Short Communication

The Influence of CYP3A5 Genotype on Dexamethasone Induction of CYP3A Activity in African Americans

Received December 9, 2007; accepted May 16, 2008

ABSTRACT:
The CYP3A5*1 allele has been associated with differences in the metabolism of some CYP3A substrates. CYP3A5 polymorphism may also influence susceptibility for certain drug interactions. We have previously noted a correlation between basal CYP3A activity and the inductive effects of dexamethasone using the erythromycin breath test (ERBT). To determine whether CYP3A5 polymorphism influences induction of CYP3A activity, we examined the effect of an antiemetic dose of dexamethasone, and the prototypical inducer rifampin, on the ERBT in African American volunteers prospectively stratified by CYP3A5*1 allele carrier status.

Mean basal ERBTs were significantly higher in CYP3A5*1 carriers (2.71 ± 0.53%) versus noncarriers (2.12 ± 0.37%, P = 0.006). Rifampin increased ERBTs in CYP3A5*1 carriers (4.68 versus 2.60%, P = 0.0008) and noncarriers (3.55 versus 2.11%, P = 0.0017), whereas dexamethasone increased ERBTs only in CYP3A5*1 noncarriers (3.03 versus 2.14%, P = 0.031). CYP3A5 polymorphism appears to influence susceptibility to induction-type drug interactions for some inducers, and CYP3A5*1 noncarriers may be more susceptible to the inductive effects of dexamethasone as a result of lower basal CYP3A activity.

Members of the cytochrome P450 3A (CYP3A) subfamily of drug-metabolizing enzymes are the most abundantly expressed cytochrome P450 enzymes in human liver and are involved in the metabolism of nearly 50% of clinically used drugs (Wilkinson, 2005). In adults, hepatic CYP3A activity reflects primarily the net contributions of CYP3A4 and CYP3A5, which share overlapping substrate specificities but differ with regard to tissue expression and transcriptional regulation (Gibson et al., 2002; Goodwin et al., 2002; Burk and Wojnowski, 2004). Recent efforts to understand interindividual variability in CYP3A activity have focused primarily on CYP3A polymorphisms because variability in the contribution of functional CYP3A5 activity could influence an individual’s susceptibility to inducer- or inhibitor-mediated drug interactions. The major CYP3A5 polymorphisms include the CYP3A5*3, *6, and *7 alleles, which are functionally inactive because of single nucleotide polymorphisms that result in either splice defects or the introduction of an early stop codon that results in reduced production of the full-length active protein (Lamba et al., 2002; Wojnowski, 2004; Xie et al., 2004). CYP3A5*1 is the only functional CYP3A5 allele known to contribute to total CYP3A activity, and the frequency of the CYP3A5*1 allele has been shown to differ among ethnic groups (Kuehl et al., 2001). The CYP3A5*1 allele has been associated with higher midazolam systemic clearance and tacrolimus dose requirements (Kuehl et al., 2001; Lin et al., 2002; Wong et al., 2004) and greater metabolism of quinidine and saquinavir (Mouly et al., 2005; Mirghani et al., 2006).

Less is known about the influence of the CYP3A5*1 allele on susceptibility for drug interactions caused by different CYP3A inducers and inhibitors. Recently, the CYP3A5*1 allele was shown to influence susceptibility to the inhibitory effects of fluconazole but only in a substrate-dependent manner (Isoherranen et al., 2008). We have previously noted a correlation between basal hepatic CYP3A activity and the inductive effects of dexamethasone in healthy volunteers using the erythromycin breath test (ERBT) (McCune et al., 2000). Whereas the effect of CYP3A5 polymorphism on the induction of CYP3A activity by glucocorticoids has not been explored, the presence of the CYP3A5*1 allele does not appear to influence induction of hepatic CYP3A activity by the potent CYP3A4 inducer rifampin when assessed by the systemic clearance of midazolam or by the ERBT (Floyd et al., 2003; Yu et al., 2004). We hypothesized that the influence of CYP3A5 polymorphism on induction-type drug interactions could vary with different inducers. Therefore, the effect of an antiemetic dose of dexamethasone on CYP3A activity, as determined by the ERBT, was compared with that of the prototypical inducer rifampin in a cohort of young, healthy African American volunteers prospectively genotyped for the presence of the CYP3A5*1 allele.

Materials and Methods

Study Participants. Healthy, unrelated volunteers self-identified as African American (n = 84) were prospectively genotyped to identify potential study participants. The volunteers, ranging in age from 18 to 45 years, were genotyped for CYP3A4*1B and CYP3A5*5, *6, and *7 alleles from a mouthwash sample using polymerase chain reaction–restriction fragment length polymorphism (Lin et al., 2002). Subjects were prospectively stratified according to CYP3A5 genotype without regard to smoking status or to the presence of the

ABBREVIATIONS: ERBT, erythromycin breath test; LD, linkage disequilibrium; PXR, pregnane X receptor.
CYP3A4*1B allele. To determine the effect of CYP3A5 genotype on rifampin induction, 14 subjects were grouped according to the presence (CYP3A5*1/*1 and *1/*3, *1/*6, or *1/*7) or absence (CYP3A5*3/*3 or CYP3A5*5, *6, or *7/*6, *7) of the CYP3A5*1 allele. Likewise, 12 subjects were similarly stratified to determine the effect of CYP3A5 genotype on CYP3A induction by dexamethasone. Before enrollment, all the subjects received a physical examination consisting of vital signs (pulse, blood pressure, respiration, and body temperature), auscultation of heart and lungs, and routine clinical laboratory tests. Individuals with a diagnosis or history of cancer, significant organ dysfunction or disease, human immunodeficiency virus, or taking medications known to induce or inhibit CYP3A were excluded. Individuals with risk factors for glucocorticoid-associated osteonecrosis, such as diagnosis or history of systemic lupus erythematosus, recent radiation exposure, recent history of major trauma, history of steroid use in the previous 12 months, histological disease, gout/hyperuricemia, or hyperlipidemia were excluded from participation in the dexamethasone treatment group. A negative tuberculin skin test was required for all the subjects. In addition, a negative pregnancy test within 7 days of ERBT administration was required for female subjects. No alcohol, herbal medications, or foods or beverages containing grapefruit juice were permitted for the duration of the study.

Study Protocol. The study protocol was approved by the University of North Carolina Biomedical Institutional Review Board, and all the subjects provided written informed consent before enrollment. This was a parallel design study that allowed crossover into the other treatment arm. After the screening visit, basal hepatic CYP3A activity was determined by the ERBT as previously described (Watkins et al., 1989), and the percentage of the erythromycin dose exhaled per hour was calculated from the measured amount of 14C-erythromycin (Isokerrnan et al., 2008). Subjects then received one of two CYP3A inducers for 5 days: 600 mg of rifampin p.o. once daily or 8 mg of dexamethasone p.o. twice daily, and then on day 6 the ERBT was repeated. The extent of induction in the ERBT was calculated by expressing the difference between ERBT values, obtained before and after each inducer, as a percentage of each subject’s baseline ERBT.

Dexamethasone and rifampin were self-administered on an outpatient basis. Compliance was assessed through study calendars in which subjects documented the time of drug self-administration. Subjects qualifying and consenting to both inducer treatments underwent a minimum 14-day washout period between receipt of rifampin and dexamethasone. During the dexamethasone phase of the study, subjects were asked to monitor daily their blood pressure with a home monitoring cuff, as well as glucose levels with a urine dipstick kit. Subjects were contacted daily by telephone during the induction phase to assess compliance, monitoring parameters, and adverse events.

Statistical Analysis. All the statistical analyses were performed using SAS-IMP (SAS Institute Inc., Cary, NC). The choice of sample size was guided by considerations of the expected statistical precision, expected power of the test procedure for the primary outcome, which was selected based on findings from our previous study (McCune et al., 2000), study feasibility, cost, and the aims of the study. A sample size of six per group provided sufficient power to detect an effect size of 50%; however, a sample size of eight per group provided sufficient power to detect an effect size of 30% (P < 0.05) was considered statistically significant, and data are presented as mean ± S.D. In the planning stage, estimated power curves were plotted as functions of plausible magnitudes of treatment effects and variance based on previous inductive effects observed with rifampin and dexamethasone (Gharabieh et al., 1998; McCune et al., 2000).

Results

CYP3A5 Genotyping. For the 84 subjects who originally consented and were genotyped for this study, the frequency of the CYP3A5*1 allele was 66%. Genotype distribution showed 24 individuals carrying the CYP3A5*1/*1 genotype (29%), 20 individuals carrying the CYP3A5*1/*3 genotype (24%), 7 individuals carrying the CYP3A5*1/*6 genotype (8.3%), and 4 carrying the CYP3A5*1/*7 genotype (4.8%). One individual each was genotyped as CYP3A5*6/*7 and CYP3A5*6/*6. Eight individuals carried the CYP3A5*3/*6 genotype (9.5%), and six carried the CYP3A5*3/*3 genotype (7.1%). The homozygous variant genotype, CYP3A5*3/*3, was carried by 13 individuals (15.5%). The frequency distributions of all the CYP3A5 alleles were in Hardy-Weinberg equilibrium (P ≥ 0.035). These allele frequencies were in agreement with previous reports involving African American subjects (Kuehl et al., 2001).

Linkage disequilibrium (LD) between CYP3A4*1B and CYP3A5*1 alleles was not observed (D' = 0.025, Haploview version 3.11, http://www.broad.mit.edu/mpg/haploview/index.php) when an additional 51 subjects genotyped for major CYP3A4 and CYP3A5 polymorphisms were pooled for a total of 135 African Americans for this analysis. This result was consistent with our observation from the HapMap data (http://www.hapmap.org) that CYP3A4*1B and CYP3A5*1 genotypes were not in LD in African Americans when CYP3A5 genotypes were visually sorted by CYP3A4*1B (data not shown).

Effect of CYP3A5 Genotype on Extent of Induction. Of the 27 volunteers who consented to participate, 21 completed the study. Of the six dropouts, two in the rifampin arm did not return for treatment. In the dexamethasone arm, two did not return for treatment, and two withdrew after initiating dexamethasone treatment. Of these latter two subjects, one experienced irretactable hiccups beginning on day 3 of dexamethasone treatment, which lasted more than 24 h but resolved 24 h after discontinuing the dexamethasone; the other subject completed the 5-day course of dexamethasone, but i.v. access could not be obtained to draw labs or administer the second ERBT. Fourteen subjects completed the rifampin arm of the study (7 CYP3A5*1 carriers, 7 noncarriers), and 12 subjects completed the dexamethasone arm (6 carriers, 6 noncarriers). Three CYP3A5*1 carriers and two noncarriers participated in both arms of the study.

To evaluate the influence of CYP3A5 genotype on basal CYP3A activity, the basal ERBT values from both rifampin and dexamethasone arms of the study were pooled. For the five subjects who completed both arms of the study, the mean of both basal ERBT values (one from the rifampin arm and one from the dexamethasone arm) was included in a weighted least-squares analysis depicted in Fig. 1. In addition, neither the level of significance nor conclusion was changed if either basal value for these five subjects was used in separate analyses. The mean basal ERBT value for all the subjects was found to be significantly higher for individuals who carried at least one CYP3A5*1 allele compared with individuals who lacked a CYP3A5*1 allele (2.71 ± 0.53 versus 2.12 ± 0.37%, P = 0.006).

Rifampin significantly increased CYP3A activity in carriers of the CYP3A5*1 allele from a baseline mean ERBT of 2.60 ± 0.66 to 4.68 ± 1.03% (P = 0.0008) and in noncarriers of the CYP3A5*1 allele from a baseline mean ERBT of 2.11 ± 0.43 to 3.55 ± 0.9% (P = 0.0017) (Fig. 2A). Similarly, an antiemetic regimen of dexamethasone increased CYP3A activity in noncarriers of the CYP3A5*1 allele from a baseline mean ERBT of 2.14 ± 0.31 to 3.03 ± 0.94% (P = 0.031) (Fig. 2B). In contrast to the effect of rifampin, an increase in CYP3A activity following dexamethasone administration was not detected in African Americans who carried the CYP3A5*1 allele (mean baseline ERBT of 2.83 ± 0.35 to 3.30 ± 0.85%, P = 0.160) (Fig. 2B). Because baseline values differed between the two CYP3A5 genotypes, the change from...
baseline to induced level of the ERBT was also compared between CYP3A5*1 carriers and noncarriers for both inducers. The change in the ERBT following rifampin treatment was similar for both carriers (2.09 ± 1.02%) and noncarriers (1.44 ± 0.81%) of the CYP3A5*1 allele. Although an approximately 2-fold difference in the change in the ERBT was observed between CYP3A5*1 carriers (0.47 ± 1.05%) and noncarriers (0.89 ± 0.91%) following administration of dexamethasone, this difference did not reach statistical significance because of greater variance observed with this inducer compared with rifampin.

Several studies have suggested that women have higher hepatic CYP3A activity (as measured by the ERBT) than men (Watkins et al., 1989, 1992; Kinirons et al., 1999). In the dexamethasone arm of this study, all the CYP3A5*1 carriers were women, whereas four of six of the CYP3A5*1 noncarriers were women. In the rifampin arm, there were six women who were CYP3A5*1 carriers and five who were noncarriers. To verify that sex effects on basal CYP3A activity did not account for differences observed with either inducer, we compared basal ERBT values between women who were either carriers or noncarriers of the CYP3A5*1 allele. Women who carried the CYP3A5*1 allele had a significantly higher mean basal ERBT value compared with women noncarriers (2.84 versus 2.01%; P = 0.003). From this post hoc analysis, we conclude that effects of the two inducers on CYP3A activity were not confounded by sex.

Discussion

The current study was conducted in African Americans because of the higher frequency of the CYP3A5*1 allele in this population, and to control for the effects of genetic admixture when subjects were stratified according to CYP3A5*1 allele carrier status (Kuehl et al., 2001; Lamba et al., 2002; Zeigler-Johnson et al., 2004; Roy et al., 2005). The presence of CYP3A5 protein has been used to explain disease associations linked to the CYP3A4*1B allele because CYP3A4*1B and CYP3A5*1 alleles appeared to be strongly linked in some populations. The CYP3A5*1 allele did not appear to be in LD with the CYP3A4*1B allele in our study, although strong LD has been observed in non-African populations (Thompson et al., 2006). Recently, the CYP3A7*2 allele was shown to be in high LD with the CYP3A5*1 allele in African Americans (Rodriguez-Antona et al., 2005; Thompson et al., 2006).

Our results suggest that African Americans carrying at least one CYP3A5*1 allele had higher basal ERBT values compared with those lacking the CYP3A5*1 allele, and differences in basal activity may be associated with differences in susceptibility for induction-type drug interactions with glucocorticoids. It has been estimated that CYP3A5 can account for 6 to 85% of total hepatic CYP3A content and may contribute to increases in basal CYP3A activity in individuals who carry a functional CYP3A5*1 allele (Wrighton et al., 1990; Kuehl et al., 1992).
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Similar to previous observations (Floyd et al., 2003; Yu et al., 2004), the CYP3A5*1 allele was not associated with differences in the ability of rifampin to induce ERBT values in our African American cohort. In contrast, induction of ERBT values by dexamethasone was only detected in noncarriers of the CYP3A5*1 allele. Differences in potencies between these two inducers could explain this observation. Compared with the potent inductive effects of rifampin on CYP3A4 protein, the inductive effects of a less potent pregnane X receptor (PXR) activator, such as dexamethasone, could be obscured if a large proportion of the total CYP3A activity was the result of functional CYP3A5 because CYP3A5 expression is constitutive and not altered by PXR activation (Bertilsson et al., 1998; Lehmann et al., 1998; Burk et al., 2004). The proximal promoter of CYP3A5 contains a consensus ER-6 response element, which may be important for its constitutive expression, although it lacks the distal PXR response element shown to enhance transcription of CYP3A4 by xenobiotics (Goodwin et al., 1998).

Einhorn et al. (2002) and Kuehl et al. (2002) define a new signaling pathway for CYP3A induction. ERBT values in our African American population were significantly lower than in Caucasians and African Americans (Kuehl et al., 2002; MacLean et al., 2002). The influence of CYP3A induction on the extent of CYP3A inhibition following a 5-day course of dexamethasone is illustrated in Table 1. The lower ERBT values in African Americans may be a result of the lack of CYP3A induction, which is thought to have a positive effect on the extent of CYP3A inhibition following a 5-day course of dexamethasone.

Acknowledgments. We thank Paul B. Watkins and Raymond C. Givens, University of North Carolina (UNC) School of Medicine, for providing 51 additional African American genotypes for the linkage disequilibrium analysis; Alison C. Lyke and Michael L. Lim, UNC School of Pharmacy, and Anthony Gachie for coordinating subject recruitment; June Park, UNC School of Pharmacy, for help with the ERBT for CYP3A phenotyping; and Nina Isoherranen for help with genotyping.

CYP3A5 polymorphism appears to influence susceptibility to induction-type drug interactions for certain inducers, and CYP3A5*1 noncarriers may be more susceptible to the inductive effects of dexamethasone because of lower CYP3A4-dependent basal activity. These results may be particularly relevant for patients undergoing chemotherapy and receiving dexamethasone for delayed nausea. Our data also suggest that the CYP3A5 genotype may have a role in our previous finding of an inverse correlation between baseline hepatic CYP3A activity and the extent of CYP3A induction following a 5-day course of dexamethasone.

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