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Variability in Drug Interaction According to Genetic Polymorphisms in Drug Metabolizing Enzymes

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ABSTRACT: **There are significant differences in the extent of drug interactions between subjects. The influence of the genetic make up of drug metabolizing enzyme activities (CYP3A5, CYP2C19 and UDP-glucuronosyl trans-ferase) on the pharmacokinetic drug interaction potential were studied *in vivo*. Nineteen healthy volunteers were grouped with regard to the CYP3A5*3 allele, into homozygous wild-type (CYP3A5*1/*1, n = 6), heterozygous (CYP3A5*1/*3, n = 6), and homozygous variant-type (CYP3A5*3/*3, n= 7) subject groups. The pharmacokinetic profile of intravenous midazolam was characterized before and after itraconazole administration (200 mg once daily for 4 days), and also following rifampin pretreatment (600 mg once daily for 10 days), with a washout period of 2 weeks in between. For omeprazole and moclobemide pharmacokinetic interaction study 16 healthy volunteers were recruited. The volunteer group comprised 8 extensive metabolizers and 8 poor metabolizers of CYP2C19, which was confirmed by genotyping. Subjects were randomly allocated into two sequence groups, and a single-blind, placebo-controlled, two-period crossover study was performed. In study I, a placebo was orally administered for 7 days. On the eighth morning, 300 mg of moclobemide and 40 mg of placebo were coadministered with 200 mL of water, and a pharmacokinetic study was performed. During study II, 40 mg of omeprazole was given each morning instead of placebo, and pharmacokinetic studies were performed on the first and eighth day with 300 mg of moclobemide coadministration. In the UGT study pharmacokinetics and dynamics of 2 mg intravenous lorazepam were evaluated before and after rifampin pretreatment (600 mg once daily for 10 days), with a washout period of 2 weeks in between. The subjective and objective pharmacodynamic tests were done before and 1, 2, 4, 6, 8, and 12 hours after lorazepam administration. The pharmacokinetic profiles of midazolam and of its hydroxy metabolites did not show differences between the genotype groups under basal and induced metabolic conditions. However, during the inhibited metabolic state, the CYP3A5*3/*3 group showed a greater decrease in systemic clearance than the CYP3A5*1/*1 group (8.5 ± 3.8 L/h/70 kg vs. 13.5 ± 2.7 L/h/70 kg, P = 0.027). The 1'-hydroxymidazolam to midazolam AUC ratio was also significantly lower in the CYP3A5*3/*3 group (0.58 ± 0.35, vs. 1.09 ± 0.37 for the homozygous wild-type group, P = 0.026). The inhibition of moclo-bemide metabolism was significant in extensive meta-bolizers even after a single dose of omeprazole. After daily administration of omeprazole for 1 week, the pharmacokinetic parameters of moclobemide and its metabolites in extensive metabolizers changed to values similar to those in poor metabolizers. In poor meta-bolizers, no remarkable changes in the pharmacokinetic parameters were observed. The area under the time-effect curves of visual analog scale(VAS), choice reaction time, and continuous line tracking test results of lorazepam was reduced by 20%, 7%, 23% respectively in induced state, and in spite of large interindividual variablilty, significant statistical difference was shown in VAS (repeated measures ANOVA, p=0.0027).**

Key words : **Drug-interaction, Genotype, Pharmacokinetics, Pharmacodynamics**

Introduction

Enzymes in the CYP3A subfamily are the most abundant CYPs in the human liver and intestine, and are involved in the metabolism of numerous drugs and a number of endogenous compounds. There are four differentially regulated CYP3A genes in humans, CYP3A4, CYP3A5, CYP3A7, and CYP3A43. Among the isoforms, CYP3A4 is generally thought to be the predominant form expressed in liver cells. However, CYP3A5, which is expressed polymorphically in the liver, may contribute as much as 50% to hepatic CYP3A activity in more than a third of a population.

Polymorphic CYP3A5 expression in the adult liver

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and small intestine is strongly correlated with a single nucleotide polymorphism, A>G, within intron 3 of the CYP3A5 gene, which is designated CYP3A5*3. This mutation creates a cryptic consensus splice site and results in the production of improperly spliced mRNA and a small quantity of properly spliced mRNA. In contrast to the correctly spliced wild type-CYP3A5 mRNA, this aberrant mRNA contains an exon derived from the intron 3 sequence of CYP3A5 and encodes a protein that is truncated at amino acid 102, which results in an activity loss.

On the basis of in vitro findings, it was suggested that the CYP3A5*3 allele may contribute to the observed interindividual variability of CYP3A activity in vivo. This is because, in general, CYP3A4 and CYP3A5 have similar catalytic specificities and, although the activity of CYP3A5 is often less than that of CYP3A4, the amount and relative contribution of this enzyme may be greater than previously thought. This may be more prominent during the induced or inhibited status of the enzyme. Since midazolam metabolism is greatly affected by the inhibitors and inducers of CYP3A, the effect of CYP3A5 polymorphism on the pharmacokinetics of midazolam during inhibited and induced metabolic states needs to be explored.

In this study, we investigated the effect of the CYP3A5 genotype on the pharmacokinetics of midazolam given intravenously to healthy subjects, in basal, inhibited, and induced CYP3A metabolic states.

Moclobemide, a relatively newer antidepressant with monoamine oxidase inhibition activity, has been recognized for its monoamine oxidase-A selectivity, which results in little interaction with foods that contain tyramine. Moclobemide produces various metabolites, some of which are supposedly related to CYP2C19, and it is also reported to be an inhibitor of CYP2C19, CYP2D6, and CYP1A2. The isozymes of the CYP2C gene subfamily account for about 20% of the CYP enzymes in the human live. CYP2C19, which is involved in the elimination of amitriptyline, clomipramine, diazepam, imipramine, lansoprazole, omeprazole, phenytoin, and others, has been one of the most frequently studied CYP isozymes because of its polymorphic activity and because of the difference in poor metabolizer (PM) frequency among ethnic groups. Probe drugs such as S-mephenytoin and omeprazole have shown bimodal distribution of their metabolic ratios in populations comprising extensive metabolizer (EM) and PM individuals. It is known that the distribution pattern varies by race; among Asians, the frequency is up to 20% PMs and 80% EMs, whereas among white persons, less than 5% are PMs and 95% are EMs.

The causes of poor metabolic capacity in certain individuals have been discovered through genetic research. As for CYP2C19, two point mutations that cause diminished metabolic activity are well known. The mutant allele with a base change of G681→A on exon 5 is named CYP2C19m1, and G636→A on exon 4 is named CYP2C19m2. Most PM phenotypes, especially in Asians, are explained by these two point mutations, and genotyping for m1 and m2 can be used to discriminate the phenotypes of CYP2C19. Omeprazole, the widely used proton pump inhibitor for peptic ulcer and related hypersecretory conditions, has long been used as a probe for the estimation of CYP2C19 activity. Omeprazole has also been known as a potent inhibitor of CYP2C19, and pharmacokinetic interactions with other CYP2C19 substrate drugs such as diazepam and phenytoin have been documented. Moclobemide is presumably metabolized by CYP2C19; we assumed that its elimination would be influenced by CYP2C19 inhibitors. The decrease in moclobemide clearance caused by cimetidine, a nonspecific CYP inhibitor, has been previously reported, but it needs to be confirmed with other specific inhibitors of CYP2C19, such as omeprazole. Therefore we performed a pharmacokinetic interaction study of moclobemide and omeprazole, with regard to CYP2C19 genetic polymorphism confirmed by individual genotypes.

Materials and Methods

Nineteen healthy volunteers were grouped with regard to the CYP3A5*3 allele, into homozygous wild-type (CYP3A5*1/*1, n=6), heterozygous (CYP3A5*1/*3, n =6), and homozygous variant-type (CYP3A5*3/*3, n= 7) subject groups. The pharmacokinetic profile of intravenous midazolam was characterized before and after itraconazole administration (200 mg once daily for 4 days), and also following rifampin pretreatment (600 mg once daily for 10 days), with a washout period of 2 weeks in between.

The effect of omeprazole on the pharmacokinetics of moclobemide was studied in 16 healthy volunteers. The volunteer group comprised 8 extensive metabolizers and

8 poor metabolizers of CYP2C19, which was confirmed by genotyping. Subjects were randomly allocated into two sequence groups, and a single-blind, placebo-controlled, two-period crossover study was performed. In study I, a placebo was orally administered for 7 days. On the eighth morning, 300 mg of moclobemide and 40 mg of placebo were coadministered with 200 mL of water, and a pharmacokinetic study was performed. During study II, 40 mg of omeprazole was given each morning instead of placebo, and pharmacokinetic studies were performed on the first and eighth day with 300 mg of moclobemide coadministration.

In the UGT study pharmacokinetics and dynamics of 2 mg intravenous lorazepam were evaluated before and after rifampin pretreatment (600 mg once daily for 10 days), with a washout period of 2 weeks in between. The subjective and objective pharmacodynamic tests were done before and 1, 2, 4, 6, 8, and 12 hours after lorazepam administration.

Results and Discussion

The concentration-time profiles of midazolam and 1'-hydroxymidazolam during the basal state did not show remarkable differences between the genotypes, and neither did the 1'-hydroxymidazolam to midazolam and 1'-hydroxymidazolam to 4-hydroxymidazolam AUC ratios.

ibited differences between groups, with the CYP3A5*3/*3 group and the CYP3A5*1/*3 group showing significantly lower values than the CYP3A5*1/*1 group ($P=0.044$ and 0.031, respectively). When this ratio was expressed in terms of percentages versus the basal values, only the homozygous variant-type group was found to have a significantly lower 1'-hydroxymidazolam to 4-hydroxymidazolam AUC ratio than the homozygous wild-type group, due to a high level of variability in the values ($P=0.038$).

Following pretreatment with rifampin, the systemic clearance of midazolam increased 2 to 2.5 fold, and this was accompanied by the increased formation of the 1'-hydroxy metabolite. However, no differences were noted between the genotype groups for either compound. The 1'-hydroxymidazolam to midazolam and 1'-hydroxymidazolam to 4-hydroxymidazolam AUC ratios showed no significant differences between the genotype groups.

The 4-hydroxymidazolam concentration-time profiles were low (<1 ng/mL) in all phases of the study, and were not remarkably different for the genotypes.

Plasma concentrations of moclobemide in PMs were higher than those in EMs, both after placebo administration (study I) and after the first dose of omeprazole (study II-1). The difference between genotypes almost disappeared on the eighth day of omeprazole administration (study II-2). As seen from the change in AUC and in elimination half-life, the inhibition of moclobemide metabolism was significant in EMs even after a single dose of omeprazole. After the administration of omeprazole for 1 week, the mean AUC of moclobemide in EMs more than doubled. There were decreased concentrations of Ro 12-8095 (the metabolite produced by CYP2C19) in EMs, by omeprazole administration for 1 week, which corresponded with the pharmacokinetic changes of the parent drug. The increase of Ro 12-5637 in EMs may be explained as a result caused by the increase in parent drug levels, as well as by possible metabolic shunting to the Ro 12-5637 pathway. After daily administrationof omeprazole for 1 week, the pharmacokinetic parameters of moclobemide and its two metabolites in EMs changed to values similar to those in PMs. To normalize the influence of the parent drug's AUC on our evaluation of metabolite pharmacokinetics, we compared the AUC ratios of metabolites to moclobemide. AUC ratios showed significant reduction of Ro 12-8095 formation in EMs; in contrast, no significant changes were observable in PMs. In PMs, the AUC and elimination half-life of moclobemide decreased at day 8 of omeprazole administration. However, the AUC ratios of the metabolites did not change significantly.

Lorazepam is a short acting benzodiazepine metabolized mainly by the UDP-glucuronosyl transferase (UGT) enzymes, mediating drug glucuronidation. Rifampin is known as a pleiotropic inducer of drug metabolizing enzyme genes, it induces various Phase I and Phase II enzymes such as CYP, UGT, etc. The pharmacodynamic profile of intravenous lorazepam 2 mg was characterized before and after rifampin pretreatment (600 mg once daily for 10 days), with a washout period of 2 weeks in between. The subjective and objective pharmacodynamic tests were done before and 1, 2, 4, 6, 8, and 12 hours after lorazepam administration. The Pharmacodynamic parameters were reduced in induced metabolic conditions by rifampin.

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