Short Communication

CYP3A4*16 and CYP3A4*18 Alleles Found in East Asians Exhibit Differential Catalytic Activities for Seven CYP3A4 Substrate Drugs

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ABSTRACT:

CYP3A4, the major form of cytochrome P450 (P450) expressed in the adult human liver, is involved in the metabolism of approximately 50% of commonly prescribed drugs. Several genetic polymorphisms in CYP3A4 are known to affect its catalytic activity and to contribute in part to interindividual differences in the pharmacokinetics and pharmacodynamics of CYP3A4 substrate drugs. In this study, catalytic activities of the two alleles found in East Asians, CYP3A4*16 (T185S) and CYP3A4*18 (L293P), were assessed using the following seven substrates: midazolam, carbamazepine, atorvastatin, paclitaxel, docetaxel, irinotecan, and terfenadine. The holoprotein levels of CYP3A4.16 and CYP3A4.18 were significantly higher and lower, respectively, than that of CYP3A4.1 when expressed in Sf21 insect cell microsomes together with human NADPH-P450 reductase. CYP3A4.16 exhibited intrinsic clearances (Vmax/Km) that were lowered considerably (by 84–60%) for metabolism of midazolam, carbamazepine, atorvastatin, paclitaxel, and irinotecan compared with CYP3A4.1 due to increased Km values with or without decreased Vmax values, whereas no apparent decrease in intrinsic clearance was observed for docetaxel. On the other hand, Km values for CYP3A4.18 were comparable to those for CYP3A4.1 for all substrates except terfenadine; but Vmax values were lower for midazolam, paclitaxel, docetaxel, and irinotecan, resulting in partially reduced intrinsic clearance values (by 34–52%). These results demonstrated that the impacts of both alleles on CYP3A4 catalytic activities depend on the substrates used. Thus, to evaluate the influences of both alleles on the pharmacokinetics of CYP3A4-metabolized drugs and their drug-drug interactions, substrate drug-dependent characteristics should be considered for each drug.

Introduction

CYP3A4, the major form of cytochrome P450 (P450) expressed in the adult human liver, is involved in the metabolism of approximately 50% of commonly prescribed drugs (Guengerich, 1999). CYP3A4 is capable of oxidizing a wide range of structurally diverse drugs as well as endogenous compounds. For example, many anticancer drugs, such as docetaxel, paclitaxel, etoposide, tamoxifen, irinotecan, vincristine, and cyclophosphamide, are known to be metabolized by CYP3A4.

The expression and catalytic activity of CYP3A are highly variable among individuals, and this variability is partially attributable to genetic factors (Ozdemir et al., 2000). Several CYP3A4 genetic polymorphisms are known to affect the metabolism of CYP3A4 substrate drugs (www.cypalleles.ki.se/cyp3a4.htm). In addition, CYP3A4 alleles were reported to exhibit large ethnic differences in their distribution. In the Japanese, four alleles with amino acid alterations, CYP3A4*6 (D277EfsX8), CYP3A4*11 (T363M), CYP3A4*16 (T185S), and CYP3A4*18 (L293P), are found at frequencies of 0.001, 0.002, 0.014 to 0.05, and 0.013 to 0.028, respectively (Lamba et al., 2002; Yamamoto et al., 2003; Fukushima-Uesaka et al., 2004). Of these alleles, CYP3A4*16 has also been detected in Korean (allele frequency, 0.002) and Mexican populations (allele frequency, 0.05) and CYP3A4*18 is distributed commonly among East Asians such as Chinese (allele frequency, 0.008 – 0.01), Koreans (allele frequency, 0.012 – 0.017), and Malaysians (allele frequency, 0.021) (Wen et al., 2004; Hu et al., 2005; Lee et al., 2007; Ruzilawati et al., 2007; Kang et al., 2009).

CYP3A4*16 and CYP3A4*18 are reported to affect both in vitro and in vivo catalytic activities toward several substrates and to be involved in the interindividual differences in the pharmacokinetics and pharmacodynamics of CYP3A4 substrate drugs. CYP3A4.16 exhibited an approximately 50% reduction in intrinsic clearance (Vmax/Km) for testosterone (TST) 6β-hydroxylation activity in vitro (Murayama et al., 2002). We recently demonstrated the substrate-dependent altered kinetics of CYP3A4.16 for midazolam (MDZ) and carbamazepine (CBZ) (Maekawa et al., 2009). The intrinsic clearance for 1’-hydroxymidazolam (1’-OH-MDZ), 4-hydroxymidazolam (4-

ABBREVIATIONS: P450, cytochrome P450; APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxy camptothecin; TST, testosterone; MDZ, midazolam; CBZ, carbamazepine; 1’-OH-MDZ, 1’-hydroxymidazolam; ATV, atorvastatin; PTX, paclitaxel; DTX, docetaxel; IRN, irinotecan; TFN, terfenadine; 4-OH-MDZ, 4-hydroxymidazolam; 3’-p-OH-PTX, 3’-p-hydroxypaclitaxel; 2-OH-ATV, 2-hydroxydocetaxel; 4-OH-ATV, 4-hydroxydocetaxel; NPC, 7-ethyl-10-(4-amino-1-piperidino) carbonyloxy camptothecin; OR, NADPH P450 reductase.
OH-MDZ), and CBZ 10,11-epoxide formation decreased by 50, 30, and 74%, respectively, compared with the wild type. In vivo, heterozygous CYP3A4*16 patients administered paclitaxel (PTX) showed significantly reduced 3'-p-hydroxypaclitaxel (3'-p-OH-PTX)/PTX area under the plasma concentration-time curve ratios (Nakajima et al., 2006). In addition, decreased metabolism of irinotecan (IRN) to the inactive metabolite 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycaumptothecin (APC) was observed with CYP3A4*16 (Sai et al., 2008).

In contrast to CYP3A4*16, CYP3A4*18 seems to be bidirectional in terms of its catalytic activity toward different substrates, although different evaluation systems were used for each study. For example, the CYP3A4.18 protein exhibited increased activity for TST and chlorpyrifos (Dai et al., 2001), but not nifedipine (Lee et al., 2005) in vitro. On the other hand, for the conventional probe drug MDZ, CYP3A4.18 showed decreased metabolism in vitro but not in vivo (Lee et al., 2007). Kang et al. (2009) demonstrated that CYP3A4*18 is the gain-of-function allele for metabolism of several CYP3A4 substrates, including sex steroids like estrogens, leading to a relative sex-hormone deficiency that may predispose older women to osteoporosis.

In this study, to evaluate the effects of CYP3A4*16 and CYP3A4*18 on the catalytic activity toward structurally diverse substrates, recombinant wild-type (CYP3A4.1) and variant CYP3A4 enzymes (CYP3A4.16 and CYP3A4.18) were expressed using baculovirus-insect cell systems. The seven substrates used in the investigation were MDZ, CBZ, atorvastatin (ATV), PTX, docetaxel (DTX), IRN, and terfenadine (TFN) (Supplemental Fig. S1).

Materials and Methods

Materials. Purified human cytochrome b5 was purchased from either Inviron (Carlsbad, CA) or Oxford Biomedical Research (Rochester, MI). MDZ and PTX were obtained from Wako Pure Chemicals (Osaka, Japan). 1'-OH-MDZ and 4-OH-MDZ were obtained from BD Gentest (Woburn, MA). CBZ, CBZ 10,11-epoxide, 3'-p-OH-PTX, and TFN and its metabolite t-buty1-hydroxysterfenadine were purchased from Sigma-Aldrich (St. Louis, MO). A second TFN metabolite, α,α-diphenyl-4-piperidinomethanol, was obtained from Fine & Performance Chemicals Ltd (Middlesbrough, UK). ATV, its metabolites 2-hydroxyatorvastatin (2-OH-ATV) and 4-hydroxyatorvastatin (4-OH-ATV), and DTX and its metabolite, DTX hydroxy (t-buty1 hydroxyl DTX), 519.2243 were kindly supplied by Yakult (M2), were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). IRN and its CYP3A4 metabolites, APC, and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycaumptothecin (NPC), were kindly supplied by Yakult (Tokyo, Japan). All other chemicals and solvents were used of the highest commercially available grade or analytical grade.

Expression of Recombinant Wild-Type and Mutant CYP3A4 Proteins. Insect cell microsomes coexpressing CYP3A4 (wild type or variants) and NADPH P450 reductase (OR) were prepared according to methods described previously (Maekawa et al., 2009). The cytochrome P450 content and OR activity in microsomes were measured (Phillips and Langdon, 1962; Omura and Sato, 1964), and Western blotting of CYP3A4 and OR was performed as described previously (Maekawa et al., 2009).

Assay for CYP3A4 Activity. To compare alterations in kinetic parameters among substrates, three batches of wild-type and two variant enzyme preparations were used for all kinetic studies. Kinetic analysis on all seven CYP3A4 substrates was performed under proper conditions for the incubation time and P450 concentrations such that linear relationships for metabolite formation were obtained.

Catalytic activities for MDZ 1'- and 4-hydroxylations and CBZ 10,11-epoxide formation were measured as described previously (Maekawa et al., 2009), with slight modifications. For other substrates (ATV, PTX, DTX, IRN, and TFN), the incubation conditions were similar to those used for MDZ and CBZ. For all substrates, CYP3A4s from insect microsomes and purified cytochrome b5 were mixed together (CYP3A4/b5 ratio, 1:4), and protein concentrations and the OR/P450 ratio in the CYP3A4 wild-type and variant reaction mixtures were adjusted to be equivalent by adding both control (uninfected) microsomes and microsomes expressing solely OR. MDZ (0.2–200 μM), CBZ (10–500 μM), ATV (5–120 μM), PTX (1–50 μM), DTX (0.25–64 μM), IRN (5–400 μM), or TFN (0.0125–160 μM) was added into aliquots of the above-mentioned enzyme preparations. The reaction was started by adding NADPH generation system and terminated by adding appropriate stop solutions containing suitable internal standard for the measurement of each metabolite. Samples were mixed well and then spun at 13,000g for 3 to 5 min.

Metabolite analyses for MDZ, CBZ, ATV, and PTX were carried out on a tandem quadrupole mass spectrometer (Micromass Quattro Premier XE; Waters, Milford, MA) interfaced with an Acquity UPLC System (Waters) equipped with an Acquity BEH C18 column (1.7 μm, 2.1 × 30 mm; Waters) kept at 50°C. Two solutions (solution A, 10 mM ammonium acetate; solution B, 90% acetonitrile containing 10 mM ammonium acetate) were used as the mobile phase. Metabolites were eluted by linear gradient, increasing solution B. Detections were performed by monitoring the transitions of m/z 242 to 203 (1'-OH-MDZ), m/z 342 to 234 (4-OH-MDZ), m/z 253 to 180 (CBZ 10,11-epoxide), m/z 575 to 440 (2-OH-ATV and 4-OH-ATV), and m/z 870 to 122 (3'-p-OH-PTX).

For IRN, TFN, and DTX, a time-of-flight mass spectrometer (Micromass LCT Premier XE; Waters) interfaced with an Acquity UPLC System, equipped with an Acquity BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters), and kept at 40°C was used for metabolite analyses. The mobile phase consisted of a mixture of acetonitrile/methanol/distilled water containing 0.1% (v/v) formic acid (14:14:72 for IRN, 21:21:58 for TFN, and 15:45:40 for DTX) delivered isocratically at a flow rate of 0.3 ml/min. Detections were performed by monitoring the M+H+ ions, m/z 824.3493 ± 0.02 (t-buty1 hydroxyl DTX), 519.2243 ± 0.02 (NPC), 619.2768 ± 0.02 (APC), 488.3165 ± 0.02 (t-buty1 hydroxyl TFN), and 268.1701 ± 0.02 (α-α-diphenyl-4-piperidinomethanol).

Kinetic parameters were calculated using the computer program designed for nonlinear regression analysis (MULTI program) (Yamaoka et al., 1986). Kinetic parameters for MDZ 4-hydroxylation, ATV 2'- and 4-hydroxylation, PTX 3'-p-hydroxylation, IRN oxidation to NPC, and DTX t-buty1 hydroxylation were determined by the hyperbolic Michaelis-Menten model (eq. 1). The substrate inhibition model (eq. 2) was used for MDZ 1'-hydroxylation, TFN C'-hydroxylation, and TFN N-demethylation, where Ks is the substrate inhibition constant. In the case of the 10,11-epoxidation of CBZ, kinetic parameters were determined by the modified two-site equation (Vmax1 = 0) (Korzekwa et al., 1998) (eq. 3).

\[
V = V_{\text{max}} S / (K_s + S) \\
V = V_{\text{max}} S / (K_s + S + S/K_s) \\
V = (V_{\text{max}} S / (K_s + K_s)(1 + S/K_s + S/K_s))
\]

Kinetic data were determined as the mean ± S.D. for three microsomal preparations derived from separate baculovirus infections, and statistical analysis was conducted by Dunnnett’s multiple comparison test in SAS (SAS Institute, Cary, NC). A p value of <0.05 was set as a statistically significant difference.

Results and Discussion

Expression of Wild-Type and Variant CYP3A4s in Insect Cells. Wild-type (CYP3A4.1) and variant proteins (CYP3A4.16 and CYP3A4.18) were coexpressed with human OR in Sf21 insect cells. Typical CO difference spectra with a maximum absorbance at 450 nm were obtained for all microsomal fraction preparations (Supplemental Fig. S2). CYP3A4.18 exhibited a larger peak at 420 nm than either CYP3A4.1 or CYP3A4.16. In three independent expression experiments, holoenzyme contents in the variant CYP3A4.16 (230.8 ± 25.2 pmol/mg microsomal protein) and CYP3A4.18 microsomes (51.3 ± 3.2 pmol/mg microsomal protein) were significantly higher and lower (p < 0.05), respectively, than that in the wild-type.
CYP3A4.1 microsomes (104.4 ± 23.9 pmol/mg microsomal protein). OR activity varied among the preparations but was not significantly different (p > 0.05) among CYP3A4.1 (1032.3 ± 88.2 nmol cytochrome c reduced/min/mg protein), CYP3A4.16 (659.4 ± 254.6 cytochrome c reduced/min/mg protein), and CYP3A4.18 (1019.1 ± 260.1 cytochrome c reduced/min/mg protein). On the other hand, total (apoenzyme and holoenzyme) CYP3A4 protein expression levels in insect cell microsomes were not significantly different (p > 0.05) between the wild type and variants by immunoblot analysis (data not shown).

**Catalytic Activities of Wild-Type and Variant CYP3A4s.** To characterize the substrate-dependent functional alterations of CYP3A4*16 and CYP3A4*18, CYP3A4 catalytic activities of wild type and variants toward the seven substrates (MDZ, CBZ, ATV, PTX, IRN, and TFN) were measured. For four of the substrates, two different metabolites were detected: 1'- and 4-OH-MDZ from MDZ, 2- and 4-OH-ATV from ATV, APC and NPC from IRN, and t-butylhydroxy-TFN and a-α diphenyl-4-piperidinomethanol (azacyclonol) from TFN. Because the level of APC formed from IRN was too low to quantify precisely under our experimental conditions, kinetic analysis for IRN was performed only for NPC formation. The kinetic profiles are shown in Supplemental Fig. S3, and kinetic parameters are summarized in Table 1. The variant-to-wild-type ratios (percent) of intrinsic clearance values (\(V_{\text{max}}/K_m\)) are compared among substrates used (Fig. 1).

**TABLE 1**

Kinetic parameters for 10 catalytic reactions using seven substrates by CYP3A4.1, CYP3A4.16, and CYP3A4.18.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(\mu M)</th>
<th>(V_{\text{max}}) pmol/min/pmol P450</th>
<th>Intrinsic Clearance ((V_{\text{max}}/K_m)) µM</th>
<th>(K_m) µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDZ 1'-hydroxylation</td>
<td>CYP3A4.1</td>
<td>1.9 ± 0.1</td>
<td>28.1 ± 2.8</td>
<td>14.8 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>2.6 ± 0.1**</td>
<td>15.0 ± 3.5**</td>
<td>5.8 ± 1.7***</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>2.0 ± 0.1</td>
<td>17.5 ± 3.2*</td>
<td>8.8 ± 1.6**</td>
</tr>
<tr>
<td>MDZ 4-hydroxylation</td>
<td>CYP3A4.1</td>
<td>23.1 ± 5.2</td>
<td>12.9 ± 0.1</td>
<td>0.58 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>51.5 ± 3.5***</td>
<td>11.7 ± 1.4</td>
<td>0.23 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>22.3 ± 3.5</td>
<td>9.2 ± 2.0*</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>CBZ epoxidation*</td>
<td>CYP3A4.1</td>
<td>21.9 ± 5.2 ((K_m))</td>
<td>15.5 ± 2.5</td>
<td>0.095 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>165 ± 15 ((K_m))</td>
<td>11.0 ± 1.0</td>
<td>0.020 ± 0.008***</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>20.0 ± 2.7 ((K_m))</td>
<td>15.7 ± 4.5</td>
<td>0.090 ± 0.011</td>
</tr>
<tr>
<td>ATV 2-hydroxylation</td>
<td>CYP3A4.1</td>
<td>24.2 ± 7.6</td>
<td>6.6 ± 1.1</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>87.4 ± 22.6**</td>
<td>8.2 ± 1.9</td>
<td>0.10 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>20.2 ± 6.8</td>
<td>3.8 ± 1.3</td>
<td>0.20 ± 0.11</td>
</tr>
<tr>
<td>ATV 4-hydroxylation</td>
<td>CYP3A4.1</td>
<td>19.6 ± 4.0</td>
<td>16.1 ± 5.0</td>
<td>0.84 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>65.4 ± 19.3**</td>
<td>8.4 ± 1.4</td>
<td>0.14 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>16.1 ± 3.4</td>
<td>11.1 ± 4.2</td>
<td>0.71 ± 0.31</td>
</tr>
<tr>
<td>PTX 3'-hydroxylation</td>
<td>CYP3A4.1</td>
<td>2.9 ± 0.1</td>
<td>0.55 ± 0.06</td>
<td>0.19 ± 0.03</td>
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<td></td>
<td>CYP3A4.16</td>
<td>12.9 ± 2.4***</td>
<td>0.55 ± 0.13</td>
<td>0.04 ± 0.02***</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>2.7 ± 0.1</td>
<td>0.24 ± 0.07**</td>
<td>0.09 ± 0.03**</td>
</tr>
<tr>
<td>DTX t-butyl hydroxylation</td>
<td>CYP3A4.1</td>
<td>2.5 ± 0.1</td>
<td>0.38 ± 0.01</td>
<td>0.16 ± 0.01</td>
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<tr>
<td></td>
<td>CYP3A4.16</td>
<td>1.8 ± 0.2**</td>
<td>0.26 ± 0.03**</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>2.3 ± 0.2</td>
<td>0.24 ± 0.04**</td>
<td>0.10 ± 0.03*</td>
</tr>
<tr>
<td>IRN oxidation to NPC</td>
<td>CYP3A4.1</td>
<td>19.3 ± 2.7</td>
<td>1.4 ± 0.2</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>34.0 ± 2.9**</td>
<td>0.9 ± 0.4</td>
<td>0.03 ± 0.01*</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>19.7 ± 2.8</td>
<td>0.7 ± 0.1*</td>
<td>0.04 ± 0.01*</td>
</tr>
<tr>
<td>TFN t-butyl hydroxylation</td>
<td>CYP3A4.1</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.6</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>3.5 ± 0.5</td>
<td>2.1 ± 0.1*</td>
<td>0.6 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.18</td>
<td>6.0 ± 1.2*</td>
<td>3.1 ± 0.5</td>
<td>0.5 ± 0.1*</td>
</tr>
<tr>
<td>TFN N'-demethylationb</td>
<td>CYP3A4.1</td>
<td>2.4 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>0.95 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>CYP3A4.16</td>
<td>2.1 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>0.72 ± 0.12</td>
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<tr>
<td></td>
<td>CYP3A4.18</td>
<td>3.4 ± 0.3*</td>
<td>1.9 ± 0.2</td>
<td>0.57 ± 0.10</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, and ***p < 0.001 versus the wild-type (Dunnett’s multiple comparison test).

*For CBZ epoxidation, \(K_m\) and \(V_{\text{max}}\) values are indicated in each column.

*b For TFN N'-demethylation, kinetic profile of CYP3A4.18 was better fitted to the Michaelis-Menten model than to the substrate inhibition model.
FIG. 1. The percent ratios of intrinsic clearance of variants to that of the wild type. Data are represented by mean ± S.D. of three different expression experiments. MDZ-1’, MDZ 1’-hydroxylation; MDZ-4, MDZ 4-hydroxylation; CBZ, CBZ 10,11-epoxidation; ATV-2, ATV 2-hydroxylation; ATV-4, ATV 4-hydroxylation; PTX-3’, PTX 3’-p-hydroxylation; DTX, DTX t-buty1 hydroxylation; IRN (NPC), IRN oxidation to NPC; TFN (C), TFN t-buty1 hydroxylation; TFN (N), TFN N-demethylation. * p < 0.05, ** p < 0.01, and *** p < 0.001 versus the wild-type (Dunnett’s multiple comparison test).

Our results were consistent with those by Miyazaki et al. (2008), who found that recombinant CYP3A4.16 expressed in Escherichia coli is markedly deficient in MDZ, TST, and nifedipine metabolisms with lower V\textsubscript{max} and increased K\textsubscript{m} relative to CYP3A4.1. Thr185 in the E helix is far away from the active site and is not located in the substrate recognition site. Further studies are necessary to elucidate the role of this residue in the binding of structurally diverse CYP3A4 substrates to the substrate recognition site.

In agreement with the lower in vitro catalytic activity of CYP3A4.16 toward PTX and IRN, CYP3A4*16 heterozygous patients administered PTX or IRN were reported to show significantly reduced metabolite-to-substrate area under the plasma concentration-time curve ratios, which are parameters for in vivo CYP3A4 activity (Nakajima et al., 2006; Sai et al., 2008). As for substrates for which the clinical significance of CYP3A4*16 has not been evaluated, this study demonstrated that ATV metabolism was markedly affected by CYP3A4.16. Because CYP3A4 (but not CYP3A5) is the major enzyme involved in the formation of the two ATV metabolites: 2- and 4-OH-ATV (Park et al., 2008), the clinical relevance of CYP3A4*16 for efficacy and/or adverse reactions of ATV should be further investigated. In contrast, CYP3A4.16 retained its catalytic activity toward DTX, and thus it is predicted that this allele does not substantially influence the metabolism of DTX in vivo.

For CYP3A4.18, the reduced intrinsic clearances were observed for MDZ 1’-hydroxylation (by 40%, p < 0.01), PTX 3’-p-hydroxylation (by 52%, p < 0.01), DTX t-buty1 hydroxylation (by 32%, p < 0.05), IRN oxidation to NPC (by 50%, p < 0.05), and TFN t-buty1 hydroxylation (by 48%, p < 0.05) compared with CYP3A4.1. Except for TFN, the lowered V\textsubscript{max} values for CYP3A4.18 resulted in lower activity in contrast to those for CYP3A4.16, which exhibited increased K\textsubscript{m} values for most substrates. On the other hand, CYP3A4.18 had similar kinetic profiles to CYP3A4.1 in their values for K\textsubscript{m}, V\textsubscript{max}, and intrinsic clearance for oxidation of CBZ (Table 1; Supplemental Fig. S3), which has the lowest molecular weight among the seven substrates (Supplemental Fig. S1).

For the substrates MDZ, PTX, and IRN, it was reported that heterozygous CYP3A4*1/CYP3A4*18 did not affect their pharmacokinetics (Nakajima et al., 2006; Lee et al., 2007; Sai et al., 2008). Because our in vitro results with CYP3A4.18 showed a partial decrease in V\textsubscript{max} values for these drugs, an in vivo correlation was not observed, at least for heterozygotes. Further studies are necessary to evaluate the clinical relevance of homozygous CYP3A4*18.

By molecular modeling studies, Kang et al. (2009) demonstrated that the L293P substitution at the beginning of the I helix caused significant secondary structural changes in the I helix and reduced protein stability. Our spectral analysis that CYP3A4.18 preparations contained more P420 than CYP3A4.1 might also be in agreement with their modeling. These possible conformational changes in CYP3A4.18 may affect substrate access depending on the substrate structure.

In conclusion, the substrate-dependent functional alterations of CYP3A4.16 and CYP3A4.18 were assessed toward seven structurally diverse substrates, MDZ, CBZ, ATV, PTX, DTX, IRN, and TFN. Compared to the wild type, CYP3A4.16 exhibited more than 60% reduced activity toward MDZ, CBZ, ATV, PTX, and IRN due to increased K\textsubscript{m} values. In contrast, CYP3A4.18 showed a moderate reduction in its catalytic activity (by 34–52%) for MDZ, PTX, DTX, and IRN due to decreased V\textsubscript{max} values. Thus, to evaluate the influences of both alleles on the pharmacokinetics of other CYP3A4-metabolized drugs and their drug-drug interactions, substrate drug-dependent characteristics should be elucidated for each drug.

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References


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Supplemental Data

*CYP3A4*16 and *CYP3A4*18 Alleles Found in East Asians Exhibit Differential Catalytic Activities for Seven CYP3A4 Substrate Drugs

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Supplemental Figure S1. Chemical structures of the 7 CYP3A4 substrates used in this study. Arrows indicate the sites of oxidation for each substrate. Average molecular weight (M_r) is shown in parentheses.
Supplemental Figure S2. Representative CO difference spectra for CYP3A4.1, CYP3A4.16 and CYP3A4.18. Insect cell microsomes containing 2 mg/ml protein were used to measure CYP3A4 content as described in Materials and Methods.
Supplemental Figure S3.
Kinetic profiles of MDZ 1’-hydroxylation (a), MDZ 4-hydroxylation (b), CBZ 10, 11-epoxidation (c), ATV 2-hydroxylation (d), ATV 4-hydroxylation (e), PTX 3’-p-hydroxylation (f), DTX t-butyl hydroxylation (g), IRN oxidation to NPC (h), TFN t-butyl hydroxylation (i) and TFN N-demethylation (j) by CYP3A4.1 (closed circle), CYP3A4.16 (closed square) and CYP3A4.18 (closed triangle). Each point represents the mean ± S.D. of three independent preparations derived from different transfections. The inset in each profile is the data in expanded form to allow better visual inspection of the shape of the curve in the range of lower substrate concentrations.