[10:30 - 11:00]

Influence of *MDR1* Genetic Polymorphisms on Levosulpiride Disposition

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Introduction

P-glycoprotein (P-gp), the product of the multidrug resistance-1 (*MDR1*) gene, is an important factor in drug disposition, and the involved processes often show considerable inter-individual variability that may be genetically determined. Some of the single nucleotide polymorphisms (SNP) of *MDR1* reported correlated with the *in vivo* activity of P-gp [Hoffmeyer et al., 2000]. Levosulpiride, the L-isomer of sulpiride, whose pharmacokinetic characteristics displays large inter-individual variations, is mainly used in the treatment of psychoses and gastro-intestinal disorders [Jenner and Marsden, 1981; Trabucchi et al., 1975], and recently several studies suggested that levosulpiride might be a substrate of P-gp *in vitro* [Watanabe et al., 2002]. Therefore, the large interindividual variability of levosulpiride was thought to correlate with *MDR1* expression and uptake of orally administered levosulpiride.

Purposes

The purposes of this study were to clarify the involvement of P-gp in the efflux of levosulpiride in knockout mice that lack the *mdr1a1b* gene and to evaluate the relationship between the genetic polymorphisms in *MDR1* gene (exon 12, 21 and 26) and levosulpiride disposition in healthy Korean subjects.

Methods

Genotyping and haplotype analysis

430 volunteers were genotyped for *MDR1*, C1236T (exon 12), G2677T (exon 21) and C3435T (exon 26) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis [Cascorbi et al., 2001]. Allele and genotype frequencies for the SNPs were assessed for deviation from Hardy-Weinberg equilibrium using chi-square analysis. The allele frequencies of exon 12 1236T, exon 21 2677T and exon 26 3435T were 55.86, 35.12 and 36.51%, respectively.

Haplotype frequencies were estimated based on the Expectation-Maximization (EM) algorithm [Tang et al., 2002]. Haplotype frequencies on *MDR1* genes were estimated using the population genetics data analysis program HapAnalyzer [http://www.ngri.re.kr/HapAnalyzer/ right.html].

Animals

Male *mdr1a/1b(-/-)* transgenic mice (FVB/NTacFBR-[KO]*mdr*1a-[KO]*mdr*1b N7) and genetically matched male wild-type *mdr1a/1b(+/+)* mice (FVB/NTacFBR) aged 8 weeks weighing 20 ~ 26 g were purchased from Taconic Farms Inc. (Germantown, NY, USA). The mice were housed individually and maintained on a 12 hr/12 hr light/dark cycle at 23±1°C and 50% relative humidity, and acclimated for 2 weeks with food and tap water *ad libitum*. After oral administration (10 μg/g) of

levosulpiride to *mdr1a/1b(-/-)* knockout and *mdr1a/1b(+/+)* wild-type mice, plasma and brain samples were obtained up to 180 minutes.

Subjects

Based on the genotype analysis for 430 subjects, 58 subjects (20 ~ 32 years, 50 ~ 84 kg) with different genotypes who gave us informed consent recruited and received a single oral dose of 25-mg levosulpiride tablet. Blood and urine samples were taken up to 36 hours after dosing.

Pharmacokinetic study

The concentrations of levosulpiride in human serum and urine as well as mouse plasma and brain homogenates were determined by HPLC method with florescence detector [Cho and Lee 2003]. We compared the distribution of levosulpiride between brain and blood in the *mdr1a/1b(-/-)* knockout and *mdr1a/1b(+/+)* wild-type mice, and their pharmacokinetic parameters of levosulpiride were calculated using WinNonlin program.

Data from three different genotype groups were compared by use of the Kruskal-Wallis test. Differences between the pharmacokinetic parameters of the two genotypic groups were determined by use of the Mann-Whitney *U* test. A $P<0.05$ was considered statistically significant.

Results

Pharmacokinetics and brain homogenate/plasma concentration ratios

The concentrations of levosulpiride in plasma and brain homogenates were significantly increased in *mdr1a/1b(-/-)* compared with those in wild-type mice. Also, the values of the $AUC_{0-\infty}$ and C_{max} of levosulpiride in plasma and brain for knockout

mice were markedly higher (5.0- and 10.0-fold for $AUC_{0-\infty}$; 5.6- and 8.7-fold for C_{max}, respectively, $P<0.001$) than those for wild-type mice, respectively.

In addition, the average brain-to-plasma concentration ratio (Kp) and blood-brain barrier (BBB) partitioning of levosulpiride were 2.3- and 2.4-fold ($P<0.000$) higher in the *mdr1a/1b(-/-)* mice compared with the *mdr1a/1b(+/+)* wild-type mice, respectively.

Genotyping and haplotype analysis

The allelic frequencies of exon 12 (C1236T), 21 (G2677T), and 26 (C3435T) in 430 healthy Korean subjects were 56.86, 35.12, and 36.51%, respectively. In exon 21 G2677T, the frequencies of the GG, GT, and TT genotypes were 40.47, 48.84, and 10.70%, respectively. In exon 26, the frequencies of the CC, CT, and TT genotypes were 38.37, 50.23, and 11.40%, respectively.

Haplotype analysis was restricted to the coding SNP of exon 12, 21, and 26. Of eight possible haplotypes, seven were present in the Korean subjects but only three occurred with high frequency, suggesting tight linkage among the loci of exon 12, 21, and 26. Three major haplotypes, C-G-C, T-G-C and T-T-T, constituted 37.21, 21.51, and 30.35% of all haplotypes, respectively, adding up to a total of 89.07%, and one haplotype, C-T-T, was absent in our population (Fig. 1).

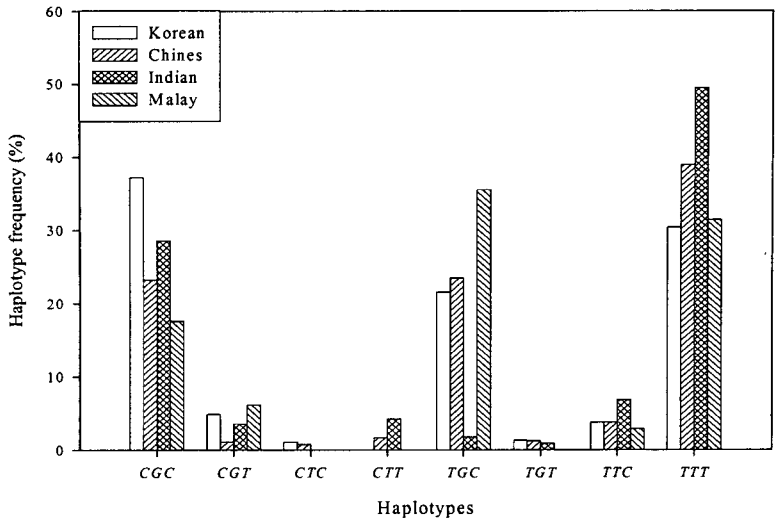

Fig. 1. *MDR1* haplotypes based on high frequency SNP loci present in Korean (our data) and other Asian population [Chowbay et al., 2002]

Effect of genetic polymorphisms on levosulpiride disposition in human

Mean serum concentration time curves of homozygous 2677TT and 3435TT subjects were higher than those of subjects with at least one wild-type allele in exon 21 and 26. There were no statistically significant differences in the PK parameters of levosulpiride obtained from the subjects with C1236T in exon 12. On the other hand, the PK parameters such as partial AUC ($AUC_{0\text{-}4h}$), $AUC_{0\text{-}\infty}$ and C_{max} of homozygous 3435TT subjects were found to be significantly higher than those of subjects with at least one wild-type allele (CC and CT subjects, P=0.021 for $AUC_{0\text{-}\infty}$; P=0.014 for $AUC_{0\text{-}4h}$; P=0.043 for C_{max}). We also found that the values of $AUC_{0\text{-}\infty}$, $AUC_{0\text{-}4h}$ and C_{max} were significantly different between homozygous 2677TT subjects and the subjects with at least one wild-type allele (GG and GT subjects, P=0.012 for $AUC_{0\text{-}\infty}$; P=0.008 for $AUC_{0\text{-}4h}$; P=0.038 for C_{max})

Also, significant differences of urinary excreted amounts in exon 21 (GG versus TT, 5.97±2.00 versus 7.94±2.02 mg; P=0.049) and 26 (CC versus TT, 5.70±208 versus 7.94±2.02 mg; P=0.039) were observed.

Conclusion

The results confirm that levosulpiride is a P-gp substrate *in vivo*, and clearly demonstrated the effect of SNP 3435C>T in exon 26 and 2677G>T in exon 21 of the *MDR1* gene on levosulpiride disposition. Collectively, these findings provide a little explanation for the influence of *MDR1* polymorphisms on the disposition of levosulpiride, although others probably contribute to levosulpiride transport in human.

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