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Variability in drug metabolizing enzyme activity in HIV-infected patients

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Abstract

Aims—To evaluate variability in cytochrome P450 (CYP) 1A2, CYP2D6, CYP3A, N-acetyltransferase 2 (NAT2), and xanthine oxidase (XO) activity in HIV-infected patients and compare this with data from uninfected, healthy volunteers.

Methods—Ten HIV-infected men and seven women on medication affecting CYP enzyme activity were phenotyped four times over 2 months using caffeine, dextromethorphan, and midazolam. Urinary caffeine and dextromethorphan metabolite ratios were used to phenotype CYP1A2, NAT2, XO, and CYP2D6 activity and midazolam plasma clearance was used to phenotype CYP3A activity. Plasma and urine samples were analyzed by validated LC/UV or LC/MS methods for midazolam, caffeine, and dextromethorphan. Noncompartmental pharmacokinetics and nonparametric statistical analyses were performed, and the data compared with those of healthy volunteer historic controls.

Results—Compared with age and sex-matched healthy volunteers, HIV-infected subjects had 18% lower hepatic CYP3A4 activity, 90% lower CYP2D6 activity, 53% lower NAT2 activity, and 22% higher XO activity. No significant difference was found in CYP1A2 activity. Additionally, 25% genotype–phenotype discordance in CYP2D6 activity was noted in HIV-infected subjects. Intraindividual variability in enzyme activity increased by 42–62% in HIV-infected patients for CYP1A2, NAT2, and XO, and decreased by 33% for CYP2D6. Interindividual variability in enzyme activity increased by 27–63% in HIV-infected subjects for CYP2D6, CYP1A2, and XO, and decreased by 38% for NAT2. Higher plasma TNF α concentrations correlated with lower CYP2D6 and CYP3A4 activity.

Conclusions—Infection with HIV or stage of HIV infection may alter Phase I and II drug metabolizing enzyme activity. HIV infection was related to an increase in variability of these drug-metabolizing enzymes. Altered metabolism may be a consequence of immune activation and cytokine exposure.

Keywords

Cytokines; Drug-metabolizing enzymes; Metabolism; HIV

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This manuscript is the first investigation into the effects of chronic HIV infection on P450 enzyme activity. These data have implications for drug dosing with agents having narrow therapeutic indices, including antiretroviral drugs

Introduction

Drug metabolism occurs primarily in the liver and intestine by Phase I and II drug metabolizing enzyme systems [1]. The activity of these enzymes is exquisitely sensitive to endogenous and exogenous factors such as genetic polymorphisms, hormones, disease, drug therapy, and diet [2–5]. Specifically, drug metabolizing enzyme activity has been found to be altered in a number of infectious and inflammatory states such as bacterial pneumonia, viral respiratory infections, surgery, and trauma [6].

During states of infection and inflammation, the activity of CYP3A, CYP2D6, CYP1A2, and N-acetyltransferase (NAT) is down-regulated (or suppressed) by 20–70% [5,7,8]. Conversely, activity of xanthine oxidase (XO) has been shown to increase by 10–50% [9,10]. These changes can be clinically important during drug therapy, as the down-regulation of enzyme activity can lead to drug toxicity (a result of decreased drug clearance), or decreased efficacy (a result of decreased conversion of prodrugs to their active form) [4,5]. The effect of infection and inflammation on drug metabolizing enzymes has been ascribed to cytokines such as IL1 β , IL6, tumor necrosis factor alpha (TNF α), and interferon (INF) α or γ [4,5].

Patients infected with HIV may have increased circulating plasma cytokine concentrations [11–14], resulting from generalized immune activation [15]. These patients are also commonly on combination antiretroviral drug regimens that primarily rely on cytokine-sensitive drug metabolizing enzymes for clearance [16]. Some of these drugs, such as protease inhibitors and non-nucleoside reverse transcriptase inhibitors, have narrow therapeutic indices. Therefore, it is important to consider the potential for adverse effects if HIV infection causes altered drug metabolism. However, sparse data exist on constitutive drug metabolizing enzyme activity in these patients [7,8]. In this study, we used a cocktail phenotyping approach to estimate the activity of, and variability in, CYP1A2, CYP2D6, CYP3A, NAT2, and XO in 17 HIV-infected subjects, and compared those results with drug metabolizing enzyme activity from uninfected volunteers.

Materials and methods

Study design and subjects

This 4-week, observational, repeated-measure study was designed to measure the activity of three important cytochrome P450 enzymes (CYP1A2, CYP2D6, and CYP3A4), NAT2, and xanthine oxidase by phenotyping men and premenopausal women infected with HIV-1. The phenotyping approaches selected were based on those meeting the majority of standard phenotyping validation criteria [17], and are ones currently accepted by regulatory bodies [18–20]. All patients gave informed consent prior to study participation, and this protocol was approved by the Biomedical Institutional Review Board of the University of North Carolina (UNC) at Chapel Hill.

Subjects were screened for eligibility and recruited from the Infectious Diseases Clinic at UNC Hospitals. Prior to participation in the study, all subjects gave a complete history and underwent a physical examination, and women who were not surgically sterile underwent a urine pregnancy test. In order to be considered for the study, males and premenopausal women were required to be between the ages of 18 and 40, have documented evidence of HIV infection (confirmed by PCR), with a plasma HIV RNA < 20,000 copies/mL. Individuals were excluded if they had: undergone previous treatment with protease inhibitors or non-nucleoside reverse transcriptase inhibitors; previous treatment with an MAO inhibitor within 14 days of dextromethorphan therapy; taken any medications on a chronic basis that had been shown to inhibit or induce hepatic microsomal enzymes; been receiving investigational agents,

immunomodulators or corticosteroid therapy; malabsorption, severe chronic diarrhea or were unable to eat one or more meals a day due to nausea, emesis or abdominal/oral-esophageal discomfort; visceral Kaposi's sarcoma or lymphoma currently requiring chemotherapy and/or radiation, serious opportunistic infections requiring immediate treatment; unexplained fever for 14 days or more; been (in the judgment of the investigator) unable to comply with protocol requirements; a history of significant hypersensitivity; known or suspected CNS disorders predisposing them to seizures; clinically relevant history of alcohol and/or drug abuse; or significant gastrointestinal, renal, hepatic, bronchopulmonary, neurological, cardiovascular, oncological or allergic disease. Patients were also excluded if their neutrophil cell count was $<1,000/\text{mm}^3$, their platelet count was $<75,000/\text{mm}^3$, their hemoglobin was $<8.0 \text{ g/dL}$, their alkaline phosphatase, AST, ALT, or bilirubin were $\geq 1.5\times$ the upper limit of laboratory normal, or their estimated creatinine clearance was $<60 \text{ mL/min}$. Women were required to have regular (over 3 months) menstrual cycles (± 3 days). In addition, women were excluded if they were pregnant or breastfeeding, planning to become pregnant during the study, or if they were taking any hormonal birth control method or sex steroids.

The data collected from the HIV-infected subjects were compared with those of age and sex-matched healthy volunteers who underwent an identical cocktail phenotyping procedure. The 17 uninfected Caucasian volunteers were selected from a group of 20 subjects whose data have been previously published [21–23], in order to closely match age and sex. These 10 men and 7 women had a median age of 33 (30–42) years. All of the healthy individuals were non-smokers and had not been exposed to second-hand smoke.

Procedure

Males began the study at any time and came for four visits on 4 consecutive weeks. Females underwent phenotyping on four visits during the mid-follicular (days 3–5) and mid-luteal (days 17–20) phases of two consecutive menstrual cycles. To define ovulation, women were given Clearblue® Easy home ovulation kits and instructed to test first morning urine for qualitative luteinizing hormone (LH) 3 days prior to predicted mid-cycle, and to continue until a positive result was noted.

All patients were required to refrain from ingesting ethanol, chocolate, charbroiled foods, caffeine-containing beverages, grapefruit or grapefruit juice, and cruciferous vegetables (e.g., broccoli, cauliflower, cabbage, Brussels sprout, kale, watercress) for 3 days prior to each inpatient visit.

Subjects were admitted to the General Clinical Research Center at 0800 for each overnight visit. At this time, a blood sample was collected from women for estrogen and progesterone concentrations, and a urine sample was collected from all subjects. At approximately 0900, an intravenous catheter was inserted into the non-dominant arm of subjects for blood sampling, and a 0.025 mg/kg IV bolus dose of midazolam was administered into the antecubital vein of the dominant arm. Subjects' vital signs and pulse oximetry were continuously monitored for the hour following midazolam administration, and 10-mL blood samples were obtained immediately before, and 5 min, 30 min, 1, 2, 4, 5, and 6 h after midazolam administration. Urine was also collected during this time to ensure no interferences for subsequent urine analysis of caffeine and dextromethorphan metabolite ratios.

At the end of the blood collection period for midazolam, each patient was given an oral dose of caffeine tablets (No Doz® tablets) at the standard dose of 2 mg/kg , rounded to the nearest 50 mg, and 30 mg of dextromethorphan (Robitussin® Pediatric solution; 30 mg). Urine was collected in one aliquot in a refrigerated plastic jar containing 2 g ascorbic acid over the next 16 h to maintain a $\text{pH} < 4$ and ensure caffeine metabolite stability.

Blood samples were collected in Vacutainer tubes containing EDTA as the anticoagulant, and kept on ice for no more than 1 h after collection. Tubes were centrifuged at 2,800 rpm and 4°C for 15 min. The resulting plasma was aliquotted into cryovials and immediately frozen at -70°C until analysis. After total urine volumes had been measured, the urine samples were thoroughly mixed, and 15-mL aliquots transferred to cryovials and stored at -70°C until analysis. For urine samples to be assayed for caffeine and its metabolites, these aliquots were transferred to cryovials and additional ascorbic acid was added if necessary to maintain a pH of 3. Samples were mixed thoroughly before freezing.

Sample and data analysis

A sample size calculation was based on phenotyping data in healthy subjects [21–23], with the following intraindividual variability in enzyme activity: CYP1A2=20%, CYP2D6= 50%, CYP3A4=10%, NAT2=10%, and XO=5%. With 10 in each group (separated by sex), this variability would allow power greater than 85% to detect at least a 25% difference in CYP1A2, a 40% difference in CYP2D6, and a 10% difference in CYP3A4, NAT2, and XO. With 7 in each group, the variability allowed power of 80% to detect the aforementioned differences.

All samples were analyzed within 12 months of collection. Plasma samples were analyzed for midazolam concentrations by LC/MS as previously described [21]. Quantification of urinary caffeine and dextromethorphan metabolites was performed by HPLC/UV [22] and HPLC/FL analysis [23] respectively. Plasma IL1, IL6, and TNF α were analyzed by Quantikine HS ELISA kits from R&D Systems (Minneapolis, MN, USA) with an Opsys MRTM microplate reader.

CYP1A2 activity, or phenotype, was determined by the urinary caffeine metabolite ratio $(1U + 1X + AFMU)/17U$, where 1U = 1 methyluric acid, 1X = 1-methylxanthine, AFMU = 5-acetylamino-6-amino-3-methyluracil, and 17U = 1,7-dimethyluric acid. NAT2 activity was determined by the urinary caffeine metabolite ratios, $AFMU/(AFMU + 1U + 1X)$. A metabolic ratio less than 0.5 is associated with slow NAT2 acetylator phenotype and a ratio greater than 0.5 is associated with a rapid NAT2 acetylator phenotype [22]. Xanthine oxidase activity was determined by the urinary caffeine metabolite ratios $1U/(1X + 1U)$. The activity of CYP2D6 was determined by the O-demethylation ratio of $DM + 3MM/DR + 3-OH$, where DM = dextromethorphan, 3MM = methoxymorphinan, DR = dextrorphan, and 3-OH = 3-hydroxymorphinan.

The activity of CYP3A was determined by midazolam plasma clearance. Midazolam plasma concentration–time data were analyzed using noncompartmental methods with WinNonlin Pro (V 5.0.1). Statistical analyses were performed with STATA 8.0 and SigmaPlot V9.0, using nonparametric methods (Mann–Whitney Rank Sum Test and Wilcoxon Sign Test) to compare activity across menstrual cycle stages, between men and women, and between HIV-infected and uninfected populations. Intra-individual variability was calculated as the coefficient of variation for the repeated measures within each subject. Inter-individual variability was calculated as the coefficient of variation for the measures between subjects using the median value of each subject's repeated measures. Spearman's rank correlation was used to investigate relationships between cytokine concentrations and enzyme activity. Data are presented as geometric means (95% confidence interval), unless otherwise noted, and are compared using geometric mean ratios and 95% confidence intervals (GMR and CI_{95%}).

CYP2D6 genotyping was performed in the HIV-infected individuals at the same time as the testing in the healthy individuals [23]. The subjects were not matched a priori for genotype, but evaluated for genotype after study completion. The test panel was complemented by the recently described reduced function allele *CYP2D6*41* for this study. Updated genotyping procedures can be found in a recent report [24]. Briefly, high-quality genomic DNA was isolated from peripheral blood mononuclear cells. Subsequently, the entire coding region was

amplified and the PCR amplicon used as a template for a series of PCR-RFLP-based genotyping assays. Testing comprised the following allelic variants: *CYP2D6**2, *CYP2D6**3, *CYP2D6**4, *CYP2D6**5, *CYP2D6**6, *CYP2D6**7, *CYP2D6**8, *CYP2D6**9, *CYP2D6**10, *CYP2D6**11, *CYP2D6**12, *CYP2D6**15, *CYP2D6**17, *CYP2D6**29, *CYP2D6**41, as well as gene duplications.

NAT2 genotyping performed for the HIV-infected individuals was determined by a TaqMan allelic discrimination method [25]. All NAT2 alleles (*NAT2**4; *NAT2**5A, *NAT2**5B, *NAT2**5C, *NAT2**5D, *NAT2**5E, *NAT2**5F, *NAT2**5G, *NAT2**5H; *NAT2**6A, *NAT2**6B, *NAT2**6C, *NAT2**6E; *NAT2**7A, *NAT2**7B; *NAT2**10; *NAT2**11; *NAT2**12A, *NAT2**12B, *NAT2**12C; *NAT2**13; *NAT2**14A, *NAT2**14B, *NAT2**14C, *NAT2**14D, *NAT2**14E, *NAT2**14F, *NAT2**14G), except very rare ones, are readily detected with this method. As previously reviewed [26], deduction of NAT2 phenotypes is assigned based on co-dominant expression of rapid and slow acetylator NAT2 alleles or haplotypes. Individuals homozygous for rapid NAT2 acetylator alleles are deduced as rapid acetylators and individuals homozygous for the slow acetylator NAT2 allele are deduced as slow acetylators. Individuals with one rapid and one slow NAT2 acetylator allele are deduced as intermediate acetylators.

Results

Subject demographics

Seventeen HIV-infected volunteers participated in the study: 10 men and 7 women, 12 African Americans and 5 Caucasians. Due to recruitment difficulties with this population, only 7 female subjects were enrolled. None of the African Americans in the study had sickle cell disease. Their median age was 36 (31–41) years, median plasma HIV RNA was 3.0 (2.6–3.7) log copies/mL, and the median CD4+ T cell count was 534 (440–671) cells/ μ L. As this study was conducted between March 1998 and May 2000, 12 subjects (70%) were not on antiretroviral therapy, 3 were on a 2',3'-dideoxyinosine/hydroxyurea (ddI/HU) regimen, and 2 were on a ZDV/3TC (zidovudine/lamivudine) regimen, as was the standard of care at the time. None of the drugs used in these treatment regimens have any effect on the CYP enzyme system. Of the 10 men enrolled in the study, 7 completed all visits (2 subjects missed one visit each, and 1 subject missed two visits). Of the 7 HIV-infected women, 4 completed all visits (3 subjects missed two visits). In total, 15 of the 17 HIV-infected subjects were genotyped, and 14 of the 17 uninfected subjects have genotypic data.

As demonstrated in previous investigations, no significant differences were found for any of the phenotypes between men and women or between the midfollicular and midluteal phases of the menstrual cycle. Therefore, the data were combined.

CYP3A4 activity

Midazolam clearance was also compared in HIV-infected subjects and the non-infected volunteer historic control group. Midazolam clearance was significantly lower in HIV-infected patients, 6.16 (5.49–6.92) mL/min/kg, compared with healthy volunteers, 7.77 (7.01–8.62) mL/min/kg (GMR and CI_{95%}: 0.79 [0.68–0.92]). This difference remained significant when only Caucasian subjects were compared (GMR and CI_{95%}: 0.71 [0.57–0.89]). No meaningful differences were found in intraindividual and interindividual variability in midazolam clearance between HIV-infected subjects and the healthy volunteers. For intraindividual variability, the median coefficient of variation (CV) for the HIV-infected patients was 11.4 (8.9–23.7)% and for healthy volunteers was 10.0 (9.2–11.1)%. For interindividual variation, HIV-infected patients had a midazolam clearance CV of 21.7%, and non-infected volunteers had a CV of 23.3%.

CYP2D6 activity

As seen in Fig. 1, the urinary DM ratio was significantly higher (GMR and $CI_{95\%}$: 10.02 [2.36–42.48]) in HIV-infected subjects (0.062 [0.02–0.24]) than in healthy volunteers (0.0066 [0.0031–0.014]), indicating lower CYP2D6 activity. Intraindividual variability in the DM ratio was greater in uninfected controls (48.9 [30.9–95.7]%) than in HIV-infected individuals (32.6 [24.9–47.4]%). The opposite was found for interindividual variability; 80.3% in HIV-infected individuals and 57.3% in healthy volunteers.

Based on the urinary ratios measured, 5 out of 17 (30%) of the HIV-infected volunteers and 1 out of 17 (6%) of the uninfected volunteers were determined to be poor metabolizers (PM) by phenotype. Genotype results for 15 of these HIV-infected subjects as well as 14 of their matched controls are listed in Tables 1 and 2. Only 1 HIV-infected patient was a CYP2D6 PM by genotype. To examine this genotype–phenotype discordance, subjects were divided into those with no functional CYP2D6 alleles, those with one fully functional CYP2D6 allele, and those with two fully functional CYP2D6 alleles (Fig. 1). Both HIV-infected and -uninfected individuals with no functional alleles appropriately showed DM urinary ratios above the anti-mode of 0.3 [27]. The HIV-infected subjects with one functional allele had lower activities than the healthy volunteers (DM urinary ratio 0.084 [0.025–0.29]; vs 0.0056 [0.0019–0.018] respectively). The GMR and $CI_{95\%}$ for this comparison was 14.16 (3.06–65.65). Two of these HIV-infected subjects were PMs by phenotype. The DM urinary ratios of the HIV-infected subjects with two functional alleles (0.035 [0.0016–0.75]) did not differ from that of the healthy volunteers, which was 0.0049 (0.0031–0.0077; $n=8$). The GMR and $CI_{95\%}$ for this comparison was 7.05 (0.54–91.86). However, 2 out of 7 HIV-infected subjects with two functional alleles were considered PMs by phenotype. The phenotype did not change from one measure to the next, but rather it remained stable across all time points tested. Finally, to remove the confounder of race in this comparison, Caucasian subjects with one functional allele were isolated in the HIV-infected and -uninfected cohort (Fig. 1). Although the number of subjects is small, the HIV-infected individuals showed significantly less CYP2D6 activity (0.17 [0.022–1.35]) than the uninfected individuals (0.0035 [0.0011–0.011]) with a GMR and $CI_{95\%}$ of 49.02 (6.40–375.27).

CYP1A2 activity

As illustrated in Fig. 2, after excluding smokers from the analysis (28), the activity of CYP1A2 was not significantly different in HIV-infected and uninfected nonsmokers (9.43 [4.60–19.32] vs 5.87 [4.17–8.27]; GMR and $CI_{95\%}$: 1.61 [0.83–3.11]).

Intra- and interindividual variability in CYP1A2 activity was found to be greater in HIV-infected nonsmokers compared with uninfected nonsmokers. For intraindividual variability, the median CV for the HIV-infected patients was 26.7% (range 20.0–37.0%), compared with 14.6% (range 10.9–23.3%) for uninfected volunteers. For interindividual variation, the CV for the urinary caffeine ratio was 203.0% in HIV-infected patients vs 106.0% in uninfected volunteers.

NAT2 activity

As demonstrated in Fig. 3a, NAT2 activity was decreased in HIV-infected subjects compared with healthy controls (0.15 [0.086–0.25] vs 0.43 [0.36–0.52], GMR and $CI_{95\%}$: 0.34 [0.20–0.57]). Intraindividual variability in the NAT2 urinary ratio (AFMU/(AFMU + 1U + 1X)) was greater in HIV-infected subjects compared with healthy volunteers (28.0% [range 24.1–34.1%] vs 10.5% [range 3.2–20.3%]). Interindividual variability in this ratio was lower in HIV-infected patients compared with non-infected volunteers (43.3% vs 72.8% respectively).

Based on the urinary AFMU/(AFMU + 1U + 1X) ratios measured, all HIV-infected patients were phenotypically NAT2 slow acetylators (ratio <0.5). However, the NAT2 genotypes of only 7 out of 15 HIV-infected patients were classified as slow acetylators, as they had one of the following genotypes; *NAT2*5B/6A*, *5C/5C*, *5B/5B* and *6A/6A* (Table 1). In contrast, 6 of the 17 uninfected volunteers (35%) were of NAT2 rapid acetylator phenotype. Genotyping was not performed on the uninfected volunteers, as their phenotyping results were as expected in this population and an excellent correlation exists between NAT2 phenotype and genotype in healthy individuals [25].

Xanthine oxidase activity

Xanthine oxidase activity was increased (GMR and CI_{95%}: 1.23 [1.12–1.34]) in the HIV-infected subjects compared with the uninfected controls, with a urinary caffeine ratio of 0.77 (0.70–0.85) vs 0.63 (0.61–0.65) respectively (Fig. 3b). Intraindividual variability was similar in HIV-infected subjects and uninfected volunteers. The median coefficient of variation for the XO urinary ratio was 10.1% (range 3.7–15.0%) in the HIV-infected subjects and 4.6 (3.4–8.2)% in the uninfected controls. Interindividual variation was higher in the HIV-infected patients (142.0%) compared with uninfected volunteers (55.1%).

Cytokine activity

Plasma concentrations of IL-6 and TNF α were measured in HIV-infected patients and healthy volunteers. For HIV-infected patients IL-6 and TNF α concentrations were 2.95 (2.26–3.84) and 5.86 (4.7–7.31) pg/mL respectively. For non-infected patients, IL-6 and TNF α concentrations were 1.05 (0.69–1.60) and 1.25 (1.01–1.54) pg/mL respectively. These concentrations were significantly higher in HIV-infected individuals than in healthy volunteers (GMR and CI_{95%} for IL6 was 2.80 (1.71–4.57), and for TNF α it was 4.70 (3.50–6.31). No significant relationship was found between IL-6 concentrations and hepatic CYP3A4, CYP2D6, CYP1A2, enzyme activity ($r < 0.4$, $p > 0.2$). However, with increasing TNF α concentrations, midazolam clearance (and thus CYP3A enzyme activity) decreased ($r = -0.66$, $p = 0.008$) as shown in Fig. 4a, and urinary dextromethorphan ratios increased (and thus CYP2D6 enzyme activity decreased; $r = 0.49$, $p = 0.06$), which is shown in Fig. 4b. Although the relationship between cytokine concentration and CYP2D6 activity did not achieve statistical significance, this is an interesting trend in the data. These relationships did not change when multiple regression analysis was performed with variables such as age, sex, ethnicity, treatment status, HIV RNA plasma concentrations, and CD4+ T cell counts (data not shown).

Discussion

The purpose of this study was to describe the variability of drug metabolizing enzyme activity in HIV-infected subjects with detectable HIV RNA, but a stable clinical presentation; in addition, it was to compare enzyme phenotype between age and sex-matched HIV-negative and HIV-positive subjects. All subjects were not on medications known to induce or inhibit CYP enzyme activity. As this study was conducted between March 1998 and May 2000, we were able to recruit subjects not taking protease inhibitors or non-nucleoside reverse transcriptase inhibitors. This presented a unique opportunity to evaluate the influence of low-level HIV replication on drug metabolizing enzymes in isolation of confounding drug therapies. A cocktail phenotyping approach was used to estimate the activities of CYP3A, CYP2D6, CYP1A2, NAT2, and XO in 17 HIV-infected volunteers. This study was conducted using the same methods and analytical techniques that had been previously performed in uninfected healthy volunteers [21–23]. These controls were used for comparison, and selected for sex and age, but not matched on race.

In this study, HIV-infected subjects had decreased activities of CYP3A, CYP2D6, and NAT2 compared with healthy volunteers, while XO activities were increased and CYP1A2 (after correcting for smoking status) were similar. CYP3A is the predominant drug metabolizing enzyme in the liver. Reduced CYP3A activity (by 30–75%) has been documented in patients following surgery and infection [29–32]. However, only one other study has evaluated CYP3A activity in HIV-infected individuals. Slain et al. [33] demonstrated that 39 HIV-infected patients had increased variability in CYP3A activity, as measured by the erythromycin breath test. However, these results were not surprising, as 50% of these patients were taking known inducers or inhibitors of CYP3A. The data obtained in the present study overcome any confounding by concomitant medications, and strengthens the estimates of variability by repeatedly measuring CYP activity in individual subjects. Our data can be added to the previous findings of infection-and inflammation-altering CYP enzyme activity by demonstrating a significant decrease in CYP3A activity (as measured by midazolam plasma clearance) in HIV-infected individuals compared with age- and sex-matched uninfected controls. CYP3A4 genotyping was not performed as polymorphisms have not been associated with changes in metabolism [34]

A significant decrease in CYP2D6 activity in HIV-infected subjects compared with non-infected volunteers was also found ($p=0.003$). One investigation in 61 HIV-positive, Caucasian male patients found 2 subjects who had genotypes consistent with a CYP2D6 extensive metabolizer phenotype, presenting as poor metabolizers [8]. Our data support this finding, as 5 of our 17 HIV-positive subjects were PM by phenotype, but only 1 of these 5 was found to be a PM by genotype. The observation of phenocopy in 24% of our HIV-infected subjects suggests that HIV infection itself may downregulate CYP2D6 activity.

Lower CYP2D6 enzyme activity has been previously associated with race and certain genetic polymorphisms [35]. Generally, CYP2D6 activity is lower in black populations compared with Caucasians (i.e., higher mean urinary ratio, also referred to a right shift in activity) [24]. This observation can be explained, in part, by the presence of alleles conferring reduced function such as *CYP2D6**17, *29 and *41 [36–38]. A study of *CYP2D6* genotyping and phenotyping with debrisoquine in 248 Caucasians and 104 African Americans found no difference in phenotype between Caucasians and African Americans without the *CYP2D6**17 allele [24, 39], and confirmed lower activity in African Americans with the *CYP2D6**17 allele. However, an investigation of 201 West Africans found a higher mean metabolic ratio associated with two functional alleles for *CYP2D6**1 and *2, compared with Caucasians [40]. Among HIV-infected patients in the present study, two African Americans carrying the *CYP2D6**17 allele did not have CYP2D6 activity that differed significantly from our healthy volunteer data. To control for the potential confounding variable of race, we separated Caucasian subjects carrying one functional allele and compared phenotypes in HIV-infected (7) and non-infected (5) subjects. Although the numbers are small, we found significantly lower CYP2D6 activity ($p=0.001$) in HIV-infected patients, supporting the hypothesis that HIV infection may downregulate CYP2D6 activity.

The lower drug metabolizing enzyme activity seen in HIV-infected subjects may also decrease the efficacy of antidepressants and antipsychotics. This is significant because studies have shown that infected individuals are more likely to suffer depression than uninfected individuals [38]. According to the National Institute of Mental Health, HIV-infected individuals are at twice the risk of suffering depression compared with uninfected individuals [41]. Most antidepressants/psychotics, including selective serotonin reuptake inhibitors like fluoxetine, sertraline, and paroxetine, as well as tricyclic antidepressants such as imipramine, desipramine, and amitriptyline, are metabolized by CYP2D6, and this may lead to increased adverse effects.

Caffeine was used to measure the activity of CYP1A2, NAT2, and XO. Once CYP1A2 activity was controlled for smoking status, it was not significantly different in HIV-infected subjects than uninfected controls. However, in non-smokers, intra- and interindividual variability in CYP1A2 activity was greater in HIV-infected individuals. Previous studies have shown that CYP1A2 activity is decreased in viral infections and inflammation [42,43]. Patients with chronic hepatitis B infection (16) and cirrhosis (12) had 47.0–78.7% lower CYP1A2 activity (as measured by phenacetin clearance) than healthy volunteers [44]. Children with upper respiratory infections have 60% lower CYP1A2 activity (as measured by theophylline clearance) compared with that found in healthy states [42]. It is not clear why the HIV-infected patients investigated in this study had increased variability. Since the caffeine dosing and urine sampling and analysis were identical in the two studies compared here, methodological differences can be ruled out. However, as exposure to second-hand smoke was not evaluated in the HIV-infected subjects, this may have confounded the results [43,45].

In HIV-infected subjects, this study found 53% lower NAT2 activity, and 22% higher XO activity than in uninfected controls. Our results also confirm previously documented data demonstrating that the NAT2 genotype–phenotype relationship is altered in HIV-infected individuals. In one study of 50 HIV-infected subjects, 12 out of 24 (50%) of NAT2 rapid acetylators by genotype were slow acetylators by phenotype [7]. A second study found that 93% of AIDS patients with acute illness were NAT2 slow acetylators by phenotype compared with 66% of non-ill AIDS patients and 62% of healthy subjects [45]. A subsequent investigation into 85 subjects (39 HIV-positive subjects, 46 healthy controls) found no difference in the frequency of NAT2 slow acetylator genotypes between AIDS patients with acute illness and healthy controls [46]. In our study, all HIV-infected individuals were found to be slow acetylators by NAT2 phenotype, while only 7 of the 15 were slow acetylators by NAT2 genotype.

A trend toward increased XO activity has been previously documented in AIDS patients with acute illness, compared with patients with stable AIDS or HIV-infected patients without AIDS [47]. It is proposed that interferon and its inducers (often present in states of inflammation and infection) increase XO activity, with the generated superoxide subsequently degrading CYP enzymes [45,47–49]. However, a causal relationship between XO activity and the downregulation of P450 enzymes has not been clearly delineated [50,51]. Our data, with increased XO activity and decreased CYP2D6 and CYP3A4 activity in HIV-infected subjects, are provocative, but further investigation is required to understand this relationship.

Finally, we investigated the relationship between circulating plasma concentrations of IL-6 and TNF α and enzyme activity. Both IL-6 and TNF α are normally produced during infection and the inflammatory response, and it has been previously demonstrated that HIV-infected individuals produce more proinflammatory cytokines [52]. Additionally, in vitro studies have demonstrated that IL1, IL6 and TNF α decrease CYP3A and CYP2D6 activity [53–56]. Although this investigation measured peripheral cytokine concentrations, a correlation was found between increased TNF α concentrations and decreased CYP3A and CYP2D6 enzyme activity.

Although the number of HIV-infected subjects in this study was limited, the population investigated was unique in that patients were selected with stable disease, and were not on medications that could influence drug metabolizing enzyme activity. The repeated measures design allowed investigation of intra-individual variability in drug metabolizing enzyme activity. In accordance with previous data, this study demonstrated that sex or menstrual cycle phase do not have a clinically significant effect on drug metabolizing enzyme activity. These results suggest that HIV infection, or stage of HIV infection, may alter drug metabolism and

exposure. This has implications for efficacy and/or toxicity of drugs with narrow therapeutic indices, such as antiretroviral agents.

The Department of Health and Human Safety guidelines state that therapy should be deferred in asymptomatic HIV-infected patients with CD4+ T cell counts >350 and RNA <100,000 [16]. According to these guidelines, patients may have active HIV RNA replication for a number of years before treatment. Therefore, there is a need for larger studies to determine whether measures of cytokine concentrations might be helpful in predicting drug metabolizing enzyme activity to assist the provider in choosing the best regimen for an infected individual.

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Fig. 1. CYP2D6 activity in HIV-infected subjects vs uninfected subjects. The *solid line* represents the antinode of 0.3. The individual data points of the Caucasian subjects are represented by the *open circle* and those of the African American subjects are represented by the *cross*. **a** Comparison of CYP2D6 activity in all HIV-infected subjects ($n=17$) and uninfected volunteers ($n=17$; $p=0.003$). **b** Comparison of functional allele frequency in HIV-infected subjects vs uninfected subjects (15 HIV-infected with genotype data, 14 uninfected with genotype data). 0 functional alleles: 1 for HIV-infected and 1 for HIV-uninfected. 1 functional allele: 7 for HIV-infected and 5 for HIV-uninfected. 2 functional alleles: 7 for HIV-infected and 8 for HIV-uninfected. **c** Comparison of CYP2D6 activity between Caucasian subjects with one functional allele (4 for HIV-infected subjects and 3 for HIV-uninfected subjects, $p=0.001$)

Fig. 2.
CYP1A2 activity in HIV-infected subjects (8 smokers, 6 nonsmokers) vs uninfected subjects (17; $p=0.2$)

Fig. 3. NAT2 and xanthine oxidase (XO) phenotype in HIV-infected subjects (17) vs uninfected subjects (17). **a** Comparison of NAT2 activity in HIV-infected and -uninfected subjects ($p=0.0007$). **b** Comparison of XO activity in HIV-infected and uninfected subjects ($p=0.0003$)

Fig. 4.

Tumor necrosis factor alpha (TNF α) concentrations in HIV-infected subjects compared with CYP3A4 and 2D6 activity. **a** Comparison of CYP3A4 activity and TNF α concentrations in all HIV-infected subjects (17). **b** Comparison of CYP2D6 activity and TNF α concentrations in all HIV-infected subjects (17)

Table 1
CYP2D6 genotype–phenotype discordance in HIV-infected subjects and uninfected subjects.

DM/DX ratio	HIV-infected subjects		African Americans		Uninfected subjects	
	Caucasian					
	DM/DX (%)	Genotype(s)	DM/DX (%)	Genotype(s)	DM/DX (%)	Genotype(s)
>0.3	3 (60)	*4*5, *1*4, *4*4I	2 (17)	*1*17, *17*4I	1 (7)	*4*4
0.1–0.3	0	NA	1 (8)	ND	0	NA
0.01–0.1	2 (40)	*3*4I, *5*4I	4 (33)	*4*9, *2*5, *1*2, *1*5	2 (14)	*2*2, *2*4
0.001–0.01	0	NA	5 (42)	*1*17, *10*17, *1*29, *2*29, ND	11 (78)	*1*2(4), *1*4(2), *2*4 (2), *2*2(2), *1*10(1)

Numbers in parentheses after genotype reflect the number of subjects found to have that genotype.

DM dextromethorphan, DX dextrothorphan, NA not available, ND no data

Table 2
NAT-2 genotype-phenotype discordance in HIV-infected subjects and uninfected subjects

NAT-2 ratio	HIV-infected subjects			Uninfected subjects	
	Caucasian		African Americans		
	AFMU/(AFMU + IU + IX)	Genotype(s)	AFMU/(AFMU + IU + IX)	AFMU/(AFMU + IU + IX)	Genotype
≥0.5 (rapid)	0	NA	1 (8)	6 (35)	ND
0.3–0.5	0	NA	5 (42)	9 (53)	ND
0.1–0.3	0	NA	5 (42)	2 (12)	ND
0.001–0.1 (slow)	5 (100)	*5B/*5B (2), *5B/*6A (3)	1 (8)	0	ND

Numbers in parentheses after genotype reflect the number of subjects found to have that genotype.
AFMU 5-acetylaminio-6-amino-3-methyluracil