Inducibility of CYP1A2 by omeprazole *in vivo* related to the genetic polymorphism of CYP1A2

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Aims To evaluate the effect of the *CYP1A2**1C and *CYP1A2**1F polymorphisms on the inducibility of CYP1A2 by omeprazole in healthy subjects.

Methods Mutations of *CYP2C19* and *CYP1A2* were identified by PCR-RFLP. Omeprazole, 120 mg day⁻¹, was given to 12 extensive metabolizers (EM) with respect to *CYP2C19* (six *CYP1A2**1F/*CYP1A2**1F and six *CYP1A2**1C/*CYP1A2**1F of *CYP1A2*) for 7 days. CYP1A2 activity was determined on three occasions, namely on day 1, day 9 and day 16 using the caffeine plasma index (the ratio of the concentrations of paraxanthine to caffeine), 6 h after oral administration of 200 mg caffeine.

Results There was a significant difference ($P = 0.002$) between the caffeine ratios for *CYP1A2**1F/*CYP1A2**1F and *CYP1A2**1C/*CYP1A2**1F genotypes on day 9, but not on day 1 or day 16 ($P > 0.05$). The changes in the ratios from day 9 to day 1 ($48\% \pm 20\%$ vs $19\% \pm 20\%$) and from day 9 to day 16 ($50\% \pm 31\%$ vs $15\% \pm 22\%$) were significantly different ($P < 0.05$) between the *CYP1A2**1F/*CYP1A2**1F and *CYP1A2**1C/*CYP1A2**1F genotypes.

Conclusion The *CYP1A2**1C and *CYP1A2**1F genetic polymorphisms influenced the induction of CYP1A2 activity *in vivo* by omeprazole.

Keywords: caffeine, CYP1A2, genetic polymorphism, induction

Introduction

CYP1A2, a member of cytochrome P450 (CYP) superfamily, plays a key role in the metabolism of a number of drugs and also participates in the activation of some procarcinogens [1]. It has also been suggested that high CYP1A2 activity is a susceptibility factor for hepatocellular carcinoma and colon cancer [2]. Thus, characterization of factors that induce *CYP1A2* gene expression may be of importance to therapeutics and to the aetiology of some cancers.

Studies show up to 70-fold variation in CYP1A2 activity [3]. At least four single nucleotide polymorphisms (SNPs) of the *CYP1A2* gene have been identified, among which *CYP1A2**1C and *CYP1A2**1F are suggested to be associated with the induction of CYP1A2 activity in smokers [4–6]. Our previous study showed that the *CYP1A2**1F/*CYP1A2**1F and *CYP1A2**1C/

*CYP1A2**1F genotypes were associated with the higher and lower inducibility of CYP1A2, respectively, in smokers and non-smokers from the Qidong area [8].

Two important alleles of *CYP2C19*, designated as *CYP2C19**2 and *CYP2C19**3, account for most poor metabolizers (PMs) with respect to CYP2C19 in Chinese populations. *CYP2C19**2 accounts for approximately 80% of defective alleles, and *CYP2C19**3 for the remainder [9]. *CYP2C19**1/*CYP2C19**1, *CYP2C19**1/*CYP2C19**2 or *CYP2C19**1/*CYP2C19**3 genotypes are normally extensive metabolizers (EMs), while *CYP2C19**2/*CYP2C19**2, *CYP2C19**2/*CYP2C19**3 or *CYP2C19**3/*CYP2C19**3 are PMs.

As a potent inhibitor of H⁺/K⁺-stimulated adenosine triphosphatase (a proton pump) in parietal cells of the gastric mucosa, omeprazole is widely used in the treatment of peptic ulceration and reflux esophagitis. Omeprazole is known to induce selectively human liver CYP1A2 activity *in vitro* and *in vivo*. Because the *in vivo* induction of CYP1A2 by omeprazole depends on the plasma concentration of the drug, and CYP2C19 participates in the oxidation of omeprazole [10], CYP1A2 activity is affected differentially by omeprazole in EMs compared with PMs with respect to CYP2C19 [11, 12].

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The objective of this study was to evaluate the association between the *CYP1A2*1C* and *CYP1A2*1F* genetic polymorphisms and *CYP1A2* inducibility in healthy subjects by using omeprazole as a substrate for the latter.

Methods

Subjects

This study was conducted according to the Declaration of Helsinki and with the approval of Ethics Committee of the Xiang-Ya School of Medicine, Central South University, Changsha, China. Seventy-nine Chinese healthy male volunteers aged 18–20 years were selected randomly in this open study after providing informed consent. All subjects were non-smokers and healthy as determined by medical history, physical examination and laboratory screening. They were required to refrain from taking any medication or alcohol for at least 2 weeks prior to the start of the study and during the course of the investigation.

Chemicals

Omeprazole was purchased from Dinuo Pharmaceuticals Inc. (Changsha, China). Caffeine (1,3,7X), paraxanthine (1,7X) and β -hydroxyethyl-theophylline (HT) (used as an internal standard) were all products of the Sigma Chemical Co. (St Louis, MO USA). The caffeine administered (200 mg per capsule) was kindly provided by the Hunan Pharmaceutical Company (Changsha, China). All of the restriction endonucleases, primers and reagents used in the genotyping analysis were supplied by Sangon Inc. (Shanghai, China).

Genotyping analysis

All volunteers took part in this test. Genomic DNA was extracted from white cells from peripheral venous blood. PCR-RFLP was used to identify the genotypes of *CYP2C19* [9] and *CYP1A2* [7] as described previously. Twelve subjects with particular genotypes were enrolled in the study; they were six *CYP2C19*1* homozygotes with the *CYP1A2*1F/CYP1A2*1F* genotype and six *CYP2C19*1* homozygotes with the *CYP1A2*1C/CYP1A2*1F* genotype.

Study protocol

Omeprazole, 120 mg day⁻¹ (three 20 mg capsules twice daily, at 08.00 h and 20.00 h), was given to the subjects from day 2 to day 8 [11, 12]. On days 1, 9 and 16 blood was collected at 6 h after oral administration of caffeine

for the analysis of drug (1,3,7X) and its metabolite paraxanthine (1,7X).

Analysis of 1,7X and 1,3,7X

Plasma samples were extracted with chloroform and isopropanol (9 : 1), following the addition of internal standard HT, and the concentrations of 1,7X and 1,3,7X were determined using a Hewlett-Packard 1050 series h.p.l.c. with a reversed-phase column (Spherisorb ODS-2, 250 mm $\times$ 4 mm i.d., 5 μ m particle size); a mobile phase of methanol : acetonitrile : 0.05% acetic acid (12 : 8 : 80) at a flow rate of 1 ml min⁻¹, a column temperature of 40 °C and a detection wavelength of 282 nm [3]. The retention times of 1,7X, HT and 1,3,7X were 5.5, 6.8 and 9.8 min, respectively. No endogenous interfering peaks were found in the blank samples. The 1,7X and 1,3,7X standard curves were linear over the range of 1–100 μ M, with correlation coefficients of 0.999 and 0.997, respectively. Recovery of 1,7X and 1,3,7X ranged from 94% to 104%. The coefficients of variation for the intraday and interday reproducibility for 1,7X and 1,3,7X were less than 10%. The method had a limit of detection of 0.1 μ M both for 1,7X and 1,3,7X.

Data analysis

The ratio of 1,7X/1,3,7X was used as an index of *CYP1A2* activity *in vivo*. The degree of induction of *CYP1A2* by omeprazole was expressed as the change in the ratios of 1,7X/1,3,7X from day 1 to day 9 (day 9/day 1) or from day 9 to day 16 (day 9/day 16). The Mann–Whitney *U*-test and Wilcoxon non-parametric test, were used with the level of significance set at $P < 0.05$. Values were expressed as mean $\pm$ s.d.

Results

Frequencies of *CYP1A2* and *CYP2C19* alleles

The frequencies of the *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* alleles in the 79 subjects were 0.60, 0.37 and 0.03, respectively. The frequencies of the *CYP1A2*1C* and *CYP1A2*1F* alleles were 0.22 and 0.66, respectively. Six genotypes were identified with respect to the *CYP1A2*1C* and *CYP1A2*1F* genetic polymorphisms. No *CYP2C19*3* homozygotes were detected.

Induction of *CYP1A2* activity by omeprazole

The ratio of 1,7X/1,3,7X of six subjects with the *CYP1A2*1F/CYP1A2*1F* genotype increased significantly ($P = 0.004$) after 7 days omeprazole treatment (day

Table 1 Influence of 7 days of omeprazole treatment on the plasma caffeine metabolic ratio (1,7X/1,3,7X).

Genotype	n	Dose of omeprazole	Day 1	1,7X/1,3,7X Day 9	Day 16	% change			95% CI on the difference	
						Day 9/ Day 1	Day 9/ Day 16	Day 16/ Day 1	Day 9/ Day 1	Day 9/ Day 16
<i>CYP1A2*1F/CYP1A2*1F</i>	6	120 mg day ⁻¹	0.46 ± 0.10 ^a	0.68 ± 0.16	0.46 ± 0.05 ^a	48 ± 20 ^b	50 ± 31 ^b	2 ± 19	22, 71	-1, 97
<i>CYP1A2*1C/CYP1A2*1F</i>	6	120 mg day ⁻¹	0.38 ± 0.10	0.44 ± 0.06 ^c	0.39 ± 0.06	19 ± 20 ^d	15 ± 22 ^d	8 ± 34	-16, 37	-51, 64

Data are mean ± s.d. ^a $P < 0.05$, compared with day 9; ^b $P < 0.05$, compared with the % change between day 16/day 1; ^c $P < 0.05$, ^d $P < 0.01$ compared with that for *CYP1A2*1F/CYP1A2*1F* genotypes.

Figure 1 Influence of 7 days of omeprazole treatment on the plasma caffeine metabolic ratio (1,7X/1,3,7X). ○: *CYP1A2*1F/CYP1A2*1F* genotypes, $n = 6$; ×: *CYP1A2*1C/CYP1A2*1F* genotypes, $n = 6$.

9), and returned to the baseline value at the end of the 1 week washout period (day 16) (Figure 1). However, the ratio did not change significantly in the six *CYP1A2*1C/CYP1A2*1F* genotypes.

There was a significant difference ($P = 0.002$) between the ratios for the *CYP1A2*1F/CYP1A2*1F* and *CYP1A2*1C/CYP1A2*1F* genotypes on day 9, but not on day 1 or day 16 ($P > 0.05$) (Table 1).

The differences in the ratios between day 9 and day 1 were $48\% \pm 20\%$ and $19\% \pm 20\%$ for the *CYP1A2*1F/CYP1A2*1F* and *CYP1A2*1C/CYP1A2*1F* genotypes, respectively. This difference between genotypes was statistically significant ($P = 0.025$), as was that for the

changes between day 9 and day 16 ($50\% \pm 31\%$ vs $15\% \pm 22\%$, $P = 0.048$). There was no significant difference ($P > 0.05$) between the *CYP1A2* genotypes with respect to the changes between day 16 and day 1 ($2\% \pm 19\%$ vs $8\% \pm 34\%$).

Discussion

The frequencies of the *CYP1A2* and *CYP2C19* alleles in our study were similar to those in previous reports [4–6, 9]. Since *CYP1A2* activity increases in EMs with respect to *CYP2C19* with high dose (120 mg day^{-1}) omeprazole, but not with 40 mg day^{-1} [11, 12], *CYP2C19*1* homozygotes, but not *CYP2C19*1/CYP2C19*2* or *CYP2C19*1/CYP2C19*3* heterozygotes, were selected for further investigation to minimize the influence of *CYP2C19* gene dose on the plasma concentration of omeprazole. The results supported a role for the *CYP1A2*1C* and *CYP1A2*1F* genetic polymorphisms in the inducibility of *CYP1A2* in EMs with respect to *CYP2C19*, who were administered omeprazole.

Transcriptionally mediated enzyme induction of omeprazole is believed to occur because of increased expression of hepatic *CYP1A2* mRNA [13]. Our study also supported this hypothesis since induction by omeprazole differed between the *CYP1A2* genotypes. Whether the mechanism involved in the induction of *CYP1A2* activity by omeprazole is the same as that occurring in cigarette smokers remains to be elucidated. Since variation was observed in the ratios for the *CYP1A2*1F/CYP1A2*1F* and *CYP1A2*1C/CYP1A2*1F* genotypes (see Table 1), the induction by omeprazole might be related to factors other than the *CYP1A2*1C* and *CYP1A2*1F* genetic polymorphisms.

When a drug can induce a specific *CYP* gene, the metabolism of other coadministered compounds may be altered, resulting in a change in response. In this study, we studied induction caused by omeprazole in 12 EMs with respect to *CYP2C19* with different genotypes for *CYP1A2*. The induction by omeprazole, 120 mg day^{-1} ,

on *CYP1A2* expression in EMs with respect to *CYP2C19* is close to that observed with 40 mg day⁻¹ in PMs with respect to *CYP2C19*, each phenotype showing similar plasma concentration of omeprazole *in vivo* [11, 12]. Using the plasma caffeine ratio as an index of CYP1A2 activity, the EMs exhibited an increase of between -11% and 60% which was similar to the results (4.2%~48.7%) reported by Rost *et al.* who used the urinary caffeine index [12]. Thus, when omeprazole and substrates of CYP1A2 are coadministered, we would not expect clinically relevant drug interactions in EMs with respect to *CYP2C19*, as long as usual therapeutic doses are used [14]. However, induction of CYP1A2 may be of importance in CYP2C19 PMs.

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