s, which was a metabolic substudy of A5202 (ClinicalTrials.gov NCT00118898). In A5202, HIV-1-infected treatment naïve adults (≥ 16 years of age), with HIV-1 RNA > 1000 copies/mL were randomized to open-label efavirenz (600 mg) or atazanavir/ritonavir (300 mg/100 mg), with double-blinded placebo-controlled tenofovir disproxil fumarate/emtricitabine (300 mg/200 mg) or abacavir/lamivudine (600 mg/300 mg) [23–25]. Randomization was stratified by HIV-1 RNA (< or ≥ 100,000 copies/mL) and by intent to participate in the metabolic substudy A5224s, in which select markers of inflammation and endothelial activation were measured longitudinally [26,27]. Enrollment inclusion criteria included participants to have a screening total bilirubin ≤ 2.5 x upper limit of normal (ULN), and aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase ≤ 5 x ULN. Human subjects committees of all sites approved the protocol, and informed consent was obtained from all participants. Primary analyses from these studies have been previously reported [23–27].

Measurements of Biomarkers

Fasting plasma samples for measurement of biomarkers were collected at study entry (baseline), week 24, and week 96. Markers of inflammation that were measured included high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and soluble receptors of TNF-α (sTNFR-I, and -II). Endothelial activation markers that were measured included soluble vascular cellular adhesion molecule (sVCAM-1) and soluble intracellular adhesion molecule (sICAM-1). Biomarker assays were analyzed as previously described [2,27]. Bilirubin was assayed at research site clinical laboratories at baseline, week 24, and week 96 as part of the parent A5202 protocol.

Measurement of Atazanavir Exposure

In A5202, plasma samples for atazanavir assay were collected at steady-state during the first 24 weeks of study follow-up. A sparse sampling study design was used to collect at least three samples per participant for population pharmacokinetic modeling, as has been previously described [28]. Adherence to medications was monitored using the ACTG self-reported adherence questionnaire. Additionally, the last three doses of antiretrovirals prior to collection of pharmacokinetic samples were reviewed by the University at Buffalo Pharmacology Specialty Laboratory to ensure adherence was documented on the case report forms. Atazanavir concentrations were quantified using a validated high-pressure liquid chromatography assay [29]. The atazanavir concentration-time data were used to construct a one-compartment population pharmacokinetic model with NONMEM VII (ICON Development Solutions, Ellicott City, MD). Individual Bayesian estimates of apparent oral clearance (CL/F) for atazanavir were generated for each study participant, such that an individual had a single CL/F value (liters/hour) representative of their exposure to atazanavir over the first 24 weeks of study participation.

Statistical Analysis

For this analysis, participant data were included if they had inflammation and endothelial activation markers measured at baseline and week(s) 24 and/or 96, and had an atazanavir plasma CL/F value estimated. Baseline characteristics, biomarker concentrations, and atazanavir CL/F were summarized by proportions or medians with interquartile ranges (IQR). Continuous measurements were compared using Wilcoxon rank-sum tests or Kruskal-Wallis tests. Spearman rank correlation tests were performed to determine the correlation between atazanavir CL/F and week 24 biomarker concentrations, while week 96 biomarker data were used for sensitivity analyses. Spearman rank correlation tests were also used to determine the correlation between atazanavir CL/F and the percent change in bilirubin levels from baseline to weeks 24 and 96 [$(\text{bilirubin}_{\text{week24}} - \text{bilirubin}_{\text{week0}})/\text{bilirubin}_{\text{week0}}$]. Pearson correlation coefficients are reported for analyses between total bilirubin and each of the biomarkers of inflammation and endothelial activation after they were log-transformed. For paired data with more than one time point for each study participant, an ordinary correlation coefficient is not appropriate because it does not take into account the lack of independence between repeated measurements of the same participant [30]. Instead, we calculated a “within participants” correlation coefficient, which removes the variation between individuals to examine, for example, whether an increase in bilirubin within the same individual is associated with a decrease in an inflammation marker. The level of significance was set at two-sided P-values <0.05. Power calculations were not performed, as this was an exploratory analysis that took advantage of data collected from previous conducted studies. Analyses were performed in either R 3.3.2 or STATA 14 (College Station, TX: StataCorp LP).

RESULTS

There were 269 study participants enrolled in A5224s, of which 130 were randomized to receive atazanavir/ritonavir-based treatment. A total of 107 participants had both biomarkers and atazanavir CL/F measured and were therefore included in this analysis. There were 82

participants who had biomarker concentrations measured at baseline and both weeks 24 and 96; 20 participants who had biomarker concentrations measured at baseline and week 24 only; and 5 participants with baseline and week 96 data only. Baseline characteristics of study participants are summarized in Table 1.

The overall median [IQR] atazanavir CL/F was 7.6 [6.7, 9.4] liters/hour. Atazanavir CL/F was significantly faster in the randomized tenofovir disproxil fumarate/emtricitabine group at 8.7 [6.8, 10.1] liters/hour compared to the abacavir/lamivudine group of 7.3 [6.5, 8.0] liters/hour (p=0.006). Atazanavir CL/F did not differ significantly by sex or race/ethnicity, and was not significantly correlated with baseline body mass index or HIV-1 RNA laboratory values.

Correlations between atazanavir clearance and biomarkers

At baseline, none of the biomarkers were significantly correlated with atazanavir CL/F ($p \geq 0.19$). The correlations between atazanavir CL/F with week 24 biomarker concentrations among all study participants, and by randomized nucleos(t)ide reverse transcriptase inhibitor (NRTI) treatment arm are presented in Table 2. There were no significant correlations between atazanavir CL/F and biomarker concentrations measured at week 24 for all study participants. Similar results were observed at week 96 (data not shown), except for sVCAM-1, in which there were weak inverse correlations with atazanavir CL/F in the overall study population (rho = −0.244, p = 0.02) and among those randomized to abacavir/lamivudine (rho = −0.326, p = 0.03).

As expected with atazanavir treatment initiation, the median [IQR] bilirubin concentrations increased at weeks 24 and 96 to 2.1 [1.3, 2.6] and 1.9 [1.1, 2.9], respectively. Percent change in bilirubin concentration from baseline to week 24 was weakly negatively correlated with atazanavir CL/F among all participants (rho = −0.251, p-value = 0.01) but appeared to differ by randomized NRTI treatment arm. Those in the tenofovir disoproxil fumarate/emtricitabine arm had moderate inverse correlation between atazanavir CL/F and percentage change in bilirubin concentration (rho = −0.370, p-value = 0.008); meaning as atazanavir exposure increased (i.e. CL/F decreased), the change in bilirubin levels increased. Those in the abacavir/lamivudine arm did not exhibit significant correlations between atazanavir CL/F and bilirubin changes (rho = −0.056, p-value = 0.69). Results were similar for the week 96 bilirubin changes (data not shown).

Correlations between biomarkers and total bilirubin

The correlations between total bilirubin concentrations and each of the inflammatory and endothelial activation marker concentrations were determined for all measurements collected at each time point (baseline, and weeks 24 and 96) to assess for the potential anti-inflammatory effects of bilirubin (Table 3). Within participants, there were moderate-to-strong inverse correlations observed between bilirubin concentrations and TNF-α (r = −0.68), sTNFR-II (r = −0.69), and sVCAM-1 (r = −0.68; p < 0.00001); moderate inverse correlations with sTNFR-I (r = −0.43) and sICAM-1 (r = −0.45; p < 0.00001); and, a weak inverse correlation with IL-6 (r = −0.21; p = 0.003). Collectively, these negative correlations suggest that increased bilirubin concentrations correlate with decreased concentrations of

markers of inflammation and endothelial activation within individuals, except with hsCRP, for which there was not a significant correlation in the overall study group. These patterns of correlation remained largely the same within each randomized NRTI arm, except for IL-6 which was driven primarily by the tenofovir disproxil fumurate/emtricitabine containing regimen (Table 3).

DISCUSSION

Inflammatory processes can alter the expression and activity of different drug-metabolizing enzymes and transporters thereby affecting the pharmacokinetics of drugs. Previous studies have shown that infections and inflammation, both acute and chronic, can reduce the metabolic clearance of cytochrome P450 (CYP) substrates by 20 – 70% [31]. These effects likely stem primarily from the altered expression of CYP and drug transporter proteins that are down-regulated during the generation of host defense mechanisms. Both atazanavir and ritonavir are substrates and inhibitors of CYP isoenzymes 3A4/5, which led us to hypothesize that a source of the atazanavir pharmacokinetic heterogeneity in AIDS Clinical Trial Group study A5202 was mediated, in part, by ongoing inflammation associated with chronic HIV-1 infection. However, among study participants who received atazanavir-based treatment, we did not generally observe significant associations between the apparent plasma clearance of atazanavir and markers of inflammation and endothelial activation that would suggest a reduction in CYP-mediated metabolism due to higher inflammatory activity.

The underlying mechanisms of immune activation during HIV infection are multifactorial and result directly from the replication of HIV itself and indirectly through other mechanisms like the reactivation of chronic infections, oxidized lipids, and the translocation of microbial products from the gastrointestinal tract to systemic circulation. One of the direct consequences of activation of the immune system is secretion of pro-inflammatory cytokines, such as IFN-α, TNF-α, IL-1, IL-6, and IL-18, which can contribute to additional immune activation and apoptosis of immune cells [32,32]. Although cytokine perturbations may be partially corrected by antiretroviral therapy, some immune and endothelial activation markers have been shown to remain elevated despite months to years of treatment [4,17,34–36]. For example, markers such as TNF-α, IL-6, hsCRP, and sVCAM-1 are elevated in HIV-infected patients receiving stable antiretroviral therapy compared to healthy individuals [37,38]. In A5224s, antiretroviral therapy with atazanavir- or efavirenz-based regimens was effective in reducing all inflammation and endothelial activation markers measured overall, except for hsCRP [27]. However, the magnitudes in reduction differed for each biomarker. For instance, levels of sTNFR-I decreased by only 12% from baseline after 96 weeks of ART, while sTNFR-II decreased by approximately 50%.

The various CYP enzyme families and drug transporters also vary dramatically in response to diverse inflammatory stimuli, as the different cytokines can display a diverse spectrum of activity towards individual enzyme forms. For example, TNF-α reduces CYP2C11 and -3A2 but has no effect on CYP2A1 and -2C6 in rats [39]. IL-1 but not IL-6 reduce CYP2E1 mRNA and protein expression but only when this enzyme is induced, leaving the constitutive form of the enzyme unchanged [40]. Contrary, IL-6, blocks rifampicin-mediated induction of CYP3A4 activity by reducing the expression of pregnane X and constitutively

activated receptors in primary human hepatocytes [41,42]. Changes in drug disposition have also been observed clinically in various disease states that involve an inflammatory response (e.g. cancer, sepsis, cardiovascular disease, arthritis) with different inflammatory markers being negatively correlated with different CYP enzyme function [12,13,43].

The lack of correlation between inflammation markers and atazanavir CL/F in this analysis may be due to elevated indirect bilirubin concentrations through the inhibition of UGT1A1-mediated bilirubin glucuronidation by atazanavir. Bilirubin has known anti-oxidant, cytoprotective, and anti-inflammatory properties, thus potentially blunting the effects of inflammation on CYP metabolism [21,22,44,45]. We did observe inverse correlations within study participants between their bilirubin concentrations and the majority of the markers of inflammation and endothelial activation. The strongest correlations were with sTNFR-II, TNF-α, and sVCAM-1. This is in agreement with prior studies reporting that bilirubin reduces TNF-α-induced gene upregulation of VCAM-1 and ICAM-1 [46].

In this study, if those with the most vigorous inflammatory conditions do indeed have impaired drug metabolism that result in higher atazanavir concentrations, they would ultimately also have greater elevations in their bilirubin levels, thereby attenuating the inflammatory-mediated alterations on metabolism we initially sought to observe. Furthermore, ritonavir exposures were not accounted for in this analysis, which may have also influenced associations between atazanavir metabolism and inflammatory processes. Thus, future analyses that will investigate the effects of inflammatory processes on drug metabolism in HIV-infected individuals should include antiretrovirals or other drugs that do not increase bilirubin or other endogenous signals of anti-inflammatory mechanisms (e.g. anti-inflammatory cytokines, transforming growth factor-β, IL-10 and IL-1 receptor antagonist, HDL). For example, an antiretroviral like darunavir, might be a more appropriate agent to study because of its substantial interindividual pharmacokinetic variability, its primary metabolism by CYP3A and absence of treatment-related hyperbilirubinemia, as well as its limited direct effects on some of the inflammatory markers that evoke metabolism and disposition changes [46].

In conclusion, atazanavir exposure was not correlated with levels of endothelial activation markers (sVCAM-1 and sICAM-1) or inflammatory markers (hsCRP, IL-6, TNF-α, sTNR-I, sTNFR-II). In contrast, bilirubin levels were positively correlated with atazanavir exposure and inversely correlated with the majority of the biomarkers (except hsCRP), possibly due to the endogenous anti-oxidant and anti-inflammatory properties of bilirubin. These anti-inflammatory effects of bilirubin have clinical implications for inflammatory disorders. For example, through disruption of endothelial VCAM-1 and ICAM-1-mediated leukocyte migration, bilirubin has demonstrated the ability to suppress atherosclerotic plaque formation [47]. This would explain, in part, the recent observations that HIV-infected individuals with elevated bilirubin have a decreased risk for heart failure, and the lack of association between atazanavir/ritonavir and cardiovascular disease compared to other protease inhibitors [48–50]. Future studies, including dedicated clinical pharmacokinetic studies, should continue to examine whether ongoing systemic inflammation in HIV-infected individuals affect the metabolism of antiretrovirals and other therapeutic agents that are important to this potentially vulnerable population to drug-disease interactions.

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Table 1

Baseline characteristics of study participants

Variable	Study Participants (n=107)
Male; No. (%)	95 (89)
Self-reported race/ethnicity; n (%)	
White, non-Hispanic	45 (42)
Black, non-Hispanic	38 (36)
Hispanic	20 (19)
Other	4 (4)
Randomized NRTI arm; n (%)	
Abacavir/Lamivudine	55 (51)
Tenofovir/Emtricitabine	52 (49)
Age in years; median (IQR)	37 (31, 43)
BMI in kg/m^2; median (IQR)	24.8 (21.7, 28.1)
CD4 in $cells/mm^3$; median (IQR)	234 (78, 321)
Total bilirubin in mg/dL; median (IQR)	0.5 (0.4, 0.7)
Biomarkers; median (IQR)	
hsCRP in μg/mL	1.7 (0.7, 4.1)
IL-6 in pg/mL	0.8 (0.6, 1.3)
TNF-α in pg/mL	11.0 (7.8, 14.5)
sTNFR-I in pg/mL	1224 (1034, 1596)
sTNFR-II in pg/mL	4930 (3573, 7964)
sVCAM-1 in ng/mL	1146 (920, 1542)
sICAM-1 in ng/mL	329 (261, 418)

Table 2

Spearman's rho correlation coefficients (p-values) between apparent oral clearance of atazanavir (ATV CL/F) and week 24 biomarker concentrations

Biomarker	ATV CL/F Overall	ATV CL/F in TDF/FTC randomized group	ATV CL/F in ABC/3TC randomized group
	Week 24	Week 24	Week 24
hsCRP	−0.117 (0.24)	−0.103 (0.47)	−0.042 (0.77)
IL-6	0.010 (0.92)	0.017 (0.91)	0.161 (0.26)
TNF-α	−0.067 (0.50)	−0.107 (0.46)	−0.074 (0.61)
sTNFR-I	−0.059 (0.56)	−0.095 (0.51)	0.012 (0.94)
sTNFR-II	−0.083 (0.41)	−0.172 (0.23)	−0.032 (0.82)
sVCAM-1	−0.110 (0.27)	−0.156 (0.27)	−0.109 (0.45)
sICAM-1	−0.055 (0.58)	−0.104 (0.47)	−0.012 (0.93)

Table 3

Within participants' Pearson's correlation coefficients (p-values) between log-transformed inflammation markers and bilirubin at baseline, and weeks 24 and 96

Biomarker	Overall	TDF/FTC	ABC/3TC
hsCRP	−0.07 (0.36)	**−0.22 (0.03)**	0.11 (0.29)
IL-6	**−0.21 (0.003)**	**−0.35 (0.0005)**	−0.07 (0.48)
TNF-α	**−0.68 (< 0.0001)**	**−0.70 (< 0.0001)**	**−0.68 (< 0.0001)**
sTNFR-I	**−0.43 (< 0.0001)**	**−0.39 (0.0001)**	**−0.46 (< 0.0001)**
sTNFR-II	**−0.69 (< 0.0001)**	**−0.66 (< 0.0001)**	**−0.72 (< 0.0001)**
sVCAM-1	**−0.68 (< 0.0001)**	**−0.63 (< 0.0001)**	**−0.73 (< 0.0001)**
sICAM-1	**−0.45 (< 0.0001)**	**−0.41 (< 0.0001)**	**−0.58 (< 0.0001)**