Atazanavir Metabolism According to CYP3A5 Status: An In Vitro-In Vivo Assessment^S

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ABSTRACT:

The current study was a follow-up to an in vivo study in which atazanavir oral clearance was shown to be dependent on genetically determined CYP3A5 expression status, but only in non-African Americans. The aim of this study was to identify atazanavir metabolites generated by CYP3A5 and to evaluate this metabolite pattern in the African-American versus non-African-American CYP3A5 expressors from the previous study. First, the in vitro metabolism of atazanavir was evaluated using human liver microsomes (HLM) and CYP3A4 and CYP3A5 isoforms. Second, the metabolite pattern generated by CYP3A5 was evaluated in human plasma samples from the previous study. Atazanavir metabolites were analyzed using liquid chromatography-tandem mass spectrometry methods. Metabolite areas under the time-concentration curves (AUCs) were normalized to atazanavir AUC to generate an AUC ratio. Sixteen metabolites were observed in human liver mi-

croosomal incubations representing five "phase I" biotransformation pathways. Mono-oxidation products (M1 and M2) were formed by CYP3A5 at a faster rate than CYP3A4 by 32- and 2.6-fold, respectively. This finding was replicated in HLM from a genetically determined CYP3A5 expressor versus nonexpressor. In the in vivo samples, the M1 and M2 AUC ratios were approximately 2-fold higher in CYP3A5 expressors versus nonexpressors ($P < 0.05$), and the difference was similar in African Americans and non-African Americans. Thus, CYP3A5 produced a unique metabolite "signature" for atazanavir in vitro and in vivo, independent of race. Therefore, other pharmacological factors are likely to explain the apparent lack of effect of genetically determined CYP3A5 expressor status on atazanavir oral clearance in African Americans from the previous study.

Introduction

Atazanavir (ATV) is a commonly prescribed human immunodeficiency virus protease inhibitor that is approved by the U.S. Food and Drug Administration for once-daily administration. ATV exhibits significant pharmacokinetic (PK) variability arising from acid-dependent absorption, drug-food/drug-drug interactions, and/or variability in the enzyme systems responsible for its clearance (Croom et al., 2009).

In a previously published study, the influence of genetically determined CYP3A5 expression on atazanavir pharmacokinetics was assessed in a group of 31 human immunodeficiency virus-negative human volunteers (Anderson et al., 2009). Subjects were administered 400 mg of atazanavir each morning for 7 days, and then a 24-h PK study was conducted after a standardized breakfast. Enrollment was balanced for genetically determined CYP3A5 expression, race, and

gender. The primary finding was that atazanavir oral clearance (CL/F) was 1.39-fold faster in people who expressed CYP3A5 versus those who did not. However, atazanavir CL/F differences were not present in African-American CYP3A5 expressors.¹ This discrepancy raised the question as to whether CYP3A5 expression was exerting a consistent effect in AAs and NAAs. We hypothesized that an analysis of ATV metabolites could shed light on these in vivo findings, and this was the basis for the present study. The aims were to evaluate atazanavir metabolism in vitro using human liver microsomes (HLM), CYP3A4, and CYP3A5 to define metabolite formation differences for CYP3A5 followed by a comparison of CYP3A5 metabolite profiles in the AA and NAA groups from the previous human PK study.

Materials and Methods

Chemicals and Reagents. Atazanavir sulfate was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Rockville, MD) 20850 (lot NIH 2 040131). Ritonavir was purchased from Apin Chemicals (Oxon, UK). HLM (20.0 mg/ml, pool of 50, mixed gender, lot 0510025), HLM from a genetically determined CYP3A5 nonexpressor (lot 0710253), HLM from a genetically determined CYP3A5 expressor (lot 0710272), human CYP3A4 (CYP010; 3A4 low reductase; batch C3A4LR004; 5.6 nmol P450/ml; 12.6 mg protein/ml; 444 pmol P450/mg protein), and human CYP3A5 (CYP015; 3A5 low reductase; batch C3A5LR008; 2.8 nmol P450/ml; 14.5 mg

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¹ The terms "expressor" and "expresser" have both been used in the scientific literature to describe persons who express CYP3A5 protein.

ABBREVIATIONS: ATV, atazanavir; PK, pharmacokinetic; CL/F, oral clearance; AA, African American; NAA, non-African American; HLM, human liver microsomes; P450, cytochrome P450; CL_{int}, intrinsic clearance; LC, liquid chromatography; MS/MS, tandem mass spectrometry; amu, atomic mass unit.

protein/ml; 193 pmol P450/mg protein) with human P450 reductase coexpressed in *Escherichia coli* were purchased from XenoTech, LLC (Lenexa, KS).

In Vitro Experiments. Incubations consisted of phosphate buffer (100 mM; pH 7.4), $MgCl_2$ (5.0 mM), and EDTA (0.1 mM) and were conducted at 37°C. Conditions used included human microsomes (1.0 mg protein/ml), CYP3A4 (100 pmol P450/ml), or CYP3A5 (100 pmol P450/ml) with atazanavir (10.0 μ M) in the presence of NADPH (1.0 mM) for various times (0.5, 5, 10, 15, and 30 min). In the case of human liver microsomes, additional incubations were conducted for 90 min. Other incubations used human liver microsomes (0.20 mg protein/ml) in the presence of atazanavir (1.0 μ M), ritonavir (0.0, 0.10, 0.25, and 0.50 μ M), and NADPH (1.0 mM) for various times (0.5, 5, 10, 20, and 30 min). In all cases, incubate samples (100 μ l) were removed and added to the quench solution (acetonitrile, 300 μ l). The resulting samples were vortexed (5 s) and centrifuged at 3000 rpm (5.0 min) and subsequently analyzed by LC-MS/MS. The data were expressed as percent disappearance of parent relative to the ~0.5-min time point sample. These results were used to calculate in vitro intrinsic clearance values (reported in units of microliters per minute per milligram of protein) as described previously using the following equation: $(0.693/\text{in vitro } t_{1/2}) \cdot (\text{microliters of incubation per milligram of microsomal protein})$ (Obach, 1999).

LC-MS/MS. Atazanavir metabolites were analyzed by LC-MS/MS (Sciex 4000 from Applied Biosystems, Foster City, CA; high-performance liquid chromatograph from Shimadzu Scientific Instruments, Inc.; Columbia, MD; and autosampler from LEAP Technologies, Carrboro, NC). For liquid chromatography a Zorbax extended-C18 250 $\times$ 4.6 mm, 5- μ m column (Agilent Technologies, Santa Clara, CA) at 40.0 $\pm$ 0.1°C with a flow rate of 0.4 ml/min was used. The mobile phase consisted of A (10 mM ammonium acetate-0.1% formic acid in water) and B (50:50 acetonitrile-methanol). Chromatography consisted of 95% A for 0.10 min; brought to 50% B at 1.50 min and held for 2.50 min; ramped to 80% B at 11.50 min, and then to 95% B at 12.00 min and held for 7.50 min, and finally brought back to 95% A at 22.50 min and held for 3.5 min (26.0 min total run time). An atazanavir sulfate stock dimethyl sulfoxide solution (20 mM) was freshly prepared and used to generate the atazanavir standard curve (linear range; 0.05–950 ng/ml). Atazanavir was monitored via electrospray ionization positive ion mode using the following conditions: 1) an ion-spray voltage was 5500 V; 2) temperature was 450°C; 3) curtain gas (set at 10) and collisionally activated dissociation (set at 5) gas were nitrogen; 4) ion source gas 1 and 2 were set at 20; 5) entrance potential was set at 10.0 V; 6) quadrupole 1 and quadrupole 3 were set on unit resolution; 7) dwell time was set at 200 ms; and 8) declustering potential, collision energy, and collision cell exit potential are voltages. Compound settings were atazanavir (parent drug) 705.4 $\rightarrow$ 168.1 *m/z* and tamoxifen (internal standard) 372.1 $\rightarrow$ 72.1 *m/z*. Atazanavir metabolite settings are provided in Table 1. Ten-microliter aliquots were injected onto the LC-MS/MS system for all standards and unknowns. The apparent metabolite concentrations (nanograms per

milliliter) were derived from the atazanavir standard curve fitted to a $1/x^2$ weighted linear regression with a high correlation coefficient ($R^2 = 0.998$). Metabolite values were estimates because metabolite reference standards were not available for calibrators and quality control samples.

In Vivo Plasma Samples. Plasma samples from the in vivo study (Anderson et al., 2009) were analyzed for atazanavir metabolites. The measurement of metabolites in the present study was reviewed by the Colorado Multiple Institutional Review Board under the original protocol and was approved. All participants provided written informed consent. Samples from 14 of the original 31 individuals were selected for these analyses; subjects were chosen to most efficiently and economically evaluate whether atazanavir metabolites were comparable in the NAA and AA subjects who expressed CYP3A5 versus those who did not. The subjects included five NAAs not expressing CYP3A5 (group 1), four NAA expressors (group 2), and five AA expressors (group 3). Time points for the atazanavir metabolite analysis included the 2, 4, 12, and 24 h postobserved dose samples to evaluate points along the entire AUC profile; the 24-h value was also used for the 0-h value, given the steady-state conditions. Atazanavir concentrations in these samples were not requantified with the LC-MS/MS method; many samples were higher than the linear range, so the reported concentrations are those generated previously using a validated high-performance liquid chromatography-UV method (Anderson et al., 2009).

To ensure a homogeneous mixture, plasma samples were allowed to warm to room temperature (10 min) followed by vortex mixing (5 s). Samples were removed (100 μ l) and transferred into individual microcentrifuge tubes (500 μ l). An extraction solution (200 μ l; 2:2:1; methanol-acetonitrile-water) containing 40 ng/ml tamoxifen (internal standard) was added. The tubes were capped, vortex-mixed (5 s), set aside for 5.0 min, and subsequently centrifuged at 13,000 rpm (5.0 min). The supernatants were transferred into individual wells of a 96-well plate, capped, and placed into the autosampler cool stack (6.0 $\pm$ 0.1°C) for analysis.

Data Analyses. Metabolite AUC values were estimated with the linear trapezoidal method. All of the atazanavir PK data were reported as part of the parent study and are summarized briefly in this report. The metabolite AUC was normalized by atazanavir AUC to produce metabolite/atazanavir AUC ratios for comparisons. Prism 4.02 (GraphPad Software, Inc., San Diego, CA) was used for graphing and statistical analysis. One-way analysis of variance, followed by a Tukey multiple comparison test, was used for comparisons.

Results

In Vitro Liver Microsome Experiments. In the presence of NADPH, atazanavir was observed to undergo five different “phase I biotransformation” pathways via human liver microsomal incubations (Fig. 1). These five pathways produced 16 metabolites (Table 1) including 1) monooxidation (M1–M4), 2) oxidative decarboxylation

TABLE 1
Atazanavir metabolite summary

Analyte	Time	Phase I Transformation	ESI(+) <i>m/z</i>	HLM	CYP3A4	CYP3A5	In Vivo
	<i>min</i>						
Atazanavir	18.0	None	705.4 $\rightarrow$ 168.1	Y	Y	Y	Y
M1	16.9	MO	721.4 $\rightarrow$ 168.1	Y	Y	Y	Y
M2	17.1	MO	721.4 $\rightarrow$ 168.1	Y	Y	Y	Y
M3	17.6	MO	721.4 $\rightarrow$ 168.1	Y	Y	Y	Y
M4	18.1	MO	721.4 $\rightarrow$ 168.1	Y	Y	N.D.	Y
M5	14.6	O-Decarb	647.4 $\rightarrow$ 168.1	Y	Y	Y	Y
M6	14.7	O-Decarb	647.4 $\rightarrow$ 168.1	Y	Y	Y (t)	Y
M7	18.0	O-Deh	703.4 $\rightarrow$ 168.1	Y	Y	Y	Y
M8	17.2	O-Deh	703.4 $\rightarrow$ 168.1	Y	N.D.	N.D.	Y
M9	17.9	MO + O-Decarb	663.4 $\rightarrow$ 168.1	Y (t)	Y	Y	Y
M10	12.4	MO + O-Decarb	663.4 $\rightarrow$ 168.1	Y	N.D.	N.D.	N.D.
M11	12.8	MO + O-Decarb	663.4 $\rightarrow$ 168.1	Y	N.D.	N.D.	N.D.
M12	13.9	MO + O-Decarb	663.4 $\rightarrow$ 168.1	Y	N.D.	N.D.	N.D.
M13	15.0	DO	737.4 $\rightarrow$ 168.1	Y	N.D.	N.D.	N.D.
M14	16.0	DO	737.4 $\rightarrow$ 168.1	Y (t)	N.D.	N.D.	N.D.
M15	16.4	DO	737.4 $\rightarrow$ 168.1	Y	Y (t)	Y (t)	N.D.
M16	16.9	DO	737.4 $\rightarrow$ 168.1	Y	N.D.	N.D.	N.D.

ESI(+), electrospray ionization positive ion mode; Y, detected; MO, monooxidation; O-Decarb, oxidative decarboxylation; O-Deh, oxidative dehydration; DO, deoxidation; (t), trace amount observed; N.D., not detected.

FIG. 1. Proposed atazanavir metabolite pathways (major and minor) commonly observed via human liver microsomal, CYP3A4, or CYP3A5 incubates and via human in vivo samples.

(M5–M6), 3) oxidative dehydration (M7–M8), 4) mono-oxidation plus oxidative decarboxylation (M9–M12); and 5) dioxidation (M13–M16) (Supplemental Fig. S1). Six predominant metabolites (major) were identified (Fig. 2), monooxidation metabolites M2 and M1 being the most prevalent. The human CYP3A4 and CYP3A5 incubations produced nine and eight metabolites, respectively, and represent all five biotransformation pathways (Table 1; Supplemental Figs. S2 and S3).

Given the significant inhibitory effect of ritonavir on atazanavir clearance in vivo, the effect of ritonavir on atazanavir intrinsic clear-

ance (CL_{int}) was probed in vitro. Atazanavir (1.0 μM at 0.2 mg of liver microsomal protein; pool of 50, mixed gender) was incubated in the presence of varying concentrations of ritonavir. Ritonavir (0.5 μM) modified the in vitro CL_{int} from 139.8 ± 4.2 (control) to 70.4 ± 10.0 $\mu\text{l/min}$ per mg of protein (0.5 μM ritonavir; $P < 0.001$) (Supplemental Fig. S4). Human CYP3A4 and CYP3A5 incubations were probed for metabolite formation differences. As summarized in Fig. 3, there were notable differences observed between CYP3A4 and CYP3A5. On a picomole per P450 comparison, CYP3A5 produced the monooxidation metabolites M1 and M2 at a faster rate than CYP3A4: 32- and 2.6-fold faster for M1 and M2 at the 30-min time point, respectively. Other differences include that 1) CYP3A4 produced approximately equal amounts of M5 and M6, whereas CYP3A5 favored the formation of M5 and 2) CYP3A4 produced M3 and M4, whereas CYP3A5 favored the formation of M3 over M4. Both CYP3A4 and CYP3A5 incubations showed the formation of M7, M9, and M15. The minor metabolites formed were not appreciably different between the two isoforms (Supplemental Fig. S5). A comparison of HLM from 1) a pool of 50 mixed gender, 2) an individual (male) with genetically determined CYP3A5 nonexpression (*3/*3), and 3) an individual (female) with genetically determined CYP3A5 expression (*1/*1) demonstrated faster formation of M1 and M2 according to the presence of CYP3A5 (Supplemental Fig. S6). Given that CYP3A5 produced the major monooxidation metabolites M1 and M2 at a faster rate than CYP3A4 (Fig. 3), these metabolites were the focus in the analysis of the in vivo plasma samples.

FIG. 2. Major atazanavir metabolites observed in human liver microsomes in vitro incubations (0–30 min): ■, M1; ▲, M2; ▼, M5 and M6; ○, M7; ●, M9. Data are presented as means $\pm$ S.D. $n = 6$.

FIG. 3. Major atazanavir metabolites observed via human CYP3A4 and CYP3A5 in vitro incubations (0–30 min): □, M1 (CYP3A4); ■, M1 (CYP3A5); △, M2 (CYP3A4); ▲, M2 (CYP3A5). Data are presented as means $\pm$ S.E.M. $n = 4$.

In Vivo Metabolites. Metabolites M1 to M9 (Table 1) were identified in in vivo samples (Supplemental Fig. S7). In terms of the major monooxidation metabolites M1 and M2, the in vivo plasma samples displayed lower assay signals for M1 relative to M2, consistent with the in vitro observations. Atazanavir PK parameters from the parent study and metabolite AUCs and AUC ratios are summarized in Table 2 (Anderson et al., 2009). On average, the atazanavir AUC was 1.8-fold higher in NAA nonexpressor individuals (group 1) compared with NAA expressor individuals (group 2; $P < 0.05$). However, the AUCs in AA expressor individuals (group 3) were 2.4-fold higher than those in the NAA expressor individuals (group 2; $P < 0.01$) and not significantly different from those of NAA nonexpressor individuals (group 1) (Table 2).

Despite these significant ATV differences in African Americans versus non-African Americans CYP3A5 expressors (groups 2 and 3), the M1/ATV (Fig. 4) and M2/ATV (Fig. 5) AUC ratios were similar ($P > 0.05$) in these two groups. The M1 ratio values for both CYP3A5 expressor groups were approximately 2-fold higher than those of the CYP3A5-nonexpressor individuals (0.24 and 0.23 versus 0.10) ($P < 0.001$, both comparisons) (Fig. 4). The M2 ratio showed a similar relationship in which the CYP3A5 expressor groups were approximately the same (mean 14.0, group 2; and mean 17.3, group 3)

(Fig. 5), and both were approximately 2-fold higher than the CYP3A5 nonexpressor individuals (mean 8.8 group 1) ($P < 0.05$ and $P < 0.001$, respectively). One non-African American who expressed CYP3A5 had an M2 ratio similar to that of the nonexpressor group, whereas all of the M1 ratios in expressors were above those in nonexpressors. This observation was consistent with the in vitro data showing that the M1 formation rate was faster via CYP3A5 compared with that for M2 (32- versus 2.6-fold, respectively). Taken together, these in vitro and in vivo data demonstrate a consistent atazanavir metabolite phenotype, in particular the M1 ratio, which was associated with CYP3A5 expressor status independent of African-American race.

Discussion

The current study was a follow-up to an in vivo study in which atazanavir oral clearance was shown to be dependent on CYP3A5 expression status, but only in non-African Americans (Anderson et al., 2009). As a follow-up to this finding, the in vitro hepatic metabolism of ATV was investigated first. As summarized in Table 1 and Fig. 1, 16 phase I metabolites were observed via human liver microsomal incubations. All observed metabolites maintained the 2-(*p*-tolyl)pyridine functionality; hence, all had the daughter ion at 168 *m/z* (Table 1; Supplemental Fig. S8). Similar daughter fragmentation patterns for atazanavir metabolites have been reported previously (ter Heine et al., 2009). The study of ter Heine et al., which evaluated metabolites in plasma from people taking atazanavir and atazanavir plus ritonavir, also showed evidence of the major metabolites elucidated in this study (M1, M2, M5, and M6) but reported a *N*-dealkylation and keto metabolite not readily observed in the present study. A keto metabolite was also reported among three metabolites in the manufacturer's briefing document (Bristol-Myers Squibb Company BMS-232632: atazanavir briefing document May 2003, http://www.fda.gov/ohrms/dockets/ac/03/briefing/3950B1_01_BristolMyersSquibb-Atazanavir.pdf). The metabolite structures were not reported in the briefing document. No metabolites that retained antiretroviral activity were described. The present study evaluated only the metabolites elucidated in the in vitro studies, so the *N*-dealkylation and keto metabolite, which were not detected in vitro, were not monitored in the in vivo samples. The 16

TABLE 2
Metabolite and ATV concentrations for in vivo samples

Subject Group ^a	ATV AUC	M1 AUC	M2 AUC	M1/ATV Ratio ^b	M2/ATV Ratio ^b	ATV CL/F
	$\mu\text{g} \cdot \text{h/ml}$	$\text{ng} \cdot \text{h/ml}$				l per h/kg
1	33.2	2.9	270	0.09	8.1	0.13
1	24.9	2.0	207	0.08	8.3	0.18
1	17.7	2.4	151	0.14	8.6	0.25
1	32.8	3.3	300	0.10	9.2	0.16
1	17.4	2.0	169	0.11	9.8	0.22
Average	25.2	2.5	219	0.10	8.8	0.19
S.D.	7.7	0.6	64	0.02	0.7	0.05
2	9.9	1.7	82	0.18	8.3	0.43
2	18.3	3.9	268	0.21	14.6	0.34
2	16.5	4.9	267	0.29	16.2	0.37
2	10.9	3.1	184	0.28	16.9	0.38
Average	13.9	3.4	200	0.24	14.0	0.38
S.D.	4.1	1.4	88	0.05	3.9	0.04
3	29.3	6.0	431	0.21	14.7	0.19
3	28.1	6.6	481	0.23	17.1	0.16
3	35.9	8.4	639	0.23	17.8	0.21
3	38.6	8.1	700	0.21	18.1	0.13
3	36.5	9.1	683	0.25	18.7	0.15
Average	33.7	7.6	587	0.23	17.3	0.17
S.D.	4.7	1.3	123	0.02	1.6	0.03

^a Group 1, non-African-American CYP3A5 nonexpressors; group 2, non-African-American CYP3A5 expressors; group 3, African-American CYP3A5 expressors.

^b For readability, the actual value was 1000-fold lower; the ATV AUC units are micrograms $\cdot$ hour per milliliter and metabolite units are nanograms $\cdot$ hour per milliliter.

FIG. 4. Metabolite **M1**/atazanavir AUC ratios in the three groups: group 1 (G1), non-African-American CYP3A5 nonexpressors (G1-NAA-NE); group 2 (G2), non-African-American CYP3A5 expressors (G2-NAA-E); and group 3 (G3), African-American CYP3A5 expressors (G3-AA-E). Data are presented as means $\pm$ S.E.M. ***, $P < 0.001$.

metabolites from HLM in the present study were generated from five main pathways: four were monooxidation products [$M + O$; $M + 16$ atomic mass units (amu): **M1–M4**], two were oxidative decarboxylation products ($M - 56$ amu: **M5** and **M6**), two were oxidative dehydration products ($M - 2$ amu: **M7** and **M8**), four were oxidation with oxidative decarboxylation ($M - 42$ amu: **M9–M12**), and four were dioxidation products ($M + 32$ amu: **M13–M16**). The observed formation of **M5** and **M6** and **M9** to **M12** warrants additional discussion. These metabolites require the destruction of a carbamate functional group (i.e., one of two present in atazanavir). Although this chemical transformation might represent general hydrolysis or is catalyzed via esterase activity, atazanavir incubates (37°C , 2 h) in the presence of HLM without NADPH did not reveal carbamate hydrolysis (data not shown). This result suggests that these metabolites were produced via a P450-catalyzed oxidative decarboxylation mechanism (Fig. 1), a P450-catalyzed pathway analogous to that proposed for loratadine to generate descarboethoxyloratadine (Yumibe et al., 1996). Six major atazanavir metabolites were identified (Fig. 2). Monooxidation products, **M1** and **M2**, were most prevalent via HLM. In addition, HLM were used to probe the effect(s) of ritonavir on atazanavir metabolism. Ritonavir ($0.50 \mu\text{M}$) was shown to decrease atazanavir ($1.0 \mu\text{M}$) in vitro CL_{int} by 2-fold (Supplemental Fig. S4), consistent with the effect of ritonavir on atazanavir CL/F in vivo (Anderson et al., 2009). A limitation to the metabolite analyses was the lack of metabolite reference standards. Therefore, the atazanavir standard curve was used to estimate metabolite concentrations, an approach to determine relative metabolite signals, but not a true quantification of metabolite concentrations.

Human CYP3A4 and human CYP3A5 incubates produced evidence for all five phase I biotransformation pathways (**M1**, **M2**, **M5**, **M6**, **M7**, **M9**, and **M15**). Similar to HLM, in human CYP3A4 and CYP3A5 incubates, metabolites **M1** and **M2** were major and were formed in a linear time-dependent fashion (Fig. 3). Notable differences between CYP3A4 and CYP3A5 were observed. Most significantly, CYP3A5 produced the major monooxidation metabolites **M1** and **M2** at a significantly faster rate than CYP3A4: ~ 32 -fold faster for **M1** and 2.6-fold faster for **M2**. A faster rate was also observed in HLM from a genetically determined CYP3A5 expressor (*1/*1) versus that from a genetically determined CYP3A5 nonexpressor (*3/*3) (Supplemental Fig. S6). It should be noted that atazanavir itself is a CYP3A inhibitor as measured by inhibition of triazolam hydroxylation in HLM (Perloff et al., 2005). The relative inhibition of atazanavir on CYP3A4 versus CYP3A5 was not evaluated in this study, and it is

not clear whether autoinhibition may influence the metabolite formation profiles. Nevertheless, the present study demonstrated metabolite formation differences between CYP3A4 versus CYP3A5 in vitro and showed consistent metabolite findings in humans receiving atazanavir.

The goal of the present study was to characterize the metabolite phenotype in individuals who express CYP3A5 and to also compare this phenotype between the AAs and NAAs who express CYP3A5. As demonstrated in Figs. 4 and 5, the **M1** and **M2** metabolite ratios were higher in those who expressed CYP3A5, regardless of race. Of these two metabolites, the **M1** ratio in the in vivo samples showed the most consistent metabolite phenotype (Fig. 4), results that were consistent with the in vitro data. The consistent metabolite findings suggest that other biological differences are driving the different atazanavir exposures in African Americans versus non-African Americans who express CYP3A5 (Table 2). *ABCB1* (encoding the P-glycoprotein efflux transporter) genotypes were evaluated in the parent study and found to be associated with atazanavir pharmacokinetics. However, the small sample sizes in the AA versus NAA groups expressing CYP3A5 ($n = 5$ and $n = 4$, respectively) limits the ability to attribute atazanavir PK differences to P-glycoprotein genetics on the background of CYP3A5 expression. Other possibilities include differences in intake or other efflux transporter function/expression (Rodríguez-Nóvoa et al., 2007; Lubomirov et al., 2010), differences in transcription factors for metabolic activity (Siccardi et al., 2008), or differences in acidity of the stomach influencing drug absorption (the latter was unlikely because standardized meals were given in the parent study and subjects taking antacids were excluded) (Zhu et al., 2010). It should be noted that the atazanavir metabolite AUC ratio is dependent on metabolite formation and metabolite clearance. The in vitro studies demonstrated **M1** and **M2** formation rate differences for CYP3A5 relative to CYP3A4. Although the study was not designed to quantify metabolite distribution volume and clearance, there was no difference in the in vivo metabolite half-life between CYP3A5 expressors versus nonexpressors (data not shown), which suggests that metabolite clearance was not influenced by CYP3A5 expression.

In conclusion, this study provides evidence for a consistent metabolite phenotype for atazanavir in genetically determined CYP3A5 expressors, regardless of race. This result is relevant for understanding atazanavir pharmacology in terms of CYP3A5 expression status observed in a previous study. This study also illustrates the apparent, and important, contribution of other factors on atazanavir pharmacokinetics even within individuals expressing CYP3A5. African-American race appears to be a predictor for the presence of these other phar-

FIG. 5. Metabolite **M2**/atazanavir AUC ratios in the three groups: group 1 (G1), non-African-American CYP3A5 nonexpressors (G1-NAA-NE); group 2 (G2), non-African-American CYP3A5 expressors (G2-NAA-E); and group 3 (G3), African-American CYP3A5 expressors (G3-AA-E). Data are presented as means $\pm$ S.E.M. *, $P < 0.05$; ***, $P < 0.001$.

macological factors. Future pharmacogenomic studies with atazanavir should include sampling from diverse populations and assessment of CYP3A5 expressor status along with other pharmacological variables to gain the most complete picture of atazanavir disposition in humans.

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Authorship Contributions

Participated in research design: Wempe and Anderson.

Conducted experiments: Wempe.

Performed data analysis: Wempe and Anderson.

Wrote or contributed to the writing of the manuscript: Wempe and Anderson.

References

- Anderson PL, Aquilante CL, Gardner EM, Predhomme J, McDanel P, Bushman LR, Zheng JH, Ray M, and MaWhinney S (2009) Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non-expressors. *J Antimicrob Chemother* **64**:1071–1079.
- Croom KF, Dhillon S, and Keam SJ (2009) Atazanavir: a review of its use in the management of HIV-1 infection. *Drugs* **69**:1107–1140.
- Lubomirov R, di Iulio J, Fayet A, Colombo S, Martinez R, Marzolini C, Furrer H, Vernazza P, Calmy A, Cavassini M, et al. (2010) ADME pharmacogenetics: investigation of the pharmacokinetics of the antiretroviral agent lopinavir coformulated with ritonavir. *Pharmacogenet Genomics* **20**:217–230.
- Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos* **27**:1350–1359.
- Perloff ES, Duan SX, Skolnik PR, Greenblatt DJ, and von Moltke LL (2005) Atazanavir: effects on P-glycoprotein transport and CYP3A metabolism in vitro. *Drug Metab Dispos* **33**:764–770.
- Rodríguez-Nóvoa S, Martín-Carbonero L, Barreiro P, González-Pardo G, Jiménez-Nácher I, González-Lahoz J, and Soriano V (2007) Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. *AIDS* **21**:41–46.
- Siccardi M, D'Avolio A, Baietto L, Gibbons S, Sciandra M, Colucci D, Bonora S, Khoo S, Back DJ, Di Perri G, et al. (2008) Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C→T) with reduced concentrations of unboosted atazanavir. *Clin Infect Dis* **47**:1222–1225.
- ter Heine R, Hillebrand MJ, Rosing H, van Gorp EC, Mulder JW, Beijnen JH, and Huitema AD (2009) Identification and profiling of circulating metabolites of atazanavir, a HIV protease inhibitor. *Drug Metab Dispos* **37**:1826–1840.
- Yumibe N, Huie K, Chen KJ, Snow M, Clement RP, and Cayen MN (1996) Identification of human liver cytochrome P450 enzymes that metabolize the nonsedating antihistamine loratadine. Formation of descarboethoxyloratadine by CYP3A4 and CYP2D6. *Biochem Pharmacol* **51**:165–172.
- Zhu L, Persson A, Mahnke L, Eley T, Li T, Xu X, Agarwala S, Dragone J, and Bertz R (2010) Effect of low-dose omeprazole (20 mg daily) on the pharmacokinetics of multiple-dose atazanavir with ritonavir in healthy subjects. *J Clin Pharmacol* doi: 10.1177/0091270010367651.

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