Pharmacogenomic/pharmacokinetic assessment of a four-probe cocktail for CYPs and OATPs following oral microdosing

Ichiro Ieiri, Masato Fukae, Kazuya Maeda, Yukie Ando, Miyuki Kimura, Takeshi Hirota, Takeshi Nakamura, Kazuhide Iwasaki, Shunji Matsuki, Kyoko Matsuguma, Eri Kanda, Mariko Deguchi, Shin Irie and Yuichi Sugiyama

Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Kyushu Clinical Pharmacology Research Clinic, Fukuoka, and Business Development Division, Contract Research Company, Shin Nippon Biomedical Laboratories, Ltd, Osaka, Japan

Key words: pharmacogenomics – pharmacokinetics – cytochrome P450 – OATPs – oral microdosing

Abstract. Objectives: To test whether the multiple phenotype and genotype relationships established using therapeutic dose, can be reproduced following oral microdosing using substrates of CYP2C9 (warfarin and glibenclamide), CYP2C19 (lansoprazole), CYP2D6 (dextromethorphan), and OATPs (glibenclamide). Methods: A cocktail of test drugs was administered orally under the microdose in liquid or capsule form, and then a therapeutic dose of dextromethorphan was administered to 17 healthy subjects whose genotypes for CYPs and OATPs had been prescreened. Concentrations of the drugs and their metabolites were measured by LC-MS/MS. Results: The AUC and t1/2 of glibenclamide following the microdosing tended to be higher and longer, respectively, in CYP2C9*1/*3 than CYP2C9*1/*1 subjects. In contrast, there were no significant differences in any of the pharmacokinetic parameters for warfarin between the two genotypes. For CYP2D6 following the therapeutic dose, there was good concordance between genotype and phenotype: however, such relationships disappeared after microdosing. For CYP2C19 following the microdosing, there were significant differences between EMs and PMs in the pharmacokinetic parameters of lansoprazole. The relative AUC0-12 ratio of lansoprazole in EMs and PMs was 1.33 – 4.3. Among test drugs, phenotypic measurements of lansoprazole accorded well with the CYP2C19 genotype at the microdose as well as therapeutic dose. Conclusions: The present study suggests that 1) the sampling strategy should be optimized according to pharmacokinetic profiles of the test drugs following oral microdosing, and 2) microdosing can be applied to the pharmacogenomic study of CYP-specific drugs.

Introduction

Drug development costs continue to increase, while the number of new approved drugs has been declining [1, 2]. One reason for this is the low success rate in clinical trials, with unfavorable pharmacokinetics (e.g., ADME) accounting for ~10% of cases where new drug candidates were dropped from the clinical testing in 2000 [3, 4]. To enhance the probability of success in drug development, the concept of microdosing was introduced into clinical trials in Europe in 2004, the US in 2006, and Japan in 2008.

In microdosing studies, pharmacokinetic profiles are characterized after a single administration of the test drug at a maximum dose of 100 μg or at a dose less than 1% of the pharmacologically effective dose [5]. Such lower doses should reduce the risk of toxicological events, especially for drug cocktails. Recent advances in LC-MS/MS measurements have made it possible to determine plasma/urine concentrations of drugs without radio-labeling, also provide a simple and safe means of microdosing in clinical trial phases.

It is well established that large interindividual differences exist in the activities of drug-metabolizing enzymes and transporters. Estimates of individual enzymatic activity are useful not only for personalized drug therapy but also for the development of new medicines. In terms of drug-metabolizing enzymes, the "cocktail" test has already
been used to estimate the individual activity of specific cytochrome P450s (CYPs) and other enzymes (e.g., N-acetyltransferase, NAT2) simultaneously [6, 7]. The following drug and enzyme sets are applicable: caffeine (CYP1A2) [7], flurbiprofen, diclofenac, and warfarin (CYP2C9) [6, 7, 8, 9]; mephenytoin and omeprazole (CYP2C19) [6, 7]; debrisoquin and dextromethorphan (CYP2D6) [6, 7]; chloroxazone (CYP2E1) [7]; and dapsone (NAT2) [7]. To date, several cocktails following the concurrent administration of CYP-selective drugs have been reported [10, 11]. Most such studies have found the cocktails to be well tolerated, however, since therapeutic doses are used, the risk of side effects remains when increasing the number and/or dose of the test drugs. Furthermore, most genes of the CYP-selective drugs are polymorphic. Subjects with non-functional allele(s) exhibit low metabolic activities, resulting in high plasma concentrations of the drugs. Safety and validated multiple phenotype tests with genotype assessments for non-functional alleles are desirable.

In the present study, we applied the concept of microdosing to the study of drug-metabolizing cocktails in order to test whether the multiple phenotype and genotype relationships established using therapeutic doses, can be reproduced with extremely low doses of following CYP-specific drugs; warfarin and glibenclamide (CYP2C9), lansoprazole (CYP2C19), dextromethorphan (CYP2D6), and glibenclamide (organic anion transporting polypeptides, OATPs) [12, 13]. To compare accuracy between individual phenotyping and genotyping results, genotypes of CYPs and OATPs were prescreened. In the microdosing phase, two dosage formulations, liquid and capsule, were used. After the microdosing phase, dextromethorphan was administered again as a therapeutic dose (30 mg) to compare the pharmacokinetic/pharmacogenomic relationship between the microdose and therapeutic dose conditions. Another purpose in the present study was to apply conventional and simultaneous LC-MS/MS determinations for phenotyping of CYPs using non-radio-labeled test probes.

Materials and methods

Participants and genotyping of CYPs and SLCOs

This study was approved by the Ethics Review Boards of Kyushu University, Tokyo University, and the Kyushu Pharmacology Research Clinic. Written informed consent was obtained from all participants before the study. 17 unrelated healthy male volunteers (age, 20 – 33 years; BMI, 16.3 – 27.8 kg/m²) were selected from study panels based on genotyping for CYPs. Genotyping for SLCOs was also done. Each participant was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs. None had taken any drugs or beverages containing grapefruit juice for at least 1 week before the study.

Genomic DNA was isolated from blood samples. Genotyping of CYP2C9, CYP2C19, CYP2D6, OATP1B1, OATP1B3, and OATP2B1 was performed according to methods described previously [14, 15, 16, 17, 18, 19]. The subjects were genotyped for CYP2C9*3; CYP2C19*2 and *3; CYP2D6*10; SLC01B1*1b and *15; SLC01B3 c.334T>G, c.699G>A, and +9158A>G; and SLC02B1*3 c.935G>A, and a 9-bp deletion at c.76. Subjects with the two non-functional alleles (*2 and *3) for CYP2C19 were identified as poor metabolizers (PMs). Homozygotes for the *1 allele, and heterozygotes for the mutant alleles were defined as homozygous extensive metabolizers (hmEMs) and heterozygous EMs (htEMs), respectively. In contrast to the *2 and *3 variants, *17 allele is known to be associated with a high metabolic activity of CYP2C19; however, since the frequency of the *17 allele is limited in Japanese population (i.e., 1.3%) [20], we did not diagnose this allele in the present study.

Study protocol

In the microdosing study, we tested two formulations, liquid and capsule, because glibenclamide shows insolubility. In the liquid formulation test, each subject received a solution comprising 10 μg of warfarin (Warfarin-K YD, Yoshindo, Toyama, Japan) and 100 μg of dextromethorphan (Wako,
Osaka, Japan) dissolved in purified water (3 ml), with 150 ml of water under fasting conditions. Because lansoprazole is entericoated, one granule (50 – 70 μg) (Takepran, Takeda, Osaka, Japan) wrapped in rice paper was administered simultaneously. Following a washout period of at least 7 days, subjects received a gelatin capsule containing 10 μg of glibenclamide (Biomol, Tokyo, Japan), 10 μg of warfarin, 100 μg of dextromethorphan, and 50 – 70 μg of lansoprazole with 150 ml of water under fasting conditions. After a 24-h washout period, they received a therapeutic dose (30 mg) of dextromethorphan (Medicon tablet, Shionogi, Osaka, Japan) with 150 ml of water. The concentration of the test drugs in a microdose solution was confirmed by the same quantitative LC-MS/MS methods used for the plasma and urine samples (n = 3).

Venous blood samples (5 ml each for the microdose and therapeutic dose tests) were obtained before and 0.5, 1, 2, 4, 8, and 12 h after dosing. The samples were immediately centrifuged at 3,000 rpm for 10 min at 4 °C, and the supernatant was stored at –20 °C until analyzed. Urine was collected for 12 h. Total urine volume and urine pH were recorded, and two aliquots of urine were stored at –20 °C for analysis. This study was registered in the UMIN Clinical Trials Registry at www.umin.ac.jp/ctr/index.htm (UMIN000003920).

Quantification of the test drugs and their metabolites in plasma and urine

All drugs and metabolites were determined by a fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS). For the simultaneous determination of warfarin and glibenclamide concentrations in plasma, 200 μl of each plasma sample from the clinical study was subjected to solid-phase extraction with a solid extraction cartridge (OASIS HLB 1cc/30 mg, Waters, Milford, MA, USA) prior to LC-MS/MS. Chromatographic separation was achieved on a Cadenza CD-C18 column (2.0 mm i.d. × 150 mm, 3 μm, Intakt Corp., Kyoto, Japan) by elution with a gradient of acetonitrile and a 20 mM ammonium acetate solution (pH 7.4) at 40 °C and at flow rate of 0.3 ml/min. Quantitation was performed with electrospray ionization (ESI) in the positive mode on an API5000 (AB Scie, Foster City, CA, USA) by selected reaction monitoring (SRM) with a transition of 387 m/z to 163 m/z for warfarin, and 494 m/z to 369 m/z for glibenclamide. For the simultaneous determination of lansoprazole and its metabolite, 5-hydroxylansoprazole, in plasma, 100 μl of each sample was subjected to solvent extraction with acetonitrile and methanol prior to LC-MS/MS. Chromatographic separation was achieved on a CAPCELL PAK C18 MG-II (2.0 mm i.d. × 50 mm, 3 μm, Shiseido, Tokyo, Japan) by elution with acetonitrile and a 20 mM acetate ammonium (pH 7.6) solution at 40 °C and a flow rate of 0.3 ml/min. Quantitation was again performed with the API5000 with a transition of 370 m/z to 234 m/z for lansoprazole, and 386 m/z to 234 m/z for 5-hydroxylansoprazole. For the simultaneous determination of dextromethorphan and its metabolite, dextorphan, in urine, 100 μl of each sample was subjected to solvent extraction with t-butyl methyl ether prior to LC-MS/MS. Chromatographic separation was achieved on a CAPCELL PAK C18 MG-II (2.0 mm i.d. × 50 mm, 3 μm) by elution with acetonitrile and a 20 mM ammonium acetate solution (pH 6.8) at 40 °C and a flow rate of 0.3 ml/min. Quantitation was performed with API5000 by SRM with a transition of 272 m/z to 215 m/z for dextromethorphan, and 258 m/z to 157 m/z for dextorphan. Validated quantitation ranges were as follows: 50 – 50,000 ng/ml for warfarin; 1 – 1,000 pg/ml for glibenclamide; and 10 – 10,000 pg/ml for lansoprazole, 5-hydroxylansoprazole, dextromethorphan, and dextorphan.

Pharmacokinetic analysis

Noncompartmental analysis

The pharmacokinetic analysis was performed using Phoenix WinNonlin 6.1 (Pharsight, Mountain View, CA, USA). The AUCo-12 of the test drugs was calculated by the linear trapezoidal rule. Cmax and tmax values were obtained directly from the data. Kel was calculated by least squares regression
from the terminal post-distribution phase of the concentration-time curve. The AUC$\text{C}_{0-\infty}$ was calculated as follows: $\text{AUC}_{0-\infty} = \text{AUC}_{0-12} + C_{\text{last}}/\text{Kel}$, where $C_{\text{last}}$ is the concentration at the last time. The CL/F and $t_{1/2}$ values were calculated as follows: $\text{CL/F} = \text{Dose}/\text{AUC}_{0-12}$ and $t_{1/2} = 0.693/\text{Kel}$.

**Population pharmacokinetics analysis**

The pharmacokinetics of lansoprazole was also analyzed by the nonlinear mixed-effects modeling approach with the software package NONMEM, version VI level 1.0 (Icon Development Solution, Ellicott City, MD, USA) with regard to the CYP2C19 polymorphisms [21]. To check the contribution of the formulation (capsule or liquid) to bioavailability (F), the data from both experiments were combined for the analysis. The first-order conditional estimation-interaction (FOCE-I) method was used throughout. After the oral administration of lansoprazole, the plasmatic profile showed linear elimination. Therefore, 1-compartment model was selected as the structure model. Inter-individual variability in all parameters and residual errors were described by the exponential error model. As candidates for the physiological covariate, body weight relative to apparent clearance (CL/F) and volume of distribution (V/F) were tested with power functions. The effect of CYP2C19 variants on apparent clearance was assessed as follows:

$\text{CL/F}_i = N_{\text{wt}} \times \text{CL/F}_{\text{wt}} + N_{\text{mut}} \times \text{CL/F}_{\text{mut}}$

where $\text{CL/F}_i$ represents the apparent clearance of the ith individual, and $N_{\text{wt}}$ and $N_{\text{mut}}$ represent the number of wild-type and mutant alleles, respectively. CL/F$_{\text{wt}}$ and CL/
Table 1. Pharmacokinetic parameters of the test drugs after the microdosing with regard to CYP- and OATPs-genetic polymorphisms.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Metabolic enzyme/transporter</th>
<th>Pharmacokinetic parameter</th>
<th>Formulation</th>
<th>Capsule phase genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liquid phase genotyping</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>wt/wt</td>
<td>wt/mt</td>
</tr>
<tr>
<td>Gilbenclamide</td>
<td>CYP2C9 (*3 allele)</td>
<td>n</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{1/2} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>CYP2C9 (*3 allele)</td>
<td>n</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>CYP2C19 (*2 and *3 alleles)</td>
<td>n</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{1/2} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-hydroxylansoprazole</td>
<td>CYP2C19 (*2 and *3 alleles)</td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilbenclamide</td>
<td>SLCO1B1 (*15 allele)</td>
<td>n</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+9158A&gt;G)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLCO1B3</td>
<td>(3347G&gt;A, 699G&gt;A)</td>
<td>n</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+3, 1457C&gt;T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC_{0-12} (pg·h/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>t_{max} (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_{max} (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean or mean ± S.D.

F_{mt} represent partial clearance in the wild-type and mutant type subjects, respectively (mt = 1 or 2). Forward inclusion and backward elimination methods were used to build the final model. The significance of the physiological covariate and the genotype's effect was assessed by comparing the objective functions (-2 log likelihood) for different models, assuming a χ²-distribution of their distance. To assess the robustness of the estimated parameters, a bootstrap analysis was performed.
Phenotyping for CYP2D6

The metabolic ratio (MR) of dextromethorphan was determined as the ratio of the molar recovery of dextromethorphan to that of dextrophan in the post-dose 12-h urine [22, 23].

Statistical analysis

Statistical differences among the data for each group were determined by analysis of variance (ANOVA), followed by Fisher's least significant difference test. The Mann-Whitney U-test was also used, as appropriate. A p value of less than 0.05 was considered statistically significant.

For both CYP2D6 and CYP2C19 genotype/phenotype studies, activity scores have been developed [24, 25, 26]. These scores are very helpful for prediction of individual metabolic capability; however, since only two (*2 and *3) and *10 variants were diagnosed for CYP2C19 and CYP2D6, respectively, we did not use these scores in the present study.

Results

All participants completed the study according to the protocol without clinically important adverse events.

CYP2C9

The mean plasma concentration-time profile of glibenclamide in the two CYP2C9 groups is shown in Figure 1A, with the corresponding pharmacokinetic parameters summarized in Table 1. The mean AUC_{0-12} (± S.D., 1,009 ± 262 vs. 801 ± 203 pg·h/ml) and AUC_{0-∞} (1,189 ± 332 vs. 839 ± 212) tended to be higher in CYP2C9*1/*3 than CYP2C9*1/*1 subjects. The elimination half-life (t_{1/2}) of glibenclamide was longer in the CYP2C9*1/*3 subjects; 3.76 ± 2.62 vs. 2.37 ± 0.54 h.

There were no differences in the mean concentration of warfarin in plasma between the two genotypes (CYP2C9*1/*1 and *1/*3) with either formulation in the microdosing tests (Figure 1B). As expected from the data shown in Figure 1B, there were no differences in any of the pharmacokinetic parameters of warfarin between the two groups (Table 1).

The AUC_{0-12} of warfarin in capsule form in each individual significantly correlated with that in liquid form (R^2 = 0.629) (Figure 2). Since warfarin and glibenclamide are both reported to be good substrates for CYP2C9, we compared pharmacokinetic parameters between the two drugs. The AUC_{0-12} of warfarin was weakly but significantly correlated with that of glibenclamide (R^2 = 0.299, p < 0.05) (Figure 2).
CYP2C19

For both liquid and capsule forms, there were significant differences between hmEMs and PMs in most of the pharmacokinetic parameters of lansoprazole (Table 1). A genedose effect relationship was observed in the mean plasma concentration-time profiles; the mean profile in hTEMs was located between that in hmEMs and in PMs (Figure 1C). The relative AUC0-12 ratio of lansoprazole in hmEMs, hTEMs and PMs administered the liquid and capsule formulations was 1:2.1:4.3 and 1:3.6:3.3, respectively. In contrast, the pharmacokinetic data for 5-hydroxylansoprazole were opposite to that observed for lansoprazole (Table 1).

CYP2D6

Figure 3 shows the mean dextromethorphan MR in relation to the genotype of CYP2D6*10 following the microdose and therapeutic dose tests. For the therapeutic dose, the mean MR (± SD) in CYP2D6*1/*1, *1/*10, and *10/*10 subjects was 0.032 ± 0.014, 0.089 ± 0.069, and 0.104 ± 0.118, respectively. Although large inter-individual differences existed, the mean MR value was larger in those homozygous for the *10 allele than those homozygous for the wild-type allele, and heterozygotes had mean values between those of the two homozygous groups. In the microdose test, both with liquid and capsule forms, the gene-dose effect relationship disappeared. Heterozygous carriers of the CYP2D6*10 allele had the highest mean MR values among the three genotypes.

Transporters

We compared the pharmacokinetic parameters of glibenclamide between different genotypes with regard to three transporter
Table 2. Estimated population parameters of lansoprazole following the microdosing with regard to CYP2C19 polymorphisms.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>RSE (%)</th>
<th>1,000 bootstrap samples</th>
<th>95% LLCI</th>
<th>95% ULCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_a$ (h)</td>
<td>1.65</td>
<td>25.7</td>
<td>1.69</td>
<td>0.83</td>
<td>3.18</td>
</tr>
<tr>
<td>Partial CL/F for wt (h)</td>
<td>4.72</td>
<td>17.0</td>
<td>4.76</td>
<td>3.27</td>
<td>6.49</td>
</tr>
<tr>
<td>Partial CL/F for mt (h)</td>
<td>1.35</td>
<td>10.3</td>
<td>1.33</td>
<td>1.10</td>
<td>1.64</td>
</tr>
<tr>
<td>V/F (l)</td>
<td>13.7</td>
<td>14.6</td>
<td>13.6</td>
<td>8.80</td>
<td>17.7</td>
</tr>
<tr>
<td>Inter-individual variability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV CL/F (CV; %)</td>
<td>35.8</td>
<td>46.6</td>
<td>33.2</td>
<td>16.1</td>
<td>51.6</td>
</tr>
<tr>
<td>IIV V/F (CV; %)</td>
<td>33.6</td>
<td>58.5</td>
<td>32.2</td>
<td>10.8</td>
<td>55.0</td>
</tr>
<tr>
<td>Residual variability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma$: Proportional</td>
<td>0.473</td>
<td>10.3</td>
<td>0.472</td>
<td>0.398</td>
<td>0.538</td>
</tr>
</tbody>
</table>

RSE = relative standard error; 95% LLCI = lower limit of 95% confidence interval; 95% ULCI = upper limit of 95% confidence interval; IIV = inter-individual variability.

gene polymorphisms, SCL01B1, SCL01B3, and SLC02B1 (Table 1), but found no significant differences.

Population pharmacokinetic analysis

No apparent systematic deviations were observed in population (Figure 4A) or individual (Figure 4B) predictions, and goodness of fit plots showed no bias except conditional weighted residuals; the residuals tended to be low in early times after dosing (Figure 4C, D). Estimated population pharmacokinetic parameters are listed in Table 2. The analysis confirmed that the individual genotypes of CYP2C19 play a significant role in the clearance of lansoprazole. The effect of the CYP2C19 genotype on the apparent clearance of lansoprazole was associated with a significant reduction in the objective function value (2040.821–2020.694). The mean value of apparent clearance in hMEMs, hTEMs and PMs was 9.44, 6.07, and 2.701/h, respectively. No obvious effect of the type of formulation on bioavailability (F) was found.

Discussion

To assess the activity of individual drug-metabolizing enzymes, cocktail-based regimens have been introduced in both the research and clinical fields. The “Cooperstown 5 + 1 cocktail”, including caffeine for the determination of CYP1A2, NAT2, and xanthine oxidase (XO) activities, omeprazole for CYP2C19, dextromethorphan for CYP2D6, midazolam for CYP3A, and warfarin for CYP2C9, provides for accurate phenotyping without limitations of safety, and assay and drug interactions [6]. Similarly, the “6-drug Pittsburgh cocktail”, comprising caffeine (CYP1A2), flurbiprofen (CYP2C9), mephenytoin (CYP2C19), debisquin (CYP2D6), chlorzoxazone (CYP2E1), and dapsone (NAT2), has also been reported; however, mephenytoin and debisquin are not recently commercially available in some countries [7].

The main purpose of the present study was to test the reproducibility of genotype and phenotype relationships established using therapeutic doses, with CYP-specific drugs following microdosing. For this purpose, appropriate probes which have good concordance between individual genotypes and phenotypes should be selected.

Several studies have investigated the effects of genetic polymorphisms of CYP2C9 on the pharmacokinetics of glibenclamide [27, 28]. Most found the pharmacokinetics to depend on the CYP2C9*3 genotype; the average clearance of glibenclamide in CYP2C9*1/*3 and CYP2C9*3/*3 subjects ranged from less than half to one sixth of that in CYP2C9*1/*1 subjects. Hypoglycemia also developed more frequently in subjects with the CYP2C9*3 allele [28]. Warfarin is also a major substrate for CYP2C9 [29]. The pharmacokinetic profile of warfarin is affected by CYP2C9 variants; CYP2C9*1/*3 and CYP2C9*3/*3 subjects exhibited respectively 63 to 66% and 90% reductions in the clearance of unbound (S)-warfarin compared with CYP2C9*1/*1 subjects, and the formation clearance of (S)-7-hydroxywarfarin in these groups was reduced to a similar extent to that of unbound (S)-warfarin [30, 31]. A recent meta-analysis, including 39 studies (7,907 patients), indicated that patients with genotypes CYP2C9*1/*2, *1/*3, *2/*2, and *3/*3 required warfarin doses that were 19.6, 33.7, 36.0, 56.7, and 78.1% lower than those in CYP2C9*1/*1 subjects, respectively [32]. Similar to CYP2C9, various drugs have been used for the phenotyping of CYP2D6 including dextromethorphan, which is relatively safe and has high affinity for CYP2D6 [33]. Assessments of phenotype with dex-
tromethorphan involve the administration of a single oral dose to the subjects, followed by the collection of urine samples. The ratio of dextromethorphan to dextrophan (molar concentrations) in urine (i.e., MR) is a good in vivo index of CYP2D6 activity [22, 34]. Despite that the frequency of PMs is extremely low (less than 1%), the CYP2D6*10 variant, which is associated with lower CYP2D6 activity, resulting in “intermediate metabolizers (IM)”, has been found at high frequencies (i.e., ~ 50%) in Asian populations [35, 36]. Indeed, our previous study indicated that the mean MR value (± SD) in *1/*1, *1/*10, and *10/*10 subjects was 0.001 (0.0005), 0.004 (0.006), and 0.03 (0.02), respectively and significantly different between the two homozygous groups [16]. Finally, we selected lansoprazole as a test drug for CYP2C19. Previous studies have shown that the AUC of lansoprazole, similar in chemical structure and metabolic pathways to omeprazole, was significantly increased in PM individuals [37, 38]. In addition to these two proton pump inhibitors, pantoprazole is really known as good substrate for CYP2C19 [39, 40]; however, unfortunately, pantoprazole is not commercially available in Japan. Taking these findings into consideration, we selected CYP-specific drugs for this study.

Glibenclamide has also been reported to be a substrate for some organic anion transporting polypeptide (OATP) [12, 13], we tested the relationship between its pharmacokinetics and transporter gene polymorphisms. SLCO1B1, SLCO2B1, and SLCO1B3 are involved in the large inter-individual differences in the pharmacokinetics of some clinical drugs [19, 41]. No obvious effect of any transporter gene polymorphisms on the pharmacokinetics of glibenclamide was demonstrated. Since the pharmacokinetics of glibenclamide tended to differ among CYP2C9 genotypes, the contribution of transporters to the overall pharmacokinetics is suggested to be weaker than that of CYP2C9. However, this was also probably a result of the small number of homozygotes and absence of homozygotes for the mutant *3 allele in Asians.

Among the substrates tested, phenotypic measurements of glibenclamide and lansoprazole were in concordance with the CYP2C9 and CYP2C19 genotypes, respectively, following the microdosing tests. The mean CL/F of glibenclamide was 27% lower in CYP2C9*1/*3 than CYP2C9*1/*1 subjects (Table 1), and in line with findings for therapeutic doses [27], in which the mean total clearance of glibenclamide in *3/*3 and *1/*3 subjects was less than 50% and 28%, respectively, of that in the *1/*1 subjects. For CYP2C19, individual phenotypes well co-segregated with the genotypes; the relative AUCs of lansoprazole in hHEM, hTEM, and PMs after microdosing were 1: 2.1: 4.1 (liquid) and 1: 3.6: 3.3 (capsule). These results are consistent with our previous study using therapeutic doses, in which the relative AUC in hHEM, hTEM, and PMs was 1: 1.7: 3.9 [38]. Initially, the gene-dose effect was unexpected, because hTEMs, who had one functional allele, could metabolize trace amounts (i.e., microdoses) of lansoprazole, showing similar pharmacokinetic values to hHEMs. The present study indicates that microdosing can be used to assess drug-metabolizing status in heterozygous individuals. The pharmacokinetics of 5-hydroxylansoprazole, a major metabolite of lansoprazole produced by CYP2C19, also co-segregated with genotype; the mean AUC of 5-hydroxylansoprazole was significantly lower in CYP2C19*3/*3 subjects than in CYP2C19*1/*1 subjects for both liquid (19.4 ± 18.2 vs. 4.1 ± 1.4) and capsule (15.1 ± 6.8 vs. 2.7 ± 1.8 h·ng/ml) formulations.

For warfarin, CYP2C9 genotype-dependent phenotypes could not be reproduced in the present microdosing study. Several factors are likely to contribute to the discrepancy. First, we measured racemic warfarin concentrations in plasma. Since CYP2C9 is known to be involved in the metabolism of (R)-warfarin, (S)-warfarin concentrations would be expected to give more accurate results in this case. Second, a 12-h study may be insufficient, given that the mean half-life is 274 h for a dose of 100 μg [42], to allow full characterization of the pharmacokinetics of warfarin. Indeed, due to its wide tissue binding (i.e., liver), volume of distribution was extremely larger (671 vs. 10 – 18 l for the therapeutic dose) after microdose administration in the CREAM trial, which probably make half-life long [42].

For dextromethorphan, however, individual phenotypes and genotypes did not match following the microdosing. Several studies have indicated the antimode of MR to be
around at 0.3 [22, 23]. As mentioned previ-
ously, the frequency of PMs in Asian popu-
lations is less than 1%; however, 3 (17.6%), 2 were CYP2D6*10/*10 and 1 was *1/*10
subjects for the liquid form and 2 (11.8%), one was CYP2D6*10/*10 and another *1/*10
for the capsule form, who had an EM geno-
type, had an extremely high value (> 0.3) for
MR and were classified as phenotypic PMs.
Several technical factors for the measure-
ment of the parent and its metabolites have
been reported to influence the variability of
MR. Omission of the deconjugation process
resulted in a 10-times lower recovery of dex-
trophan in urine, leading to a large over-
ap in MR between EMs and PMs [23, 43, 44].

In conclusion, among the four drugs test-
ed, a genotype/phenotype mismatch was ob-
erved for warfarin and dextromethorphan in
microdosing tests. Technical issues such as
the duration of sampling and pre-treatment
methods (e.g., deconjugation), may be in-
volved in the mismatch. In contrast, it is sug-
gested that lansoprazole and glibenclamide
can be used for CYP2C19- and CYP2C9-
based microdosing pharmacogenomic/phar-
macokinetic assessments.

Acknowledgments

This study is part of a Research Project for the “Establishment of Evolutional Drug Development with the Use of Microdose Clinical Trials” supported by the New Energy and Industrial Technology Development Organization in Japan.

References

[1] DiMasi JA, Hansen RW, Grabowski HG. The
price of innovation: new estimates of drug de-
[2] Kola I, Landis J. Can the pharmaceutical industry
drug discovery and development. Nat Rev Drug
clinical study — why necessary and how useful?
[5] Combes RD, Berridge T, Connelly J, Eve MD,
Garner RC, Toon S, Wilcox P. Early microdose
drug studies in human volunteers can minimise
animal testing: Proceedings of a workshop organ-
ised by Volunteers in Research and Testing. Eur J
[6] Chandnovski S, Nafziger AN, Ledeer JS, Gaedigk A,
Kearns GL, Sellers E, Zhang Y, Kashuba AD,
Rowland E, Bertino JS Jr. Combined phenotypic
assessment of cytochrome p450 1A2, 2C9, 2C19,
2D6, and 3A, N-acetyltransferase-2, and xanthine
oxidase activities with the “Cooperstown 5+1
cocktail”. Clin Pharmacol Ther. 2003; 74: 437-
447.
[7] Zgheib NK, Frye RF, Tracy TS, Romkes M, Branch
RA. Validation of incorporating furbufiropeno into
2006; 80: 257-263.
Rost KL, Kovar A. Investigation of sarizotan’s
impact on the pharmacokinetics of probe drugs for
major cytochrome P450 isoenzymes: a combined
cocktail trial. Eur J Clin Pharmacol. 2006; 62:
277-284.
Pottiers F, Donazzolo Y, Boulenc X. Pharmacoki-
etic assessment of a five-probe cocktail for CYPs
1A2, 2C9, 2C19, 2D6 and 3A. Br J Clin Pharma-
[10] Ma JD, Nafziger AN, Villano SA, Gaedigk A, Ber-
tino JS Jr. Maribavir pharmacokinetics and the
effects of multiple-dose maribavir on cytochrome
P450 (CYP) 1A2, CYP 2C9, CYP 2C19, CYP
2D6, CYP 3A, N-acetyltransferase-2, and xan-
thine oxidase activities in healthy adults. Anti-
D, Robinovitz M, Shaikh OS, Branch RA. Liver
disease selectively modulates cytochrome P450-
2006; 80: 235-245.
Koyabe O, Ohzumi H, Sawada Y. Citrus juices in-
hbit the function of human organic anion-trans-
porting polypeptide OATP-B. Drug Metab Dis-
[13] Zheng HX, Huang Y, Frassetto LA, Benet LZ. Elu-
cidating rifampin’s inducing and inhibiting effects
on glyburide pharmacokinetics and blood glucose
in healthy volunteers: unmasking the differential
effects of enzyme induction and transporter inhibi-
tion for a drug and its primary metabolite. Clin
[14] Ieiri I, Mamiya K, Urnue A, Wada Y, Kimura M,
Irie S, Amamoto T, Kubota T, Yoshioka S, Nak-
mura K, Nakano S, Tashiro N, Higuchi S. Stere-
oselective 4-hydroxylation of phenytoin: rela-
tionship to (S)-mephentoin polymorphism in
445.
Mamiya K, Yoshioka S, Irie S, Amamoto T, Nak-
mura K, Nakano S, Higuchi S. Pharmacokinetics
of omeprazole (a substrate of CYP2C19) and
comparison with two mutant alleles, C gamma
P2C19m1 in exon 5 and C gamma P2C19m2 in
[16] Ieiri I, Yamada S, Seto K, Morita T, Kameda T,
Mamiya K, Tashiro N, Higuchi S, Otsubo KA. A
CYP2D6 phenotype-phenotype mismatch in Japa-


