Neuroscience 169 (2010) 378–387

INFLUENCE OF *ABCB1* GENETIC POLYMORPHISMS ON THE PHARMACOKINETICS OF LEVOSULPIRIDE IN HEALTHY SUBJECTS

H. Y. CHO,[a] H. D. YOO[b] AND Y. B. LEE[b]*

[a]*Clinical Trials Management Division, Korea Food & Drug Administration, 194 Tongilro, Eunpyeong-gu, Seoul, 122-704, Republic of Korea*

[b]*College of Pharmacy and Institute of Bioequivalence and Bridging Study, Chonnam National University, 300 Yongbong-Dong, Buk-Gu, Gwangju, 500-757, Republic of Korea*

Abstract—The purposes of this study were to clarify the involvement of P-glycoprotein in the absorption of levosulpiride in knockout mice that lack the *Abcb1a*/*1b* gene, and to evaluate the relationship between genetic polymorphisms in *ABCB1* (exon 12, 21 and 26) and levosulpiride disposition in healthy subjects. The plasma and brain samples were obtained after oral administration (10 μg/g) of levosulpiride to *abcb1a*/*1b*(−/−) and wild-type mice (n=3∼6 at each time point). The average brain-to-plasma concentration ratio and blood-brain barrier partitioning of levosulpiride were 2.3- and 2.0-fold higher in *Abcb1a*/*1b*(−/−) mice than in wild-type mice, respectively. A total of 58 healthy Korean volunteers receiving a single oral dose of 25 mg levosulpiride participated in this study. The subjects were evaluated for polymorphisms of the *ABCB1* exon 12 C1236T, exon 21 G2677A/T (Ala893Ser/Thr) and exon 26 C3435T using polymerase chain reaction restriction fragment length polymorphism. The PK parameters (AUC_{0-4h}, $AUC_{0-\infty}$ and $C_{max.}$) of *ABCB1* 2677TT and 3435TT subjects were significantly higher than those of subjects with at least one wild-type allele (P<0.05). The results indicate that levosulpiride is a P-glycoprotein substrate *in vivo*, which is supported by the effects of SNPs 2677G>A/T in exon 21 and 3435C>T in exon 26 of *ABCB1* on levosulpiride disposition.

Key words: genotype, knockout mice, SNP, P-glycoprotein, blood-brain barriers, haplotype.

Levosulpiride, the levo-enantiomer form of racemic sulpiride, 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy benzamide, is a selective dopamine D_2 receptor antagonist at the trigger zone, both in the central nervous system and in the gastrointestinal tract (Jenner and Marsden, 1981; Gerlach, 1991; Rossi and Forgione, 1995). Levosulpiride is used to treat gastric or duodenal ulcers, irritable colon due to psychosomatic stress, and various vertigo syndromes, as well as depression, schizophrenia and psychopathology of senescence (Catapano et al., 1992; Distrutti et al., 2002).

Mizuno et al. showed that the sulpiride is mainly absorbed from the intestine and excreted by the kidney, mostly as an unchanged compound, after oral administration (Mizuno et al., 1986). The bioavailability of levosulpiride is low (20–30%) after oral administration of 100–200 mg doses and exhibits great inter- and intra-individual variability (Wiesel et al., 1980; Mauri et al., 1994). The low bioavailability in humans is probably due to incomplete absorption (Bateman, 1982). The time to peak plasma concentration is approximately 3 h, while the plasma elimination half-life ranges from 6 to 19 h depending on the dosage and route of administration (Wiesel et al., 1980; Bressolle et al., 1984).

According to *in vitro* studies, Watanabe et al. demonstrated that absorption of sulpiride across human intestinal Caco-2 cells was mediated by the transport systems of P-glycoprotein (P-gp) and organic cation (Watanabe et al., 2002a,b, 2004). But, their studies provided only indirect evidence that sulpiride may be a substrate of P-gp, because the direct efflux (e.g. using inside-out vesicles or a monolayer of transfected MDCKII cells) was not shown. Baluom et al. also reported that, in the rat model used, bioavailability of sulpiride was increased by co-administration of P-gp inhibitors such as verapamil and quinidine (Baluom et al., 2001). Their results suggest that one of the reasons for the poor oral bioavailability of sulpiride in humans after oral administration is P-gp efflux on the brush-border membrane.

P-gp is a member of the adenosine triphosphatase-binding cassette (ABC) superfamily of transporters (Higgins, 1992). The *ABCB1* transporter affects the efflux of a wide variety of drugs, such as immunosuppressants (Kuypers et al., 2008), anti-viral agents (Lee et al., 1998), antipsychotics and antidepressants (Shinkai et al., 2008; Uhr et al., 2008). Mammalian P-gps are encoded by a family of closely related genes, including two in humans (*ABCB1* and *ABCB3*) and three in mice (*Abcb1a*, *Abcb1b* and *Abcb2*) (Thiebaut et al., 1987; Croop et al., 1989; Hsu et al., 1989; Devault and Gros, 1990). Knockout mice in which either the *Abcb1a* gene or both the *Abcb1a* and *1b* genes are disrupted have been especially useful in determining the importance of P-gp in a particular drug's disposition, since the transporter is essentially or completely absent in these animals. Mice deficient in *Mdr1a* or

*Corresponding author. Tel: +82-62-530-2931; fax: +82-62-530-5106.
E-mail address: leeyb@chonnam.ac.kr (Y. B. Lee).
Abbreviations: ABC, adenosin triphosphate-binding cassette; ANOVA, analysis of variances; AUC_{0-2h}, area under the concentration-time curve from 0 to 2 h; $AUC_{0-\infty}$, area under the concentration-time curve from zero to infinity; BBB, blood-brain barrier; bp, base pair; Cl/F, relative total clearance; $C_{max.}$, maximum concentration; CYP, cytochrome P450; EDTA, ethylenediamine tetra acetic acid; HPLC, high performance liquid chromatography; IRB, Institutional Review Board, IS, internal standard; LLOQ, lower limit of quantitation; MDR1, multi drug resistance 1; OCT, organic cation transporter; OCTN, novel organic cation transporter; PCR, polymerase chain reaction; PEPT, oligopeptide transporter; P-gp, P-glycoprotein; PK, pharmacokinetic; RFLP, restriction fragment length polymorphism; SD, standard deviation; SNPs, single nucleotide polymorphisms; $T_{max.}$, time of maximum concentration; $t_{1/2}$, half-life; V/F, volume of distribution; λ_z, terminal phase rate constant.

0306-4522/10 $ - see front matter © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2010.04.065

Mdr1a/b are widely used as powerful tools for assessing the role of P-gp *in vivo* (Schinkel et al., 1995).

In the last several years, single nucleotide polymorphisms (SNPs) within the *ABCB1* gene have been identified and characterized. The three most frequent SNPs in the *ABCB1* gene are C1236T in exon 12, G2677T/A in exon 21 and C3435T in exon 26 (Cascorbi et al., 2001; Marzolini et al., 2004). These SNPs could affect the pharmacokinetics (PKs) and pharmacodynamics of drugs that are P-gp substrates (Hoffmeyer et al., 2000; Kim et al., 2001; Kroetz et al., 2003; Zhou, 2008). However, the effects of *ABCB1* polymorphisms on P-gp substrates have not been consistently reported; digoxin (Hoffmeyer et al., 2000; Johne et al., 2002), tacrolimus (Macphee et al., 2002; Tada et al., 2005) and cyclosporine (Min and Ellingrod, 2002; Yates et al., 2003) et al. Therefore, the effect of the *ABCB1* variant allele on P-gp activity is still controversial.

Levosulpiride has been suggested as a substrate of P-gp by means of *in vitro* studies using Caco-2 cells, which provide indirect evidence. However, the involvement of P-gp in the *in vivo* tissue-distribution, absorption, and elimination of levosulpiride has not yet been characterized. We hypothesized that the PKs and absorption of levosulpiride were influenced by P-gp *in vivo*. First, we sought to clarify the role of P-gp in the disposition of levosulpiride using *Abcb1a/ 1b* knockout mice. Second, we determined the SNPs in exon 12 (C1236T), exon 21 (G2677A/T) and exon 26 (C3435T) of *ABCB1*, and investigated the relationship between the *ABCB1* polymorphisms and levosulpiride disposition in genotype groups of healthy subjects.

EXPERIMENTAL PROCEDURES

Materials

Levosulpiride (99.77%) was provided by the Research Institute of Kuhnil Pharmaceuticals (Seoul, Korea). Tiapride hydrochloride (IS) was purchased from Sigma Co. (St. Louis, MO, USA). Normal saline (Choongwae Pharma Co., Seoul, Korea), heparin sodium (25,000-IU/mL, Green Cross, Seoul, Korea), and diethyl ether (Dae-Jung, Inchon, Korea) were used for animal experiments. Methanol and acetonitrile (high performance liquid chromatography (HPLC) grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and dichloromethane, potassium dihydrogenate phosphate (Yakuri Pure Chemical Co., Ltd., Osaka, Japan), and sodium borate buffer (pH 8.5, Sigma Co., St. Louis, MO, USA) were used for HPLC assay. The other chemicals were of HPLC grade or the highest quality available. HPLC-grade water was obtained from a Milli-Q water purification system (Millipore Co., Milford, MA, USA) and used throughout the study. The mobile phase components such as acetonitrile and potassium dihydrogenate phosphate were filtered through 0.45-μm pore size membrane filters prior to mixing and were ultrasonically degassed after mixing.

Experimental procedures with Abcb1a/1b knockout mice

The plasma and brain samples were obtained after oral administration (10 μg/g) of levosulpiride to *Abcb1a/1b*(−/−) and wild-type mice (n=3∼6 at each time point). Animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and approved by the institutional review board (IRB) of the Animal Care and Use Committee of Chonnam National University (CNU), Gwangju, Korea. All efforts were made to minimize the number of animals used and their suffering. Eight-weeks-old male *Abcb1a/1b*(−/−) transgenic mice (FVB/NTacFBR-[KO] *Abcb1a*- [KO]*Abcb1b* N7) and genetically matched male wild-type *Abcb*1a/*1b*(+/+) mice (FVB/NTacFBR) weighing 20–26 g were purchased from Taconic Farms, Inc. (Germantown, NY, USA). These mice, developed by Schinkel et al. (Schinkel et al., 1997), are functionally deficient in the blood-brain barrier and accumulate much higher amounts of certain drugs in their brains. The genetic authenticity of each parent of these mice was verified by polymerase chain reaction (PCR) amplification of *Abcb1a/1b* from genomic DNA (Schinkel et al., 1997). The mice were housed individually and maintained on a 12-h/12-h light/dark cycle at 23±1 °C and 50% relative humidity, and acclimated in the College of Pharmacy at CNU Animal Resources facility for 2 weeks with food and tap water *ad libitum*. Before the experiment, mice were fasted overnight but given free access to water.

The mice in each group were randomly tagged. Levosulpiride was dissolved in weak acetic acid solution (acetic acid 0.5 ml in H_2O 100 ml), and a dose of 10 μg/g was orally administered in an entire volume of 10 μl/g mouse. Each mouse was subjected to only one blood sampling from the inferior vena cava with a heparinized syringe under light ether anesthesia at 10, 20, 30, 45, 60, 90, 120 and 180 min. Trunk blood (about 1 ml) was collected in EDTA-coated tubes (K3 EDTA Vacutainer®, 13×15 mm, Becton Dickinson, Maylan, UK) and centrifuged at 3000 g for 2 min for the determination of plasma levosulpiride concentration. After blood sample collection, the brain was immediately dissected, washed with saline, blotted on paper towels, weighed and then homogenized in ten-fold volume of sodium borate buffer (pH 8.5). The plasma and brain homogenates were kept at −70 °C until assayed.

Levosulpiride disposition in healthy subjects

A total of 58 healthy Korean male volunteers participated in this study. The subjects' age ranged from 20 to 32 years (mean±SD, 23.7±2.5 years) and BMI from 17.1 to 28.2 kg/m^2 (mean±SD, 22.5±2.6 kg/m^2) for the PK study. Based on medical history, physical examination, and routine biochemical laboratory test results, all subjects were considered healthy. All subjects gave informed written consent to undergo genotyping and PK studies. The study protocol was approved by the IRB of Institute of Bioequivalence and Bridging Study, CNU, Gwangju, Korea. This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practice. Each subject was physically normal and had no previous history of significant illness or hypersensitivity to any drugs. Furthermore, the health status of subjects was judged normal on the basis of a physical examination with screening of blood chemistries and urinalysis before the subjects were admitted to the study. Finally, they were asked to refrain from taking alcohol, nicotine, caffeine and any other drugs for at least 1 week before and throughout the study period.

On the study day, each subject received a single oral dose of 25-mg levosulpiride tablet with 240 mL of water after an overnight fast. Blood samples were obtained through an indwelling cannula in Vacutainer® (8 mL, Becton Dickinson and Company, Franklin Lakes, NJ, USA) tubes before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h after drug administration. Following centrifugation (3000×g, 20 min, 4 °C), plasma samples were transferred to polyethylene tubes and immediately stored at −70 °C until analysis. Concentrations of levosulpiride in serum were analyzed using a validated HPLC method.

Analysis of levosulpiride in plasma and brain

Plasma (or brain homogenates) samples were analyzed for levosulpiride content using a validated HPLC method based on a