with single amino acid substitutions of P34S, G42R, R296C, and S486T.

2.3. Assay of progesterone hydroxylase activity

Progesterone hydroxylase activity was measured by the method described previously [15] with a minor modification. The incubation mixture consisted of microsomes from cells containing recombinant P450s (10–20 pmol/ml), 5, 10, 20, 50, 100, or 200 μM progesterone, 1 mM NADPH, and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 0.5 ml. After a 3-min preincubation at 37 °C, the reaction was started by adding NADPH. Incubation was carried out at 37 °C for 10 min and the reaction was terminated by the addition of 2 ml of ethyl acetate. The mixture was shaken and centrifuged at 1900×*g* for 5 min. The organic phase (1.5 ml) was evaporated under reduced pressure, and residue was dissolved immediately in 250 μl of 50% methanol. The HPLC system consisted of a Tosoh model DP-8020 pump (Tosoh, Tokyo, Japan), Tosoh model CO-8020 column heater, Tosoh model AS-8021 autosampler, an SPD-6-AV UV-detector (Shimazdu Corporation, Kyoto, Japan) set at 240 nm, and an analytical column TSK-gel ODS-80Ts (5 μm, 2.0×150 mm; Tosoh). The column temperature was set at 40 °C. The mobile phase was eluted at a flow rate of 0.3 ml/min as follows. The mobile phase was water as eluent A and methanol as eluent B, and the initial eluent profile was 50% B and then the eluent B was linearly increased to 65% over 20 min.

2.4. Assay of dopamine formation from p-tyramine

Dopamine formation from *p*-tyramine was measured by the method described previously [14] with a minor modification. The incubation mixture consisted of microsomes from cells containing recombinant P450s (10–20 pmol/ml), 0.05, 0.1, 0.2, 0.5, 1, or 2 mM, 0.05, 0.1, 0.2, 0.5, 1, 2, or 4 mM (for the G42R mutant), or 0.05, 0.1, 0.2, 0.5, 1, 2, 4, 10, or 20 mM (for CYP2D6.10C) *p*-tyramine, 1 mM NADPH, and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 0.5 ml. After a 3-min preincubation at 37 °C, the reaction was started by adding NADPH. Incubation was carried out at 37 °C for 10 min and the reaction was terminated by the addition of 20 μl of 60% perchloric acid. After the mixtures were shaken and centrifuged at 1900×*g* for 10 min, dopamine in the supernatant was determined by HPLC. The HPLC system described above was used except that a Tosoh model FS-8011 fluorometric detector, and an analytical column TSK-gel ODS-120T (5 μm, 4.6×250 mm; Tosoh) were employed. The fluorescence intensity was determined at an excitation wavelength of 280 nm and emission wavelength of 340 nm. The column temperature was set at 40 °C, and flow rate was 0.7 ml/min. The mobile phase was a 6.8:93.2 (v/v) mixture of acetonitrile and an aqueous solution containing 160 mM ammonium dihydrogen phos-

Table 1
Kinetic parameters for the progesterone hydroxylation by CYP2D6 and its variants

P450	6β-Hydroxylation			16α-Hydroxylation			21-Hydroxylation		
	K_m (μM)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (μl/min/nmol P450)	K_m (μM)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (μl/min/nmol P450)	K_m (μM)	V_{max}/K_m (nmol/min/nmol P450)	V_{max}/K_m (μl/min/nmol P450)
CYP2D6.1	23±5	0.39±0.03	17.3±4.2	16±8	0.079±0.013	5.0±2.7	34±23	0.12±0.03	3.5±2.5
CYP2D6.2 (R296C/S486T)	19±8	0.19±0.02	9.8±4.3	17±8	0.068±0.010	4.0±1.8	49±21	0.091±0.018	1.8±0.9
CYP2D6.10A (P34S/S486T)	11±7	0.061±0.010	5.4±3.3	–	<0.02[a]	–	–	<0.02[a]	–
CYP2D6.10C	50±37	0.10±0.03	2.0±1.6	–	<0.02[a]	–	–	<0.02[a]	–
P34S	36±15	0.19±0.03	5.3±2.4	–	<0.02[a]	–	–	<0.02[a]	–
G42R	35±21	0.068±0.017	2.0±1.3	–	<0.02[a]	–	–	<0.02[a]	–
R296C	44±6	0.38±0.02	8.6±1.2	53±21	0.11±0.02	2.1±0.9	15±8	0.053±0.008	3.6±2.0
S486T	21±6	0.29±0.02	13.8±3.7	22±11	0.089±0.015	4.1±2.2	21±9	0.073±0.011	3.6±1.6

Progesterone at 5–200 μM was incubated with CYP2D6 and its variants (10–20 pmol/ml) and 1 mM NADPH at 37 °C for 10 min after a 3-min preincubation. Values are the means±S.D. of the data set using a nonlinear kinetic analysis from mean values obtained in duplicate at each substrate concentration.

[a] The activity at 5–500 μM as a substrate concentration was <0.02 nmol/min/nmol P450.

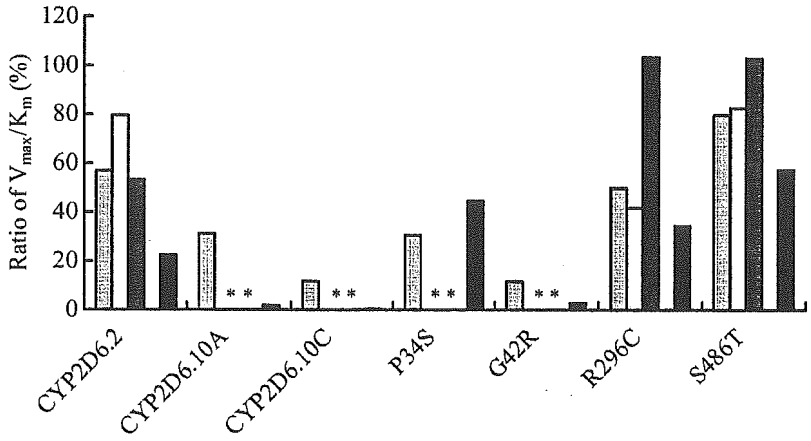

Fig. 1. Ratio of V_{max}/K_m for the metabolism of progesterone and *p*-tyramine by CYP2D6 variants. Ratios of V_{max}/K_m for 6β-hydroxylation (shaded column), 16α-hydroxylation (open column), 21-hydroxylation (striped column) of progesterone and dopamine formation from *p*-tyramine (closed column) were calculated by dividing the V_{max}/K_m for the variant by that for CYP2D6.1. *The 16α-hydroxylated and 21-hydroxylated metabolites for CYP2D6.10A, CYP2D6.10C and the P34S and G42R mutants were not detected (less than 0.02 nmol/min/nmol P450) even using 500 μM progesterone.

phate, 60 mM citric acid, 150 mM disodium EDTA, 10 mM dibutylamine, and 6 mM sodium 1-octanesulfonate.

2.5. Data analysis

In preliminary experiments, the linearity of the reaction with the protein concentration and incubation time was confirmed for each set of assay conditions. All data were analyzed using the mean of duplicate determinations. V_{max} and K_m values for progesterone hydroxylation and dopamine formation from *p*-tyramine were determined by fitting to Michaelis-Menten kinetics by nonlinear regression analysis (Microcal Origin, version 5.0J, Origin LabCorp, Northamptom, MA, USA).

3. Results

3.1. Progesterone hydroxylation by CYP2D6 and its variants

Kinetic parameters for progesterone hydroxylase activities of CYP2D6 and its variants are summarized in Table 1. The V_{max} value of CYP2D6.1 was highest for the 6β-hydroxylation followed by 21-hydroxylation and 16α-hydroxylation, whereas there were no marked differences between the K_m values for the three reactions. Although the K_m values for the 6β-hydroxylation by the CYP2D6 variants except for CYP2D6.10C were similar to those of CYP2D6.1, the V_{max} values for CYP2D6.2, CYP2D6.10A, and the P34S and G42R mutants, were less than half of those for CYP2D6.1. CYP2D6.10C had a higher K_m and a lower V_{max} than CYP2D6.1, whereas the V_{max} values as well as the K_m values for the R296C and S486T mutants were similar to those for the wild-type. The V_{max}/K_m values for CYP2D6.10A, CYP2D6.10C, and the P34S and G42R mutants were 12–31% of that for CYP2D6.1 (Fig. 1).

The 16α-hydroxylated and 21-hydroxylated metabolites for CYP2D6.10A, CYP2D6.10C, and the P34S and G42R mutants were not detected (less than 0.02 nmol/min/nmol P450) even using 500 μM progesterone. In addition, the K_m value for the 16α-hydroxylation and the V_{max} value for the 21-hydroxylation by the R296C mutant were 333% and 45%, respectively, of those for CYP2D6.1.

Table 2
Kinetic parameters for the dopamine formation from *p*-tyramine by CYP2D6 and its variants

P450	K_m (mM)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (μl/min/nmol P450)
CYP2D6.1	0.13±0.02	8.0±0.3	61±9
CYP2D6.2 (R296C/S486T)	0.33±0.07	4.5±0.3	14±3
CYP2D6.10A (P34S/S486T)	1.0±0.4	1.1±0.2	1.1±0.5
CYP2D6.10C	16.2±0.5	3.9±0.1	0.24±0.01
P34S	0.91±0.13	25.0±1.6	27±4
G42R	1.6±0.3	2.6±0.2	1.6±0.4
R296C	0.28±0.01	5.9±0.1	21±1
S486T	0.21±0.05	7.4±0.5	35±8

p-Tyramine at 50–2000 μM, 50–4000 μM (for G42R mutant) or 50–40000 μM (for CYP2D6.10C) was incubated with CYP2D6 and its variants (10–20 pmol/ml) and 1 mM NADPH at 37 °C for 10 min after a 3-min preincubation. Values are the means±S.D. of the data set using a nonlinear kinetic analysis from mean values obtained in duplicate at each substrate concentration.

3.2. Dopamine formation from p-tyramine by CYP2D6 and its variants

Kinetic parameters for dopamine formation from *p*-tyramine by CYP2D6 and its variants are shown in Table 2. Although a mutation at 486 (S486T) had no marked effect on the K_m and V_{max} values, the K_m values for CYP2D6.2 and the R296C mutant were 2.1–2.5 times higher than those for CYP2D6.1 without affecting the V_{max} values. CYP2D6.10A had an 8-fold higher K_m and a 7-fold lower V_{max} than CYP2D6.1, and CYP2D6.10C exhibited an 124-fold higher K_m and a 51% reduction in V_{max} relative to the wild type. The P34S mutant had a 7-fold higher K_m and a 3-fold higher V_{max} than CYP2D6.1, and the G42R mutant had a 12-fold higher K_m and a 3-fold lower V_{max} than the wild type. Therefore, the V_{max}/K_m for CYP2D6.2 and the P34S and R296C mutants were 23–45% of those for CYP2D6.1, and the values for CYP2D6.10A, CYP2D6.10C and the G42R mutant, were 0.3–2.6% of those for the wild-type (Fig. 1).

4. Discussion

Progesterone exists in the brain and has various functions in the nervous system as a neurosteroid [2,19]. Although it is well known that CYP3A4 is one of the major metabolizing enzymes for progesterone hydroxylation in human liver [35], we have reported that progesterone 2β- and 21-hydroxylation in rat brain microsomes are catalyzed by CYP2D [15] and that the 21-hydroxylation of allopregnanolone as well as progesterone and 17α-progesterone is catalyzed by CYP2D isoforms in the brain [9,20], suggesting that CYP2D is involved in the regulation of endogenous neuroactive steroids in brain tissues. In addition, tyramine, one of the trace amines, is present in the brain, especially in the basal ganglia or limbic systems, which are thought to be related to an individual behavior and emotion [33], and CYP2D6 polymorphism has some relationship with an individual behavior [3,24]. In this study, we have demonstrated that the V_{max} and/or K_m values for the metabolism of progesterone and *p*-tyramine by CYP2D6.2, CYP2D6.10A, and CYP2D6.10C were different from those for CYP2D6.1, and that the G42R, P34S, and R296C substitutions affected these metabolic activities (Tables 1 and 2). Additionally, the V_{max}/K_m values for all of the variants except for progesterone 16α-hydroxylation by CYP2D6.2 and progesterone hydroxylations by the S486T mutant were less than 57% of those for CYP2D6.1 (Fig. 1). The G42R substitution is found in a CYP2D6*12 allele in combination with R296C and S486T [6,26]. Furthermore, it has been shown that, when an individual behavior was compared between extensive and poor metabolizers of debrisoquine, a typical probe substrate of CYP2D6, using the Eysenck personality questionnaire and the Karolinska Scales of personality inventory in 769 healthy Swedes, poor metabolizers had significantly lower scores in the Karolinska psychascthenia scales and a higher frequency of extreme responses than extensive metabolizer [3]. Comparison of the debrisoquine hydroxylation capacity and the Karolinska scales of personality in 225 healthy subjects in Spain indicated that poor metabolizers of debrisoquine are more anxiety-prone and less successfully socialized than extensive metabolizers [24]. These studies suggest that there may be a relationship between an individual behavior and the activity of the enzyme hydroxylating debrisoquine (CYP2D6). Although the patients are phenotyped but not genotyped in these papers, it has been reported that the study to assess the relationship between CYP2D6 genotype (including *CYP2D6*10* allele) and debrisoquine phenotype in African-Americans and Caucasians in Los Angels shows the positive identification of 88% of phenotypic poor metabolizers by genotyping [23]. Therefore, the present results suggest that the polymorphism of CYP2D6, including *CYP2D6*2, CYP2D6*10* and *CYP2D6*12*, might affect not only the metabolic activities toward exogenous compounds in the liver [25,34,37] but also an individual behavior and the nervous system through endogenous compounds, such as neuroactive steroids and tyramine, in the brain.

For all of the metabolic activities investigated, the V_{max} values for the G42R mutant were lower than those for CYP2D6.1 (wild-type), and the K_m values for the mutant were higher than those for the wild-type except for progesterone 6β-hydroxylation. On the other hand, the substitution at Pro34 decreased the V_{max} value for progesterone 6β-hydroxylation and increased both the V_{max} and K_m values for dopamine formation from *p*-tyramine. Tsuzuki et al. [37] reported that the G42R substitution but not the P34S substitution increased K_m and decreased V_{max} for debrisoquine 4-hydroxylation, whereas the G42R substitution increased both V_{max} and K_m and the P34S substitution gave only an increased K_m for bunitrolol 4-hydroxylation. Therefore, the present findings suggest that Gly42 is essential for the metabolic activities toward not only exogenous substrate but also endogenous compounds such as progesterone, a non-nitrogen containing compounds, and *p*-tyramine, and that the P34S substitution also affects the metabolism of progesterone and *p*-tyramine.

Gotoh [11] predicted six potential substrate recognition sites (SRS) in the CYP2 family, and the SRSs span residues 100–125, 211–218, 239–247, 294–312, 367–377 and 477–484 for CYP2D6.1. In this study, although the metabolic activities were affected only minimally by the S486T substitution, a mutation of 296 (R296C) of CYP2D6 decreased the V_{max}/K_m for progesterone hydroxylations and dopamine formation from *p*-tyramine. Although it has been reported that the R296C mutation is of little importance for debrisoquine 4-hydroxylation and bunitrolol 4-hydroxylation [37], it is possible to speculate that Arg296, which is included in SRS 4, also might be important to some extent to the metabolism of progesterone and tyramine.

Similarly, V_{max}, K_m and V_{max}/K_m of debrisoquine 4-hydroxylation and bunitrolol 4-hydroxylation by CYP2D6.2 (R296C/S486T) are similar to those by CYP2D6.1 [37], whereas consistent changes in the kinetic characterizing dextromethorphan, bufuralol, and debrisoquine biotransformation by CYP2D6.2 relative to CYP2D6.1 are observed for all three substrates, with an increase in K_m and V_{max} such that V_{max}/K_m values are the same or slightly greater for CYP2D6.2 [34]. In addition, it has been reported that the V_{max}/K_m of CYP2D6.2 toward dextromethorphan, fluoxetine, and codeine decreased levels to less than 35% that of CYP2D6.1 [41], and that the V_{max} for codeine *O*-demethylation catalyzed by CYP2D6.2 are significantly higher than for CYP2D6.1 [31]. In the present study, the V_{max}/K_m values for dopamine formation from *p*-tyramine by CYP2D6.2 were 23% of those for CYP2D6.1, whereas the K_m and V_{max} values for the 21-hydroxylation and 16α-hydroxylation by CYP2D6.2 were comparable with those for CYP2D6.1. Therefore, it is possible to speculate that the effect of the R296C/S486T variant is substrate-dependent.

In summary, our results suggest that the polymorphism of CYP2D6 might influence an individual behavior and the nervous system through endogenous compounds, including neuroactive steroids and tyramine, in the brain.

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ORIGINAL ARTICLE

CYP3A5 genotype did not impact on nifedipine disposition in healthy volunteers

T Fukuda[1,4]
S Onishi[1,4]
S Fukuen[1]
Y Ikenaga[1]
M Ohno[1]
K Hoshino[1]
K Matsumoto[1]
A Maihara[2]
K Momiyama[2]
T Ito[3]
Y Fujio[1]
J Azuma[1]

[1]*Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, Japan;* [2]*Japan Clinical Laboratories, Inc., Bioassay Division, Japan;* [3]*Osaka Pharmacology Research Clinic, Japan*

Correspondence:
J Azuma, Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.
Tel: +81 6 6879 8258
Fax: +81 6 6879 8259
E-mail: azuma@phs.osaka-u.ac.jp

[4]These two authors contributed equally to this work

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ABSTRACT

CYP3A5 expression is regulated by single-nucleotide polymorphisms (SNPs). The CYP3A5 genotype might contribute to a marked interindividual variation in CYP3A-mediated metabolism of drugs. Nifedipine is a typical substrate of CYP3A4 and CYP3A5 *in vitro*. The aim of this study was to elucidate the influence of the CYP3A5 genotype on nifedipine disposition in healthy subjects. A single capsule containing 10 mg of nifedipine was administered to 16 healthy male Japanese subjects (eight subjects: *CYP3A5*1/*3*; eight subjects: *CYP3A5*3/*3*). Blood samples were collected to analyze the pharmacokinetics of serum nifedipine and nitropyridine metabolite (M-I). The area under the plasma concentration–time curve (AUC), the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of nifedipine, and the ratio of the nifedipine AUC to M-I AUC showed large intragroup variations, but no significant differences between the two genotypes. Based on the present findings, the functional relevance of CYP3A5 polymorphism should be re-evaluated in clinical trials.
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Keywords: CYP3A5; nifedipine; polymorphism; clinical; pharmacokinetics

INTRODUCTION

Cytochrome *P*450 3A (CYP3A) is abundantly expressed in human liver and small intestine, [1,2] and contributes to the metabolism of 50% of prescribed drugs. The activities of CYP3A in the general population show interindividual variations in CYP3A-mediated metabolism of drugs.[2] Recently, single-nucleotide polymorphisms (SNPs) were identified in intron 3 (*A–G*: *CYP3A5*3*) and exon 7 (*G–A*: *CYP3A5*6*) of the *CYP3A5* gene.[3] In addition, *CYP3A5*5* and *CYP3A5*7* were reported as a defective allele of CYP3A5, which gave a substantial impact on CYP3A5 expression.[4,5] These SNPs cause a frame-shift mutation or alternative splicing and protein truncation, and result in the absence of CYP3A5, suggesting that only people with at least one *CYP3A5*1* allele express large amounts of CYP3A5 protein. Therefore, these findings suggest that polymorphic CYP3A5 expression might be one factor contributing to the marked interindividual variation observed in CYP3A-mediated metabolism of drugs.

We previously reported the frequencies of CYP3A5-related SNPs in 200 healthy Japanese subjects.[6] As a result, the allele frequency of *CYP3A5*3* was approximately 70%, but *CYP3A5*6* was not detected in the Japanese population. Accordingly, these findings suggested that about 40% of Japanese express relatively high levels of metabolically active CYP3A5 protein.

Table 1 Enzyme kinetic analyses of the conversion of M-I using baculovirus-expressed human = *P*450s

	Km (μM)	V_{max} (pmol/min/pmol P450)	V_{max}/Km (μl/min/pmol P450)
CYP3A4	3.0	3.1	1.0
CYP3A5	6.5	3.3	0.5

To obtain clinical evidence of CYP3A5 polymorphism, we focused on nifedipine, a typical substrate of CYP3A4 and CYP3A5,[7–9] because a large individual difference was observed in nifedipine disposition,[10] which was thought to be regulated by a genetic background rather than environment.[11] Consistently, using baculovirus-expressed human CYP3A5 and CYP3A4, we confirmed their contribution to the metabolism of nifedipine (Table 1). These findings suggested that CYP3A5 contributes to the metabolism of nifedipine with kinetics similar to CYP3A4, implying that the interindividual differences in nifedipine disposition might be explained in part by CYP3A5 polymorphism. Thus, in the present study, we evaluated the influence of the CYP3A5 genotype on nifedipine disposition in healthy subjects to examine the polymorphic activities of CYP3A5 *in vivo*.

RESULTS

The subjects were genotyped and divided into two groups, *CYP3A5*1/*3* and *CYP3A5*3/*3* (Table 2). No subject had the other CYP3A5 alleles, *CYP3A5*5*, *CYP3A5*6* and *CYP3A5*7*. First, the plasma concentration profiles of nifedipine and M-I were compared between **1/*3* and **3/*3* groups. Unexpectedly, the time profiles of both plasma nifedipine and M-I were not significantly different between the two genotypes (Figure 1). Moreover, plasma nifedipine and M-I showed a large intragroup variation. Next, the typical pharmacokinetic parameters of nifedipine, such as the area under the plasma concentration–time curve from 0 to 12 h after administration ($AUC_{0-12\,h}$), the peak plasma concentration (C_{max}), terminal half-life ($t_{1/2}$) and clearance (CL/F) were calculated (Table 3). The $AUC_{0-12\,h}$ values showed large intragroup variations without significant differences between the two genotypes (218.8 ± 80.9 ng h/ml in *CYP3A5*1/*3* subjects, 178.7 ± 92.8 ng h/ml in *CYP3A5*3/*3* subjects; mean ± SD). Furthermore, the ratio of the nifedipine $AUC_{0-12\,h}$ to the M-I AUC(4.77 in *CYP3A5*1/*3* subjects, 3.62 in *CYP3A5*3/*3* subjects; mean) also showed large intragroup variations with no significant differences between the two genotypes (Figure 2). The differences in the C_{max}, $t_{1/2}$ and CL/F of nifedipine between the two groups were not significant.

Finally, we measured systolic and diastolic blood pressure and pulse rate to estimate the significance of CYP3A5 polymorphism in the pharmacodynamics of nifedipine. Consistent with the pharmacokinetics, there were no significant differences in the pharmacodynamics between the two genotypes (Figure 3 and Table 4).

Table 2 Characteristics of the subjects in the study

Subject no.	*Age (years)*	*Height (cm)*	*Weight (kg)*	*BMI (%)*
*CYP3A5*1/*3 (n = 8)*				
1	23	174.2	65.0	97.3
2	21	171.2	65.4	102.1
3	22	169.0	57.5	92.6
4	23	177.7	58.9	84.2
9	22	185.3	64.9	84.5
10	21	180.1	72.3	100.3
11	23	177.7	62.6	89.5
12	23	163.3	49.8	87.4
Mean	22.3	174.8	62.1	92.2
SD	0.9	6.9	6.7	7.0
*CYP3A5*3/*3 (n = 8)*				
5	22	170.8	56.1	88.0
6	32	182.4	70.9	95.6
7	21	166.7	60.9	101.4
8	20	179.5	66.1	92.4
13	22	179.4	61.2	85.6
14	21	175.0	62.9	93.2
15	22	173.5	57.7	87.2
16	25	171.8	71.6	110.8
Mean	23.1	174.9	63.4	94.3
SD	3.9	5.3	5.7	8.4

DISCUSSION

In the present study, we examined the effects of CYP3A5 genotype on nifedipine pharmacokinetics, and demonstrated that an interindividual variation of plasma nifedipine concentration was not over-ridden by the CYP3A5 genotype. The interindividual variation was not beyond our conception and was almost similar to that described in the previous report following the administration of a 10-mg capsule.[12] The present finding suggests that CYP3A5 polymorphism is unlikely to be responsible for interindividual variation in the plasma level of nifedipine because the remaining CYP3A5 alleles, *CYP3A5*5*, *CYP3A5*6* and *CYP3A5*7*, were not found in the present subjects.

With respect to nifedipine metabolism, nifedipine disposition is slightly affected by the expression of intestinal CYP3As because grapefruit juice influences nifedipine disposition significantly but to a lesser extent than felodipine or nisoldipine,[10,13,14] suggesting that nifedipine is mainly metabolized not in the intestine but in the liver. In addition, it is hypothesized that P-glycoprotein (P-gp) is responsible for the large interindividual difference in

CYP3A-mediated drug disposition, since P-gp exists in the similar tissue to CYP3A4. However, this hypothesis is not the case with nifedipine disposition because nifedipine is not a substrate of P-gp.[15,16] Therefore, nifedipine pharmacokinetics must be crucially determined by the total liver CYP3A activities.

It was reported that nifedipine, as well as midazolam, were not only metabolized by CYP3A4 but also by CYP3A5 *in vitro*.[7–9,17] Prior to clinical study, we conducted kinetic study on the formation of M-I using recombinant microsomes (CYP3A4 and CYP3A5) because previous reports provided the oxidation activity at a single high concentration of nifedipine. We confirmed the contribution of CYP3A5 toward the metabolism of nifedipine at relatively low concentrations.

We, however, observed a discrepancy between the *in vitro* and *in vivo* contribution of CYP3A5 to nifedipine metabolism in the present study. Interestingly, a similar result has been obtained in the case of midazolam, a typical CYP3A5 substrate. Namely, midazolam pharmacokinetics was also hardly influenced *in vivo* by the genotype of CYP3A5,[18] although midazolam is metabolized *in vitro* by CYP3A5 rather than CYP3A4.[3,17,19] Several possibilities can be proposed to explain these discrepancies between the *in vitro* and *in vivo* data.

It was previously reported that total CYP3A activity showed an interindividual variation,[2] and the ratio of CYP3A4 to CYP3A5 might also vary interindividually in the liver.[20] Recently, Westlind-Johnsson *et al*[21] reported that CYP3A5 did not contribute to total CYP3A activity using

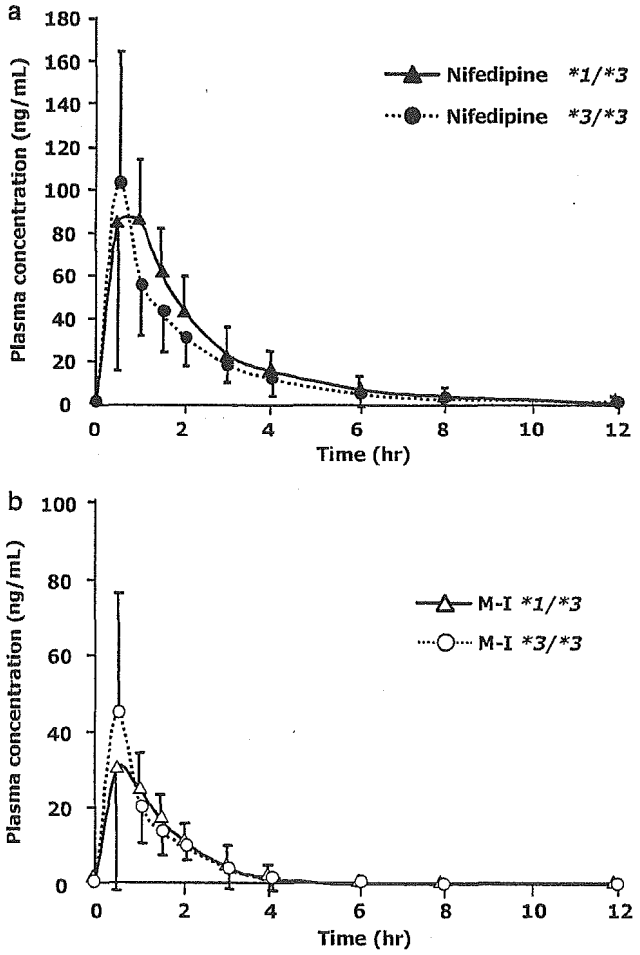

Figure 1 Plasma concentration–time curves of nifedipine (a) and M-I (b) in the *CYP3A5*1/*3* and *CYP3A5*3/*3* subjects. Values represent the means with SD ($n=8$).

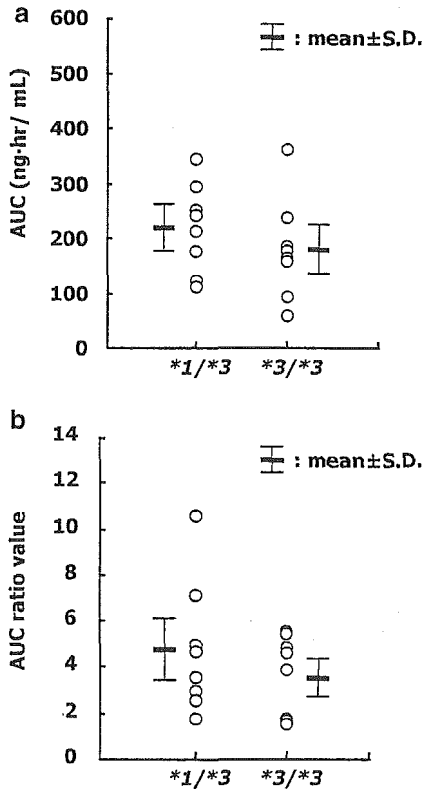

Figure 2 Individual (dot) and mean (line) values with SD of the $=AUC_{0-12h}$ of nifedipine (a) and the ratio of the nifedipine $=AUC_{0-12h}$ to the M-I $=AUC_{0-12h}$ (b) in the two genotypes.

Table 3 Pharmacokinetic parameters of nifedipine after oral administration to subject with *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes (mean±SD)

Genotype	C_{max} *(ng/ml)*	$t_{1/2}$ *(h)*	AUC_{0-12} *(ng h/ml)*	*CL/F (ml/min)*
*CYP3A5*1/*3* ($n=8$)	116±43.3	1.77±0.66	219±80.9	877±375
*CYP3A5*3/*3* ($n=8$)	111±53.8	1.81±1.09	178±92.8	1246±837

liver samples. That is, they indicated that Kuehl *et al*[3] might have overestimated the level of CYP3A5 protein, possibly due to problems with the method of quantification. On the other hand, Williams *et al*[19] reported that the Km value of nifedipine was much lower for CYP3A4 than CYP3A5 under the detailed conditions with cytochrome *b*5. Cytochrome *b*5 was suggested to be an essential component in CYP3A4-catalyzed nifedipine oxidation in human liver microsomes.[22] Although these data seem to support the present findings *in vivo*, the same was not consistent in the case of midazolam. Based on all these observations, we speculated that the amount of CYP3A5 protein in the liver is much lower than CYP3A4, although we should consider the limitations of our study design. This speculation might be demonstrated by using CYP3A5-specific substrates, although no drugs metabolized specifically by CYP3A5 have been reported yet. With respect to mRNA expression, it was reported that CYP3A genes exhibit a degree of tissue-specific expression and CYP3A5 is predominantly expressed in the adrenal grand, prostate and kidney.[23] Therefore, CYP3A5 polymorphism might have a physiological and pharmacological effect, which is related to the extrahepatic tissues. This possibility remains to be verified in further studies.

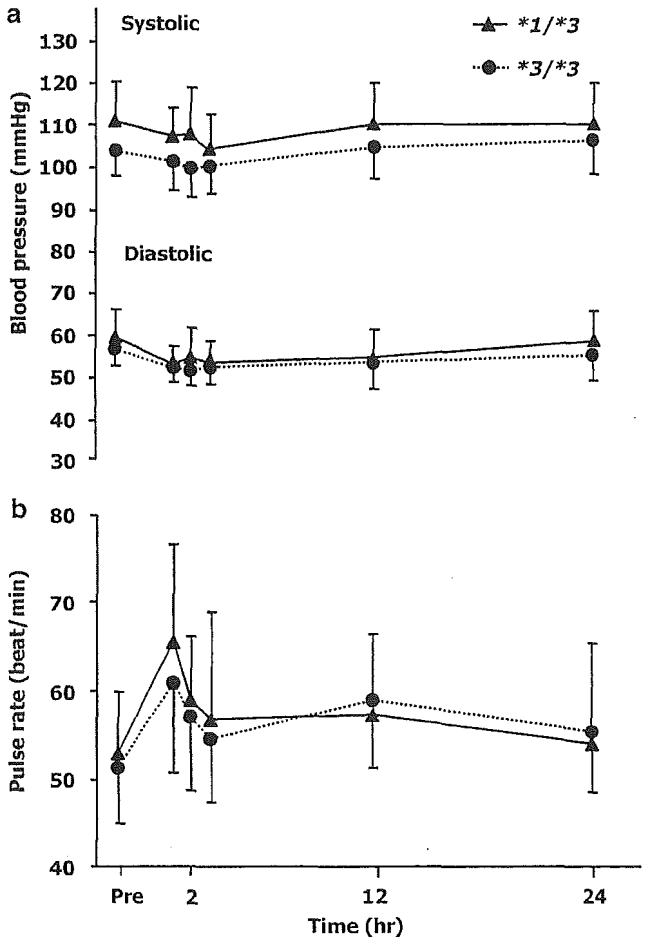

Figure 3 Blood pressure (a) and pulse rate (b) after nifedipine administration. Values represent the means with SD ($n=8$).

Interindividual differences in nifedipine pharmacokinetics remain to be elucidated genetically. Here, we propose that polymorphic regulation of CYP3A4 gene transcription, including the polymorphisms of the promoter activities and transcriptional factors, may have to be taken into account to explain the variation of nifedipine pharmacokinetics. However, a polymorphism, which plays a significant role in the activity of CYP3A4, has not yet been identified in the 5′-regulatory region of the CYP3A4 gene.[24] Therefore, we have focused on human PXR (hPXR) as a factor effecting CYP3A expression and identified splicing variants of hPXR as a possible factor in interindividual variation caused in CYP3A activity.[25] Interestingly, we have found that mRNA expression of wild-type hPXR is well correlated with mRNA expression of CYP3A4 in liver sample (unpublished data), which is consistent with the recent report.[21]

In summary, we revealed that nifedipine disposition *in vivo* is not affected by the *CYP3A5*3* allele. Owing to a discrepancy between the *in vitro* and *in vivo* contribution of CYP3A5, the functional relevance of CYP3A5 in humans should be re-evaluated by clinical studies for each drug.

MATERIALS AND METHODS

In vitro Screening for the Contributions of CYP3A4 and CYP3A5

Nifedipine and phenytoin were purchased from Wako Pure Chemicals Co. (Osaka, Japan) and oxidized nifedipine (ULTRAFINE) was obtained from Funakoshi (Tokyo, Japan). Microsomes from baculovirus-infected insect cells expressing human CYP3A4 and CYP3A5 with NADPH cytochrome *P*450 reductase (GENTEST) were obtained from Daiichi Pure Chemicals Co. (Tokyo, Japan). NADPH was purchased from Oriental Yeast Co. (Tokyo, Japan). Other reagents and

Table 4 Systolic and diastolic blood pressures change rate (%) after administration of nifedipine

	1 h after administration of nifedipine		*2 h after administration of nifedipine*	
	Systolic	*Diastolic*	*Systolic*	*Diastolic*
*CYP3A5*1/*3* ($n=8$)	3.0±7.2	9.3±10.4	2.7±5.7	6.9±11.3
*CYP3A5*3/*3* ($n=8$)	2.3±2.8	6.9±5.2	3.8±3.5	8.5±3.7

Values were the mean±SD of blood pressure changes (%). Each blood pressure change (%) was calculated by the formula ((measured–baseline)/baseline × 100).

organic solvents were obtained from Nakarai Tesq Chemical Industries (Kyoto, Japan).

All incubation, extraction and other handling of samples were carried out in amber vials. A mixture (0.50 ml) containing 10 pmol of *P*450 protein from baculovirus-expressed human CYP3A4 and CYP3A5 and 1 mM NADPH in 0.1 M potassium phosphate buffer (pH 7.4) was incubated with nifedipine (final concentration: 1, 2, 5, 10 and 40 μM) at 37°C after 2 min of preincubation without NADPH. The incubation was continued with gentle shaking at 37°C for 30 min and the reaction was stopped with ethyl acetate (3 ml). A measure of 50 μl of methanol and 12.5 μl of 20 mM phenytoin (internal standard) were added to each sample. The organic layers were transferred to other vials after centrifugation at 1500*g* for 10 min and evaporated to dryness. The residues were dissolved in 200 μl of the mobile phase. A measure of 50 μl were analyzed by high-performance liquid chromatography (HPLC) with a reverse-phase column (Mightysil RP-18 GP250, $4.6 \times 250\,mm^2$, Kanto Chemical Industries Ltd, Kyoto, Japan). The column temperature was set at 40°C. The mobile phase was composed of 40% acetonitrile (pH 3.0 with perchloric acid) and was delivered at a constant flow rate of 1.2 ml/min. Nifedipine, M-I and phenytoin were detected by a UV detector (Nanospace, SHISEIDO, Tokyo, Japan) with a wavelength at 254 nm. In determining kinetic parameters, nifedipine concentration ranged from all points.

Subjects

Institutional Review Board approval of the study protocol was obtained. In all, 16 healthy male Japanese volunteers participated in this study (Table 2). The subjects gave written informed consent to participate.

A total of 33 healthy volunteers was screened by the genotyping test to find the eight *CYP3A5*1/*3* subjects and eight *CYP3A5*3/*3* subjects. The genotyping test of CYP3A5 was conducted according to the previous study.[6,26] All subjects were healthy as assessed by medical history, physical examination, hematologic tests, blood chemistry and urinalysis, and the results of a positive test for hepatitis B and C, human immunodeficiency virus and syphilis.

Eight *CYP3A5*1/*3* and eight *CYP3A5*3/*3* subjects who showed normal results on routine laboratory tests described above and the negative results of virus tests were selected.

Genotyping Test

Genomic DNA was isolated from peripheral leukocytes using QIAGEN blood kit. The genotypes of each individual at the *CYP3A5*3* and *CYP3A5*6* alleles were determined using PCR-restriction fragment length polymorphism analysis according to the previous report.[6] For the analysis of the *CYP3A5*3* allele, the forward (CYP3A5 6956Fm; 5′-CTT TAA AGA GCT CTT TTG TCT CTC A-3′) and reverse (CYP3A5 7155R; 5′-CCA GGA AGC CAG ACT TTG AT-3′) primers were used (GenBank accession no. AC005020). For the analysis of the *CYP3A5*6* allele, the forward (CYP3A5 14505F; 5′-GTG GGT TTC TTG CTG CAT GT-3′) and reverse (CYP3A5 14741R; 5′-GCC CAC ATA CTT ATT GAG AG-3′) primers were also created based on the published sequence. These PCR reactions were carried out in 25 μl of solution consisting of 2.5 μl of 10 × PCR buffer, 0.2 mM of each dNTP, 0.4 μM of each primer, 90 ng of genomic DNA as a template and 1 U of AmpliTaq Gold (Perkin-Elmer, Branchburg, NJ, USA). After initial denaturation at 95°C for 10 min, the amplification for the *CYP3A5*3* or **6* alleles was performed using 37 cycles of 94°C for 30 s, 56°C (**3*) or 58°C (**6*) for 30 s and 72°C for 30 s, followed by 72°C for 5 min for final extension. After PCR amplification, 5 μl of each PCR product was digested for a minimum of 2 h at 37°C with 5 U of *Dde*I before electrophoresis using a 3% agarose gel.

*CYP3A5*5* and *CYP3A5*7* alleles were also determined according to the method of van Schaik *et al*[26] with some modifications.

Study Design

A single oral dose of 10 mg of nifedipine (Adalat® Bayer, Germany) with 200 ml of water was administered to the subjects at 01000 after overnight fasting. Blood samples (7 ml each) were collected before administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after administration. During this study, adverse effects were assessed on an ongoing basis as subjects offered information. Blood pressure and pulse rate were measured before administration and at 1, 2, 3, 12 and 24 h after administration. A 12-lead electrocardiogram was recorded before administration and at 2.5 and 24 h after administration. Hematologic tests, blood chemistry and urinalysis were performed before administration and at 24 h after administration.

Grapefruit juice, St John's Wort, alcohol, caffeine-containing beverages, tobacco and exercises were not allowed during the study.

Assays

Blood samples were collected in heparinized tubes and adequately protected from light and centrifuged to obtain serum. The samples were stored frozen at −20°C until analyzed. Plasma concentrations of nifedipine and M-I were measured by HPLC as described previously with minor modifications.[27–29] Briefly, nifedipine and M-I were extracted with dichloromethane/pentane (3 : 7, v/v) and separation of the compounds was achieved using an Inertsil ODS-3 column (3 mM particles, $4.0 \times 100\,mm^2$, GL Sciences Inc., Tokyo, Japan). The compounds were detected at a wavelength of 230 nm using a UV detector (SPD-10Avp, SHIMADZU, Tokyo, Japan).

Pharmacokinetic Parameters

The peak plasma concentration (C_{max}) and the time to reach the C_{max} (T_{max}) were obtained as measured values. The apparent first-order elimination rate constant (K) of nifedipine was determined by linear regression analysis of the slope of the terminal phase using the last three or four points on the log plasma drug concentration–time curve. The terminal elimination half-life ($t_{1/2}$) was calculated from the relation $t_{1/2} = 0.693/K$. The area under the plasma concentration–time curve from 0 to 12 h after administra-

tion (AUC_{0-12h}) was calculated by the linear trapezoidal method. The area under the drug concentration–time curve from time to infinity (AUC_{∞}) was determined by the linear trapezoidal method with extrapolation to infinity. Clearance (CL/F) was calculated as dose/(AUC_{∞}). The plasma nifedipine/M-I ratio was calculated from the AUC_{0-12h} value of nifedipine and M-I.

Data Analysis

The pharmacokinetic parameters and the plasma nifedipine/M-I ratios were first analyzed by one-way ANOVA. If the overall F ratio was significant, further comparison of the means was performed with the Student's *t*-test. Statistical analysis of the pharmacodynamic variables of systolic and diastolic blood pressure, and pulse rate made under resting conditions, were performed using ANOVA with repeated measurements. Time was used as a repeated variable. A probability level of $P < 0.05$ was considered to be statistically significant.

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DUALITY OF INTEREST

None declared

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1st DIA Annual Japan Workshop for Pharmacogenomics in Tokyo

1st DIA Annual Japan Workshop for Pharmacogenomics, September 2–3, 2004, Tokyo, Japan

Mieko Tamaoki[1†], Hiroshi Gushima[2] & Kiichiro Tsutani[3]

†Author for correspondence

[1]Yamanouchi Pharmaceutical Co. Ltd, Product Evaluation Department, Tokyo, Japan

Tel.: +81 3 5916 5537; Fax: +81 3 5916 5616; E-mail: tamaoki@yamanouchi.co.jp

[2]Biofrontier Partners, Inc., Tokyo, Japan

[3]The University of Tokyo, Department of Pharmacoeconomics, Graduate School of Pharmaceutical Sciences, Tokyo, Japan

The Drug Information Association (DIA)-sponsored *First Annual Japan Workshop for Pharmacogenomics* (PGx) was held on September 2 and 3, 2004, at Shinjuku in Tokyo, Japan [101]. This was one of the two workshops started in 2004 together with regulatory affairs (Figure 1). This workshop, which was also organized in collaboration with the Council for International Organizations of Medical Sciences (CIOMS), provided a platform to introduce the activities of the CIOMS Working Group on Pharmacogenetics (CIOMS PGt WG), which was established in 2002 [102]. In addition, general trends and the regulatory and ethical aspects of PGx in the USA, Europe, Asia and Japan were reported and discussed. This report summarizes the outline of the workshop and the individual topics that were presented. Although presentations of the findings of basic and clinical research were also reported, this paper refers only to the regulatory, ethical and social dimensions of PGx.

1st DIA Annual Japan Workshop for Pharmacogenomics

The Program Committee Chairperson for this first DIA workshop on PGx in Japan was Kiichiro Tsutani, who is a Professor at the Graduate School of Pharmaceutical Science of the University of Tokyo. Other committee members included Junichi Azuma (Professor of Osaka University), who represented academia at the meeting; Tohru Masui (Senior Researcher of the National Institute of Health Science) and Chie Kojima (Manager of the Pharmaceutical and Medical Devices Agency [PMDA]), who both represented regulatory agencies; and Hiroshi Gushima (Scientific Adviser of Yamanouchi and Biofrontier Partners), six members of the Japan Pharmaceutical Manufacturers Association (JPMA), and Mieko Tamaoki (Yamanouchi), who all represented the pharmaceutical industry. These individuals were responsible for developing the scientific program, selecting the guest speakers, and organizing the workshops.

The meeting was divided into four sessions. In Session 1, which was entitled 'CIOMS Initiatives', six PGx experts from academia, regulatory agencies and industry in the USA, Europe and Japan introduced PGx-related regulatory perspectives and trends in the USA and Europe and the main activities of the CIOMS PGt WG since it was established in 2002.

In Session 2, entitled 'Collaboration between Regulators and Industry', leaders in PGx from US and European industry presented the activities of the Pharmacogenomics Working Group (PWG) and experience with the application of PGx in clinical research [103].

The third session, entitled 'PGx in Asia', centered on the current trends and status of PGx research in the Republic of Korea, Taiwan, and China, where some areas of PGx research have advanced relatively further than in Japan.

In the final session, entitled 'PGx in Japan', presentations were made by speakers representing academia, regulatory agencies, and industry. First, Azuma introduced cases of PGx-based clinical trials from the perspective of the physician desiring to promote individualized medicine. This was followed by presentations on the activities of the Japan Pharmacogenomics Consortium (JPGC) [104], and activities of the Pharma SNP Consortium (PSC) and other JPMA projects. Furthermore, Yoshiaki Uyama (Principal Reviewer at the PMDA, Japan) discussed regulatory issues concerning PGx in Japan.

In addition to these presentations, the keynote address was delivered by Kiyoshi Kurokawa, the President of the Science Council of Japan. Prior to the workshop, those not familiar with PGx were also given the opportunity to attend tutorials by Ryuichi Kato (Emeritus Professor of Keio University Medical School) and Hirotoshi Echizen (Professor of Meiji Pharmaceutical University).

Session 1

The workshop commenced with a presentation by Juhana E Idänpään-Heikkilä (Secretary General of the CIOMS), who provided an update on the activities of the CIOMS PGt WG.

The CIOMS is an international, non-governmental, non-profit organization established jointly by the World Health Organization (WHO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) in 1949. Its membership is comprised of both national and international organizations, including the Science Council of Japan. The main objective of this collaboration is to promote international activities in biomedical sciences. It plans and executes long-term programs, which

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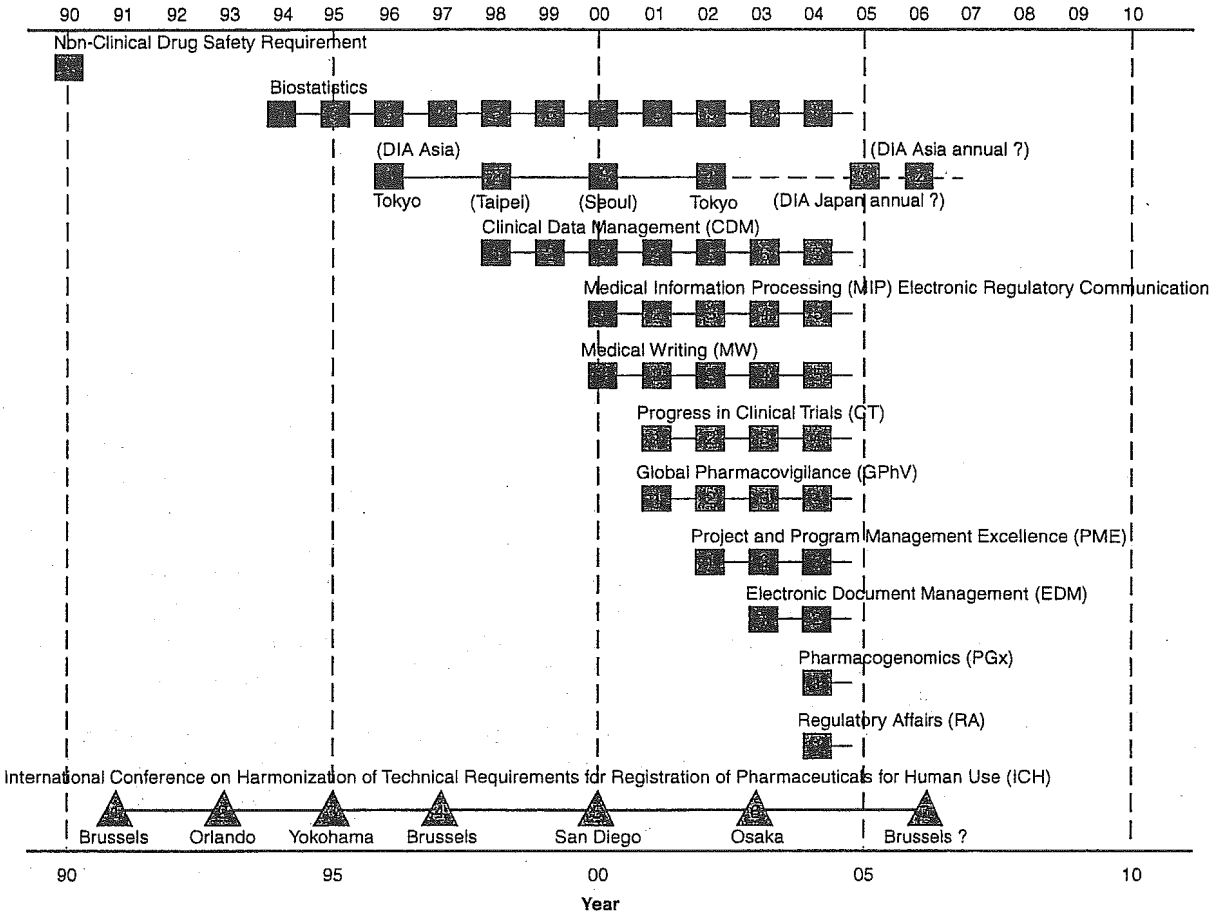
Figure 1. DIA activities in Japan.



DIA meetings/workshops have also been held in other Asia Pacific countries.
? Denotes a possible event.
DIA: Drug Information Association.

include: bioethics; the international harmonization of health policies, ethics, and human values; the development and proper use of medicines; and the management of the international nomenclature of diseases. One of the most important activities of the CIOMS is the publication of the *International Ethical Guidelines for Biomedical Research Involving Human Subjects* (the revised guidelines were issued in August 2002) [1]. This document is known as a 'must read' for every person involved in clinical research, and is of equal importance to the Declaration of Helsinki. Although recommendations issued by the CIOMS are not compulsory for member countries, there are some cases illustrating their influence on regulatory authorities via the *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH) activities, and, thus, certain attention should be paid to these trends.

The CIOMS PGt WG was established to provide opportunities for the examination and evaluation of the influence of PGx on future medical treatment, drug development, regulatory control, society, economy and insurance systems for experts from academia, regulatory agencies, and industry. This is in response to the general perception of PGx as having potential value and increasing practical application of genome information for the development and proper use of medicines. A new report, entitled '*Pharmacogenetics – towards improving treatment with medicines*', was published in early 2005 [2]. This report consists of 12 chapters and an appendix summarizing the current state of PGx research in Australia, Canada, China, Chinese Taipei, Europe, Japan, the Republic of Korea, and Singapore.

The CIOMS PGt WG held its preparatory meetings in January and September 2001. Thereafter, the group formally started its activities at a London-based conference held in February 2002, followed by a conference in Bonn,

Germany, in August 2002, Washington DC in February 2003, Warsaw in September 2003, and Windsor, UK, in April 2004. In addition to these meetings, the group has exchanged opinions via e-mail and other means of communication and prepared reports. At this conference, the group introduced its reports on PGx and held a workshop as a follow-up meeting held jointly with the DIA in Japan.

The CIOMS PGt WG itself is composed of members from academia, regulatory agencies and industry in Japan, the USA, and Europe: 3 members from academia, 15 from 11 different regulatory agencies, and 14 from 11 private enterprises (including venture companies). The Japanese members include Noboru Takahashi and Chie Kojima of the Japanese Ministry of Health, Labour, and Welfare (MHLW), and Tsutani, Gushima and Tamaoki.

In the session entitled 'CIOMS Initiatives' held in the afternoon on the initial day of the workshop, the activities of the CIOMS were introduced and the following chapters of the *Pharmacogenetics – towards improving treatment with medicines* report were presented: communication and education, ethical issues, abnormal drug response: opportunities for risk reduction through PGt, improvements in existing therapies, and regulatory perspectives in PGt.

The presentation focusing on 'Communication and Education' of PGx was prepared jointly by Tomas R Weihrauch (Bayer) and Tsutani. The latter delivered the speech. Tsutani pointed out that, for all stakeholders, the use of clear, relatively simple and common language is crucial. He added that all communications and educational efforts should enhance the stakeholders' understanding of PGx, which will lay the groundwork for its support, acceptance, and implementation.

David A Lepay (US Food & Drug Administration [FDA]) delivered a presentation entitled 'PGx, Ethics, and Clinical Trials: a Regulator's Perspective', in which he commented on the desired rules of ethics and the conduct of clinical trials from a regulatory point of view. His conclusions, based on the FDA's perspective, were that PGx data should be viewed as a part of medical data and no separate regulations are necessary. He did indicate that the current Good Clinical Practice (GCP) principles concerning informed consent (IC), as well as the institutional review board (IRB), are all applicable to PGx trials. Lepay stressed that the important point to note was that IC is the process by which a candidate patient exchanges study-related information with the investigator and other study personnel, and the patient must not only be asked to sign the form but also be fully informed of the study through the exchange of information. Lepay emphasized that an uniform procedure for obtaining IC cannot be applied to all clinical trials.

Rashmi R Shah (Medicines & Healthcare Products Regulatory Agency [MHRA], UK) spoke of the promise of using PGx approaches to ensure and improve drug safety. Shah cited cases where the dose on the labeling had been changed for 73 (21%) of 354 drugs approved by the FDA during 1980–1999 [3]. He also stated that the dose had been reduced to ensure safety for 58 (79%) of those drugs. When the dose approved by the agency was compared with the therapeutic dose reported in the literature for 48 drugs, almost all were found to be efficacious at doses lower than the approved dose [4]. The identification of the optimal dose still remains a critical issue, irrespective of the use of PGx approaches. The optimal dose should be established based not on the maximum tolerated dose, but on other careful considerations. The manifestation of therapeutic effects, adverse drug reactions (ADRs) and drug interactions is often dependent on genotypes, but clinical problems do not always involve genes. Shah commented that when judged to be clinically useful, genotyping approaches should be employed in identifying target patients or finding the optimal dose for individual patients.

Lembit Rägo (WHO) gave a presentation entitled 'Improving Existing Therapies'. Rägo stated that PGx is expected to be useful in:

- promoting the rational use of drugs
- extending therapeutic indications of approved drugs
- re-evaluating drugs withdrawn from the market or terminated during research
- omitting trial and error in prescribing drugs or abolishing fixed prescriptions
- reducing the overall cost to society

He also pointed out that there are many remaining issues that need to be resolved, including the need to encourage investment in the research of older, previously approved drugs and the time-consuming approach used for obtaining public consensus on gene analysis, as well as the involvement of other non-genetic factors, such as environmental contributory factors and ethical problems. Nonetheless, Rägo considers the use of PGx studies valuable for re-evaluating current drug therapy by providing a basis for revising labeling, updating treatment guidelines, and recording PGx data as patient medical information. These approaches should be useful in substantially reducing medical expenses and improving medical quality.

The presentation on PGx-related regulatory perspectives was jointly prepared by Marisa Papaluca Amati (European Medicines Agency [EMEA]) and Lawrence J Lesko (FDA). Amati addressed the workshop attendees, and pointed out the following findings and issues surrounding healthcare and medicine:

- the existence of a dropout rate of more than 80% for new investigational drugs during the clinical stage due to safety or efficacy concerns
- the insufficient number of new drug products launched onto the market
- the possibility that pharmaceutical company product pipelines are being depleted
- changes in labeling within 2 years after license approval
- withdrawal of blockbuster products from the market each year due to serious ADRs
- lawsuits or other issues facing the industry
- a strong need for new drugs in clinical practice

According to Amati, both the EMEA and the FDA believe that PGx may be

useful in improving such situations. However, since there are ethical as well as scientific and technological issues that must be resolved before PGx research can be put into practice, the agencies are currently trying to participate in PGx workshops and to join in the international activities of CIOMS, OECD and other organizations in order to maintain unofficial dialog with industry and provide non-binding proposals to the parties concerned.

Regulatory recommendations currently available include the Committee for Proprietary Medicinal Products (CPMP) Position Paper on Terminology in PGt (November 2002), the CPMP Concept Paper on Pharmacogenetics 'Briefing Meetings' (January 2003), and the FDA Guidance for Industry and Pharmacogenomic Data Submissions (November 2003) [105–107]. At the time of writing, regulatory advisory documents that are expected to be issued include the Biobanks and Specific Issues Relevant to Pharmacogenetics (December 2004), Pharmacokinetic Studies and Pharmacogenetics (1st quarter, 2005), Explanatory Note on Briefing Meetings (Including Formats for Pharmacogenetics Submissions; December 2004), and FDA Drug/Test Development Guidance (December 2004).

In conclusion, Amati added that the agencies are willing to promote the utility of genomic data for the improvement of medical therapy through dialog with any persons or parties concerned.

Session 2

Iman Barilero (Johnson & Johnson) gave a presentation entitled 'Experiences from the PWG'. The PWG, which is a voluntary body made up of 22 companies, including Eisai Co. Ltd from Japan, has discussed and evaluated the following PGx-related issues for further action: terminology, IC, and disclosure of genetic information. The group has already published reports of its conclusions concerning terminology and IC, and has submitted a report discussing the disclosure of genetic information [5–7]. Barilero mentioned that regulatory agencies are taking positive actions for applying PGx technology to new drug research and the industry welcomes and supports the agencies in preparing and issuing PGx guidances. The industry, added Barilero, would also support international cooperation among agencies.

Lara Hashimoto (Roche) discussed the issues surrounding the use of PGx in clinical trials. Roche established the Roche Sample Repository (RSR) in 1998 and is collecting blood samples from around the world [108]. The company has announced that it has obtained approval for sample collection from more than 1000 ethical committees in 37 countries. The purpose of this sample collection is for exploratory PGx research, and the research aims are limited to research on drug therapy and diseases related to approved indications. The samples and clinical information to be obtained are anonymized to guarantee confidentiality and stored for a maximum of 15 years. The sampling procedures, genotyping laboratories and storage facilities conform to GCP standards. Major hurdles for sample collection are the dynamics of rapidly changing genetic information, regulatory controls over biological sample banking, the ethical environment, and the processes for obtaining IC from study subjects.

The IRB protocol approval rate greatly varies among countries according to government policy and the national sentiment regarding PGx. When the 2003–2004 approval figures were compared with 2000, the rate had drastically fallen in Denmark, Norway, Italy, Portugal, Spain, and the UK, but, in contrast, the rate rose from 0 to 100% in France. No approval rating has been recorded yet in Sweden or Finland. Roche worries that negative feelings or attitudes toward PGx may prevail among investigators in Sweden and Finland if such a situation continues. Looking at the situation in Asia, Roche has not yet tried to collect samples in Japan because it is certain that IRBs would reject such an application. The samples from China, with the exception of the Hong Kong Special Administrative Region, have not yet been shipped abroad because their exportation requires approval from the Ministry. In Taiwan, the IRBs generally grant approval but the government disapproves of the sampling. Thus, Roche has been limited in collecting clinical samples in Asian countries.

Roche also reported that it was conducting surveys of public opinion concerning expressions used in the IC document from 50 people each in the UK, Japan, and the USA. The company intends to revise its IC document based on the survey results. To date, the survey has been completed in the UK, is close to completion in Japan, and is ongoing in the USA. In the UK, Roche received many questions concerning the publication of study results and the protection of confidentiality, whereas in Japan, many people misinterpreted clauses concerning withdrawal from the study and the sample preservation period. Generally, the Japanese asked more questions and were more critical of PGx studies than UK individuals.

In response to these survey results, Roche said that it would rewrite clauses in the Japanese IC document using clearer and more concise language, be more careful concerning cultural differences in language nuances, prepare a training CD-ROM and kits for guiding investigators and coordinators, and take other appropriate actions.

A recent news article reported that GlaxoSmithKline have started collecting and preserving DNA samples in some of its Japanese clinical trials that were held in 2003 [8]. Thus, it is apparent that trends toward such PGx-related activities are underway, even in Japan.

Session 3

During this session, speakers from the Republic of Korea, Taiwan and China reported on the status of PGx research in their respective countries/areas, stating that progress is underway for the establishment and improvement of a PGx research base, the conduct of PGx research, and the clinical application of PGx with the aid of strong government support. They added that there have been certain achievements in these areas already.

Sang-Goo Shin (Seoul National University, Republic of Korea) reported that

the Korean Ministry of Health and Welfare strongly supports the Korean PGx Research Network (KPRN) Program, which is led by Shin. He added that the Ministry of Science Technology has established a PGx Center at Inje University. The KPRN is an academia-led organization of approximately 300 members and has conducted many activities to promote PGx.

Herng-Der Chern (Center for Drug Evaluation, Taipei, Taiwan) reported that the Super Control Genomic Database has been established in Taiwan and that 3312 clinical samples have already been collected from normal Han Chinese subjects. Taiwan plans to start a pilot study to establish a disease-oriented genomic database by 2005. Chern introduced a case study example of PGx research that identified SNPs related to Stevens-Johnson syndrome caused by carbamazepine [9]. He also discussed the achievements of a venture company that succeeded in identifying 10 SNPs in eight genes involved with the elicitation of the efficacy of interferon in the treatment of chronic hepatitis C, and was looking for practical applications of this knowledge.

Hong-Hao Zhou (PGt Research Institute, Central South University, China) reported the establishment of the PGt Research Institute in Changsha, the Human Genome Project Centers in Shanghai and Beijing [109,110], and the Demonstrative Laboratory for PGt and PGx in Changsha. Zhou also reported that the following projects and research are currently being conducted in China: the International HapMap Project, Chinese PGx Research, the Project on the Relationship of Genomics and Severe Diseases, the Bioinformatics of Gene Functions and Drug Designing, the Demonstrative Laboratory of PGt, PGx and the Modernization of Chinese Herbs, the Research Center for Medication in Minorities, and the Individualization of Drug Therapy for Patients with Hypertension.

Session 4

Pharmaceutical companies in Japan organized consortia for the establishment of a PGx research base, and some of the activities of the organization were introduced at this workshop. Kenichi Imagawa (Otsuka, and Vice Chair of the JPGC) introduced the activities of the JPGC. The JPGC is an organization that was created in July 2003 by the 10 biotechnology and pharmaceutical companies mainly based in the Osaka area. The organization was formed to establish and promote a base for initiating PGx-based clinical trials and to standardize the procedures necessary for the conduct of trials in Japan. Currently, the JPGC is performing clinical research to investigate the relationship between platelet response to aspirin and genetic differences among patients. The JPGC is scheduled to continue research activities until May 2005 and intends to establish the PGx Clinical Trial Support Center.

Kozo Watanabe (Otsuka, and the Head of the PGx Promotion Working Group of the JPMA) introduced four projects that have been proven to work and which the JPMA considers necessary for preparing for the era of genome-based drug discovery. These projects are the PSC Project, the Pharmaceutical Consortium for Protein Structure Analysis (PCProt) [111], the Toxicogenomics Project [112], and the Proteome Factory Consortium Project. Watanabe also touched upon other activities that require efforts by the JPMA to promote PGx research. Based on the importance of a prompt establishment of a PGx research base, well-balanced ethics and science, and the collaboration of academia, regulatory agencies, and industry, Watanabe proposed the following measures to facilitate the initiation of PGx clinical trials:

- the preparation of ethical guidelines for conducting PGx clinical trials
- the development of a PGx research-promoting environment (including the establishment of a PGx research promotion center)
- the clarification of regulatory views on the acceptability of exploratory PGx data for drug evaluation in license applications
- an appeal to the regulatory agencies about the need to establish consultation systems

Yoshiaki Uyama (Principal Reviewer at the PMDA) mentioned regulatory perspectives on PGx in his presentation entitled 'How to Use PGx for Drug Development: Designs of Clinical Studies Using PGx, Issues for Conducting PGx Studies in Japan, Establishment of Guidance for Appropriate Pharmacogenomic Approach in Clinical Trials and Review in Japan'. Uyama commented that the application of the enrichment approach to clinical trials using genetically selected responders may reduce the size of the study population necessary for clinical trials and increase the power of detection. However, data obtained in this manner are specific to selected patients, making overall drug evaluation difficult. Furthermore, patients to be employed in this approach are not representative of actual patients in a clinical setting. As long as there are such patients, the approval of a drug based on the enrichment approach may possibly lead to a undesirable off-label use of such a compound. Uyama also mentioned that in order for PGx data to be used in the license application, the data must be obtained using validated methodology, verified by prospective studies, and evaluated for the risks and benefits in patient groups other than selected responders. He further commented that it is important for PGx to be accepted by the public; that care be taken in safeguarding the patient's privacy; that ethical reviews and coding procedures be handled carefully; that participants be provided with sufficient study-related information with adequate time for consideration; and that there is greater education of the public. Moreover, from an economical point of view, the cost of genotyping should be compared with potential clinical benefits. It will be necessary to harmonize PGx approaches internationally in the future, and ICH would be an appropriate place to start discussions in this regard. Uyama closed his speech by commenting that the appropriate application of PGx to new drug development will only become possible when the MHLW, the PMDA, industry, academia and patients begin to closely cooperate and exchange opinions.

Highlights

- A new report entitled '*Pharmacogenetics – towards improving treatment with medicines*' was issued by the Council for International Organizations of Medical Sciences (CIOMS), providing a platform upon which to introduce the activities of the CIOMS Working Group on Pharmacogenetics in early 2005.
- The US Food & Drug Administration's perspectives are that pharmacogenomic (PGx) data should be viewed as a part of medical data and separate regulations are unnecessary. They also feel that the current Good Clinical Practice principles concerning informed consent and the institutional review board are applicable to PGx trials.
- The basic principle of the regulatory agencies is to promote the utility of genomic data for the improvement of medical therapy through dialog with persons or parties concerned, although the stage of policy formation and its adoption varies among countries.
- The industry welcomes and supports the regulatory agencies in preparing and issuing PGx guidances, and would also support international cooperation among agencies.
- The necessity and importance of international harmonization were pointed out by many speakers in this workshop.
- In the Republic of Korea, Taiwan, and China, progress is underway for the establishment and improvement of a PGx research base, conduct of PGx research, clinical application of PGx, and development of a network under strong government support. There have been achievements in these areas already.
- Pharmaceutical companies in Japan have organized consortia for the establishment of a PGx research base.
- The industry has such a strong interest in PGx that the environment will be ripe for accepting PGx-based clinical trials in Japan.

Expert opinion

In 2001, the Ministry of Education, Culture, Sports, Science, and Technology, MHLW and Ministry of Economy, Trade and Industry jointly issued the *Ethics Guidelines for Human Genome/Gene Analysis Research* in Japan [113]. It states that clinical trials conducted in accordance with the Pharmaceutical Affairs Law are not subject to these guidelines. Furthermore, GCP guidelines do not contain any clauses concerning PGx studies. This has led to confusion and an extra burden on the clinical staff for the realization and execution of trials. In contrast to the speed and scale of trials undertaken by foreign pharmaceutical companies, as demonstrated in this workshop, Japanese companies have been reluctant to proceed with PGx trials, with some exceptions. Their reluctance stems from a fear of heavy burden and high risks because of the uncertainty of public acceptance concerning genotyping and genetic data handling, as well as the absence of regulatory guidance on the acceptability of PGx data for approval review, despite it being clear that PGx studies are beneficial for patients in most cases. There were, however, nearly 150 people attending this workshop, and the attendees represented almost all of the research-oriented drug manufacturers and other companies actually working in PGx-related fields based in Japan, evidencing that the industry has a strong interest in PGx.

In early June 2004, in view of the slow progress of PGx research, the MHLW issued the draft notification '*Submitting Clinical Trials Information in which Pharmacogenomic Approaches Were Used by the Regulatory Agency for Making a Guidance Document for Pharmacogenomic Approaches on Pharmaceutical Developments*', and collected public comments on the issue [114]. This notification was aimed at gathering information for the preparation of guidelines for PGx-based studies to facilitate the adequate conduct of clinical and other trials for the development of drugs that are to be prescribed using PGx tests. In response to the issue of the notification, the JPMA commented that it would welcome the issue of new guidelines since the clarification of standards concerning clinical trials would have significant merits for the industry in assisting various decision-making processes and removing complexities arising from ambiguities in judgment criteria. Additionally, the JPMA submitted a total of 18 items of requests or comments concerning the schedule for issuing the guidelines, harmonization with other regions, and the methodology of information supply. The Ministry announced that it received dozens of public comments. The guideline is to be finalized within 2004.

In Japan, discussion of the policies concerning privacy protection has been continued by relevant ministries in accordance with the enforcement of the Privacy Protection Act, which is expected to be enacted in April 2005. In the field of medicine, discussion was centered on the protection of a patient's privacy at medical institutions and the protection of the privacy of study subjects who participate in clinical studies. Consequently, the *Ethics Guidelines for Human Genome/Gene Analysis Research* was revised. It was also reviewed in response to the current progress in genetic research, as indicated by the increasing amount of research that requires the linkage of clinical information with genomic and gene analysis information.

In view of such regulatory trends, it is expected that PGx-based clinical trials will become accepted in Japan.

Outlook

The realization of PGx-based research may require different approaches, such as research on new drugs, drugs already marketed, therapeutic efficacy, and ADRs. Although it may be difficult to proceed uniformly with all these areas, at least academia, regulatory agencies and industry have agreed with the idea that PGx should be utilized for improving healthcare. It is expected that further discussion will be conducted regarding the regulatory, ethical, scientific and technological issues.

The necessity and importance of international harmonization were pointed out by many speakers in this

workshop. The discussion between people of various positions from different countries that was enabled at this gathering is considered to be valuable and meaningful for the formation of a common global concept.

This DIA workshop on PGx in collaboration with the CIOMS was able to be held for the first time in Asia, and in Japan, in particular, through the cooperation between academia, regulatory agencies and industry. However, in the USA and Europe, similar PGx workshops have been held several times already, and discussions and the exchange of opinions has occurred between academia, regulatory agencies and industry on many occassions. In particular, the FDA generally holds such workshops before and after issuing new guidances in order to gather public opinion. There are high expectations that this sort of forum will continue to play an important role in facilitating the exchange of opinions among academia, regulatory agencies, and industry, as well as in policy formation by agencies based in Asia, and in Japan, in particular, similar to those that occur in the USA and Europe.

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医薬品開発におけるファーマコゲノミクスの役割
――米国FDAファーマコゲノミクス・ドラフトガイダンスを中心に

渡辺 耕三，玉起美恵子，了戒 純一
WATANABE Kozo, TAMAOKI Mieko and RYOKAI Junichi
日本製薬工業協会・研究開発委員会

はじめに

米国食品医薬品局（以下，FDA）は，“ファーマコゲノミクスデータ提出”に関するドラフトガイダンス[1]を2003年11月3日に公開し，ファーマコゲノミクス（以下，PGx）研究の医薬品開発への適用についての明確な方針や道筋を示した。

FDAはここに至るまでに，企業などと意見交換を重ねており，2002年5月にはIND（Investigational New Drug：新薬治験許可申請）資料に関してPGxデータの提出を促進するために“Safe Harbor”の概念を提唱し，これらのPGxデータを規制には使用しないことを示して企業のPGx研究を推進しようとした。

また，FDAはこのドラフトガイダンスの公開に先立ち，2003年4月には“遺伝性のDNAマーカー，変異および発現パターンについてのMultiplex試験；企業およびFDA審査官のためのドラフトガイダンス”[2]を公開しており，2004年中にドラフトの公開が予定されている“臨床研究と体外診断薬”に関する2つのガイダンスとあわせた4つのガイダンスで企業の本格的なPGxデータ提出に向けて準備を整えようとしている。

そこで本稿では，“ファーマコゲノミクスデータ提出”に関するドラフトガイダンスを解説し，医薬品開発におけるPGxの役割についてドラフトガイダンス記載の応用例を参考にして概説する。

ファーマコゲノミクス・ドラフトガイダンス公開の目的

今回のドラフトガイダンス公開の目的は，PGx分野の科学的な発展と医薬品の開発にPGxデータの使用を促すことである。その背景には，PGxデータは薬剤応答性（有効性および副作用）の個人差を確認できる情報源としての潜在的能力があり，個々人にあった治療，すなわち，最大の有効性と最小のリスクを求めるテーラーメイド医療に役立つものとFDAは期待している。にもかかわらず，新薬の承認審査過程でFDAがPGxデータをどのように取り扱うかが示されていないために，企業は医薬品開発の段階でPGx試験に乗り出すことに躊躇していることがある。そこで，FDAはこのガイダンスを出すことで，許認可決定におけるPGxデータ取り扱いの基本方針を明らかにし，企業がPGx試験に安心して取り組めるような環境整備を図りたいと考えていると推測される。

この方針に基づき，FDAは“任意PGxデータ提出”（VGDS；Voluntary Genomic Data Submission），すなわち「企業が試験デザインなどの意思決定や科学的な論証にPGxデータを使用したいとき，薬剤ラベルや承認申請資料の科学的根拠に使

用するとき，あるいは試験結果が既知で根拠が確実なバイオマーカーもしくは根拠が確実と思われるバイオマーカー*になるときにはINDやNDA（New Drug Application：新薬承認申請）時のPGxデータの提出を必須とするが，それ以外のときにはPGxデータのINDやNDA時の提出は必須とせず，任意提出を推奨する」という“Safe Harbor”の概念を引き継ぐ新しいシステムを提案している（表1）。

VGDSにはもう一つの目的がある。それは，

表1 PGxデータの提出が必須の場合（これ以外の場合は概要の提出，あるいは任意提出となる）
――PGxデータ提出に関するドラフトガイダンスからの抜粋――

Ⅰ．完全な報告書として提出が必須の場合

＜新薬治験許可申請（IND）段階のPGxデータの提出＞

1. PGx試験結果を，臨床試験や安全性の確認のための動物試験の方針決定に用いる場合。例えばその結果が，用量設定，組み入れ基準，安全性モニタリングあるいは対象の層別化に影響を与える場合。
2. 企業が，例えば薬剤の安全性，有効性，用法用量，薬理作用に関する科学的な論証としてPGx試験結果を用いる場合。

＜新薬承認申請（NDA），生物製剤の承認申請（BLA），承認事項一部変更時のPGxデータの提出＞

3. 薬剤ラベル（添付文書）に使用されるPGx試験，または承認申請資料の科学的根拠の一部として使用することを企業が意図したPGx試験については，完全な報告書として提出すること。報告書は，新薬承認審査資料の該当部分に試験方法と完全なデータについての情報を含むこと。事例として，
   - 用法用量，安全性，患者選択，有効性について，企業が科学的な論証とする目的でPGx試験結果を用いている場合。
   - 添付文書にPGx試験結果を記載することを企業が申し出た場合。
   - 添付文書に記載された用法用量，安全性，有効性を示すためにPGx試験が必須である場合。

Ⅱ．略式報告書として提出が必須の場合

＜新薬治験許可申請（IND）段階のPGxデータの提出＞

4. 試験結果が，ヒトにおける生理学的・病態生理学的・薬理学的・毒性学的あるいは臨床状態やその転帰について既知で根拠が確実なバイオマーカーになっているか，または，動物実験の安全性結果について既知で根拠が確実なバイオマーカーである場合であっても，バイオマーカー（例：ヒトP450 2D6の場合）の情報が上記1あるいは2の目的で使用されない場合は，その情報はINDでは略式報告書として提出できる。

＜新薬承認申請（NDA），生物製剤の承認申請（BLA），承認事項一部変更時のPGxデータの提出＞

5. 試験結果が，関連した動物またはヒトにおける生理学的・病態生理学的・薬理学的・毒性学的あるいは臨床状態やその転帰について既知で根拠が確実なバイオマーカーである場合であっても，その結果を企業が科学的な論証として使用しない，または添付文書に記載しない場合には，略式の報告書をFDAに提出すること。
6. 試験結果が，関連した動物またはヒトにおける生理学的・病態生理学的・薬理学的・毒性学的あるいは臨床状態やその転帰について，根拠が確実と思われるバイオマーカーとなっている場合，NDAまたはBLAの申請資料で略式の報告書として提出すること。

＜既承認薬剤の追加審査資料の提出＞

7. 既知で根拠が確実なあるいは根拠が確実と思われるバイオマーカーに関する非臨床あるいは臨床PGx試験の結果は，年次報告書のなかで概要または略式の報告書として提出しなければならない。

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*：バイオマーカーは以下の3つに分類される。

Known valid biomarker（既知で根拠が確実なバイオマーカー）：よく確立された実行特性をもつ分析試験系で測定され，試験結果の生理的，毒性的，薬理的もしくは臨床的意義について医学もしくは科学コミュニティーにおいて広く合意されたバイオマーカー。

Probable valid biomarker（根拠が確実と思われるバイオマーカー）：よく確立された実行特性をもつ分析試験系で測定され，試験結果の生理的，毒性的，薬理的もしくは臨床的意義を説明しうると思われる科学的枠組みがあるバイオマーカー。既知で根拠が確実なバイオマーカーまでは到達しないバイオマーカー。

Exploratory or research pharmacogenomic data（探索的もしくは研究的PGxデータ）