THE CANINE CYP1A2 DEFICIENCY POLYMORPHISM DRAMATICALLY AFFECTS THE PHARMACOKINETICS OF 4-CYCLOHEXYL-1-ETHYL-7-METHYLPYRIDO[2,3-D]-PYRIMIDINE-2-(1H)-ONE (YM-64227), A PHOSPHODIESTERASE TYPE 4 INHIBITOR

Daisuke Tenmizu, Kiyoshi Noguchi, Hidetaka Kamimura, Hisakazu Ohtani, and Yasufumi Sawada

Drug Metabolism Research Laboratories, Astellas Pharma Inc., Tokyo, Japan (D.T., K.N., H.K.); and Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan (H.O., Y.S.)

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ABSTRACT:

In a previous study, it was shown that the novel canine single nucleotide polymorphism (SNP) CYP1A2 1117C>T yields an inactive enzyme. In this study, the effect that this SNP has on the pharmacokinetics of 4-cyclohexyl-1-ethyl-7-methylypyrido[2,3-d]pyrimidine-2-(1H)-one (YM-6427) was investigated. Plasma concentrations of the unchanged drug and five of its metabolites (MM-1 to MM-5) were determined after either intravenous or oral administration of YM-6427 to genotyped dogs (C/C, C/T, and T/T groups). Liver microsomes were prepared from these dogs to determine the in vitro metabolic clearance of YM-6427. After a single oral administration, the maximum plasma concentration and absolute bioavailability of YM-6427 in the T/T group were 17.1 times and 27.2 times higher than those in the C/C group, respectively, whereas the pharmacokinetics of YM-6427 after intravenous administration were not affected by genotype. The metabolic profiles in the T/T group were quite distinct from the others; i.e., the main metabolite was MM-2 in the C/C group, whereas MM-1 and MM-5 were the main metabolites in the T/T group. The formation clearances of MM-2 and MM-3 in the microsomes derived from T/T type dogs were significantly lower, whereas those of MM-1, MM-4, and MM-5 were not affected. A statistically significant correlation was observed between the in vivo and in vitro metabolic intrinsic clearances (r = 0.82, p < 0.001). The canine CYP1A2 1117C>T SNP proved to be responsible for a substantial portion of the interindividual variability in the pharmacokinetics of YM-6427.

Cytochrome P450 (P450) is a superfamily of enzymes that plays an important role in the oxidative metabolism of a wide variety of xenobiotics as well as endogenous compounds (Nelson et al., 1996). The metabolic activity of P450 is affected by several factors such as inhibition by concomitant drugs, induction, and genetic polymorphism (Pelkonen et al., 1998). The P450 polymorphisms have been investigated extensively in clinical settings, and it is well known that mutated alleles of CYP2C9, CYP2C19, and CYP2D6 cause altered phenotype (Pelkonen et al., 1998). In this study, the effect that this SNP has on the phosphodiesterase type 4 inhibitor, YM-6427 was investigated. Plasma concentrations of the unchanged drug and five of its metabolites (MM-1 to MM-5) were determined after either intravenous or oral administration of YM-6427 to genotyped dogs (C/C, C/T, and T/T groups). Liver microsomes were prepared from these dogs to determine the in vitro metabolic clearance of YM-6427. After a single oral administration, the maximum plasma concentration and absolute bioavailability of YM-6427 in the T/T group were 17.1 times and 27.2 times higher than those in the C/C group, respectively, whereas the pharmacokinetics of YM-6427 after intravenous administration were not affected by genotype. The metabolic profiles in the T/T group were quite distinct from the others; i.e., the main metabolite was MM-2 in the C/C group, whereas MM-1 and MM-5 were the main metabolites in the T/T group. The formation clearances of MM-2 and MM-3 in the microsomes derived from T/T type dogs were significantly lower, whereas those of MM-1, MM-4, and MM-5 were not affected. A statistically significant correlation was observed between the in vivo and in vitro metabolic intrinsic clearances (r = 0.82, p < 0.001). The canine CYP1A2 1117C>T SNP proved to be responsible for a substantial portion of the interindividual variability in the pharmacokinetics of YM-6427.

A novel canine CYP1A2 1117 C>T SNP that yields a stop codon (H11022*/H11549*) was reported in a previous study (Tenmizu et al., 2004a). The T-allele frequency was 0.39, which suggests that 10 to 15% of dogs would not express active CYP1A2 protein. It has also been shown that a subsequent reduction in the metabolic activity of CYP1A2 is reflected in the metabolic ratio (ratio of plasma parent drug to the major metabolite MM-2) after oral administration of 4-cyclohexyl-1-ethyl-7-methylypyrido[2,3-d]pyrimidine-2-(1H)-one (YM-6427), a phosphodiesterase type 4 inhibitor, to dogs. In fact, only a few studies have shown the possibility that interindividual variations in the pharmacokinetics of some drugs in dogs may be attributable to P450 polymorphisms (Paulson et al., 1999; Azuma et al., 2002; Mise et al., 2004).

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SNP on the pharmacokinetics of YM-64227 has yet to be investigated. In this study, the plasma concentrations of metabolites and the unchanged drug after intravenous and oral administration to genotyped dogs were investigated to clarify in detail the effect of canine CYP1A2-deficient polymorphism on the metabolism of YM-64227. In addition, to compare intrinsic in vivo and in vitro metabolic clearances, the in vivo metabolic clearance of YM-64227 was determined using liver microsomes prepared from dogs that were used in the in vivo pharmacokinetics study.

Materials and Methods

Chemicals. YM-64227, its five metabolites (MM-1 to MM-5) (Fig. 1), and the internal standard 4-(3-chlorophenyl)-1-ethyl-7-(1-hydroxyethyl)pyrido[2,3-d]pyrimidin-2(1H)-one were synthesized in the Chemistry Research Laboratories of Astellas Pharma Inc. (Ibaraki, Japan). NADPH-generating system solutions A and B were purchased from BD Biosciences (San Jose, CA). Purified water from a Milli-Q system (Millipore Co., Bedford, MA) was used throughout the study. All other chemicals used were commercially available and of the highest purity.

Animals. Male beagle dogs (body weight: 8.2 to 12.5 kg) were supplied by Covance Research Product (Kalamazoo, MI). They were kept in climate-controlled rooms (temperature: 23 ± 1°C; relative humidity: 55 ± 1%) with a light/dark cycle of 13:11 h. They had free access to both pellet diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water during the studies. This study was approved by the animal ethical committee of the Astellas Pharma Inc. Dogs were genotyped as C/C, C/T, or T/T at the 1117 nucleotide site of canine CYP1A2 by allele-specific primer polymerase chain reaction and direct sequence analysis, as described previously (Tennizui et al., 2004a).

Pharmacokinetics Study of YM-64227 in Genotyped Dogs. YM-64227 solution was prepared in a vehicle of 2% ethanol, 2% benzyl alcohol, 4% Cremophor, and 92% water for injection was administered intravenously at 0.1 mg/kg to dogs (n = 5 per each group genotyped as C/C, C/T, or T/T) after overnight fasting. Blood (ca. 5 ml/time point) was collected from the forelimb vein using a heparinized syringe after 0, 0.1, 0.25, 0.5, 1, 2, 4, and 8 h. In another study, YM-64227 suspended in 0.5% methylcellulose solution was administered orally at 0.3 mg/kg to the same dogs after an overnight fasting. Plasma was collected by centrifuging the blood. Plasma was stored frozen at -20°C until it was analyzed to determine the concentrations of YM-64227 and its metabolites. Concentrations of YM-64227 and its metabolites were quantified using HPLC (Tennizui et al., 2004b). In brief, sample preparation used the liquid-liquid extraction using tert-butyl methyl ether. Separation was achieved on a COSMOSIL-packed phenyl ethyl column (5 μm, 250 mm × 4.6 mm i.d.; Nacalai Tesque Inc., Kyoto, Japan). The mobile phase consisting of (A) 80% 50 mM acetic acid/20% acetonitrile and (B) 20% 50 mM acetic acid/80% acetonitrile were used for gradient elution. YM-64227 and its metabolites were detected fluorimetrically at 400 nm with emission at 330 nm.

The pharmacokinetic parameters for YM-64227 and its metabolites were calculated using noncompartmental modeling and WinNonlin Professional version 3.3 (Pharsight, Mountain View, CA). The pharmacokinetic parameters of C/C, C/T, and T/T dogs were compared using the Tukey test and SAS system version 8.2 (SAS Institute, Cary, NC).

Protein Binding to YM-64227 in Dog Plasma. Aliquots (0.04 ml) of 100 mM Na/K-phosphate buffer containing YM-64227 were added to 2-ml aliquots of dog plasma to yield concentrations of 2 and 2000 ng/ml. After incubation for 10 min at 37°C, a 0.5-ml aliquot was taken from each sample to measure the total concentration, and the remainder was transferred to an ultrafiltration tube (VIVASPIN2; Sartorius AG, Goettingen, Germany). These tubes were centrifuged at 1870g for 15 min at 37°C, and then a 0.5-ml aliquot of filtrate was removed and used to measure the unbound plasma concentration. The YM-64227 concentrations in the plasma and filtrate samples were quantified using HPLC.

Blood-to-Plasma Concentration Ratio (R_B) of YM-64227 in Dogs. The R_B of YM-64227 was determined using heparinized whole blood (Lin et al., 1982). Aliquots (0.05 ml) of 100 mM Na/K-phosphate buffer containing YM-64227 were added to 5-ml aliquots of dog blood preincubated at 37°C to yield concentrations of 1, 10, and 100 ng/ml. After incubation for 5 min at 37°C, the blood samples were centrifuged for 5 min at 1870g to obtain the plasma fraction. Concentrations of YM-64227 were quantified using HPLC.

Calculation of CL_int,in vivo, CLoral was calculated using dividing CLoverall by Foral. CLoverall was then calculated from eq. 1, using a Q_a value of 1620 ml/h/kg (Bischoff et al., 1971; Dedrick et al., 1973; Montandon et al., 1975) and assuming that F_a was 1.0 and CL_a was 0.0. The R_B value of YM-64227 was 0.66 in dogs (estimated from this study).

\[ CL_{a} = \frac{(1 + a \cdot F_{a} / Q_{a} \cdot R_{B})}{(1 + a \cdot F_{a} / Q_{a})} \]  

\[ F_a = (1 + a \cdot (\sqrt[3]{a} - 1 \cdot 2d_{2} + 1 \cdot (1 + a)) / 2d_{2}) \]  

\[ a = (1 + 4 \cdot R_{B} \cdot D_{a} / V_{d})^{1/2} \]  

\[ R_{B} = (f_{a} \cdot D_{a} \cdot CL_{a,in vivo} / V_{d}) \]  

A D_a of 0.17 (Roberts and Rowland, 1986b; Iwatsubo et al., 1996) was used to calculate CLa,in vivo. The f_a value of YM-64227 used for 0.036 in dogs (estimated from this study).

YM-64227 Metabolism in Genotyped Canine Liver Microsomes. Canine liver microsome preparation and the CYP1A2 1117 C/H11022 polymorphism on the metabolism of YM-64227. In addition, to compare intrinsic in vivo and in vitro metabolic clearances, the in vivo metabolic clearance of YM-64227 was determined using liver microsomes prepared from dogs that were used in the in vivo pharmacokinetics study.
The plasma concentration-time profiles of YM-64227 after intravenous administration to dogs at 0.1 and 0.3 mg/kg. Each point represents the mean ± S.D. of five animals. (■) indicates C/C type dogs, (▲) indicates C/T type dogs, and (○) indicates T/T type dogs.

**TABLE 1**

Pharmacokinetic parameters of YM-64227

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C/C Homo (n = 5)</th>
<th>C/T Hetero (n = 5)</th>
<th>T/T Homo (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 (h)</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Vmax (ml/kg)</td>
<td>1444 ± 167.9</td>
<td>1115 ± 341.6</td>
<td>1178 ± 271.9</td>
</tr>
<tr>
<td>CLtot (ml/h/kg)</td>
<td>1399 ± 72.5</td>
<td>1179 ± 357.4</td>
<td>1567 ± 184.4</td>
</tr>
<tr>
<td>AUCv (h·ng/ml)</td>
<td>2120 ± 109.9</td>
<td>1787 ± 541.5</td>
<td>2374 ± 279.5</td>
</tr>
<tr>
<td>AUCv,real (h·ng/ml)</td>
<td>71.6 ± 3.9</td>
<td>91.3 ± 7.8</td>
<td>64.5 ± 7.2</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>1.4 ± 0.6</td>
<td>3.8 ± 1.7</td>
<td>24.0 ± 14.8**</td>
</tr>
<tr>
<td>Freal (%)</td>
<td>0.5 ± 0.3</td>
<td>1.4 ± 0.7</td>
<td>13.6 ± 9.5**</td>
</tr>
</tbody>
</table>

Statistically significant differences from the C/C type were determined using the Tukey test and are indicated as *P < 0.05 and **P < 0.01.
*Terminal elimination half-life for the intravenous administration of YM-64227.

The plasma concentration-time profiles of the metabolites after intravenous administration to dogs at 0.1 mg/kg. Each point represents the mean ± S.D. of five animals. (■) indicates C/C type dogs, (▲) indicates C/T type dogs, and (○) indicates T/T type dogs.

**Results**

**Pharmacokinetics Study of YM-64227 in Genotyped Dogs.** The plasma concentration-time profiles of YM-64227 after single intravenous and oral administrations are shown in Fig. 2. The pharmacokinetic parameters of YM-64227 obtained from the in vivo studies in dogs are summarized in Table 1. No significant changes were observed in the YM-64227 pharmacokinetic parameters among the C/C homo, C/T hetero, or T/T homo groups after the administration of a single intravenous dose of YM-64227 (dose: 0.1 mg/kg). The CLint,in vivo values for the C/C, C/T, and T/T groups were 2120, 1787, and 2374 ml/h/kg, respectively, which were slightly higher than the canine hepatic blood flow rate (1620 ml/h/kg; Dedrick et al., 1973). The Cmax after a single oral administration (dose: 0.3 mg/kg) and absolute bioavailability in the T/T group were 17.1 times and 27.2 times higher than those for the C/C group, respectively. The CLint,in vivo values for the C/C, C/T, and T/T groups were 299.7, 221.0, and 116.0 l/h/kg, respectively. The CLint,in vivo for the T/T group was 38.7% of that for the C/C group.

The plasma concentration-time profiles of the metabolites after a single intravenous administration are shown in Fig. 3. The Cmax and AUCv,real values of MM-2 in the C/C and C/T groups were highest among the five metabolites (Table 2). The plasma concentrations of MM-1, MM-3, MM-4, and MM-5 in the C/C and C/T groups were low, and their Cmax and AUCv,real values were almost the same. For the T/T group, however, the plasma concentrations of MM-1, MM-4, and
FIG. 4. Plasma concentration-time profiles of YM-64227 metabolites after oral administration to dogs at 0.3 mg/kg. Each point represents the mean ± S.D. of five animals. (■) indicates C/C type dogs, (▲) indicates C/T type dogs, and (●) indicates T/T type dogs.
MM-5 were higher than those for the C/C group. The \( C_{\text{max}} \) and AUC\(_{\text{iv}} \) of MM-1, MM-4, and MM-5 were 10.4 and 44.6, 4.6 and 5.8, and 25.9 and 187.0 times higher than those for the C/C group, respectively (Table 2). The \( T_{1/2} \) for MM-1 and MM-5 in the T/T group were 4.8 and 2.8 times longer than those in the C/C group. Although the AUC\(_{\text{iv}} \) for MM-3 in the T/T group was 2.4 times higher than that in the C/C group, no significant difference was observed in the \( T_{1/2} \) for MM-3 between the C/C and T/T groups.

The plasma concentration-time profiles of metabolites after a single oral administration are shown in Fig. 4. The plasma MM-2 concentrations in the C/C and C/T groups were highest among the five metabolites as well as after intravenous dosing (Table 3). In the T/T group, the plasma MM-5 concentration was highest among the metabolites. The \( C_{\text{max}} \) and AUC\(_{\text{oral}} \) for the T/T group were 32.6 and 118.2 times higher than those for the C/C group. The \( C_{\text{max}} \) and AUC\(_{\text{oral}} \) of MM-1 in the T/T group were 3.4 times and 20.5 times higher than those for the C/C group. Although AUC\(_{\text{iv}} \) for MM-1, MM-4, and MM-5 in the T/T group were 10.4 and 44.6, 4.6 and 5.8, and 25.9 and 187.0 times higher than those for the C/C group, respectively. No significant differences were observed in the pharmacokinetic parameters of MM-3 between the C/C and T/T groups.

**YM-64227 Metabolism in Genotyped Canine Liver Microsomes.** Michaelis-Menten plots and Eadie-Hofstee plots of each metabolite formation from YM-64227 in genotyped canine liver microsomes are shown in Fig. 5. Because the contribution of multiple components in some of the metabolic pathways was suggested by the Eadie-Hofstee plots, either the mono- or biphase model was fitted to the metabolic activity of each metabolite using nonlinear least-squares regression. The best model was selected based on the Akaike’s information criterion values. The \( V_{\text{max}}, K_m, C_{\text{int}}, C_{\text{Lint,s}}, \) and \( C_{\text{Lint,ns}} \) values were calculated. Furthermore, the slope of the linear portion of the Michaelis-Menten plots was calculated using linear regression and defined as \( C_{\text{int,ns}} \). The kinetic parameters obtained from the in vitro experiments using liver microsomes are summarized in Table 4. The MM-1 formation activities of the C/C, C/T, and T/T groups had a monophasic pattern with a nonsaturable component. No significant differences in the \( C_{\text{int,ns}} \) were observed among the three groups. As for the MM-2 formation activities in all of the groups, contributions of both the saturable and nonsaturable component were demonstrated. Although differences in the \( C_{\text{int,ns}} \) were not observable among groups, the \( C_{\text{int,s}} \) for the T/T group was significantly lower than that for the C/C group, at only 8.6% of the C/C group value. The contribution of both the saturable and nonsaturable component was shown in the MM-3 formation activities of the C/C and C/T groups. Although no difference in \( C_{\text{int,ns}} \) among the three groups was observed, the saturable component was absent in the T/T group. Therefore, the \( C_{\text{int,ns}} \) for the T/T group was 12.6% of that for the C/C group. The contribution of both a saturable and nonsaturable component was detected in the MM-4 and MM-5 formation activities of all three groups. The \( C_{\text{int,ns}} \) values for MM-4 and MM-5 did not differ among the three groups.

**Correlation between \( C_{\text{int,ns}} \) and \( C_{\text{int,ns}} \).** Although the \( C_{\text{int,ns}} \) values were approximately 9.8 times higher than the \( C_{\text{max}} \) values, there was a statically significant correlation between them \( (r = 0.82, p < 0.001) \). The \( C_{\text{int,ns}} \) and \( C_{\text{int,ns}} \) values in the C/C and C/T groups were higher than those in the T/T group.

**Discussion**

The CYP1A2 1117 C>T SNP caused remarkable interindividual variability in the pharmacokinetics of YM-64227 in dogs. After intravenous administration of YM-64227, the \( C_{\text{Ltot,blood}} \) values were 1787 to 2374 ml/h/kg, which are slightly greater than the canine hepatic blood flow rate (1620 ml/h/kg; Dedrick et al., 1973). In contrast, the \( C_{\text{max}} \) and AUC\(_{\text{oral}} \) values in the T/T group were significantly greater than those in the C/C group. These findings suggest that the clearance of YM-64227 in dogs is “hepatic blood flow-limited” and the deficiency in CYP1A2 mainly affects first-pass metabolism.

After single intravenous and oral administrations of YM-64227, MM-2 was found to be the main metabolite in the C/C and C/T groups. However, the main metabolites found in the T/T group were MM-1 and MM-5, whose concentrations were significantly higher than those in the C/C and C/T groups. In addition, the AUC\(_{\text{iv}}\), AUC\(_{\text{oral}}\), and \( T_{1/2} \) values for MM-1, MM-4, and MM-5 in the T/T group were significantly greater than those in the C/C group, except for the \( T_{1/2} \) for MM-4 after intravenous administration. With regard to
MM-3, a significant difference was observed only in the AUC\textsubscript{\textit{CYP1A2}} of the T/T group. Taken together, the relative amounts and pharmacokinetic profiles of the metabolites in the C/C and C/T groups were remarkably different from those in the T/T group. It was shown that a deficiency of CYP1A2 enzyme activity leads to a decrease in MM-2, and an increase in MM-1, MM-4, and MM-5. MM-3 was slightly affected.

In an in vitro metabolism study using microsomes prepared from dogs used in the pharmacokinetic study, the contribution of both the saturable and nonsaturable components was observed in the formation of MM-2 in all groups. The CL\textsubscript{\textit{int,in vitro}} was not significantly different among the three groups whereas the CL\textsubscript{\textit{int,in vitro}} for the T/T group was more than 10-fold lower than that for the C/C and C/T groups. This finding was in good agreement with the fact that the hepatic metabolism of YM-64227 was well reflected in the in vitro metabolism study. Another possible explanation is that the formation of MM-3 is much faster than the subsequent metabolism of MM-3. Therefore, the CL\textsubscript{\textit{int,in vitro}} for MM-3 reflected the CL\textsubscript{\textit{int,in vivo}} value from CL\textsubscript{\textit{int,in vitro}} for drugs that undergo metabolism and in vitro studies is that CYP1A2 plays a significant role not only in the formation of MM-3 from YM-64227 but also in the subsequent metabolism of MM-3. Therefore, a decrease in the formation of MM-3 might be compensated by a decrease in the elimination of MM-3. Furthermore, in vitro metabolism study, another possible explanation is that the formation of MM-3 is much faster than the elimination of MM-3. Therefore, the CL\textsubscript{\textit{int,in vitro}} for MM-3 reflected mainly the formation of this metabolite in the C/C and C/T groups, and a CYP1A2 deficiency in the T/T group resulted in the decrease in the CL\textsubscript{\textit{int,in vitro}} for MM-3.

A statistically significant linear relationship was observed between CL\textsubscript{\textit{int,in vivo}} and CL\textsubscript{\textit{int,in vitro}} (\(r = 0.82, p < 0.001; \text{Fig. 6}\)), indicating that the hepatic metabolism of YM-64227 was well reflected in the pharmacokinetic profile. However, the in vitro study underestimated the in vivo intrinsic clearance by 9.8-fold. We searched for the cause of the difference between in vivo and in vitro. The binding of YM-64227 to the liver microsome and the extrahepatic metabolic clearance in the small intestine, lung, and kidney microsomes were negligible. An in vivo study in rats using radiolabeled YM-64227 showed that the oral absorption of YM-64227 is almost complete and that there is no significant accumulation in the liver (in-house data). In rats, the in vivo-in vitro correlation factor was 7.1, as it was in dogs (in-house data). It may be difficult to quantitatively estimate the CL\textsubscript{\textit{int,in vivo}} value from CL\textsubscript{\textit{int,in vitro}} for drugs that undergo metabolism

\[\text{\textit{V}}_{\text{m},n} \times (\text{nmmol/min/mg protein})\]

\[\text{\textit{K}} \times (\mu M)\]

\[\text{CL\textsubscript{\textit{int,in vitro}} (\mu l/min/mg protein)}\]

\[\text{CL\textsubscript{\textit{int,in vivo}} (\mu l/min/mg protein)}\]

\[\text{MM-3, a significant difference was observed only in the AUC\textsubscript{\textit{CYP1A2}} of the T/T group. Taken together, the relative amounts and pharmacokinetic profiles of the metabolites in the C/C and C/T groups were remarkably different from those in the T/T group. It was shown that a deficiency of CYP1A2 enzyme activity leads to a decrease in MM-2, and an increase in MM-1, MM-4, and MM-5. MM-3 was slightly affected. In an in vitro metabolism study using microsomes prepared from dogs used in the pharmacokinetic study, the contribution of both the saturable and nonsaturable components was observed in the formation of MM-2 in all groups. The CL\textsubscript{\textit{int,in vitro}} was not significantly different among the three groups whereas the CL\textsubscript{\textit{int,in vitro}} for the T/T group was more than 10-fold lower than that for the C/C and C/T groups. This suggests that CYP1A2 is involved in the saturable component. Therefore, the CL\textsubscript{\textit{int,in vitro}} value, defined as the sum of CL\textsubscript{\textit{int,s}} and CL\textsubscript{\textit{int,n}}, for the T/T group was more than 10-fold lower than that for the C/C and C/T groups. This finding was in good agreement with the fact that the plasma concentrations of MM-2 in the T/T group were significantly lower than those in the other groups. The contribution of one nonsaturable component was observed in the formation of MM-1. The kinetics of MM-4 and MM-5 formation was biphasic, consisting of a saturable and nonsaturable component. The CL\textsubscript{\textit{int,in vitro}} values for these metabolites were not significantly different among the three groups, which suggest that CYP1A2 does not mediate the biotransformation of YM-64227 into these metabolites. These findings suggest that the formation of MM-1, MM-4, and MM-5 is not different in the C/C, C/T, and T/T groups, but the elimination of these metabolites in the T/T group is decreased. A possible explanation for this discrepancy is that CYP1A2 mediates the subsequent metabolism of MM-1, MM-4, and MM-5. Both the saturable and nonsaturable components were observed in the formation of MM-3 in the C/C and C/T groups, whereas only the nonsaturable component was observed in the T/T group, which suggests that CYP1A2 is fully responsible for the saturable component. Therefore, the CL\textsubscript{\textit{int,in vitro}} for MM-3 in the T/T group was around 10-fold lower than that in the C/C and C/T groups. Thus, plasma concentrations of MM-3 in the T/T group were expected to be lower than those in the C/C and C/T groups. However, no marked differences were observed in MM-3 plasma concentration among the three groups. A possible explanation for the conflict between the in vivo and in vitro studies is that CYP1A2 plays a significant role not only in the formation of MM-3 from YM-64227 but also in the subsequent metabolism of MM-3. Therefore, a decrease in the formation of MM-3 might be compensated by a decrease in the elimination of MM-3. Furthermore, in vitro metabolism study, another possible explanation is that the formation of MM-3 is much faster than the elimination of MM-3. Therefore, the CL\textsubscript{\textit{int,in vitro}} for MM-3 reflected mainly the formation of this metabolite in the C/C and C/T groups, and a CYP1A2 deficiency in the T/T group resulted in the decrease in the CL\textsubscript{\textit{int,in vitro}} for MM-3. A statistically significant linear relationship was observed between CL\textsubscript{\textit{int,in vivo}} and CL\textsubscript{\textit{int,in vitro}} (\(r = 0.82, p < 0.001; \text{Fig. 6}\)), indicating that the hepatic metabolism of YM-64227 was well reflected in the pharmacokinetic profile. However, the in vitro study underestimated the in vivo intrinsic clearance by 9.8-fold. We searched for the cause of the difference between in vivo and in vitro. The binding of YM-64227 to the liver microsome and the extrahepatic metabolic clearance in the small intestine, lung, and kidney microsomes were negligible. An in vivo study in rats using radiolabeled YM-64227 showed that the oral absorption of YM-64227 is almost complete and that there is no significant accumulation in the liver (in-house data). In rats, the in vivo-in vitro correlation factor was 7.1, as it was in dogs (in-house data). It may be difficult to quantitatively estimate the CL\textsubscript{\textit{int,in vivo}} value from CL\textsubscript{\textit{int,in vitro}} for drugs that undergo metabolism

\[\text{\textit{V}}_{\text{m},n} \times (\text{nmmol/min/mg protein})\]

\[\text{\textit{K}} \times (\mu M)\]

\[\text{CL\textsubscript{\textit{int,in vitro}} (\mu l/min/mg protein)}\]

\[\text{CL\textsubscript{\textit{int,in vivo}} (\mu l/min/mg protein)}\]
that is limited by blood flow (Iwatsubo et al., 1997). Another explanation for the discrepancy between the in vitro and in vivo clearances may be because one or more of the following assumptions is erroneous: 1) the blood concentration and the concentration in the liver are in rapid equilibrium, 2) only unbound drug is involved in the elimination and transport across membranes, and 3) metabolic enzymes and transporters are homogenously distributed along the blood flow pathways in the liver (Iwatsubo et al., 1996; Naritomi et al., 2001).

In the previous study, it was first reported that CYP1A2 1117 C>T SNP causes a stop codon (Tenniziu et al., 2004a). The T-allele frequency was 0.39, which suggests that 10 to 15% of the dogs would not express the CYP1A2 protein. In this study, we reported the results from in vivo and in vitro studies using genotyped dogs and liver microsomes to clearly demonstrate the influence of the SNP. Therefore, attention should be paid to canine CYP1A2 polymorphism when using dogs to investigate the pharmacokinetics of drugs that are metabolized by CYP1A2. In addition, a considerable number of detailed studies on human CYP1A2 polymorphisms have been reported. In particular, CYP1A2*1C, *1F, *1K, and *7 showed an altered enzyme activity (http://www.imm.ki.se/CYPalleles/default.htm). Phenotype study using the caffeine test demonstrated that CYP1A2 has a poor metabolization frequency of only 5 to 15% (Tantcheva-Poor et al., 1999). However, no genetic factor has been identified that would cause interindividual variation in the caffeine test; thus, lifestyle factors such as cigarette smoking and food choice (Pantuck et al., 1972; Kotake et al., 1982) are thought to influence them. A clinically significant SNP that could confer obvious interindividual variation has not yet been discovered for human CYP1A2.

In conclusion, the CYP1A2 deficiency polymorphism 1117 C>T dramatically decreased the metabolic clearance of YM-64227 in dogs. The CYP1A2 polymorphism caused a remarkable increase in the plasma concentration of the unchanged drug after oral administration of YM-64227, but intravenous administration did not have the same effect because the hepatic elimination of YM-64227 is hepatic blood flow rate-limited. CYP1A2 polymorphism dramatically altered the metabolic profile of YM-64227. Attention should be paid to this canine CYP1A2 polymorphism in future studies to understand the impact of interindividual variability for the disposition of drugs in dogs.

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References

TENMIZU ET AL.


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Address correspondence to: DaiMune Tenmizu, Drug Metabolism Research Laboratories, Astellas Pharma Inc., 1-8, Azusawa 1-Chome, Itabashi-ku, Tokyo 174-8511, Japan. E-mail: daisuke.tenmizu@jp.astellas.com

806