polymerase. PCR was performed with an initial step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, at 66°C for 30 s and at 72°C for 25 s, with a final extension at 72°C for 7 min. Under these conditions, amplification of DNA fragments produced single product. The PCR product was detected with ABI PRISM 7700 Sequence detector (Applied Biosystems) using fluorescent dye SYBR Green I (Molecular Probes).

Owing to the low frequency, the alleles, except *CYP2A6*4* and **9*, were defined as *CYP2A6*1*.

Statistical analysis

According to the genotypes, subjects were divided into two groups, high- and low-activity group, as described previously.[5] In brief, subjects with the **4/*9* and **4/*4* genotypes were considered to have less than 50% of the enzyme activity of **1/*1* and, therefore, defined as CYP2A6 low-activity group, whereas those with the **1/*1*, **1/*9*, **1/*4* and **9/*9* genotypes were defined as CYP2A6 high-activity group. All comparisons were carried out between CYP2A6 high-activity group and CYP2A6 low-activity group. Differences in the number of cigarettes, daily nicotine intake and score for nicotine dependence were tested using Mann–Whitney *U*-test. The χ^2 test was used to assess the time to the first cigarette of the day. The frequencies of withdrawal symptoms were also analyzed with χ^2 test. To assess the association of the *CYP2A6* genotypes with the withdrawal symptoms, we calculated ORs and their 95% CIs. An association was reported as statistically significant if the respective null hypothesis of OR = 1 was rejected at $P<0.05$ or when the respective 95% CIs did not include the value 1.

Duality of interest

None declared.

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No positive association between adrenergic receptor variants of α_{2c}Del322–325, β_1Ser49, β_1Arg389 and the risk for heart failure in the Japanese population

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Aims
We investigated the correlation of adrenergic receptor polymorphisms, α_{2c}Del322–325, β_1Ser49Gly and β_1Arg389Gly, with the risk of heart failure in the Japanese population.

Methods
These polymorphisms were analysed by polymerase chain reaction-restriction fragment length polymorphism in patients with chronic heart failure due to idiopathic dilated cardiomyopathy (DCM) and compared with the control group.

Results
There were no differences or any trends in the allele and genotype frequencies of the β_1Ser49Gly and β_1Arg389Gly polymorphisms. The allele frequency of the α_{2c}Del322–325 variant was lower in patients than in controls (0.11 *vs.* 0.04, $P = 0.011 < 0.017$, by Bonferroni correction), while the genotype frequency just failed to reach significance ($P = 0.022 > 0.017$, by Bonferroni correction).

Conclusions
In this population, the variants β_1Ser49, β_1Arg389, and α_{2c}Del322–325 do not appear to be risk factors for chronic heart failure due to DCM. The α_{2c}Del322–325 variant may in fact confer some protection.

Introduction

Neurohumoral factors play important roles in cardiac remodelling, determining the prognosis of heart failure. In particular, the sympathetic nervous system is activated in patients with chronic heart failure (CHF) [1] and sustained stimulation of the adrenergic system exerts direct adverse effects on cardiac function [2]. In spite of the importance of the adrenergic system, the effects of polymorphic mutation of adrenergic receptors on CHF remain to be fully elucidated.

In the present study, we focus on the presynaptic α_{2c} adrenergic receptor (AR) polymorphism with the deletion of four consecutive amino acids, α_{2c}Del322–325, and polymorphic amino acid variants of β_1AR, Ser49Gly and Arg389Gly. These polymorphic changes result in alteration of AR function. The presynaptic

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α_2AR negatively regulates the release of norepinephrine from cardiac sympathetic nerves [3] and α_{2c}Del322–325 polymorphism shows a 'loss-of-function' phenotype [4]. The postsynaptic β_1AR polymorphism, β_1Ser49Gly, affects receptor sensitivity and promotes the downregulation of the receptor to agonists *in vitro* [5]. The change of β_1AR from Arg to Gly at the 389 amino acid residue leads to the decrease in G-protein coupling [6]. Considering the importance of the adrenergic system as a modulator of cardiac remodelling, it could be proposed that polymorphisms of adrenergic receptor genes may be closely related to the risk of heart failure.

Recently, Small *et al.* proposed that the polymorphisms of β_1Arg389Gly and α_{2c}Del322–325 are synergistically related to the risk of CHF in a black population [7]. However several concerns, including the aetiology of heart failure and the absence of analysis of β_1Ser49Gly frequency, have been raised against this study [8]. We have investigated the clinical significance of α_{2c}Del322–325, β_1Ser49Gly, and β_1Arg389Gly for the risk of heart failure due to dilated cardiomyopathy (DCM) in the Japanese.

Methods

Subjects

The study subjects consisted of 91 unrelated consecutive patients with CHF due to idiopathic DCM (males 79.5%, age 58.4 ± 13.7 years, ejection fraction 34.6 ± 15.8%) who attended or were admitted to Hokkaido University Hospital, Kyoto Katsura Hospital, Osaka Prefectural Medical Centre for Respiratory and Allergic Diseases, Aizenbashi Hospital or Osaka City University Medical School Hospital. The ratio of the patients, classified as NYHA class I, II, III or IV, was 13.9, 46.8, 15.1, 24.1%, respectively. Patients with ischaemic cardiomyopathy were excluded. One hundred and nineteen subjects (all males, aged from 20 to 40 years) who had no history or symptoms of cardiovascular disease were chosen as controls. This study was approved by the institutional review committee. All subjects gave their informed consent to participate.

Genotyping

Genomic DNA was extracted from samples of peripheral blood leucocytes using the QIAamp DNA Blood Maxi Kit (Qiagen K.K., Tokyo, Japan) according to the manufacturer's protocol. Genotyping of α_{2c}Del322–325, β_1Ser49Gly and β_1Arg389Gly polymorphisms was performed as described previously [4–6] with minor modifications.

Statistical analysis

Values were expressed as means ± SD. χ^2 test of independence was used to test for associations between heart failure and allele. The 2×3 exact probability test was used to evaluate associations between heart failure and genotype. All of the analyses were corrected by Bonferroni correction. $P < 0.017$ was considered to be significant. Statistical analysis was performed with StatView Version 5.0 software (SAS Institute, Cary, NC, USA).

Results

The allele and genotype frequencies of α_{2c}Del322–325, β_1Ser49Gly and β_1Arg389Gly polymorphisms in the patients and controls are shown in Table 1. The allele

Table 1

Distribution of α_{2c} and β_1 adrenergic receptor (AR) variants in controls and patients with heart failure

Alleles and subjects	Allele Frequency	*P*-value	Genotype Frequency			*P*-value
α_{2c}Del322–325			WT/WT	WT/Del	Del/Del	
Controls	0.11	0.011	95/119 (79.8%)	23/119 (19.3%)	1/119 (0.8%)	0.022
Patients with heart failure	0.04		84/91 (92.3%)	7/91 (7.7%)	0/91 (0%)	
β_1Arg389			Gly/Gly	Gly/Arg	Arg/Arg	
Controls	0.81	0.82	5/119 (4.2%)	35/119 (29.4%)	79/ 119 (66.4%)	0.94
Patients with heart failure	0.80		5/91 (5.5%)	26/91 (28.6%)	60/91 (65.9%)	
β_1Ser49			Gly/Gly	Gly/Ser	Ser/ Ser	
Controls	0.84	0.90	3/119 (2.5%)	33/119 (27.7%)	83/119 (69.7%)	0.58
Patients with heart failure	0.84		4/91 (4.4%)	21/91 (23.1%)	66/91 (72.5%)	

P-values for comparisons of allele frequency or genotype frequency between controls and patients with heart failure were determined by 2×2 χ^2 or by 2×3 exact probability test, respectively. P-value <0.017 (0.05/3) was considered to be significant.

Table 2
Combined genotypes of β_1AR and the risk for heart failure

β_1Ser49Gly	β_1Arg389Gly	Controls	Patients with heart failure	Odds ratio for heart failure (95%CI)	*P*-value
		No. of subjects			
		119	91		
≧ 1Gly	≧ 1Gly	6	6	1.00	–
≧ 1Gly	Arg/Arg	30	19	0.63 (0.18–2.25)	0.35
Ser/Ser	≧ 1Gly	34	25	0.74 (0.21–2.55)	0.43
Ser/Ser	Arg/Arg	49	41	0.84 (0.25–2.79)	0.50

Subjects with at least one β_1Gly49 allele and at least one β_1Gly389 allele served as the reference group. Odds ratios and P-values between the reference group and each other group were determined by 2 × 2 χ^2 test. P-value < 0.017 (0.05/3) was considered to be significant.

frequency of the α_{2c}Del322–325 variant was lower in patients with CHF than in controls (0.04 *vs.* 0.11, $P = 0.011 < 0.017$, by Bonferroni correction) and the genotype frequency was not significant but showed the *P*-value nearly equal to the borderline of significance ($P = 0.022 > 0.017$, by Bonferroni correction). The allele and genotype frequencies of the β_1Arg389 and β_1Ser49 variants in the patients with heart failure were consistent with those of controls.

Combined genotypes of β_1Arg389 and β_1Ser49 variants were not associated with the risk of heart failure (Table 2).

Discussion

The frequency of β_1Arg389Gly and β_1Ser49Gly polymorphisms did not differ from those in the control group, nor were there any trends, suggesting that these polymorphisms are not associated with susceptibility to CHF. Similarly, the combined genotype of β_1Arg389Gly and β_1Ser49Gly was not associated with the risk of CHF. However, we cannot definitely exclude the possibility that lack of association is derived from a β error problem, although the number of samples in our study was more than that in the previous study [7].

The allele frequency of the α_{2c}Del322–325 variant was statistically lower in CHF than in the controls. However, it is uncertain that the α_{2c}Del322–325 variant is a negative risk factor clinically, because the genotype frequency of this variant was of borderline significance, probably due to the low frequency of the homozygous genotype for α_{2c}Del322–325 variant in the Japanese population. Thus, considering the limitation of low genotype frequency of this polymorphism, a reasonable interpretation of our results is that α_{2c}Del322–325 variant is not a positive risk factor for CHF due to DCM in the Japanese population.

Previously, it was reported that allele frequency of α_{2c}Del322–325 positively correlated with heart failure in both the white and black populations [7]. It remains to be clarified why our data are not consistent with the previous study [7]. One possibility is that the inconsistency might be derived from the difference in the cause of heart failure. The previous study included patients with ischaemic cardiomyopathy, while we excluded ischaemia because some adrenoceptor polymorphisms are related to hypertension, a risk factor for ischaemic heart disease [9, 10]. Another possibility is that the pathophysiological significance of the adrenoceptor polymorphism is closely related to the severity of heart failure. Importantly, the ratio of the patients classified as NYHA III or IV is lower in the present study than that in the previous report [7]. It may be hypothesized that there is a racial difference in the severity of CHF. Indeed, the previous study reported that Japanese patients with congestive heart failure show low mortality [11].

In addition to healthy controls, who are all males aged between 20 and 40 years, we analysed 189 diabetes patients (58% male with an age 60.4 ± 9.8 years) who did not suffer from CHF, as an age-matched control. It was found that there were no differences in the allele and genotype frequencies of α_{2c}Del322–325 among healthy controls, male diabetes patients, and female diabetes patients (data not shown). Moreover, the allele frequency of α_{2c}Del322–325 was lower in the patients with CHF than in those with diabetes (0.04 and 0.10, respectively). Thus it is unlikely that α_{2c}Del322–325 polymorphism affected survival through other causes, resulting in the influence on allele frequency of samples.

Recent studies demonstrated that a genetic variability of β_2AR, Thr164Ile, is closely related to heart failure. In patients with congestive cardiac failure, patients with homozygous genotype Ile/Ile show high mortality compared with those with other genotypes [12]. However, we could not statistically confirm the previous findings, probably because of low frequency of the mutation.

In summary, the α_{2c}Del322–325, β_1Ser49 and β_1Arg389 variants do not appear to be risk factors for CHF due to DCM in a Japanese population, and the α_{2c}Del322–325 variant may be protective. Considering the contradiction with the previous report, it is proposed that there may be a racial difference in the clinical importance of this polymorphism. Further efforts should be made to address any possible racial differences in the responsiveness of heart failure from different causes to β-blockers.

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Regular Article

The Frequency of Candidate Alleles for CYP2D6 Genotyping in the Japanese Population with an Additional Respect to the −1584C to G Substitution

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Full text of this paper is available at http://www.jssx.org

Summary: The −1584C/G single nucleotide polymorphism (SNP) in the promoter region of *CYP2D6* was suggested to have the potential to influence CYP2D6 activity. In this report, we demonstrated the frequencies of −1584C to G substitution-related alleles, such as *CYP2D6*2*, *CYP2D6*21*, *CYP2D6*35* and *CYP2D6*41*, in the Japanese population. The frequencies of *CYP2D6*2*, **41* and **21* were 0.102, 0.026 and 0.005, respectively. We also showed a relationship between the SNP and other common alleles, *CYP2D6*4*, **5*, **10*, **14* and **18*. Interestingly, the SNP was detected in all three subjects carrying *CYP2D6*14*. This finding suggests the −1584G is included in the *CYP2D6*14* allele, which is a null-allele characteristic to the Japanese population. This report presents practical information on *CYP2D6* alleles that should be considered in the pharmacokinetic study of CYP2D6 substrates in the Japanese population.

Key words: CYP2D6; frequency; *CYP2D6*41*; *CYP2D6*35*; *CYP2D6*21*; Japanese

Introduction

CYP2D6 metabolizes many clinically important drugs including antidepressants, neuroleptics, β-blockers and antiarrhythmics.[1] There is a wide interethnic variation in the frequency of the *CYP2D6* genotypes. In a previous study, we reported the frequencies of *CYP2D6* genotypes in a Japanese population.[2] However, some alleles have since been reported which were not included in our study.

In the present study, we focused on the −1584C/G substitution, because the subjects with −1584G were suggested to have higher CYP2D6 enzyme activity than those with −1584C.[3] The locations of the mutations in common *CYP2D6* alleles are displayed in **Fig. 1** according to the CYP nomenclature committee (http://www.imm.ki.se/CYPalleles/cyp2d6.htm), with some modifications. Since the single nucleotide polymorphism (SNP) in −1584 seems to be mainly associated with *CYP2D6*2*, the committee has designated *CYP2D6*2* with −1584G as *CYP2D6*2A* and *CYP2D6*2* with −1584C as *CYP2D6*41*, according to Zanger *et al.*[3] Recent studies have suggested that individuals with *CYP2D6*41* have lower CYP2D6 enzymatic activity *in vivo* than those with *CYP2D6*2A*,[4] possibly as a consequence of lower expression of CYP2D6 protein.[3] The −1584G substitution is also found in the *CYP2D6*35* allele, which has a 31G to A substitution in addition to the SNPs of the *CYP2D6*2* allele, but does not have the gene conversion mutation from *CYP2D7* in intron 1 of *CYP2D6*. Although the allele has been identified in many duplication-negative "Ultra rapid" metabolizers,[5] the activity of recombinant CYP2D6.35 is comparable to that of the wild-type.[6] Furthermore, the −1584G is found in the *CYP2D6*21* allele. However, these effective alleles have been classified as *CYP2D6*2* according to previous detection criteria. Therefore, the consideration of these alleles may result in a better understanding of the phenotype-genotype correlation.

In view of the importance of the −1584C/G substitution-related alleles in the Japanese population, we examined the frequencies of *CYP2D6*41* and **35*, possibly included in *CYP2D6*2*, **21* and other alleles in the present study.

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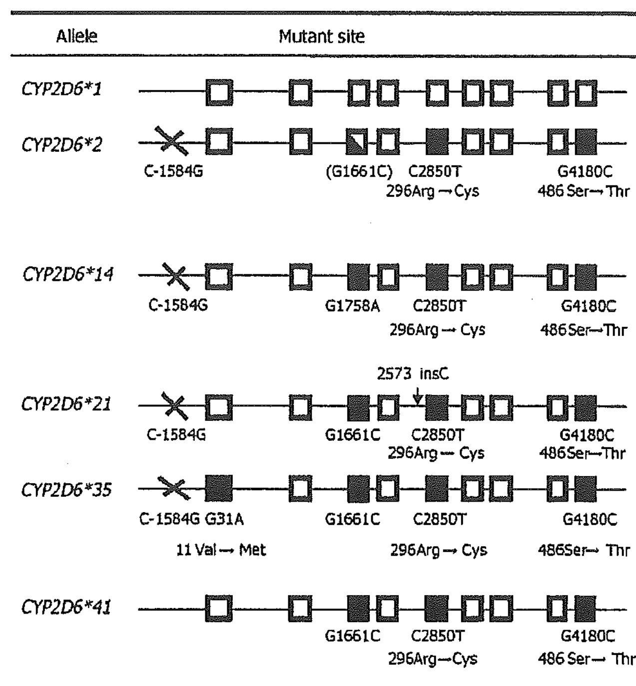

Fig. 1. Schematic representation of the position of mutations on the *CYP2D6* allele
The nature of the mutations and the positions where nucleotide changes occur are indicated. The structures are illustrated with closed boxes (exon containing at least one mutation) and open boxes (exon with wild-type sequence). The position of mutations is in accordance with the numbering used in the CYP2D6 allele nomenclature (http://www.imm.ki.se/CYPalleles/cyp2d6.htm).

Methods

All subjects in the present study were healthy males who had enrolled for several clinical trials. We regenotyped 206 subjects whose data had previously been reported.[2] An additional 79 subjects were newly included, and consequently a total of 285 subjects were examined for the *CYP2D6* genotype. This study was approved by the ethic committees of Osaka University. Written informed consent for genetic analysis was obtained from all subjects. Genomic DNA was isolated from peripheral lymphocytes from each subject.

In our previous study, each *CYP2D6* genotype was defined from the results of PCR-RFLP methods for 100C/T, 1846G/A, 2850C/T and 4180G/C[2] and from the results of *Xba*I and *Eco*RI-RFLP methods or the long-PCR method for the *CYP2D6**5* allele.[7,8]

A PCR-RFLP method for the −1584C to G substitution was developed for it's direct detection. A mismatch PCR-RFLP assay was based on a recognition site for the restriction enzyme *Sma*I by utilizing an oligonucleotide mismatch primer (CYP2D6*41 Mut.R; 5′-TTG TAT TTT TTG TAG AG<u>C</u> CC -3′; the letter with the underline is the mismatch nucleotide). This antisense primer introduces a *Sma*I recognition site by extension when a cytosine is present at the first base. In contrast, in the present of a guanine no recognition site for *Sma*I is introduced. The PCR reaction was carried out in a 25-microl reaction volume containing 1.5 mM $MgCl_2$, 10 mM Tris/HCl (pH 8.3), 0.2 mM of each dNTP, 10 pmol of each primer (CYP2D6*41F; 5′-TTC AAG ACC AGC CTG GAC AAC -3′ and CYP2D6*41Mut.R), 30 ng genomic DNA and 1U AmpliTaqGold™DNA polymerase (Applied Biosystems). An initial denaturation step at 95°C for 10 min was followed by 35 cycles of 95°C for 30 sec, 60°C for 1 min and 72°C for 30 sec, and a final elongation step of 72°C for 5 min. PCR products were then digested with *Sma*I and separated on 4% agarose gel. Product from the −1584C allele was not cut and remained 53 bp in length, while the −1584G allele was cut into 32 and 21 bp.

The 31G/A substitution was detected to find the *CYP2D6**35* allele according to Lovlie *et al.*[5] In brief, a 341-bp fragment covering the SNP was amplified by a set of primers (CYP-511; 5′-AGG TTC ACT CAC AGC AGA GGG-3′ and CYP-518; 5′-CCT GGT CGA AGC AGT ATG GTG-3′), then digested with *Nla*III. The wildtype (31G) was cut into 305 and 36 bp fragments, while the mutant (31A) allele generated 193, 112 and 36 bp fragments. The PCR reaction product was purified and directly sequenced by DNA sequencer ABI 310 using dye-terminater chemistry to confirm the nucleotide substitution and search for other related substitutions. Then, to determine the haplotype, the fragment was cloned using the TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. Additionally, after genotyping, some clones with insertions were randomly selected and directly sequenced.

The presence of the *CYP2D6**21* allele was determined by an allele specific PCR method using genotyping kit "-SNP Typing Kit-Cytochrome P450 2D6*21 (2573C insert)" (TOYOBO, Japan) according to the manufacturer's instructions.

Results

The −1584G alleles were detected in the subjects previously determined as having the *CYP2D6**2* genotype. The frequency of −1584G substitution in the Japanese population was estimated to be 0.114 and *CYP2D6**41* allelic frequency 0.026 (**Table 1**).

Interestingly, there were four unusual subjects among all of the subjects with the −1584G allele. One subject was heterozygous for −1584G and had substitutions classified as *CYP2D6**1/*10*. Two subjects were also heterozygous for −1584G although both had *CYP2D6**1/*14*. The last was homozygous for −1584G in spite of *CYP2D6**2/*14* carrier according to the previous classification.

The insertion of cytosine at 2573 was detected in three

Table 1. The allele frequency of *CYP2D6* in Japanese compared with frequencies in other ethnic populations

(Number of subjects)	Allele frequency (95% confidential interval; lower/upper)				
	−1584C	−1584G			
	*CYP2D6*41*	*CYP2D6*2*	*CYP2D6*2×n*	*CYP2D6*21*	*CYP2D6*35*
Japanese (n=285)	0.026 (0.016/0.043)	0.102 (0.082/0.132)	—#	0.005 (0.002/0.015)	0.000 (0.000/0.005)
Caucasian (n=203)[9]	0.094	0.172	—	—	0.074
Caucasian (n=206)[10]	0.102	0.187	0.010	—	—
African-American (n=193)[9]	0.114	0.047	—	—	0.010

Allelic frequency of *CYP2D6*2*×n and *CYP2D6*1*×n was reported to be 0.01 (n=206)[2].

subjects, which indicates that they had the *CYP2D6*21* allele. Consistent with the allelic information on *CYP2D6*21*, the heterozygous for −1584G was detected from all three. Therefore, one subject was genotyped as *CYP2D6*10/*21* and the other two as *CYP2D6*1/*21*. As a result, the frequency of *CYP2D6*21* allele was estimated to be 0.005 in the present study.

One subject was found to be heterozygous for the 31G to A substitution with *CYP2D6*1/*10* according to the previous classification. In the same subject, we also detected an allele carrying 100T and 31A by subcloning analysis. However, the allelic information on *CYP2D6*35* showed no link between the 31A and the 100T. Therefore, we did not consider the subject to be a *CYP2D6*35* carrier, but provisionally as a *CYP2D6*1/*10* carrier, which may be a new type of allele related to *CYP2D6*10* with 31A.

Discussion

In the past, we have classified *CYP2D6*2* as the wild-type enzymic status according to the phenotype test. However, we have, on occasion, encountered subjects with a lower enzyme activity than that expected of the *CYP2D6*2* genotype. In the Caucasian population, subjects with −1584G substitutions including *CYP2D6*2* have been reported to achieve higher CYP2D6 activity *in vivo* than those with −1584C, possibly as a consequence of greater expression of the CYP2D6 protein.[3,4] In addition, irrespective of the genotype, individuals with −1584C/C expressed less CYP2D6 protein than individuals with at least one −1584G allele.[3] There has been no study concerning the −1584C/G substitution in the Japanese population.

In the present study, we confirmed that the −1584G allele was also detected in the Japanese population although its frequency is lower than in other ethnic groups (**Table 1**). Therefore, our results did not disprove the presence of a possible inconsistency between the phenotype and genotype of CYP2D6 found in several previous studies for Japanese, regarding the effect of this SNP on the activity of CYP2D6.

We also examined the 31G to A substitution for presence of *CYP2D6*35* as a related-allele with the −1584C/G substitution. In this study, the heterozygous for 31A was found in only one subject with *CYP2D6*1/*10*. We subcloned the sample and detected the **10* allele with the 31A mutation as a minor novel allele. This was not regarded as *CYP2D6*35*. Although the 31G/A substitution is not likely to influence CYP2D6 enzyme activity and protein expression level *in vitro*,[6] the *CYP2D6*35* allele is found in many ultra rapid metabolizers.[5,9] The present findings suggest that *CYP2D6*35* allele frequency in the Japanese population is very low, compared with some other ethnic populations, which is consistent with the fact that the ultra rapid metabolizers are rare in Japanese[2] and occur in less than about 10% of Caucasians.

The result from four unusual subjects suggests that the −1584G substitution might be associated with *CYP2D6*14* and possibly *CYP2D6*1* or **10* in addition to *CYP2D6*2*, **21* and **35*. Zanger *et al.* also mentioned that one subject with *CYP2D6*1/*1* possessing −1584G was detected in their study,[3] supporting the present result. On the other hand, Gaedigk *et al.* suggested that CYP2D6 poor metabolizers (PMs) can be detected by the selection of −1584G carriers, as −1584G is exclusively linked to functional allelic variants.[9] Their study seems to be of value for the simple clinical use of the SNP information. However, the connection between the **14* allele and the −1584G substitution was found in the present study in addition to **21*. Since *CYP2D6*14* is a null-allele characteristic to the Japanese population, these connections may limit the genotyping strategy to Japanese.

In conclusion, we examined the distribution of −1584C/G substitutions in the Japanese population mainly associated with *CYP2D6*2*, and found the frequency showed interethnic variation. Since the −1584C/G substitution-related alleles potentially effect CYP2D6 enzyme activity, these alleles should be consi-

dered in *CYP2D6* genotyping. The present study found that the −1584C/G substitution has a potential link to other alleles, and further studies on the related alleles in various ethnic populations are necessary.

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Regular Article

Influence of Itraconazole Co-administration and CYP2D6 Genotype on the Pharmacokinetics of the New Antipsychotic ARIPIPRAZOLE

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Summary: The results of *in vitro* studies indicated that ARIPIPRAZOLE, a newly developed antipsychotic, is mainly metabolized by the human cytochrome P450 isozymes CYP3A4 and CYP2D6. The objective of the present study was to investigate the influence of itraconazole (hereafter referred to as ITZ) co-administration (CYP3A4 inhibition) on the pharmacokinetics of ARIPIPRAZOLE administered to 24 healthy adult male volunteers in a fasting condition. The influence of CYP3A4 inhibition was also examined by CYP2D6 genotype.

All subjects were administered a single oral dose of ARIPIPRAZOLE alone in Period I and a single oral dose of ARIPIPRAZOLE following administration of ITZ at 100 mg/day for 7 consecutive days in Period II. The pharmacokinetic parameters of ARIPIPRAZOLE and its main metabolite OPC-14857 were determined.

Co-administration of ITZ increased the C_{max}, $AUC_{336\,hr}$, and $t_{1/2,z}$ of ARIPIPRAZOLE and OPC-14857 by 19.4%, 48.0%, and 18.6% and by 18.6%, 38.8%, and 53.4%, respectively.

By co-administration of ITZ, the CL/F of ARIPIPRAZOLE in extensive metabolizers was decreased by 26.6%, with an even greater decrease (47.3%) in intermediate metabolizers. For the co-administration period, the CL/F of ARIPIPRAZOLE in intermediate metabolizers was about half of that in extensive metabolizers. For C_{max}, there was no significant difference between extensive metabolizers and intermediate metabolizers, and the percent change by co-administration of ITZ was less than 20% in both extensive metabolizers and intermediate metabolizers.

For OPC-14857, the t_{max} in intermediate metabolizers was longer than that in extensive metabolizers, with the difference being amplified by co-administration of ITZ. The $AUC_{336\,hr}$ showed similar increases by co-administration of ITZ in all genotypes. The urinary 6β-hydroxycortisol/cortisol concentration ratio following ITZ administration for 7 consecutive days was about half of that before the start of ITZ administration, indicating that CYP3A4 metabolic activity was inhibited by administration of ITZ. The influence of CYP3A4 inhibition on the pharmacokinetics of ARIPIPRAZOLE was not considered to be clinically significant. On the other hand, definite differences in pharmacokinetics were observed between CYP2D6 genotypes.

Key words: ARIPIPRAZOLE; pharmacokinetics; CYP3A4 inhibition; CYP2D6 genotypes

Introduction

In recent years the investigation of drug interaction has come to be essential for appropriate evaluation of the safety and efficacy of drugs and for appropriate use of drugs after market launch. Especially in the psychiatric field, where it is quite common to provide multiple-drug combination therapy, obtaining adequate information on drug interaction becomes very important.

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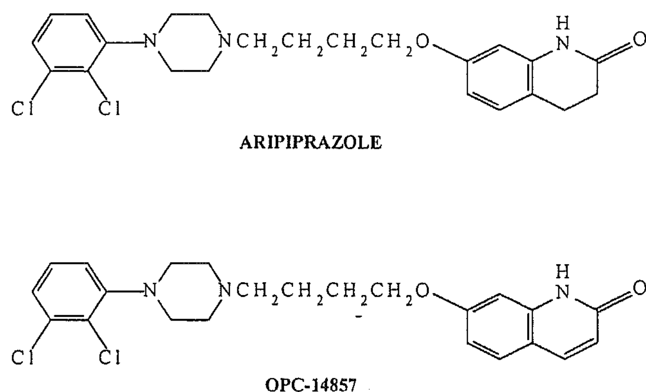

Fig. 1. Chemical structures of ARIPIPRAZOLE and its main metabolite OPC-14857.

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	14	14	11
		(17)	(17)	(17)	(12)
	Period I	1.5±0.3	56.6±20.2	304±72	93.9±24.1
		(1.4±0.4)	(56.5±18.9)	(287±79)	(92.7±23.4)
	Period II	1.8±0.3**	80.6±36.0*	396±78**	135.6±74.4
		(1.8±0.5**)	(87.5±38.8**)	(395±107**)	(138.5±71.6*)
	Ratio (II/I)	1.17±0.15	24.0±33.9[a)]	1.33±0.22	1.42±0.56
		(1.24±0.28)	(31.1±36.8[a)])	(1.41±0.34)	(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

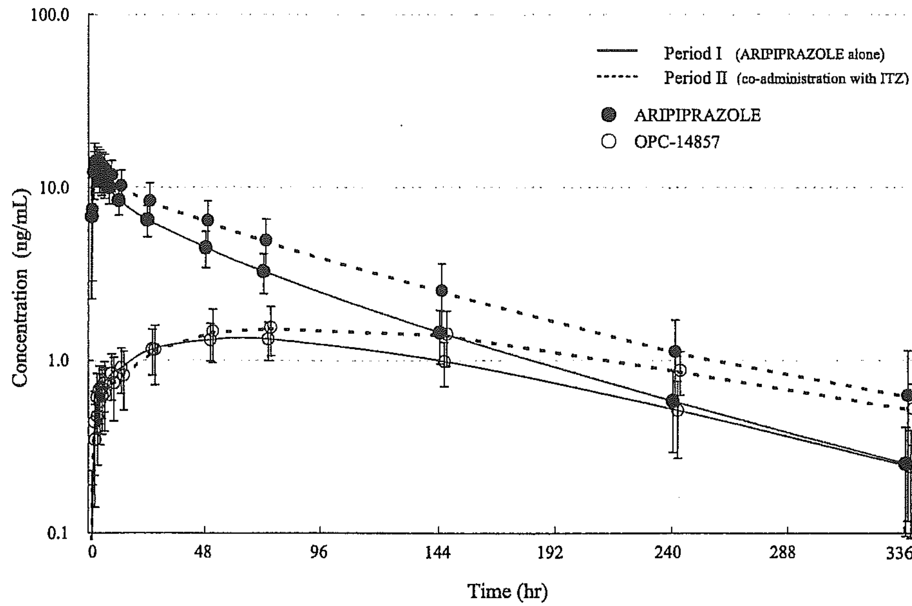

Fig. 2. Timecourses of mean plasma concentrations of ARIPIPRAZOLE and OPC-14857. (mean ± SD, n = 24).

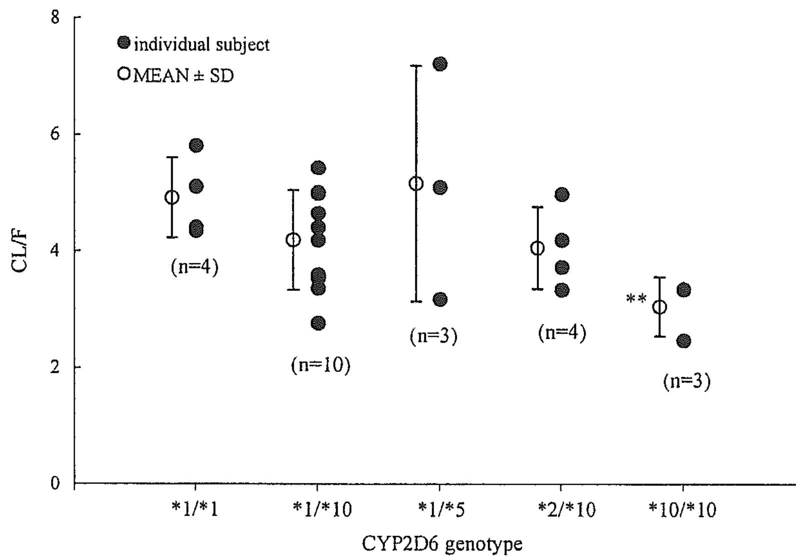

Fig. 3. ARIPIPRAZOLE plasma clearance in individual subjects by CYP2D6 genotype in Period I. **$p < 0.01$ (Fisher's least significant difference test vs. *1/*1 in Period I)

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

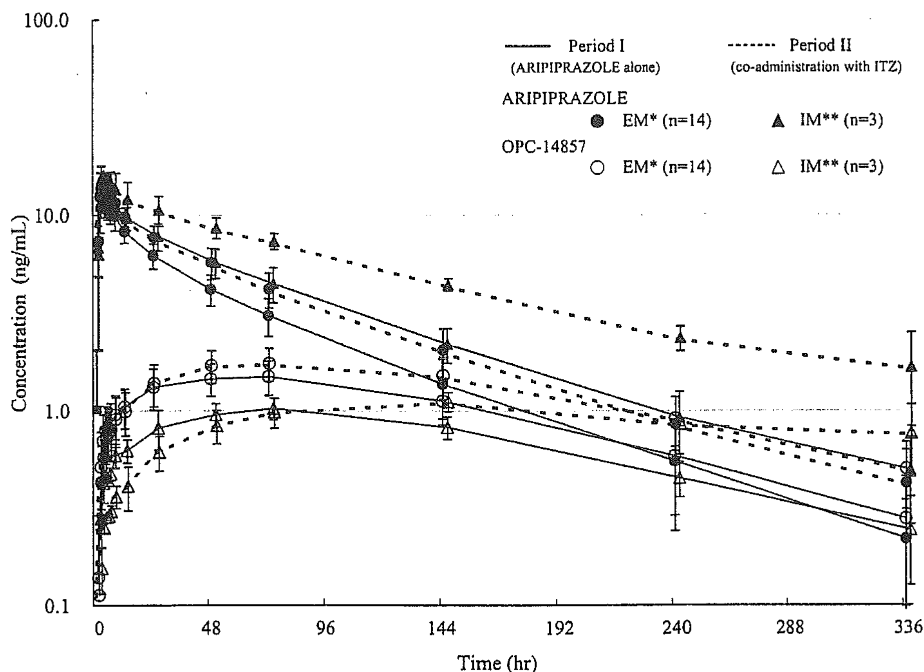

Fig. 4. Timecourses of mean plasma concentrations of ARIPIPRAZOLE and OPC-14857 by CYP2D6 genotype (mean ± SD)
EM*: CYP2D6 extensive metabolizers IM**: CY2D6 intermediate metabolizers

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

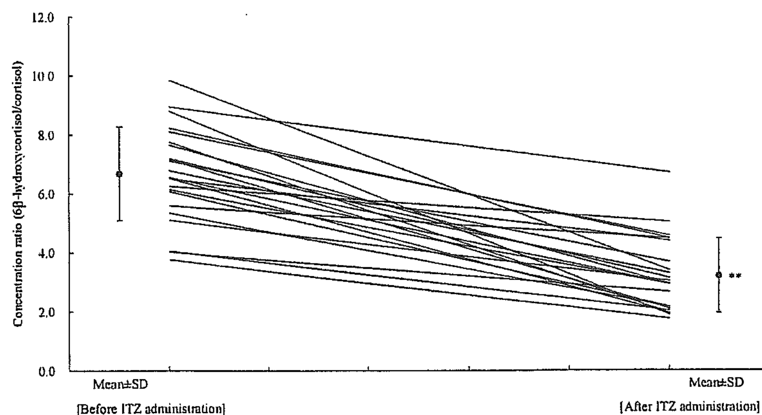

Fig. 5. Changes in urinary 6β-hydroxycortisol/cortisol ratios between before and after ITZ administration by individual subject (N=23). **$p<0.01$ (paired t-test vs. before ITZ administration)

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.

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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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