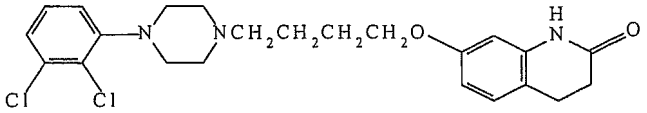
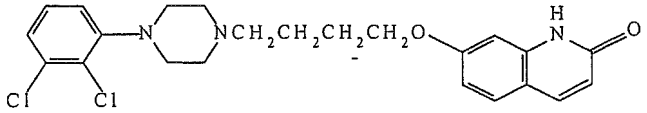


ARIPIPRAZOLE



OPC-14857

Fig. 1. Chemical structures of ARIPIPRAZOLE and its main metabolite OPC-14857.

ARIPIPRAZOLE (chemical structure shown in **Fig. 1**) is a new antipsychotic developed by Otsuka Pharmaceutical Co., Ltd..[1] The results of *in vitro* studies indicated that ARIPIPRAZOLE is mainly metabolized by the human cytochrome P450 isozymes CYP3A4 and CYP2D6.[2] It has been reported that CYP3A4 and CYP2D6 are the metabolic enzymes for numerous compounds and also that there are many compounds that inhibit these enzymes.

Although individual differences in hepatic levels of CYP3A4 enzyme protein have been reported to vary as much as 40 fold,[3] almost no gene mutations affecting CYP3A4 metabolic activity or ethnic differences have been reported. For CYP2D6, however, a number of polymorphisms and the existence of ethnic differences in the types and distribution of polymorphisms have been reported.[3,4] Therefore, it was considered important to investigate the pharmacokinetics of ARIPIPRAZOLE in Japanese subjects when CYP2D6 becomes the main metabolic enzyme as a result of co-administration of a CYP3A4 inhibitor.

Considering the above-mentioned points, the present study was planned for the purpose of investigating the influence of CYP3A4 inhibition on the pharmacokinetics of ARIPIPRAZOLE in Japanese subjects.

As mentioned above, the metabolic activity of CYP2D6 was expected to influence the pharmacokinetics of ARIPIPRAZOLE when CYP3A4 was inhibited by ITZ. In the present study, therefore, CYP2D6 genotyping of each subject was performed and the influence of CYP3A4 inhibition on the pharmacokinetics of ARIPIPRAZOLE was examined by CYP2D6 genotype.

In addition, the pharmacokinetics of OPC-14857 (chemical structure shown in **Fig. 1**), which is the main metabolite of ARIPIPRAZOLE in humans, were also evaluated, since the results of pharmacological studies indicated that OPC-14857 has pharmacological activity equivalent to that of ARIPIPRAZOLE and the results of preclinical studies indicated that CYP3A4 is involved in the production and elimination of OPC-14857.[2]

As the urinary 6β-hydroxycortisol/cortisol concentration ratio is known to be an indicator of CYP3A4 metabolic activity,[5] the inhibition of CYP3A4 metabolic activity by ITZ was confirmed by comparing the urinary 6β-hydroxycortisol/cortisol concentration ratio between before and after ITZ administration.

Methods

Study design and subjects: This study was designed as an open-label add-on study in reference to the Guidance for Drug Interaction Studies (Notification No. 813 issued by the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare of Japan, on June 4, 2001). As an inhibitor of CYP3A4 metabolic activity, ITZ (Itrizole® Capsule 50, Janssen Pharmaceutical)[6] was chosen from the *in vivo* CYP3A4 inhibitors listed in the aforementioned Notification No. 813, since the product is an oral formulation and has a lower incidence of serious adverse reactions than other CYP3A4 inhibitors. The dose of ITZ in the present study was set at 100 mg a day, as it has been reported that administration of ITZ at a clinical dose of 100 mg inhibits CYP3A4 metabolic activity.[7,8]

This study was completed in 24 healthy adult male volunteers. All subjects were administered a single dose of ARIPIPRAZOLE alone in Period I (administration alone period) and a single dose of ARIPIPRAZOLE during steady-state administration of the CYP3A4 inhibitor ITZ in Period II (co-administration period). ARIPIPRAZOLE was orally administered at 3 mg once under a fasting condition on the 1st day of each period (Periods I and II). ITZ was administered at 100 mg/day once daily after breakfast for 21 consecutive days from 7 days before to the 14th day after Period II administration of ARIPIPRAZOLE, except on the day of ARIPIPRAZOLE administration, when the two drugs were co-administered under a fasting condition. The washout period between ARIPIPRAZOLE administration in Period I and the start of ITZ administration was 35 days.

Subjects were admitted to the study site from the day before ARIPIPRAZOLE administration to the 4th day of Period I (5 days) and from 2 days before the start of ITZ administration to the 4th day of Period II (13 days).

Prior to the screening examination, the principal investigator or attending investigator gave each subject a full explanation of the study using the informed consent form and written information for subjects and obtained consent from each subject in writing.

Demographic and other baseline characteristics for the population for analysis of the influence of CYP3A4 inhibition (n = 24) are shown in **Table 1.**

Procedures: This clinical study was conducted at the

Table 1. Demographic and other baseline characteristics (population for analysis: n = 24)

Characteristic	Mean	SD	Min	Median	Max
Age	23.2	2.4	21	22.5	32
Height (cm)	172.90	6.12	161.7	173.25	184.9
Weight (kg)	61.76	5.44	50.9	62.15	73.7

Research Institute of Osaka Pharmacology Research Clinic. The subjects, all of whom were judged to be eligible for the study after a screening examination, were required to abstain from food and beverages from the completion of dinner on the day before ARIPIPRAZOLE administration until 4 hr postdosing in each period (Periods I and II).

Venous blood sampling (5 mL) for determination of plasma drug concentrations was performed 15 times each in Periods I and II (at 2 hr before and 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 144, 240, and 336 hr after ARIPIPRAZOLE administration) and once at 2 hr before the start of ITZ administration, for a total of 31 timepoints. Venous blood (10 mL) for CYP2D6 genotyping was also collected from each subject prior to ARIPIPRAZOLE administration in Period I.

To confirm that CYP3A4 was inhibited by ITZ by using the urinary 6β-hydroxycortisol/cortisol concentration ratio as a indicator, urinary cortisol and 6β-hydroxycortisol concentrations were measured in cumulative 24-hr urine collected on the day before ITZ administration and on the 7th day of ITZ administration (day before Period II ARIPIPRAZOLE administration).

During the study period, subjects were instructed to adhere to the following restrictions: 1) to abstain from food and beverages other than that provided at the study site from dinner on the day of admission until discharge, 2) to abstain from consuming grapefruit or grapefruit products from 1 week before the first ARIPIPRAZOLE administration until completion of examinations on the 15th day of Period I and from 1 week before the start of ITZ administration until completion of examinations on the 15th day of Period II, since such substances have been reported to inhibit CYP3A4 drug-metabolizing enzyme activity,[9] and 3) to abstain from consuming dietary supplements containing Saint John's Wort from 2 weeks before the first ARIPIPRAZOLE administration until completion of examinations on the 15th day of Period I and from 2 weeks before the start of ITZ administration until completion of examinations on the 15th day of Period II, since that substance has been reported to stimulate CYP3A4 drug-metabolizing enzyme activity.

CYP2D6 genotyping: For CYP2D6 genotyping, 10 mL of venous blood was collected from each subject using a heparinized blood collection tube before Period I ARIPIPRAZOLE administration and CYP2D6 genotypes were examined using PCR-RFLP and Long-PCR methods. Genotyping was performed for CYP2D6*2, CYP2D6*4, CYP2D6*5, CYP2D6*10, CYP2D6*14, CYP2D6*18, and CYP2D6*36, and all genotypes other than those were regarded as CYP2D6*1. CYP2D6 genotypes were classified into the following 5 categories according to the anticipated metabolic enzyme activity of the genotype.

1) Extensive metabolizer: CYP2D6 genotype identified as homozygous *1 or a zygote of *1 and some other active allele (*1/*1, *1/*10, etc.)

2) Intermediate metabolizer: CYP2D6 genotype identified as a zygote of *10 and some other allele except *1 and *2 (*5/*10 ,*10/*10, etc.)

3) Poor metabolizer: CYP2D6 genotype identified as a homo- or heterozygote of a defective gene (*4/*4, *5/*5, *4/*5, etc.)

4) *2 Group: CYP2D6 genotype identified as a homo- or heterozygote of *2 (*1/*2, *2/*2, *2/*5, *2/*10, etc.: These alleles were all classified as *2 Group because a CYP2D6*2 variant assigned as CYP2D6*41 (decreased activity) was reported[10] after genotyping was performed in this study and the activities of these zygotes including CYP2D6*2 could not be assessed.)

5) Other: CYP2D6 genotype other than those above (*1/*5, etc.)

For performing the CYP2D6 genotyping and the handling of data obtained in the study, reference was made to "Fundamental Principles of Research on the Human Genome" issued by the Bioethics Committee, Council of Science and Technology, on June 14, 2000, and to "Ethical Principles of Human Genome Research and Gene Analysis" jointly issued by the MHLW, the Ministry of Education, Culture, Sports, Science, and Technology, and the Ministry of Economy, Trade, and Industry on March 29, 2001.

Determination of plasma drug concentrations: Plasma concentrations of ARIPIPRAZOLE and its main metabolite OPC-14857 were determined from 0.4 mL of plasma by Sumika Chemical Analysis Service, Ltd., using LC-MS/MS (liquid chromatography tandem mass spectrometry).

The lower limit of quantitation (LLOQ) for both analytes was set at 0.1 ng/mL. For the concentration range of 0.1 to 100 ng/mL, intra-assay precision was $<5.1\%$ CV for ARIPIPRAZOLE and $<15.9\%$ CV for OPC-14857.

Determination of urinary cortisol and 6β-Hydroxycortisol concentrations: Cumulative 24-hr urine samples were mixed well and aliquots were prepared and kept frozen until transfer to the laboratory. Urinary cortisol and 6β-hydroxycortisol concentrations were measured

by Daiichi Pure Chemicals Co., Ltd., using LC-MS/MS.

The precision and accuracy of the system for the determination of cortisol and 6β-hydroxycortisol were examined using cortisol samples at 10, 20, and 100 ng/mL and 6β-hydroxycortisol samples at 50, 100, and 500 ng/mL. Precision and accuracy ranged from 0.0% to 10.0% and from −10.0% to 0.0%, respectively, and those values satisfied the quality standards (±20% for precision and ±20% for accuracy).

Pharmacokinetic analysis: Pharmacokinetic parameters of ARIPIPRAZOLE (C_{max}, t_{max}, $AUC_{336\,hr}$, AUC_{∞}, $t_{1/2,z}$, and CL/F) and OPC-14857 (C_{max}, t_{max}, $AUC_{336\,hr}$, AUC_{∞}, and $t_{1/2,z}$) were calculated for each subject by a noncompartmental method using WinNonlin® (Ver. 3.3, Pharsight Corporation).

Statistical analysis: Descriptive statistics (mean and standard deviation) of the plasma concentrations of ARIPIPRAZOLE and OPC-14857 at each blood sampling timepoint were calculated. Obtained plasma drug concentrations were summarized by CYP2D6 genotype.

Descriptive statistics of each pharmacokinetic parameter of ARIPIPRAZOLE and OPC-14857 were calculated for Period I (ARIPIPRAZOLE alone) and Period II (co-administration with ITZ) by genotype. Descriptive statistics of the Period I/II ratio for each parameter were also calculated.

To evaluate the differences between the mean values of each pharmacokinetic parameter by CYP2D6 genotype, analysis of variance (one-way ANOVA) and Fisher's least significant difference test were performed.

For analysis of the influence of CYP3A4 inhibition, descriptive statistics of the ratio and difference of each pharmacokinetic parameter between for administration of ARIPIPRAZOLE alone and for co-administration of ARIPIPRAZOLE with ITZ were calculated and Student's paired t-test was performed with $\alpha = 0.05$.

Descriptive statistics of the urinary 6β-hydroxycortisol/cortisol concentration ratio, and the ratio between the concentration ratio on the day before the start of ITZ administration and that on the 7th day of ITZ administration (final day of administration of ITZ alone) were calculated and Student's paired t-test was performed.

Results

Study subjects: In CYP2D6 genotyping, 14 subjects were classified as extensive metabolizers (4 for *1/*1 and 10 for *1/*10), 3 as intermediate metabolizers (*10/*10), 4 as *2 Group (*2/*10), and 3 as "Other" (*1/*5). There were no subjects classified as poor metabolizers.

Pharmacokinetic analysis: The pharmacokinetic parameters of ARIPIPRAZOLE and OPC-14857 were compared between Period I (administration alone period) and Period II (co-administration period). The major pharmacokinetic parameters of ARIPIPRAZOLE and OPC-14857 by CYP2D6 genotype are respectively shown in **Table 2** and **Table 3**.

Of the mean pharmacokinetic parameters of ARIPIPRAZOLE in all subjects, CL/F was decreased by 32.5% and C_{max}, $AUC_{336\,hr}$, and $t_{1/2}$ were respectively increased by 19.4%, 48.0%, and 18.6% by co-administration of ITZ. Of the mean pharmacokinetic parameters of OPC-14857 in all subjects, C_{max}, $AUC_{336\,hr}$, and $t_{1/2,z}$ were respectively increased by 18.6%, 38.8%, and 53.4% by co-administration of ITZ. All of those changes were judged to be statistically significant by paired t-test.

The timecourses of the mean plasma concentrations of ARIPIPRAZOLE and OPC-14857 are shown in **Fig. 2**.

The ratio of the $t_{1/2,z}$ of OPC-14857 between the administration alone period and the co-administration period could not be determined in 9 subjects because linearity for at least 3 points could not be obtained in the terminal phase for the co-administration period.

Regarding total exposure, the combined $AUC_{336\,hr}$ of ARIPIPRAZOLE and OPC-14857 was increased by approximately 45% by co-administration of ITZ.

Analysis of variance (one-way ANOVA) was performed to investigate the relationship between the pharmacokinetic parameters of ARIPIPRAZOLE for Period I and the CYP2D6 genotype. Among those parameters, AUC and CL/F showed significant differences between CYP2D6*1/*1 and CYP2D6*10/*10.

The relationship between CL/F and CYP2D6 genotype is shown in **Fig. 3**. CL/F for *1/*10, *1/*5, and *2/*10 were similar to that for *1/*1 and no significant differences were observed. Data for CYP2D6*1/*5 is therefore included in extensive metabolizers in **Table 2** and **Table 3**.

The timecourses of the mean plasma concentrations of ARIPIPRAZOLE and OPC-14857 by CYP2D6 genotype are shown in **Fig. 4**. In the comparison of pharmacokinetic parameters by CYP2D6 genotype, the mean plasma ARIPIPRAZOLE concentrations in intermediate metabolizers were higher than those in extensive metabolizers in both the administration alone period and the co-administration period. The CL/F of ARIPIPRAZOLE in extensive metabolizers was higher than that in intermediate metabolizers. The C_{max}, t_{max}, $AUC_{336\,hr}$, and $t_{1/2,z}$ in extensive metabolizers were lower than those in intermediate metabolizers. By co-administration of ITZ, CL/F was decreased by 26.6% in extensive metabolizers and the change was statistically significant. Although the decrease in intermediate metabolizers was even greater (47.3%), the change was not judged to be statistically significant due to the small number of subjects. In the co-administration period, the

Table 2. Major pharmacokinetic parameters of ARIPIPRAZOLE by CYP2D6 genotype

CYP2D6 genotype		C_{max} (ng/mL)	t_{max} (hr)	$AUC_{336\,hr}$ (ng·hr/mL)	$t_{1/2,z}$ (hr)	CL/F (L/hr)
All Subjects	Number	24	24	24	24	24
	Period I	13.2±2.8	2.9±1.3	726±176	71.9±10.4	4.23±1.08
	Period II	15.5±2.9**	3.2±1.2	1075±335**	85.0±17.6**	2.86±0.86**
	Ratio (II/I)	1.19±0.19	0.3±1.4[a)]	1.48±0.27	1.19±0.18	0.68±0.12
Extensive metabolizers (including *1/*5)	Number	14 (17)	14 (17)	14 (17)	14 (17)	14 (17)
	Period I	13.5±2.7 (13.0±3.2)	2.9±1.4 (2.9±1.3)	692±145 (683±163)	69.9±9.8 (69.4±9.0)	4.36±0.85 (4.50±1.09)
	Period II	15.5±2.5** (15.2±2.9**)	3.4±1.2 (3.3±1.2)	929±171** (961±271**)	79.4±10.7** (79.6±10.6**)	3.18±0.62** (3.15±0.72**)
	Ratio (II/I)	1.17±0.15 (1.20±0.16)	0.5±1.8[a)] (0.4±1.7[a)])	1.36±0.19 (1.42±0.23)	1.15±0.14 (1.16±0.14)	0.73±0.09 (0.71±0.10)
Intermediate metabolizers *10/*10	Number	3	3	3	3	3
	Period I	14.5±2.0	3.7±2.1	960±162[#]	79.1±11.7[#]	3.01±0.51[#]
	Period II	16.8±1.1	3.7±2.1	1626±92**	116.1±30.5	1.58±0.19*
	Ratio (II/I)	1.18±0.20	0.0±0.0[a)]	1.72±0.21	1.45±0.19	0.53±0.03
*1/*1	Number	4	4	4	4	4
	Period I	15.0±2.3	2.3±0.5	612±78	61.2±8.8	4.88±0.69
	Period II	16.8±2.6	2.8±1.5	781±96*	73.5±3.3	3.77±0.49*
	Ratio (II/I)	1.13±0.17	0.5±1.7[a)]	1.28±0.11	1.22±0.23	0.78±0.07
*1/*10	Number	10	10	10	10	10
	Period I	12.8±2.7	3.2±1.6	724±155	73.4±8.2[##]	4.16±0.85
	Period II	15.0±2.4**	3.7±1.1	989±159**	81.7±11.8**	2.94±0.50**
	Ratio (II/I)	1.18±0.14	0.5±1.9[a)]	1.39±0.21	1.11±0.09	0.72±0.09
*1/*5	Number	3	3	3	3	3
	Period I	10.8±5.1	2.7±0.6	639±270	66.9±2.2	5.13±2.02
	Period II	14.1±5.0**	2.7±0.6	1109±599	80.5±12.5	3.02±1.27
	Ratio (II/I)	1.36±0.18	0.0±1.0[a)]	1.69±0.24	1.20±0.18	0.58±0.08
*2/*10	Number	4	4	4	4	4
	Period I	13.0±0.6	2.5±0.6	735±118	77.1±13.4[#]	4.02±0.70
	Period II	15.4±4.0	2.5±0.6	1143±279	84.5±3.5	2.59±0.78
	Ratio (II/I)	1.19±0.32	0.0±0.8[a)]	1.57±0.40	1.12±0.16	0.65±0.18

Mean±SD
[a)]Difference (Period II-Period I)
**$p<0.01$, *$p<0.05$ (paired t-test vs. Period I)
[##]$p<0.01$, [#]$p<0.05$ (Fisher's least significant difference test vs. *1/*1 in Period I)

CL/F of ARIPIPRAZOLE in intermediate metabolizers was about half of that in extensive metabolizers. The difference in C_{max} between extensive metabolizers and intermediate metabolizers and the changes in C_{max} by co-administration of ITZ were both small.

Plasma OPC-14857 concentrations in intermediate metabolizers were lower than those in extensive metabolizers in both the administration alone period and the co-administration period (except at 336 hr postdosing). The t_{max} of OPC-14857 in intermediate metabolizers was longer than that in extensive metabolizers, with the difference being amplified by co-administration of ITZ. The C_{max} and $AUC_{336\,hr}$ of OPC-14857 in extensive metabolizers were higher than those in intermediate metabolizers, and the increase in $AUC_{336\,hr}$ by co-administration of ITZ was similar among all genotypes. The $t_{1/2,z}$ of OPC-14857 in the co-administration period could not be determined in 5 of 17 subjects classified as extensive metabolizers, all 3 subjects classified as intermediate metabolizers, and 1 of 4 subjects classified as *2 Group because linearity for at least 3 points could not be obtained in the terminal phase for the co-administration period.

Urinary cortisol concentrations: Descriptive statis-

Table 3. Major pharmacokinetic parameters of OPC-14857 by CYP2D6 genotype

CYP2D6 genotype		C_{max} (ng/mL)	t_{max} (hr)	$AUC_{336\,hr}$ (ng·hr/mL)	$t_{1/2,z}$ (hr)
All Subjects	Number	24	24	24	15
	Period I	1.4±0.3	57.0±17.1	269±74	92.9±21.7
	Period II	1.6±0.5**	103.0±62.7**	365±103**	143.4±65.4**
	Ratio (II/1)	1.19±0.26	46.0±58.3[a)]	1.39±0.31	1.53±0.52
Extensive metabolizers (including *1/*5)	Number	14 (17)	14 (17)	14 (17)	11 (12)
	Period I	1.5±0.3 (1.4±0.4)	56.6±20.2 (56.5±18.9)	304±72 (287±79)	93.9±24.1 (92.7±23.4)
	Period II	1.8±0.3** (1.8±0.5**)	80.6±36.0* (87.5±38.8**)	396±78** (395±107**)	135.6±74.4 (138.5±71.6*)
	Ratio (II/I)	1.17±0.15 (1.24±0.28)	24.0±33.9[a)] (31.1±36.8[a)])	1.33±0.22 (1.41±0.34)	1.42±0.56 (1.48±0.58)
Intermediate metabolizers *10/*10	Number	3	3	3	0
	Period I	1.0±0.1[##]	72.0±0.0[#]	216±26	—
	Period II	1.1±0.1	208.0±110.9	291±18	—
	Ratio (II/I)	1.09±0.11	136.0±110.9[a)]	1.36±0.13	—
*1/*1	Number	4	4	4	3
	Period I	1.8±0.2	42.0±23.0	319±71	75.4±7.4
	Period II	2.0±0.3	78.0±45.4	396±97*	98.6±9.8*
	Ratio (II/I)	1.09±0.06	36.0±24.0[a)]	1.24±0.07	1.31±0.08
*1/*10	Number	10	10	10	8
	Period I	1.4±0.2[#]	62.4±16.8[##]	298±75	100.9±24.7
	Period II	1.7±0.3**	81.6±34.3	396±75**	149.5±84.1
	Ratio (II/I)	1.21±0.16	19.2±37.2[a)]	1.36±0.25	1.47±0.67
*1/*5	Number	3	3	3	1
	Period I	1.1±0.4[##]	56.0±13.9	206±70[#]	78.7
	Period II	1.7±1.1	120.0±41.6	390±229	170.0
	Ratio (II/I)	1.55±0.54	64.0±36.7[a)]	1.83±0.53	2.16
*2/*10	Number	4	4	4	3
	Period I	1.2±0.3[##]	48.0±0.0	233±44	94.1±16.8
	Period II	1.2±0.2	90.0±36.0	295±49	163.2±31.3*
	Ratio (II/I)	1.03±0.23	42.0±36.0[a)]	1.30±0.32	1.73±0.13

—: Unable to be determined
Mean±SD
[a)]Difference (Period II-Period I)
**$p<0.01$, *$p<0.05$ (paired t-test vs. Period I)
[##]$p<0.01$, [#]$p<0.05$ (Fisher's least significant difference test vs. *1/*1 in Period I)

tics of the urinary 6β-hydroxycortisol/cortisol ratios are shown in **Table 4** and the changes in urinary 6β-hydroxycortisol/cortisol ratios between before and after ITZ administration by individual subject are shown in **Fig. 5.**

In all subjects, the urinary 6β-hydroxycortisol/cortisol ratio was lower after ITZ administration. The ratio ranged from 3.74 to 9.82 (mean: 6.652) before the start of ITZ administration and from 1.72 to 6.67 (mean: 3.167) after repeated oral administration of ITZ for 7 consecutive days. The mean ratio decreased approximately 50% after administration of ITZ, and the change was judged to be statistically significant by Student's paired t-test. The correlation coefficient between the postdosing/predosing ratio for the urinary 6β-hydroxycortisol/cortisol ratio and that for the CL/F of ARIPIPRAZOLE was 0.23, and no definite correlation was shown.

Safety

Regarding safety, there were no significant differences in the incidences of adverse events and adverse drug

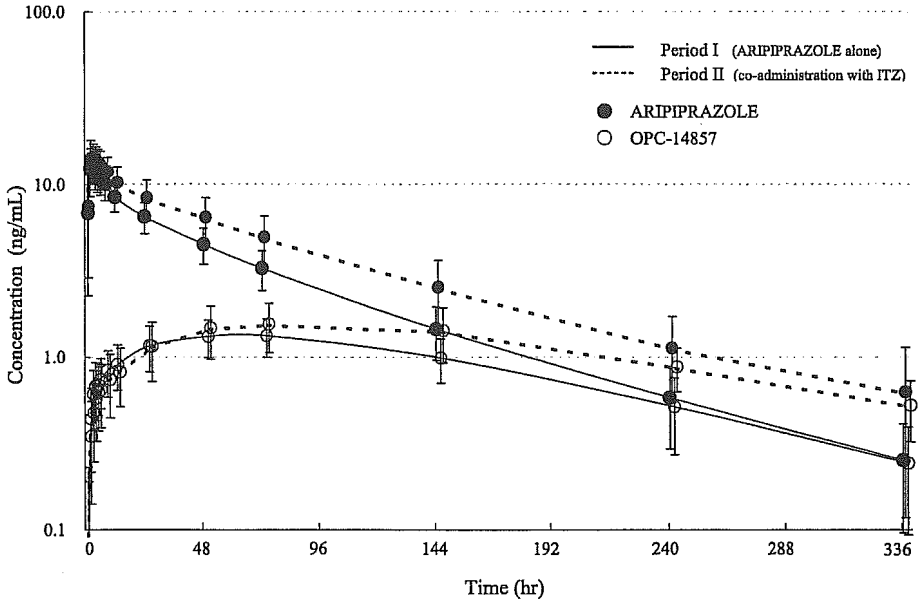


Fig. 2. Timecourses of mean plasma concentrations of ARIPIPRAZOLE and OPC-14857. (mean ± SD, n = 24).

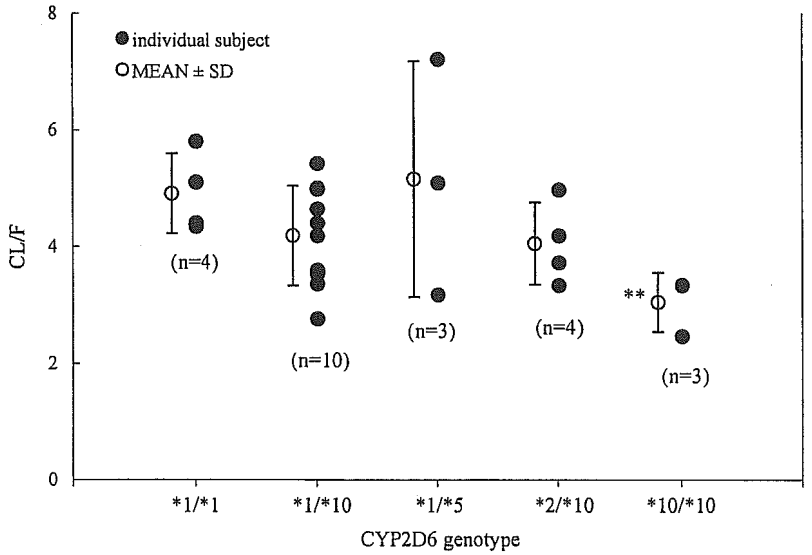


Fig. 3. ARIPIPRAZOLE plasma clearance in individual subjects by CYP2D6 genotype in Period I.
**$p < 0.01$ (Fisher's least significant difference test vs. *1/*1 in Period I)

reactions between administration of ARIPIPRAZOLE alone and co-administration with ITZ, and neither adverse events specific to co-administration with ITZ nor clinically significant issues for co-administration with ITZ were observed. It was thus considered that there were no clinically significant issues regarding the safety of ARIPIPRAZOLE when co-administered with itraconazole.

Discussion

1) Influence of CYP3A4 inhibition on the pharmacokinetics of ARIPIPRAZOLE

The purpose of this study was to investigate the influence of ITZ co-administration (CYP3A4 inhibition) on the pharmacokinetics of ARIPIPRAZOLE.

The inhibition of CYP3A4 metabolic activity by ITZ was confirmed using the urinary 6β-hydroxycortisol/cortisol concentration ratio as an indicator of CYP3A4 activity. Cumulative 24-hr urine was used for the assessment, since the urinary 6β-hydroxycortisol/cortisol concentration ratio is known to have a circadian rhythm.[11] The urinary 6β-hydroxycortisol/cortisol

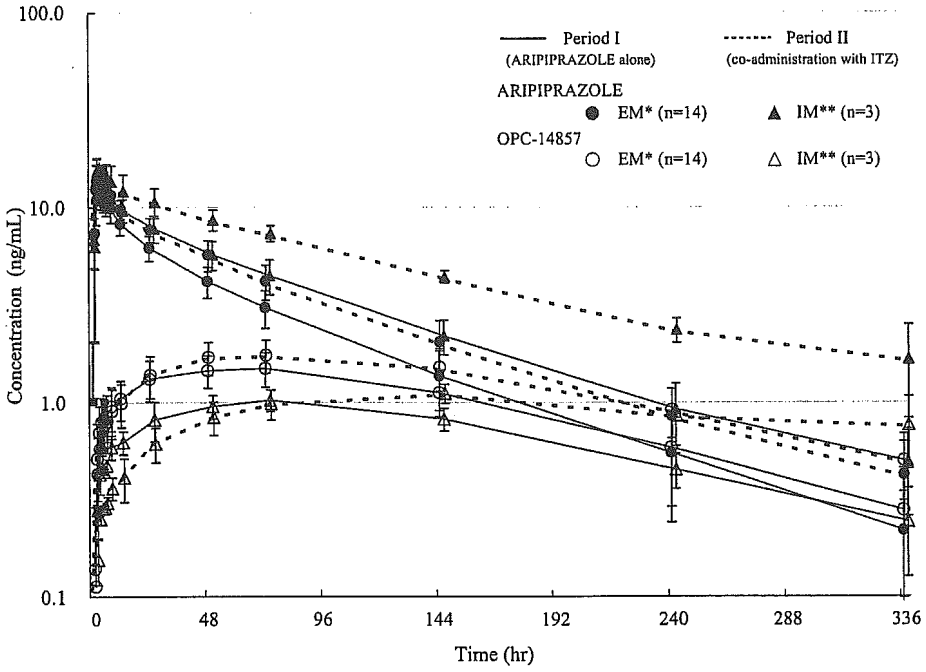


Fig. 4. Timecourses of mean plasma concentrations of ARIPIPRAZOLE and OPC-14857 by CYP2D6 genotype (mean ± SD)
EM*: CYP2D6 extensive metabolizers IM**: CY2D6 intermediate metabolizers

Table 4. Descriptive statistics of urinary cortisol concentrations and urinary 6β-hydroxycortisol/cortisol concentration ratios before and after ITZ administration

	Unit	Sampling point[a)]	N	Mean	Standard deviation	Minimum	Median	Maximum
Cortisol	ng/mL	Predosing	23	23.9	13.5	10	21.0	68
		Postdosing	23	22.5	11.3	7	20.0	53
6β-hydroxycortisol	ng/mL	Predosing	23	149.5	66.7	66	145.0	299
		Postdosing	23	63.2	24.5	21	58.0	111
6β-hydroxycortisol/cortisol		Predosing	23	6.652	1.590	3.74	6.530	9.82
		Postdosing	23	3.167**	1.256	1.72	2.900	6.67
		Ratio[b)]	23	0.4863	0.1679	0.211	0.4730	0.802

[a)]Predosing: Before ITZ administration; Postdosing: After ITZ administration for 7 consecutive days
[b)]Postdosing/predosing ratio between 6β-hydroxycortisol/cortisol ratio before and after ITZ administration
**$p < 0.01$ (paired t-test vs. predosing)

concentration ratio decreased by co-administration of ITZ in all subjects, with a mean decrease of 51%. The changes in the urinary 6β-hydroxycortisol/cortisol concentration ratio are considered to have indicated inhibition of CYP3A4 by ITZ, and the determination of the urinary 6β-hydroxycortisol/cortisol concentration ratio is considered to be meaningful as an indicator of CYP3A4 inhibition.

A weak correlation (correlation coefficient: 0.23) was observed between the postdosing/predosing ratio for the urinary 6β-hydroxycortisol/cortisol ratio and that for the CL/F of ARIPIPRAZOLE, and no significant difference in the postdosing/predosing ratio for the urinary 6β-hydroxycortisol/cortisol ratio was observed among CYP2D6 genotypes.

Plasma concentrations of ARIPIPRAZOLE and its main metabolite OPC-14857 in the elimination phase in the co-administration period were higher than those for the administration alone period, and $t_{1/2}$ was delayed by co-administration of ITZ. The increase in plasma ARIPIPRAZOLE concentration and the delay in $t_{1/2,z}$ by co-administration of a CYP3A4 inhibitor indicated decreased hepatic clearance, since almost no ARIPIPRAZOLE or OPC-14857 was excreted in the urine. This result demonstrated that CYP3A4 is involved in the metabolism of ARIPIPRAZOLE, which

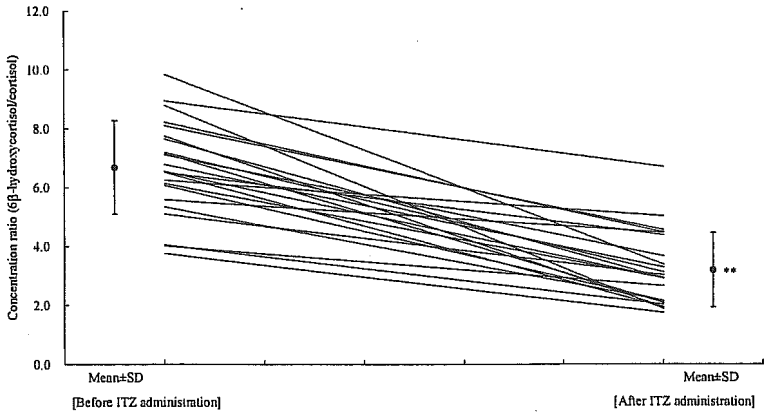


Fig. 5. Changes in urinary 6β-hydroxycortisol/cortisol ratios between before and after ITZ administration by individual subject (N=23). **$p<0.01$ (paired t-test vs. before ITZ administration)

is consistent with the results of an *in vitro* study.[2)]

ARIPIPRAZOLE is known to have good absorption from the gastrointestinal tract, and absolute bioavailability is 87%.[2)] Therefore, the influence of CYP3A4 in the gastrointestinal tract on the pharmacokinetics of ARIPIPRAZOLE during the absorption phase is relatively small. The t_{max} of ARIPIPRAZOLE was about 3 hr, indicating that absorption from the gastrointestinal tract was relatively fast.

The plasma concentration of OPC-14857 during the later phase of elimination was increased in the co-administration period, and the production of OPC-14857 from ARIPIPRAZOLE was considered to be inhibited by co-administration of ITZ. This also indicated that the metabolism of OPC-14857 was inhibited by CYP3A4 inhibition. The delayed increase in the plasma OPC-14857 concentration and the associated delay in t_{max} in the co-administration period were consistent with the results expected from the decreased rate of production of OPC-14857 from ARIPIPRAZOLE.

2) Pharmacokinetics of ARIPIPRAZOLE by CYP2D6 genotype

In the ARIPIPRAZOLE administration alone period, plasma ARIPIPRAZOLE concentrations in intermediate metabolizers were higher than those in extensive metabolizers. On the other hand, plasma OPC-14857 concentrations in intermediate metabolizers were lower than those in extensive metabolizers. The tendencies observed in the present study were similar to those observed in a comparison of pharmacokinetics by CYP2D6 genotype in another single dosing study of ARIPIPRAZOLE at 6-mg in healthy adult male volunteers (submitted for publication).

For the ITZ co-administration period in the present study, plasma ARIPIPRAZOLE concentrations in intermediate metabolizers were higher than those in extensive metabolizers, while the increases in plasma OPC-14857 concentrations in intermediate metabolizers were smaller and slower than those in extensive metabolizers. The extent of decrease in the elimination rates for the ARIPIPRAZOLE and OPC-14857 plasma concentrations by co-administration of ITZ was greater in intermediate metabolizers than in extensive metabolizers. Therefore, the influence of CYP3A4 inhibition on the elimination rate was considered to be greater in intermediate metabolizers than in extensive metabolizers.

In subjects classified as *2 Group, the pharmacokinetics more closely resembled those in extensive metabolizers than those in intermediate metabolizers. It was thus assumed that there were no CYP2D6*41 subjects in the group.

In a drug interaction study in which ARIPIPRAZOLE was co-administered with quinidine (a CYP2D6 inhibitor) in healthy volunteers in the US,[2)] the CL/F/BW of ARIPIPRAZOLE in CYP2D6 extensive metabolizers was decreased by approximately 50% (from 45.6 mL/hr/kg to 22.0 mL/hr/kg) by co-administration of quinidine, and a similar CL/F/BW was seen in poor metabolizers (27.0 mL/hr/kg). The results of that study indicated that CYP2D6 was almost completely inhibited by quinidine. Using the data for CL/F/BW in extensive metabolizers and poor metabolizers, the ratio of hepatic clearance of ARIPIPRAZOLE by CYP3A4 and CYP2D6 in CYP2D6 extensive metabolizers was estimated to be approximately 1:1.

Assuming that the ratios of hepatic clearance in CYP2D6 extensive metabolizers in the US and Japan are similar and that the ratio of hepatic clearance by CYP3A4 between CYP2D6 extensive metabolizers and CYP2D6 poor metabolizers is also similar, the ratio of hepatic clearance of ARIPIPRAZOLE by CYP3A4 and CYP2D6 in intermediate metabolizers is estimated to be

3:1 (2.3 L/hr vs. 0.8 L/hr). Using the data for the decrease in the hepatic clearance of ARIPIPRAZOLE by co-administration of ITZ (1.35 L/hr in extensive metabolizers and 1.44 L/hr in intermediate metabolizers), the decrease in hepatic clearance by CYP3A4 was estimated to be approximately 60% (from 2.3 L/hr to 0.9 L/hr) in both CYP2D6 extensive metabolizers and CYP2D6 intermediate metabolizers.

These results indicated that in CYP2D6 extensive metabolizers, CYP2D6 and CYP3A4 are approximately equally responsible for the metabolism of ARIPIPRAZOLE, OPC-14857 is the main metabolite produced, and CYP3A4 is the main metabolic enzyme involved in the metabolism of OPC-14857.

In conclusion, when administered at clinical doses, ITZ, which is known to strongly inhibit CYP3A4, would increase the AUC of ARIPIPRAZOLE by 50% at maximum, which is unlikely to cause clinically significant issues.

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Marked hyperglycemia after androgen-deprivation therapy for prostate cancer and usefulness of pioglitazone for its treatment

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Abstract

Here we demonstrate 2 patients who showed marked hyperglycemia after androgen-deprivation therapy for prostate cancer and the efficacy of the thiazolidinedione pioglitazone on their glycemic control. Case 1 was a 61-year-old man diagnosed with prostate cancer who had type 2 diabetes mellitus for 7 years. His glycemic control had been good for the previous 5 years because of diet therapy and acarbose administration. He was given the gonadotropin-releasing hormone agonist leuprolide acetate and the androgen receptor antagonist flutamide for the treatment of prostate cancer. After the second injection of leuprolide acetate, fasting glucose and hemoglobin A1c (HbA1c) levels were found to be markedly elevated (22.8 mmol/L and 10.5%, respectively). Case 2 was an 81-year-old man whose fasting glucose and HbA1c had been normal 10 months ago. He was injected with leuprolide acetate for the treatment of prostate cancer. Six months after starting the leuprolide treatment, the patient complained of thirst and weight loss and was diagnosed with diabetes mellitus with a fasting glucose of 19.4 mmol/L and HbA1c of 9.9%. The correct homeostasis model assessment evaluation indexes for pancreatic β-cell function (HOMA-%β) and for insulin sensitivity (HOMA-%S) were reduced in these 2 patients compared with control men. Their serum testosterone and 17β-estradiol concentrations were depressed. After improvement of hyperglycemia by insulin treatment, their glycemic control remained good after treatment with pioglitazone without use of insulin. The values of HOMA-%β and HOMA-%S increased to control ranges. Insulin resistance after the androgen-deprivation therapy might lead to marked hyperglycemia in these patients.

1. Introduction

A gonadotropin-releasing hormone (GnRH) agonist/antagonist alone or in combination with an antiandrogen often leads to either partial or full remission of prostate cancer [1]. GnRH agonists cause an initial surge in luteinizing hormone (LH) along with a rise in testosterone that is followed by down-regulation of the LH receptors in the pituitary, inhibition of LH release, and decrease of sex hormones secretion from the testes. Because the initial rise in testosterone may exacerbate the disease, an antiandrogen is sometimes coadministered to block the flare. The combined use of an antiandrogen has the additional potential to block the effects of adrenal androgens. Although these medical therapies are shown to be associated with hot flashes, decrease of libido, bone loss, and fatigue [2,3], their effects on glucose metabolism are not well defined. Here we report 2 cases with prostate cancer who showed marked hyperglycemia after treatments with combined GnRH agonist and an androgen receptor antagonist or with a GnRH agonist alone. The analysis by homeostasis model assessment (HOMA) indicated that both insulin sensitivity and insulin secretion were impaired in these patients. The thiazolidinedione drug pioglitazone was effective in maintaining good glycemic control in these patients.

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2. Subjects and methods

2.1. Patients

2.1.1. Case 1

A 61-year-old Japanese man was diagnosed with prostate cancer (stage T3b N_0 M_1) in August 2002. At age of 54 years, he was diagnosed with type 2 diabetes mellitus and advised by a dietitian to take a diet consisting of 1800 kcal/d: the percentages of each nutrient in the total calories were 50% to 60% of carbohydrates, 25% to 30% of fat, and 15% to 20% of protein. At that time, he was started on a α-glucosidase inhibitor acarbose (300 mg/d). Fasting plasma glucose levels remained 6.1 to 7.4 mmol/L and hemoglobin A1c (HbA1c) levels 5.3% to 6.4% (normal range, 4.3%-5.8%) during the previous 5 years, and thus, his glycemic control had been good. His height was 175.3 cm and body weight was 71.1 kg (body mass index 23.0 kg/m^2). For the treatment of prostate cancer, subcutaneous injection of leuprolide acetate (3.75 mg/mo) and oral administration of flutamide (250 mg/d) were started in September 2002. He did not change his dietary habits and his body weight did not change after the diagnosis of prostate cancer. He continued to work as usual and had no event that appeared to worsen his glycemic control, such as physiological or psychological stress, infections, and other medications. At 3 weeks after the second injection of leuprolide acetate, his glycemic control was found worse: fasting glucose and HbA1c levels were 18.2 mmol/L and 8.0%, respectively. He was administered nateglinide (270 mg/d), but fasting glucose and HbA1c reached 22.8 mmol/L and 10.5%, respectively, in December 2002 (Fig. 1). Serum testosterone and 17β-estradiol levels decreased to 0.45 nmol/L (control range in men, 10-35 nmol/L) and 40 pmol/L (control range in men, 70-110 pmol/L), respectively. After ceasing the administration of flutamide, we started injection of insulin lispro before each meal on admission. Plasma glucose levels declined on a maximal dose of 40 U/d at day 7 after insulin treatment, when we coadministered pioglitazone (15 mg/d). Thereafter, we could reduce the doses of insulin lispro. In February 2003, we stopped the insulin injection and increased the dosage of pioglitazone to 30 mg/d. We readministered flutamide and continued the injection of leuprolide acetate (11.25 mg per 3 months). In December 2003, fasting glucose levels remained below 7 mmol/L and HbA1c declined to 5.2% (Fig. 1).

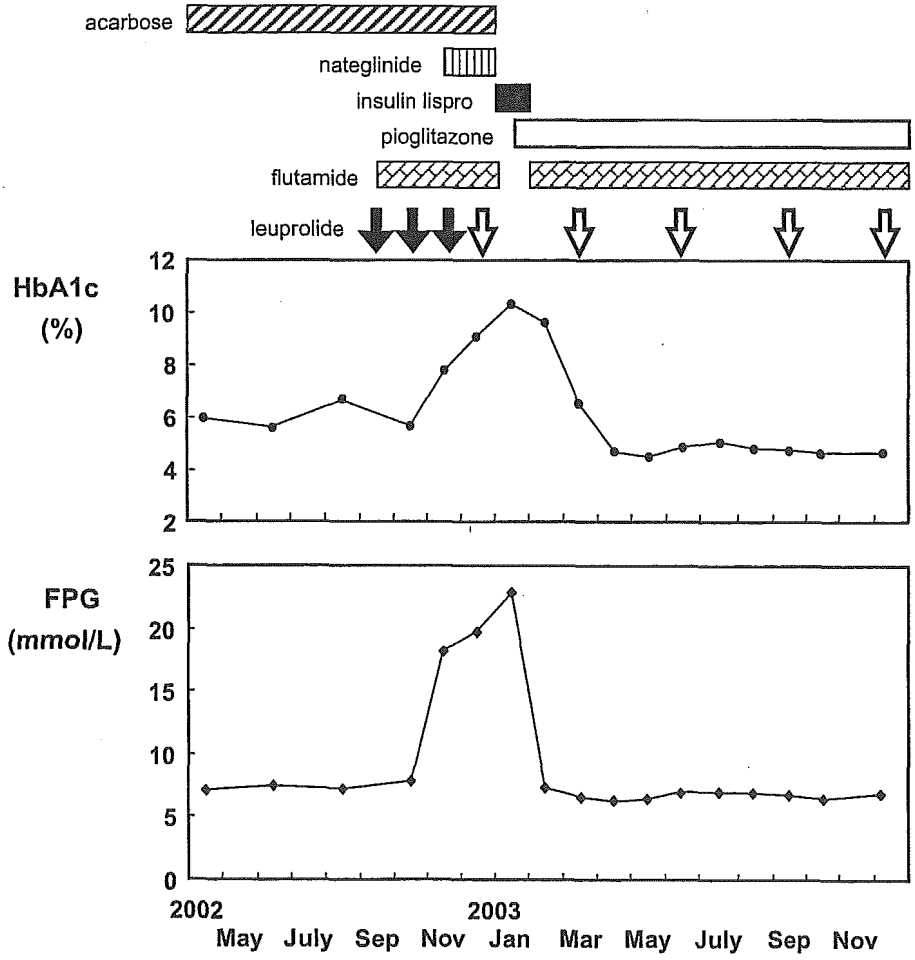

Fig. 1. Longitudinal changes of fasting plasma glucose (FPG) and HbA1c levels in case 1. Black arrows indicate subcutaneous injection of 3.75 mg leuprolide acetate; white arrows, 11.25 mg leuprolide acetate.

2.1.2. Case 2

An 81-year-old Japanese man was diagnosed with prostate cancer (stage T3a N_0 M_0) in June 2003. He has never been diagnosed with diabetes mellitus. Fasting glucose and HbA1c levels were 6.1 mmol/L and 5.1%, respectively, in April 2003. His height was 161.5 cm and body weight was 47.0 kg (body mass index 18.0 kg/m^2). The patient was started on subcutaneous injections of leuprolide acetate (3.75 mg/mo) for the treatment of prostate cancer in August 2003. After the third injection, the dose of leuprolide acetate was changed to 11.25 mg each 3 months. In March 2004, he complained of thirst and weight loss (−3.0 kg during 6 months); laboratory tests revealed marked hyperglycemia (19.4 mmol/L) and elevated HbA1c (9.9%) levels. Both serum testosterone and 17β-estradiol were undetectable (<0.42 nmol/L and <37 pmol/L, respectively). He was admitted to Osaka University Hospital and was given injections of insulin lispro before each meal. Plasma glucose concentration gradually decreased at a maximal dose of 24 U/d of insulin lispro (Fig. 2). By coadministration of pioglitazone (15 mg/d), we were able to reduce the dose of insulin lispro and thereafter stop the insulin treatment, followed by coadministration of the α-glucosidase inhibitor voglibose. The injection of leuprolide acetate (11.25 mg every 3 months) was continued. His glycemic control remained good in June 2004: fasting glucose was 6.7 mmol/L and HbA1c was 7.0%.

2.2. Control subjects

One hundred and forty-four Japanese men aged 58 to 68 years (60 ± 2 years, mean ± SD), who were confirmed to have normal glucose tolerance by the 75-g oral glucose tolerance test, were studied as control subjects. Their body mass index was 23.4 ± 2.5 kg/m^2.

2.3. Determination of pancreatic β-cell function and insulin sensitivity

Pancreatic β-cell function and insulin sensitivity were estimated by calculation from fasting plasma glucose and serum insulin levels using the HOMA method [4]. The

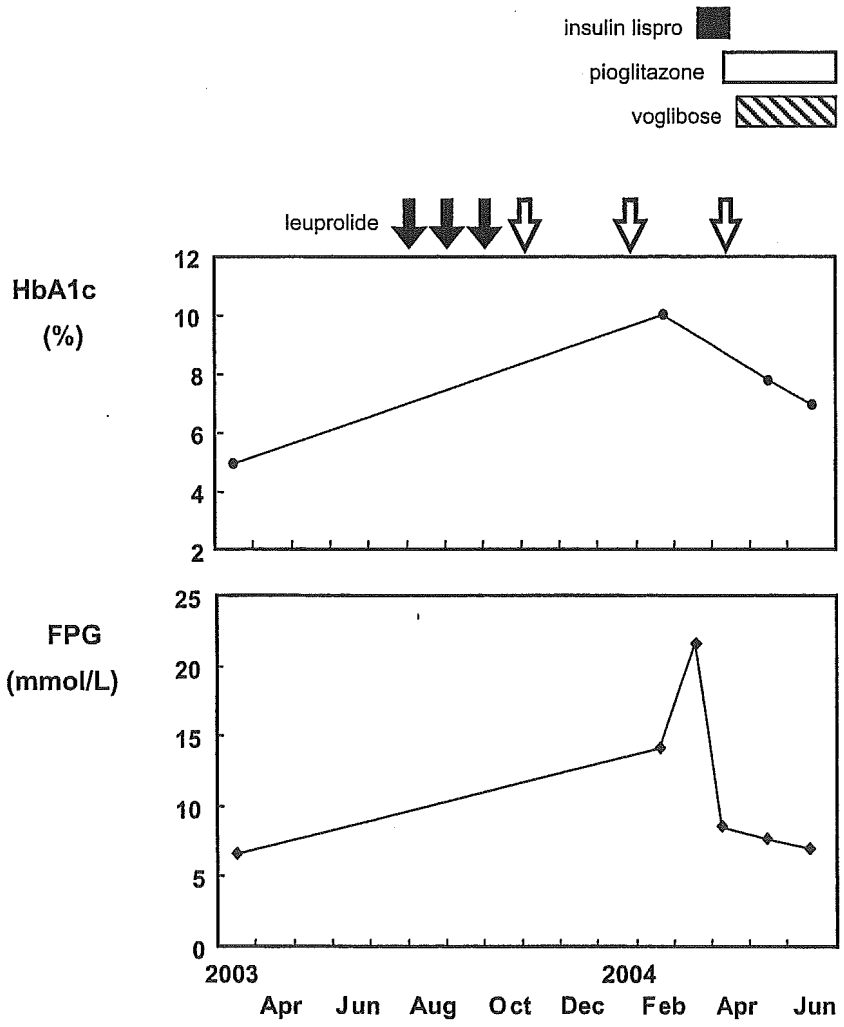

Fig. 2. Longitudinal changes of fasting plasma glucose (FPG) and HbA1c levels in case 2. Black arrows indicate subcutaneous injection of 3.75 mg leuprolide acetate; white arrows, 11.25 mg leuprolide acetate.

Table 1
Clinical and biochemical parameters of study subjects

	Fasting plasma glucose (mmol/L)	Fasting serum insulin (pmol/L)	HOMA-%β (%)	HOMA-%S (%)
Case 1				
December 2002	22.8	79	13	11
March 2003	6.3	43	58	100
June 2003	6.9	57	59	74
December 2003	7.1	50	51	84
Case 2				
March 2004	21.9	129	22	9
June 2004	6.7	34	43	125
Control subjects	5.5 ± 0.4	46 ± 17	78 ± 23	117 ± 52
Range	4.5-6.1	16-101	37-139	44-294

Control subjects are 144 Japanese men aged 58 to 68 years (60 ± 2 years) who have normal glucose tolerance. HOMA-%β and HOMA-%S were determined by the correct HOMA evaluation.

estimates of β-cell function and insulin sensitivity have been shown to correlate with estimates obtained by hyperglycemic and euglycemic clamp techniques, respectively [4]. The values for β-cell function (HOMA-%β) and insulin sensitivity (HOMA-%S) were assessed by the correct HOMA evaluation using a computer program reported by Levy et al [5].

3. Results

HOMA-%β and HOMA-%S were determined by the correct HOMA evaluation [5] in control subjects. Their HOMA-%β was 78% ± 23% (range, 37%-139%) and HOMA-%S was 117% ± 52% (range, 44%-294%).

Table 1 shows fasting glucose, fasting insulin, HOMA-%β, and HOMA-%S in 2 cases before and after treatments for diabetes mellitus. HOMA-%β and HOMA-%S in case 1 were 13% and 11%, respectively, before starting insulin treatment. Both estimates were below the ranges in the control subjects. After treatment with insulin followed by pioglitazone alone, HOMA-%β increased to 58% at 3 months, 59% at 6 months, and 51% at 12 months, which were within the control ranges (Table 1). HOMA-%S also increased to the ranges in the control subjects (100% at 3 months, 74% at 6 months, and 84% at 12 months). In case 2, HOMA-%β and HOMA-%S were 22% and 9% before treatment of insulin, both of which were below the control ranges. Both parameters increased to the control ranges (HOMA-%β 43%; HOMA-%S 125%) at 3 months after treatments with insulin followed by administration of pioglitazone and voglibose (Table 1).

4. Discussion

There is growing evidence indicating that sex hormones influence risk factors for diabetes mellitus. It has been shown that serum testosterone concentrations are inversely related to insulin resistance in men [6-8]. In addition, low levels of testosterone had been shown to predict the development of type 2 diabetes mellitus in men [9]. The effects of postmenopausal hormone replacement therapy are lower fasting glucose and insulin levels in nondiabetic women [10] and improved glycemic control in type 2 diabetic women [11], although disparate results had been reported [12,13]. Recently, the Heart and Estrogen/progestin Replacement Study (HERS) has shown that the postmenopausal hormone therapy reduced the incidence of diabetes mellitus [14]. Thus, androgens and estrogens may have favorable effects on glucose metabolism in men and in postmenopausal women, respectively.

The 2 male patients presented here demonstrated marked hyperglycemia after androgen-deprivation therapy for prostate cancer. Both cases had reduced pancreatic β-cell function as well as reduced insulin sensitivity, as determined by the correct HOMA evaluation. In case 1, a patient with type 2 diabetes mellitus whose glycemic control has been good using acarbose treatment alone was administered the GnRH agonist leuprolide acetate and the androgen receptor antagonist flutamide. Case 2, who had never been diagnosed with diabetes mellitus, was given leuprolide acetate but not antiandrogens. Leuprolide acetate causes a decrease in serum levels of androgens and estrogens. Thus, reduction of serum androgens and/or serum estrogens may contribute to marked hyperglycemia in these patients. The thiazolidinedione pioglitazone, an insulin-sensitizing drug, was very effective in maintaining good glycemic control after lowering of blood glucose levels by insulin therapy. These observations suggest that insulin resistance occurred in our patients with reduced β-cell function after androgen-deprivation therapy and it resulted in the exacerbation of hyperglycemia.

Coddington et al [15] reported a type 1 diabetic female patient who showed deterioration in glucose control after leuprolide treatment of endometriosis. This suggests the involvement of a decrease in serum estrogens in worsening of glycemic control. Furthermore, a male patient with aromatase deficiency, who had decreased serum estrogens but not androgens, has been shown to have insulin resistance [16]. In addition, androgens had been shown to impair insulin action in women [17]. Taken together, the reduction of serum estrogen levels rather than serum androgen levels may have lead to the reduction of insulin sensitivity, causing marked hyperglycemia in our cases.

At present, we have no information on the prevalence of alterations of glucose metabolism in prostate cancer patients who are given androgen-deprivation therapy, and this needs to be studied in the future. The onset of overt diabetes may depend on β-cell function as well as insulin sensitivity of the patients. Because androgen-deprivation therapy has become the mainstay of prostate cancer treatment, physicians should pay attention to carbohydrate metabolism in patients receiving androgen-deprivation therapy.

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Pharmacokinetic Evaluation of Anticonvulsants in a Patient with Porphyria

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The aim of this study was to establish the appropriate regimen of anticonvulsants for a female patient with porphyria by pharmacokinetic evaluation of the influence of anticonvulsants on porphyria. The pharmacokinetics of phenytoin, carbamazepine, clonazepam, and clobazam were estimated by the Bayesian method. The urinary 6β-hydroxycortisol/cortisol (6β-OHF/F) ratio was also evaluated as an index of hepatic CYP3A4 induction.

The phenytoin concentrations in the toxic area fitted the predicted value for *CYP2C9*1/*3* better than that for *CYP2C9*1/*1* (her genotype). The concomitant phenytoin altered the clearance of carbamazepine considerably. The clearances of clonazepam and clobazam were not altered, although hepatic CYP3A4 induction was implied from the value of the urinary 6β-OHF/F ratio.

From the pharmacokinetic evaluations, the following were concluded : (1) phenytoin was not the proper medication for this patient, (2) carbamazepine can be used safely within a relatively small dose, 500 mg/day, (3) the combination of clonazepam and carbamazepine can be used, and (4) a concomitant small dose of clobazam with carbamazepine can also be used.

Key words : anticonvulsant, porphyria, cytochrome P450, Bayesian method, urinary 6β-hydroxycortisol/cortisol ratio

Introduction

Although many anticonvulsants are known to deteriorate porphyria, some patients with porphyria have seizures and require anticonvulsant therapy. Part of the deteriorating mechanism has been conjectured to cause an imbalance of heme protein biosynthesis, which is due to the induction of cytochrome P450 (CYP) by anticonvulsants. We previously demonstrated that the measurement of the human urinary 6β-hydroxycortisol/cortisol (6β-OHF/F) ratio is a useful indicator of safe medication in a patient with hereditary coproporphyria[1]. The previous results implied that hepatic CYP induction was profoundly related to her condition of porphyria.

In the present paper, the pharmacokinetic parameters of the anticonvulsants used in the patient during two hospitalizations (phenytoin, carbamazepine, clonazepam, and clobazam) were retrospectively evaluated by the Bayesian method[2,3]. We established the effective and safe dose of anticonvulsants for her seizures based on the alteration of the estimated pharmacokinetic parameters and the measurement of the urinary 6β-OHF/F ratio.

Case

A female in her twenties was diagnosed with rare dual porphyria involving partial δ-aminolevulinate dehydratase deficiency with epilepsy. She had been treated with sodium valproate, phenytoin and carbamazepine when she was admitted to our hospital on June 24, 1998. Sodium valproate was discontinued because of abdominal side effects. For

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better control of the epilepsy, phenytoin was increased from 100 to 125 mg/day and carbamazepine was gradually increased from 800 to 1200 mg/day. However, her condition deteriorated, and the serum concentrations of phenytoin were within the toxic range. The frequency of her seizures decreased eventually by discontinuing phenytoin, gradually reducing carbamazepine, and adding clonazepam. She was discharged from our hospital after four months.

She was re-hospitalized 2 years later (March 22, 2001) for reevaluation of the medication for epilepsy. She had been treated with carbamazepine, clobazam, and zonisamide. Zonisamide was discontinued. The clobazam dose, 15 mg/day, was not changed. The carbamazepine dose was gradually increased from 450 to 500 mg/day. The frequency of her seizures decreased 2 weeks after receiving modified treatment. The urinary 6β-OHF/F ratio gathered over 24 hours was measured and compared with the value from before she left our hospital the first time[1].

Methods

The patient's genotype of the metabolic enzymes of phenytoin had been judged to be *CYP2C9*1/*1* and *CYP2C19*1/*2*[1]. The pharmacokinetic parameters of phenytoin were estimated from serum phenytoin concentrations in the toxic area, with the use of subpopulation parameters of *CYP2C9*1/*1* and *CYP2C9*1/*3*[4]. The typical predicted dose of phenytoin was calculated from equation (1) (below), based on these estimated parameters. The percentage of deviation between the predicted dose and the administered dose was calculated from equation (2) (below).

Each alteration of carbamazepine and clonazepam clearance was estimated to evaluate the pharmacokinetic influence of these agents on porphyria. In the second hospitalization, the alteration of carbamazepine and clobazam clearances was estimated. The alteration of the N-desmethylclobazam/clobazam ratio was also evaluated, because clobazam is metabolized to N-desmethylclobazam by CYP3A4. The original data used for this study were obtained as described previously[1].

Her pharmacokinetic parameters of anticonvulsants were estimated by the software PEDA (parameter estimation and dosage adjustment)[5] incorporating the Bayesian method, using the population pharmacokinetic parameters described in the literature[4,6-8]. The phenytoin dose was calculated by the following equation from the observed concentration of phenytoin :

$$Dose_{,pre} = V_{max}C_{ss}/(K_m + C_{ss}) \quad (1)$$

where V_{max}, K_m, $Dose_{,pre}$, and C_{ss} are the maximal elimination rate of the Michaelis-Menten equation (mg/day), the Michaelis-Menten constant (μg/mL), the serum concentration of phenytoin at a steady state (μg/mL), and the predicted dose (mg/day), respectively. The deviation between the dose predicted from equation (1) and the actual dose was calculated by the following equation :

$$Dev = (Dose_{,act} - Dose_{,pre})/Dose_{,act} \quad (2)$$

where Dev, $Dose_{,act}$, and $Dose_{,pre}$ are the deviation, the actual dose, and the predicted dose, respectively. The plasma concentrations of carbamazepine, clonazepam, and clobazam were calculated by the following equations :

$$C = D \cdot K_a/Vd/(K_a - K_e) \cdot \{Exp(-K_e t) - Exp(-K_a t)\} \quad (3)$$

$$C_{ss} = D/CL \quad (4)$$

where D, C, and C_{ss} are the dosage (mg), carbamazepine or clobazam concentration in plasma (μg/mL), and plasma clonazepam concentration at a steady state (μg/mL), respectively ; and K_e, K_a, Vd, t, and CL are the elimination rate constant (hr^{-1}), absorption rate constant (hr^{-1}), apparent volume of distribution normalized with bioavailability (L), time from initial administration (hr), and apparent total body clearance normalized with bioavailability (L/hr), respectively. From this point on, the term "clearance" will be used for "apparent total body clearance normalized with bioavailability". Equation (3) was used for carbamazepine and clobazam, and equation (4) was used for clonazepam.

Results

The pharmacokinetic parameters of phenytoin in *CYP2C9*1/*1* were estimated by the Bayesian method, as follows : $V_{max} = 3.8$ mg/kg/day and $K_m = 5.6$ μg/mL. The typical predicted dose of phenytoin was calculated to be approximately 141 mg/day. The percentage of deviation was approxi-

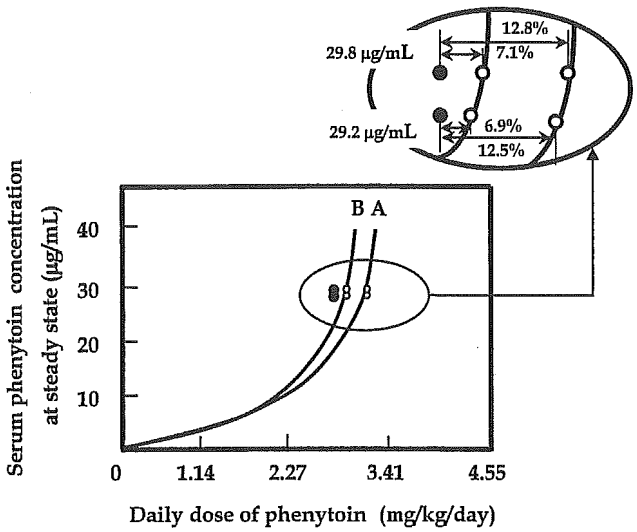

Fig. 1 Profile between serum concentration and dose of phenytoin

Curves A and B were the estimated curves from the subpopulation parameters[6] of *CYP2C9*1/*1* with *CYP2C19*1/*2* and *CYP2C9*1/*3* with *CYP2C19*1/*2*, respectively, by the Bayesian method. Closed circles (29.8, 29.2 μg/mL) represent serum phenytoin concentrations on July 31 and August 3, below 125 mg/day, respectively. Open circles are the predicted dose of phenytoin.

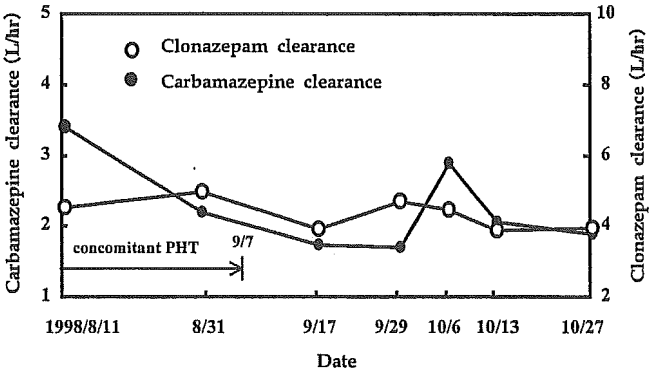

Fig. 2 Alteration of carbamazepine and clonazepam clearances

Open and closed circles represent carbamazepine and clonazepam clearances, respectively.

Table Alteration of the N-desmethylclobazam/clobazam ratio and the clearance of clobazam and carbamazepine

	April 3, 2001	May 11, 2001
Carbamazepine clearance (L/hr)	2.54	2.22
Clobazam clearance (L/hr)	2.25	2.25
Clobazam (μg/mL)	0.26	0.26
N-desmethylclobazam (μg/mL)	3.52	3.95
N-desmethylclobazam/ clobazam ratio	13.8	15.49

mately 11%. The estimated parameters in *CYP2C9*1/*3* were also calculated, as follows: V_{max}=3.3 mg/kg/day and K_m=4.1 μg/mL. The typical estimated dose was approximately 129 mg/day. The percentage of deviation was about 3%. Therefore, the predicted dose for *CYP2C9*1/*3* better fits the model used [equation(1)] than that for *CYP2C9*1/*1* (Fig. 1).

Carbamazepine clearance was altered between 1.7 and 3.4 L/hr, whereas clonazepam clearance was almost unaltered (Fig. 2). Although the carbamazepine dose was decreased by 80%, from 1200 to 1000 mg/day, the plasma concentration increased by 1.2 times when carbamazepine was combined with phenytoin, in the relationship between the daily dose and plasma concentration of carbamazepine. The clearance decreased by 50% based on the alteration. However, clonazepam clearance did not show a remarkable alteration even when clonazepam was combined with carbamazepine and phenytoin.

The alteration of carbamazepine and clobazam clearances and the N-desmethylclobazam/clobazam ratio after re-hospitalization are shown in Table. The carbamazepine and clobazam clearances remained almost unaltered. The N-desmethylclobazam/clobazam ratio was unaltered. The measured value of the urinary 6β-OHF/F ratio was 20.2.

Discussion

1. Discrepancy between the patient's genotype and phenotype of phenytoin metabolism

The patient was an extensive metabolizer of CYP2C9, which is the main metabolizing enzyme of phenytoin[1]. Her genotype was *CYP2C9*1/*1*, whereas her phenotype as indicated by the behavior of her serum phenytoin concentration was identical to that of *CYP2C9*1/*3*. *CYP2C9*3* is generally known to be a genetic polymorphism that decreases the enzyme activity. In addition, she had no inhibitor of CYP2C9 in her medications. The reason underlying the discrepancy between her genotype and phenotype of phenytoin metabolism remains an

area of considerable interest. One proposal is that phenytoin as an inducing agent of CYP3A4 might have destroyed the equilibrium of her abnormal heme pathway and depleted CYPs. Her metabolism of phenytoin might have been saturated by the reduction of CYP synthesis.

2. Alteration of the clearance of carbamazepine and clonazepam

The decrease in carbamazepine clearance (Fig. 2) leads us to conjecture that the suppression of CYP biosynthesis was brought on by a mechanism similar to that of the metabolic saturation of phenytoin by the concomitant phenytoin. In addition, an increase in carbamazepine clearance was transiently observed (Fig. 2), which might have been due to a high blood concentration of carbamazepine resulting from the improvement of the equilibrium of CYP biosynthesis, because a month had passed after the discontinuation of phenytoin. The results suggest that phenytoin markedly affected CYP biosynthesis in this patient with porphyria. The influence on CYP might have caused saturation of phenytoin metabolism and an excessive decrease in carbamazepine clearance.

The clonazepam clearance (CL/F) was estimated to be approximately 4.3 L/hr as the mean value (Fig. 2). The total body clearance (CL_{tot}) is calculated to be 4.4 L/hr because the bioavailability of clonazepam is reported to be approximately 0.98[9]. The product of the value of unbound fraction and the hepatic intrinsic clearance was calculated to be 4.4 L/hr from equation (5) when the absorption ratio was regarded to be 1.0 according to the high bioavailability (see "Appendix"). The general value of hepatic blood flow is about 90 L/hr. Therefore, the hepatic extraction ratio of clonazepam was calculated to be approximately 0.05 from equation (7) (see "Appendix"). Clonazepam is considered to be a metabolic capacity-limited agent, according to the calculated value of the hepatic extraction ratio. Carbamazepine is a similar type agent. Carbamazepine clearance seems to be excessively affected by the alteration of hepatic CYP3A4 activity, whereas the clonazepam clearance was not altered in spite of concomitant phenytoin.

The metabolic pathway of clonazepam proceeds by nitroreduction, acetylation, and hydroxylation. The acetylation is reported to be affected by the polymorphic N-acetyltransferase (NAT) that determines the acetylation phenotype of the individual[10]. This patient was considered to be an intermediate acetylator since her genotype was *NAT2*4/*6*. There has been no report of a relationship between the NAT2 genotype and a phenotype of clonazepam pharmacokinetics. The influence of the NAT2 genotype on the alteration of clonazepam clearance was unclear.

Seree et al, suggested that the nitroreduction of clonazepam is catalyzed by *CYP3A4*[11]. Therefore, clonazepam metabolism might also be affected by the suppression of CYP biosynthesis (Fig. 2). However, the alteration of clonazepam and carbamazepine clearances was different. Binding plasma proteins of phenytoin, clonazepam, and carbamazepine is 90-95%, 80-90%, and 70-80%, respectively[12,13]. This result implies that the difference in the affinity ratio of binding to plasma proteins affects the alteration of these clearances.

3. Pharmacokinetic evaluation of anticonvulsants after the second hospitalization

The patient's medication after the second hospitalization was evaluated according to the alteration of carbamazepine clearance, clobazam clearance, and the N-desmethylclobazam/clobazam ratio (Table). The carbamazepine and clobazam clearances and N-desmethylclobazam/clobazam ratio were unaltered after re-hospitalization. However, the measured value of the urinary 6β-OHF/F ratio was slightly high compared with the value (15.4) before the patient left our hospital the first time[1].

Her condition had not improved with a carbamazepine dose of 450 mg/day (see "Case"), whereas the frequency of seizures decreased by increasing the carbamazepine dose to 500 mg/day. Carbamazepine was considered to be effective and safe at a dose of 500 mg/day. The dosage regimen after the second hospitalization was considered to have been appropriately modified as a result.

4. Conclusions

From the pharmacokinetic evaluations for this patient, the following conclusion was established : phenytoin was not a proper medication. Carbamazepine was effective and safe, within a moderate

dose. In addition, the clearance of clonazepam was not affected by carbamazepine. Therefore, the concomitant use of carbamazepine and clonazepam was also considered effective. Clobazam and carbamazepine might be used safely for controlling seizures of porphyria within relatively small doses, at 15 mg/day for the former and at 500 mg/day for the latter.

The influence of anticonvulsants on porphyria was pharmacokinetically evaluated by estimating the clearance of anticonvulsants, in addition to the measurement of urinary 6β-OHF/F. We obtained information on effective and safe medications for porphyria on the basis of estimation of pharmacokinetic parameters.

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Appendix

The total body clearance (CL_{tot}) for a drug is generally expressed as follows, when a drug is mostly eliminated in the liver :

$$CL_{tot}=f_{ub}\cdot CL_{int,h}/F_a \quad (5)$$

where F_a is the absorption ratio of the drug ; f_{ub} is the unbound fraction in the blood ; and $CL_{int,h}$ is the hepatic intrinsic clearance. Furthermore, the hepatic clearance (CL_h) and hepatic extraction ratio (ER_h) of drugs are expressed as follows :

$$CL_h=Q_h\cdot f_{ub}\cdot CL_{int,h}/(Q_h+f_{ub}\cdot CL_{int,h}) \quad (6)$$
$$ER_h=f_{ub}\cdot CL_{int,h}/(Q_h+f_{ub}\cdot CL_{int,h}) \quad (7)$$

where Q_h is the hepatic blood flow ; f_{ub} is the unbound fraction in the blood ; and $CL_{int,h}$ is the hepatic intrinsic clearance. A drug with an ER_h of more than 0.8 is classified as an agent of the hepatic flow-limited type[14,15]. A drug with an ER_h of less than 0.2 is classified as an agent of the metabolic capacity-limited type.

An oral drug absorbed in the gut passes the liver via the portal vein. Only a drug which passes to the liver flows in circulating blood. Therefore, such a drug can be described by the following equation :

$$AUC_{po}=F_h\cdot F_a\cdot AUC_{iv} \quad (8)$$

where F_h is the hepatic availability ; F_a is the fraction absorbed into the portal vein from the gut ; and AUC_{iv} and AUC_{po} are the areas under the blood concentration-time curve after intravenous bolus and oral dosing, respectively.

AUC_{po} is expressed as a function of dosage (D) and total body clearance after oral dosing ($CL_{tot,po}$) as follows :

$$AUC_{po}=D/CL_{tot,po} \quad (9)$$

The following equation is obtained from equations (8) and (9) :

$$CL_{tot,po}=CL_{tot,iv}/(F_h\cdot F_a) \quad (10)$$

F_h is described by the following equation according to the literature[14,15] :

$$F_h=Q_h/(Q_h\cdot f_{ub}\cdot CL_{int,h}) \quad (11)$$

$CL_{tot,iv}$ assumes $CL_{,h}$ as the hepatic clearance, and equation (5) is obtained from equations (6), (10), and (11).