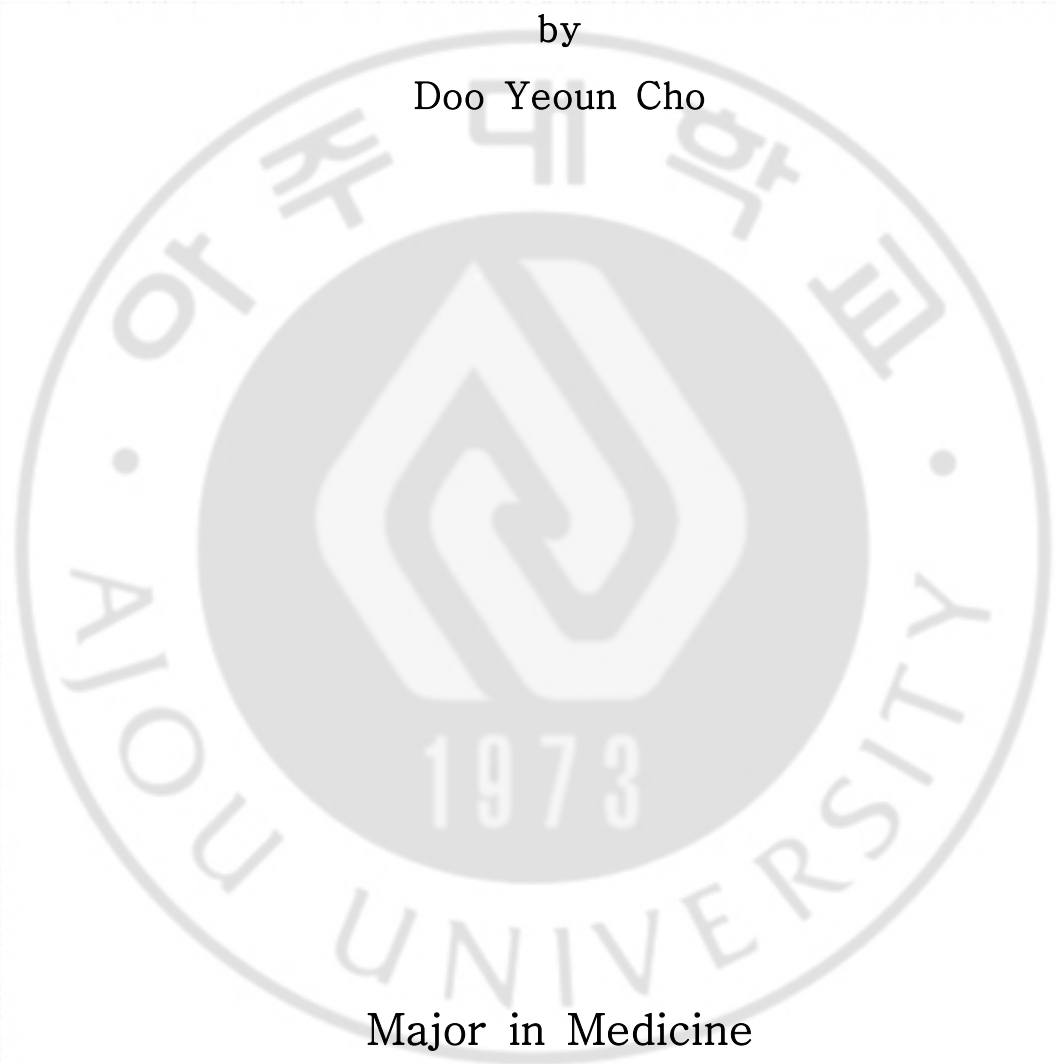


CYP2D6 Inhibitory Activities of Sarpogrelate  
hydrochloride and Drug–Drug Interaction

by

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Major in Medicine

Department of Medical Sciences

The Graduate School, Aju University

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Supervised by

Bom Taek Kim, M.D., Ph.D.

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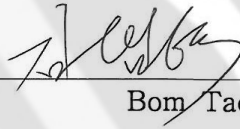
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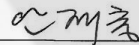
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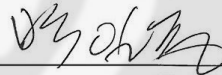
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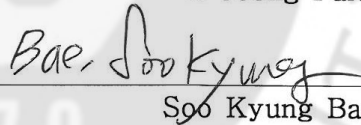
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## CYP2D6 Inhibitory Activities of Sarpogrelate hydrochloride and Drug-Drug Interaction

Sarpogrelate has inhibitory effects on serotonin-induced platelet aggregation, thrombus formation, vasoconstriction and vascular smooth muscle cell proliferation, all of which are mediated by 5-HT<sub>2A</sub> receptors, and consequently reduces the ischemic symptoms associated with peripheral artery disease. Despite the wide use and excellent pharmacological actions of sarpogrelate, to date there is no information regarding the potential inhibitory effects of sarpogrelate and M-1 (an active metabolite of sarpogrelate) on CYP isoforms or drug - drug interactions.

Using a cocktail assay, the effects of sarpogrelate and M-1 on nine CYP isoforms were measured by specific marker reactions in human liver microsomes. Nine healthy male subjects genotyped for *CYP2D6*\*1/\*1 or \*1/\*2 were included in a randomized, open-label, three-treatment three-period, crossover study. A single oral dose of metoprolol (100 mg) was administered with water (treatment A) and sarpogrelate (100 mg bid.; a total dose of 200 mg; treatment B), or after pretreatment of sarpogrelate for 3 days (100 mg tid.; treatment C). Plasma levels of metoprolol and  $\alpha$ -hydroxymetoprolol were determined using a validated liquid chromatography - tandem mass spectrometry (LC-MS/MS) method. Changes in heart rate and blood pressure were monitored as pharmacodynamic responses to metoprolol.

Sarpogrelate potently and selectively inhibited CYP2D6-mediated dextromethorphan *O*-demethylation with an IC<sub>50</sub> ( $K_i$ ) value of 3.05  $\mu$ M (1.24  $\mu$

M), in a competitive manner. M-1 also markedly inhibited CYP2D6 activity; its inhibitory effect with an  $IC_{50}$  ( $K_i$ ) value of 0.201  $\mu$ M (0.120  $\mu$ M) was more potent than that of sarpogrelate. Co-administration of sarpogrelate increased the area under the plasma concentration - time curve from time zero to the last measurement ( $AUC_t$ ) of metoprolol increased by 53% (geometric mean ratio [GMR], 1.53; 90% confidence interval [CI], 1.09 - 2.32) and by 51% (1.51; 1.04 - 2.30), respectively. Similar patterns were observed for the increase in  $C_{max}$  of metoprolol induced by sarpogrelate. However, the pharmacodynamics of metoprolol did not differ significantly among the three treatment groups.

Sarpogrelate and M-1 were potent and selective competitive inhibitors of CYP2D6 *in vitro*. Especially, inhibition of CYP2D6 by M-1 was ten-fold more potent than that of sarpogrelate. Sarpogrelate weakly inhibited a sensitive CYP2D6 substrate, metoprolol, by increasing metoprolol exposure by less than two-fold, but sarpogrelate had few effects on the pharmacodynamics of metoprolol. Higher systemic exposure to metoprolol if co-administered with sarpogrelate is not expected to be clinically meaningful. Extrapolation of these results to clinical practice suggests that no special monitoring is necessary if administering sarpogrelate with sensitive CYP2D6 substrates.

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Key words: Sarpogrelate, CYP2D6 inhibition, Drug-drug interaction, Metoprolol, Pharmacokinetics, Pharmacodynamics

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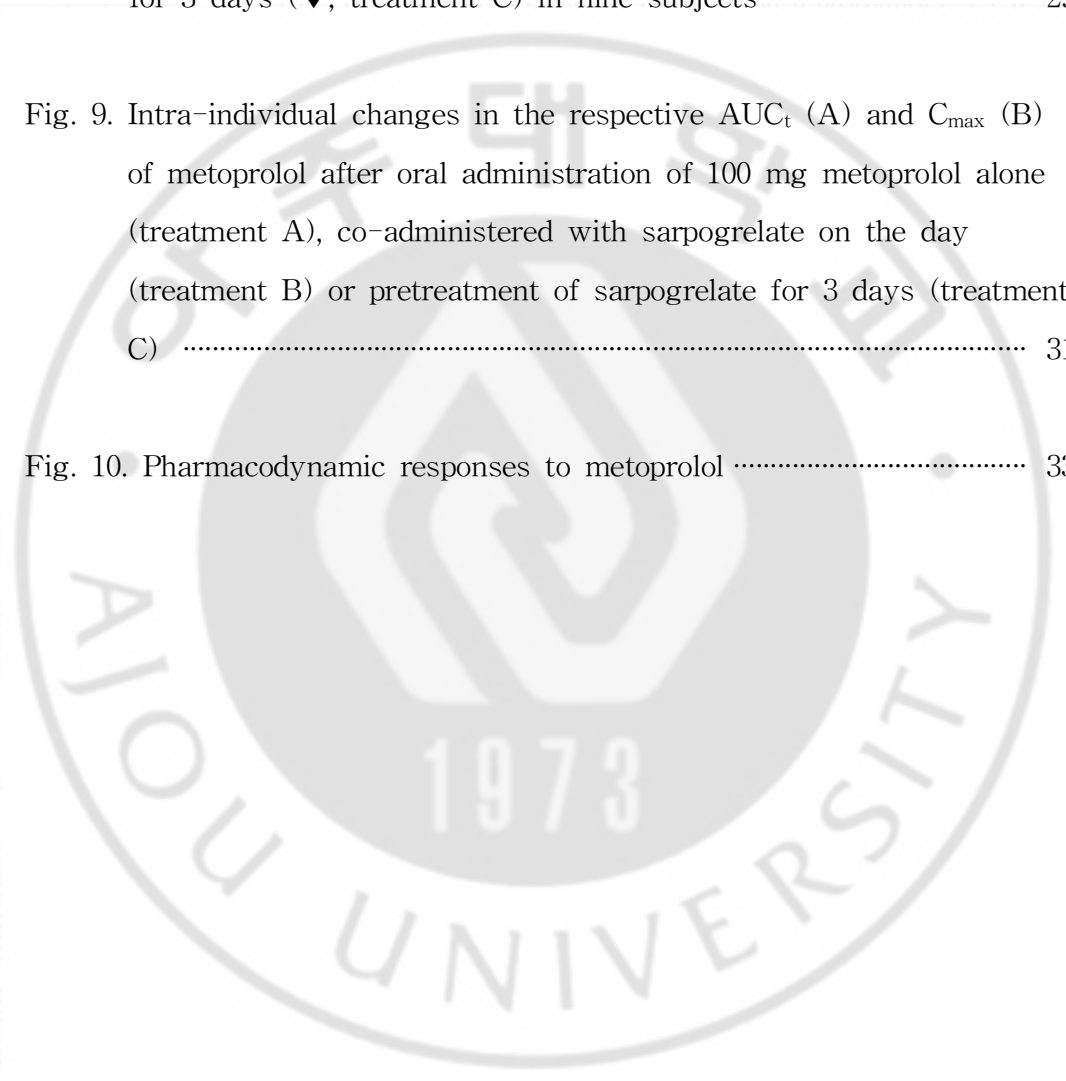
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## ABBREVIATION

5-HT, 5-hydroxytryptamine

AIC, Akaike Information Criterion

AUC<sub>t</sub>, area under the plasma concentration–time curve from time zero to t

AUEC, area under the effect curve

CI, confidence interval

C<sub>max</sub>, maximum plasma concentration

CYP, cytochrome P450

DBP, diastolic blood pressure

ECG, electrocardiography

GMR, geometric mean ratio

HIV, human immunodeficiency virus

HMT, α-hydroxymetoprolol

HR, heart rate

IC<sub>50</sub>, the 50% inhibitory concentration

$k_e$ , elimination rate constant

KF, potassium fluoride

$K_i$ , inhibitory constant

$K_m$ , Michaelis constant

LC-MS/MS, liquid chromatography–tandem mass spectrometry

MET, metoprolol

NADP, nicotinamide adenine dinucleotide phosphate

SBP, systolic blood pressure

SD, standard deviation

t<sub>max</sub>, time to reach C<sub>max</sub>

## I. INTRODUCTION

Sarpogrelate ((R,S)-1-{2-[2-(3-methoxyphenyl)ethyl]phenoxy}-3-(dimethylamino)-2-propyl hydrogen succinate chloride; Fig. 1) is a specific 5-hydroxytryptamine (5-HT)  $2_A$  receptor antagonist. It was approved in Japan for the treatment of peripheral artery disease in 1993 (Hara et al, 1999) and is used widely in Japan, China, and South Korea (Kim et al, 2014).

Sarpogrelate inhibits 5-HT induced platelet aggregation and vasoconstriction in smooth muscle cells (Rashid et al, 2003; Nishihira et al, 2006). Additionally, it has beneficial effects for diabetes mellitus (Pietraszek et al, 1993; Ogawa et al, 1999), angina pectoris (Kinugawa et al, 2002), restenosis after coronary stent (Fujita et al, 2003; Saini et al, 2004), and pulmonary hypertension (Saini et al, 2004), although the precise mechanisms underlying these effects are still unknown. Sarpogrelate is metabolized to ( $\pm$ )-1-{2-[2-(3-methoxyphenyl)ethyl]-phenoxy}-3-(dimethylamino)-2-propanol hydrochloride (M-1; Fig. 1) by esterase (Nagatomo et al, 2004; Saini et al, 2004). M-1, an active metabolite of sarpogrelate, has greater inhibitory effects than those of sarpogrelate *in vitro* (Pertz and Elz, 1995). In general, one 100 mg tablet of sarpogrelate is taken three times per day after meals (Kim et al, 2013). After oral administration of 100 mg sarpogrelate to healthy male subjects, sarpogrelate was absorbed rapidly from the gastrointestinal tract with a mean maximum plasma concentration ( $C_{max}$ ) of 1.99  $\mu$ M at 0.7 hour, and was eliminated rapidly from plasma with a half-life ( $t_{1/2}$ ) of 0.8 hour (Kim et al, 2014). The active metabolite, M-1, reached a  $C_{max}$  of 0.137  $\mu$ M at 0.9 hour and exhibited slower elimination than that shown by

sarpogrelate, with a  $t_{1/2}$  of 4.4 hour (Kim et al, 2013).

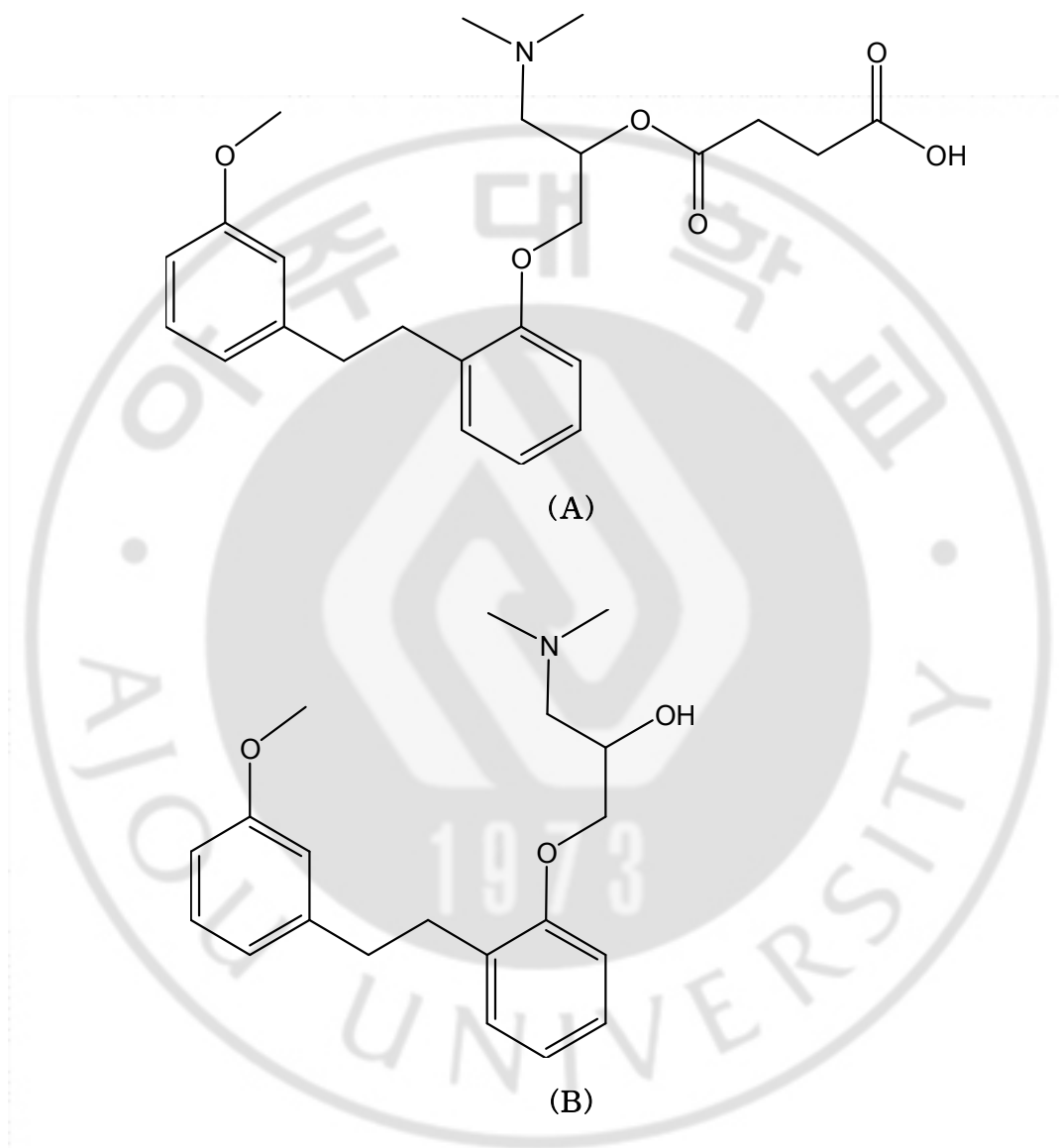


Fig. 1. Chemical structures of sarpogrelate (A) and its active metabolite, M-1 (B).

Metoprolol (1-(isopropylamino)-3[*p*-( $\beta$ -methoxyethyl)phenoxy]-2-propanol) is a selective  $\beta_1$ -receptor blocker. It is used widely for the treatment of angina pectoris, hypertension, and coronary artery disease (Regardh et al, 1983; Olsson et al, 1985; Wikstrand et al, 1991). It undergoes significant first-pass metabolism, with approximately 85% of the dose converted mostly into an inactive metabolite,  $\alpha$ -hydroxymetoprolol, *via* cytochrome P450 (CYP) 2D6 (McGourty et al, 1985; Lennard et al, 1986; Tucker et al, 2001; Wang et al, 2008). Metoprolol is also used as a CYP2D6 probe substrate for clinical studies of drug - drug interactions.

Despite the wide use and excellent pharmacological actions of sarpogrelate, to date there is no information regarding the potential inhibitory effects of sarpogrelate and M-1 on CYP isoforms or drug - drug interactions.

The purpose of the present study was to evaluate the inhibitory effects of sarpogrelate and M-1 on the nine CYP isoforms using a cocktail assay. In addition, the effect of sarpogrelate on the pharmacokinetics and pharmacodynamics of metoprolol (a typical CYP2D6 substrate *in vivo*) in healthy subjects were evaluated to assess the potential of sarpogrelate to cause drug-drug interactions with other concomitantly administered drugs.

## II. MATERIALS AND METHODS

### A. CYP isoforms inhibition study

#### 1. Chemicals and Reagents

Pooled human liver microsomes from a mixed pool of 24 donors (male: 17 and female: 7), *S*-benzylrivanol, and 1'-hydroxybufuralol were purchased from BD Gentest (Woburn, MA, USA). Sarpogrelate and M-1 were obtained from Kunwha pharmaceutical company (Seoul, Korea). Acetaminophen, bufuralol, chlorpropamide, chlorzoxazone, coumarin, dextrorphan, diethyldithiocarbamate, furafylline,  $\alpha$ -hydroxymetoprolol, ketoconazole, metoprolol, phenacetin, propranolol, quercetin, quinidine, rosiglitazone, *S*-mephenytoin, sulfaphenazole, tolbutamine, 1, 1', 1''-phosphinothioylidynetrisaziridine (thiotepa), potassium fluoride (KF),  $\beta$ -nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and MgCl<sub>2</sub> were purchased from Sigma - Aldrich Corporation (St. Louis, MO, USA). Bupropion, dextromethorphan, 6-hydroxybupropion, 6-hydroxychlorzoxazone, 7-hydroxycoumarin, 4'-hydroxymephenytoin, 1'-hydroxymidazolam, *p*-hydroxyrosiglitazone, 4-hydroxytolbutamide, and midazolam were obtained from Toronto Research Chemicals (North York, ON, Canada). Solvents were high-performance liquid chromatographic (HPLC) grade (Burdick & Jackson Company, Morristown, NJ, USA) and other chemicals were of the highest quality available.

## 2. Screening of Reversible Inhibitory Effects of Sarpogrelate and M-1

Cocktail assays in which several enzyme activities are determined in parallel by liquid chromatography–tandem mass spectrometry (LC–MS/MS) are particularly useful. The inhibitory potencies of sarpogrelate and M-1 were determined as described previously with slight modification (Bae et al., 2013). Phenacetin *O*-deethylase, coumarin 7-hydroxylase, bupropion 6-hydroxylase, rosiglitazone *p*-hydroxylase, tolbutamide 4-hydroxylase, *S*-mephenytoin 4-hydroxylase, dextromethorphan *O*-demethylase, chlorzoxazone 6-hydroxylase, and midazolam 1'-hydroxylase activities were determined as probe activities in human liver microsomes for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5, respectively. Sarpogrelate or M-1 (concentration: 0–50  $\mu$ M), and all substrates were dissolved in acetonitrile and serially diluted with acetonitrile to the required concentrations to give a final organic solvent concentration of 1.0% in the incubation mixture. Concentrations of P450-selective substrates were used close to their reported  $K_m$  (Michaelis constant) values (Kim et al, 2005; Bae et al, 2013). Briefly, the incubation mixtures containing pooled human liver microsomes (final concentrations: 0.25 mg/ml), each P450-selective substrate, and an NADPH-generating system (1.3  $\mu$ M NADP<sup>+</sup>, 3.3  $\mu$ M glucose 6-phosphate, 3.3  $\mu$ M MgCl<sub>2</sub>, and 0.4 unit/ml glucose-6-phosphate dehydrogenase) were preincubated for 5 minutes at 37°C. The reaction was initiated by adding an aliquot of sarpogrelate or M-1 and incubated for 15 minutes at 37°C in a shaking water bath. When sarpogrelate as an inhibitor was incubated, a 10-mL aliquot of 1 M KF in 0.1 M phosphate buffer (pH 7.4) was added before incubation to inhibit esterase



activity (Clarke & Waskell, 2003). After incubation, reactions were stopped by addition of 50  $\mu$ L of ice-cold acetonitrile containing 2  $\mu$ M chlorpropamide as an internal standard, and they were chilled and centrifuged (13,000 rpm, 8 minutes, 4°C). The supernatant was then diluted 100-fold with acetonitrile and then injected into the LC-MS/MS system. All incubations were performed in triplicate, and mean values were used for analysis.

### **3. Determination of the $K_i$ of Sarpogrelate and M-1 for CYP2D6**

Based on the  $IC_{50}$  values, the  $K_i$  values of sarpogrelate and M-1 for CYP2D6 were determined. Briefly, dextromethorphan, a specific substrate for CYP2D6, was incubated with sarpogrelate, M-1 or quinidine, a well-known typical CYP2D6 inhibitor. For determination of  $K_i$  values, dextromethorphan concentrations used were 2.5, 5, and 10  $\mu$ M. The concentrations of quinidine, sarpogrelate, and M-1 were as follows; 0-1  $\mu$ M for quinidine, and 0-10  $\mu$ M for sarpogrelate and M-1. All incubations were performed in triplicate, and mean values were used for the analysis. Other procedures were similar to those of the inhibition studies.

### **4. Time-Dependent Inhibitory Effects of Sarpogrelate and M-1**

The  $IC_{50}$  shift assay is one of most efficient and convenient methods of evaluating the time-dependent inhibitory effects of sarpogrelate and M-1. Changes in enzymatic activity are usually detected with and without preincubation of the test compound for a defined period. A shift in  $IC_{50}$  to a lower value following preincubation indicates time-dependent inactivation (Obach et al, 2006). Pooled human liver microsomes (1 mg/mL) were incubated with sarpogrelate or M-1 (0-50  $\mu$ M) in the absence or presence of

an NADPH-generating system for 30 minutes at 37°C (*i.e.*, the “inactivation incubation”). After inactivation incubation, aliquots (10 µL) were transferred to fresh incubation tubes (final volume 100 µL) containing an NADPH-generating system and each CYP-selective substrate cocktail set. When sarpogrelate was studied, a 10 mL aliquot of 1 M KF was added into both inactivation and incubation mixtures. The reaction system (100 µL total volume) was incubated for 15 minutes at 37°C in a shaking water bath. After incubation, reactions were stopped by addition of 50 µL ice-cold acetonitrile containing 2 µM chlorpropamide, as an internal standard, and they were chilled and centrifuged (13,000 rpm, 8 minutes, 4°C). The supernatant was then diluted 10-fold with acetonitrile and injected into the LC-MS/MS system.

## 5. LC-MS/MS Analyses

Metabolites of nine CYP-selective substrates were analyzed using a tandem quadrupole mass spectrometer (QTrap 5500 LC-MS/MS; Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization interface, as reported previously (Bae et al, 2013). Single reaction monitoring mode using specific precursor/product ion transition was used for quantification. Peak areas for all of the analytes were integrated automatically using the Analyst software (version 1.5.2; Applied Biosystems, Foster City, CA, USA). The mass transitions used for quantification of 1'-hydroxybufuralol or  $\alpha$ -hydroxymetoprolol were optimized based on the conditions used in a previous study (VandenBrink et al, 2012).

## 6. Data Analyses

For reversible inhibition and time-dependent inhibition screening, the CYP-mediated activities in the presence of the inhibitor, sarpogrelate or M-1, were expressed as percentages of the corresponding control values at 0  $\mu\text{M}$  of sarpogrelate or M-1. From plots of percent inhibition *versus* inhibitor concentrations, corresponding  $\text{IC}_{50}$  values were calculated by nonlinear regression using the WinNonlin software version 4.0 (Pharsight, Mountain View, CA, USA). The apparent kinetic parameters for inhibitory potential ( $K_i$  values) were estimated from the fitted curves using the WinNonlin software. The inhibition data were fit to different models of enzyme inhibition (competitive, non-competitive, uncompetitive or mixed) by nonlinear least-squares regression analysis (WinNonlin software). The most appropriate inhibition model selected based upon the goodness of fit criteria of a visual inspection of the data, correlation of determination ( $R^2$ ) and corrected Akaike Information Criterion. For visual inspection, data are presented as Dixon plots and Lineweaver - Burk plots.

## B. Pharmacokinetics of metoprolol associated with CYP2D6 genotypes

### 1. Subjects and Study design

Of the seven healthy subjects, four had the *CYP2D6\*1/\*1* genotype, and the remaining three had the *CYP2D6\*10/\*10* genotype. The validated LC-MS/MS method was used to quantify the concentrations of metoprolol and  $\alpha$ -hydroxymetoprolol following oral administration of 100 mg metoprolol (Betacloc<sup>®</sup>, Yuhan Pharmaceutical Inc., Korea) as authorized by the clinical trial center of Ajou University Hospital (Suwon, Korea). All participants provided written informed consent. Approximately 2 mL of blood was collected at 0 (pre-treatment), 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours after oral administration. The blood samples were immediately centrifuged (2,000 *g* for 10 minutes at 4°C), and the plasma samples (1.0 mL) were stored at -80°C until LC-MS/MS analysis.

### 2. Pharmacokinetics and Data Analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin Professional version 5.2 (Pharsight, Mountain View, CA, USA) to determine the total area under the plasma concentration-time curve from time zero to the last measured time ( $AUC_t$ ) by the trapezoidal rule extrapolation method. The maximum plasma concentration ( $C_{max}$ ) and time to reach  $C_{max}$  ( $T_{max}$ ) were taken directly from the experimental data. Statistical analysis of the pharmacokinetics of metoprolol and  $\alpha$ -hydroxymetoprolol between the two groups (*CYP2D6\*1/\*1* and *CYP2D6\*10/\*10*) was performed using a *t*-test.  $p < 0.05$  was considered to be statistically significant.

## C. Effect of Sarpogrelate on the pharmacokinetics and pharmacodynamics of metoprolol

### 1. Subjects

Healthy male subjects who fulfilled the following criteria were eligible for the study: age, 19 - 55 years; weight,  $\geq 55$  kg; body weight within  $\pm 20\%$  of ideal weight. All subjects were determined to be in good health based on medical history and results of a detailed physical examination, routine clinical laboratory tests (hematology, blood biochemistry, prothrombin time, bleeding time, and urinalyses), serology (hepatitis B surface antigen; anti-hepatitis C virus antibody; anti-HIV antibody; and Venereal Disease Research Laboratory test), and 12-lead electrocardiography (ECG) conducted within 3 weeks of the study. Subjects with the *CYP2D6*\*1/\*1 or \*1/\*2 genotype, as determined by genotyping analyses, were included in this study. Exclusion criteria were as follows: presence/history of cardiovascular, pulmonary, renal, endocrine, hematological, gastrointestinal, central nervous system, psychiatric, or malignant disease; a history of alcohol abuse ( $>21$  units/week) or excessive smoking ( $>20$  cigarettes/day), or unwillingness to abstain from drinking/smoking for the duration of the study; use of any other investigational drug within 3 months before administration of the study drug; donation of whole blood within 2 months or any blood products within 1 month before administration of the study drug; use of drugs that are inducers of CYP activity (*e.g.*, phenobarbital) or inhibitors of CYPs within 1 month before administration of the study drug; and use of prescription drugs or herbal remedies within 2 weeks, or use of over-the-counter medication within 1 week before administration of the study drug.

The study protocol was approved by the Ethics Review Board of Ajou University Hospital (Suwon, South Korea) in accordance with the ethical standards for studies in humans established by the Declaration of Helsinki and its amendments, and the applicable guidelines for Good Clinical Practice. This study was registered with ClinicalTrials.gov (NCT02097511). Before participating in the study, the subjects were given detailed written and oral information about the study, and asked to provide written informed consent before being screened for eligibility.

## 2. Determination of CYP2D6 genotypes

Blood samples were collected from 50 volunteers for CYP2D6 genotyping analyses. CYP2D6 genotype analyses were carried out by DNA Link, Inc. (Seoul, South Korea). Briefly, genomic DNA was extracted from peripheral blood using standard methods (QIAamp DNA Blood Mini kit; Qiagen, Hilden, Germany). Presence of *CYP2D6\*2* (functional allele), *CYP2D6\*10* (allele with reduced activity), *CYP2D6\*41* (allele with reduced activity), and *CYP2D6\*5* (null allele), which are frequently found in Asians with clinical significances (Lee et al, 2009; Kim et al, 2010; Yoo et al, 2011), was tested for each subject. Presence of *CYP2D6\*2* (285 C>T), *\*10* (100 C>T), or *\*41* (2988 G>A) allele was determined using multiplex single-base extension by SNaPshot analyses using ABI PRISM<sup>®</sup> SNaPshot<sup>™</sup> Multiplex kit (Applied Biosystems, Foster City, CA, USA) (Lee et al, 2009). Analyses were carried out using GeneMapper<sup>®</sup> v4.0 (Applied Biosystems, Foster City, CA, USA). The *CYP2D6\*5* allele was identified using the long polymerase chain reaction methods as described previously (Kim et al., 2010). If no variations were detected on an allele, it was defaulted to a wild-type (*CYP2D6\*1*)

assignment.

### 3. Study Design

This randomized, open-label, three-treatment, three-period, crossover study was conducted at the clinical trial center of Ajou University Hospital (Suwon, South Korea). Nine healthy subjects with the *CYP2D6*\*1/\*1 or \*1/\*2 genotype were randomly assigned to a protocol-specified treatment sequence by means of a computer-generated randomization process. The randomization involved three treatment sequences (ABC, BCA, and CAB) according to a Latin-square design. The treatments administered were as follows: metoprolol 100 mg (Betaloc<sup>®</sup>; Yuhan Corporation, Yongin, Korea) alone at 09:00 AM (treatment A; metoprolol alone); metoprolol 100 mg at 09:00 AM, with sarpogrelate 100 mg (Anplag<sup>®</sup>; Yuhan Corporation, Yongin, Korea) twice (at 09:00 AM and 03:00 PM) in the day (treatment B; co-administration of sarpogrelate on the day); and metoprolol 100 mg at 09:00 AM with sarpogrelate 100 mg twice (at 09:00 AM and 03:00 PM) on the day after pretreatment with sarpogrelate 100 mg three times daily for 3 days at 6 hour intervals (treatment C; pretreatment of sarpogrelate for 3 days). There was a washout period of 7 days between treatments. The subjects fasted overnight before metoprolol administration, and were allowed water *ad libitum* 2 hours after dosing. Each dose was administered with 240 mL of water. All subjects received a standard meal at 4 and 10 hours after each 09:00 AM dose. Following each dose, the subjects remained in the clinical trial center for 24 hours (day 2 of each treatment period), at which time they were discharged. No medications, herbal medicines, alcohol, citrus juice, grapefruit juice, or beverages containing caffeine were allowed for the duration of the study.

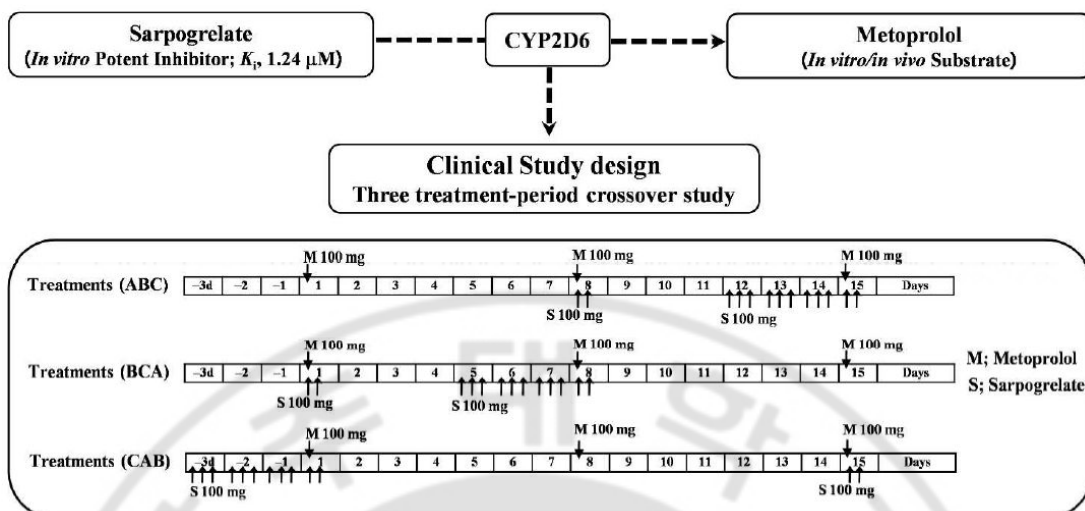


Fig. 2. Clinical study design

#### 4. Sample collection

Samples of venous blood (3 mL) were drawn from a venous catheter in the forearm and collected into lithium heparin tubes at 0 (just before drug administration) and 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 24 hours after metoprolol dosing. After centrifugation of blood samples, plasma samples (1.5 mL) were transferred immediately to polyethylene tubes and stored at  $-80^{\circ}\text{C}$  until analyses. In addition, heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded after 5 minutes rest in the sitting position at 0 (pre-dose) as well as 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after metoprolol administration by using Dash 5000 Vital Signs Patient Monitoring system (GE Healthcare, Little Chalfont, UK). Measurements were taken twice per time point at 1 minute interval. The average value was used for analyses and given as the change from the individual pre-dose value.



## 5. Bioanalytical methods

Plasma concentrations of metoprolol and  $\alpha$ -hydroxymetoprolol were analyzed using LC-MS/MS according to the previous described method (Bae et al, 2014). The system comprised a 1,260 high-performance liquid chromatography (HPLC) setup (Agilent Technologies, Wilmington, DE, USA) coupled with an API 3200 Triple Quadrupole Mass Spectrometer (AB Sciex, Foster City, CA, USA). Briefly, metoprolol,  $\alpha$ -hydroxymetoprolol, and the internal standard (chlorpropamide) were extracted from plasma (50  $\mu$ L) using ethyl acetate. Chromatographic separation was undertaken on a Luna CN column with an isocratic mobile phase comprising distilled water and methanol containing 0.1% formic acid (60:40, *v/v*) at a flow rate of 0.3 mL/minute. The total run time was 3.0 minutes per sample. Detection and quantification were done using a mass spectrometer in selected reaction-monitoring mode with positive electrospray ionization at  $m/z$  268.3 $\rightarrow$ 116.2 for metoprolol,  $m/z$  284.0 $\rightarrow$ 116.0 for  $\alpha$ -hydroxymetoprolol, and  $m/z$  277.0 $\rightarrow$ 111.0 for chlorpropamide. The optimized ion spray voltage and temperature were set at 5500 V and 600°C, respectively. The typical ion-source parameters, declustering potentials, collision energies, and entrance potential were 30 V, 20 V, and 5 V for metoprolol, 60 V, 25 V, and 5 V for  $\alpha$ -hydroxymetoprolol, and 60 V, 45 V, and 5.5 V for chlorpropamide, respectively. Nitrogen gas was used for the nebulizer, curtain, and collision-activated dissociation gas at pressures of 20, 10, and 6 psi, respectively. The linear ranges of concentration for metoprolol and  $\alpha$ -hydroxymetoprolol were 2 - 1000 and 2 - 500 ng/mL, respectively, with a lower limit of quantification of 2 ng/mL for both analytes. The coefficient of variation for the precision and accuracy of the assay met the acceptance

criteria for bioanalyses. All analytes were stable under various conditions of storage and handling, and relevant crosstalk and matrix effects were not observed.

## 6. Pharmacokinetic and Pharmacodynamic Assessments

The pharmacokinetic parameters of metoprolol and  $\alpha$ -hydroxymetoprolol were calculated by non-compartmental analytical methods using WinNonlin Professional version 5.2 (Pharsight Corporation, Mountain View, CA, USA). The maximum plasma concentration ( $C_{\max}$ ) and the time to reach  $C_{\max}$  ( $t_{\max}$ ) were obtained directly from experimental data. The apparent terminal  $t_{1/2}$  was calculated to be  $0.693/k_e$ , whereas the elimination rate constant ( $k_e$ ) was estimated from the least-squares regression slope of terminal plasma concentrations. The area under the plasma concentration - time curve from time zero to the last measurement ( $AUC_t$ ) was calculated according to the linear up/log down trapezoidal method. The area under the plasma concentration - time curve from time zero to infinity was calculated to be  $AUC_{0-\infty} = AUC_t + C_{12h}/k_e$ , where  $C_{12h}$  was the plasma concentration measured 12 hours after metoprolol administration. As a pharmacodynamic test, the area under the effect curve from 0 to 12 hours ( $AUEC_{0-12h}$ ) for HR, SBP, and DBP was calculated using the trapezoidal rule.

## 7. Statistical Analyses

The size of the study sample was not based on a power calculation but was considered to be adequate to characterize a potential interaction with sufficient accuracy based on previous experience gained in similar studies (Stout et al, 2010; Karonen et al, 2011; Misaka et al, 2013).

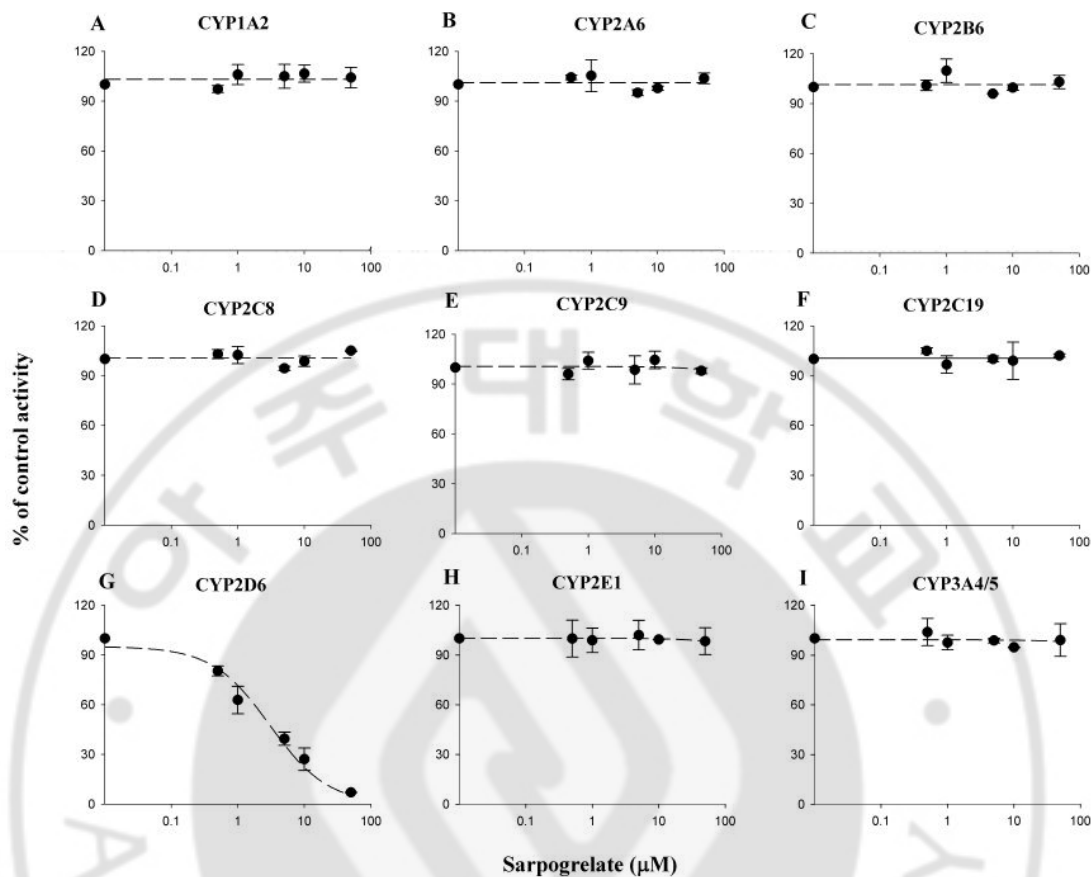
To evaluate the effect of sarpogrelate on the pharmacokinetics of metoprolol, analysis of variance (ANOVA), with treatment as an effect, was undertaken on the log-transformed  $AUC_t$  and  $C_{max}$  of metoprolol and  $\alpha$ -hydroxymetoprolol by using the general linear mixed-effects model in SAS version 9.1.3 (SAS Institute, Cary, NC, USA). The geometric mean ratios (GMRs) and 90% confidence intervals (CIs) for these ratios (treatment B; metoprolol with co-administration of sarpogrelate on the day *versus* treatment A; metoprolol alone, or treatment C; metoprolol after pretreatment of sarpogrelate for 3 days *versus* treatment A; metoprolol alone) were estimated. No significant pharmacokinetic drug interaction was concluded if the 90% CI for the ratios was within the no-effect range of 0.80 - 1.25 (US Food and Drug Administration, 2012). The  $t_{1/2}$  and  $t_{max}$  were compared using a non-parametric analysis with the Wilcoxon signed rank test;  $p < 0.05$  was considered significant. Statistical analyses were carried out using SAS version 9.1.3. Pharmacokinetic data are the mean  $\pm$  standard deviation (SD) except for  $t_{max}$ , which is the median with range.

The influence of co-administration of single or multiple doses of sarpogrelate on the pharmacodynamics of metoprolol was assessed through measurement of  $AUEC_{0-12h}$  for HR, SBP, and DBP and analyzed using general linear model repeated-measures ANOVA as described above for the pharmacokinetic analyses. Log-transformation was applied to these parameters before analyses.

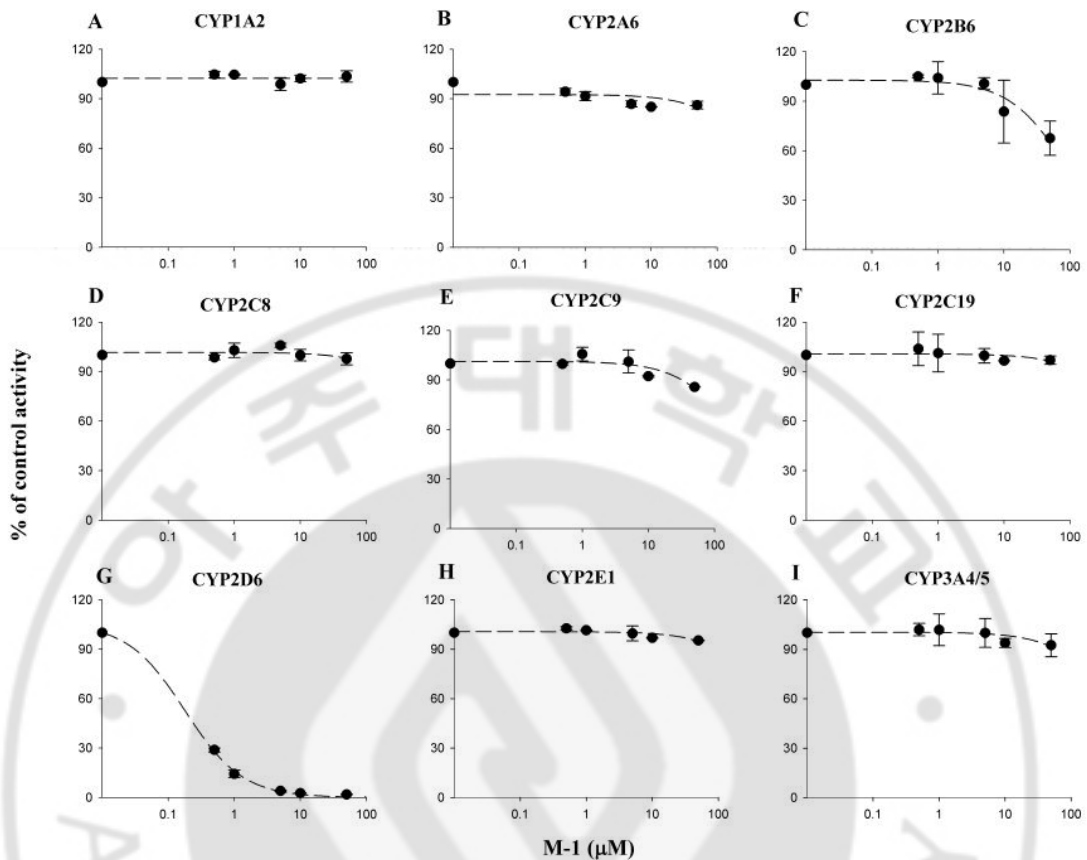
### III. RESULTS

#### A. CYP isoforms inhibition study

The inhibitory effects of sarpogrelate and M-1 on the activities of nine CYP isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5) at microsomal protein concentrations of 0.25 mg/mL are shown in Figs. 3 and 4, respectively. The  $IC_{50}$  values of sarpogrelate and M-1 at a microsomal protein concentration of 0.25 mg/mL are listed in Table 1. Of the nine CYP isoforms tested, CYP2D6-catalyzed dextromethorphan *O*-demethylation was most strongly inhibited by sarpogrelate and M-1, with  $IC_{50}$  values of 3.05 and 0.201  $\mu$ M, respectively (Table 1). However, sarpogrelate and M-1 showed no apparent inhibition of the other eight CYPs tested (Table 1, Figs. 3 and 4); the remaining activities at the tested highest concentration (50  $\mu$ M) were greater than 90%.



**Fig. 3.** IC<sub>50</sub> curves of sarpogrelate for CYP activities using the cocktail assay. CYP1A2 for phenacetin *O*-deethylase (A), CYP2A6 for coumarin 7-hydroxylase (B), CYP2B6 for bupropion 6-hydroxylase (C), CYP2C8 for rosiglitazone *p*-hydroxylase (D), CYP2C9 for tolbutamide 4-hydroxylase (E), CYP2C19 for *S*-mephenytoin 4-hydroxylase (F), CYP2D6 for dextromethorphan *O*-demethylase (G), CYP2E1 for chlorzoxazone 6-hydroxylase (H), and CYP3A4/5 for midazolam 1'-hydroxylase (I). Data are the mean ± SD of triplicate determinations. The dashed lines represent the best fit to the data using non-linear regression.



**Fig. 4.** IC<sub>50</sub> curves of M-1 for CYP activities using the cocktail assay. CYP1A2 for phenacetin *O*-deethylase (A), CYP2A6 for coumarin 7-hydroxylase (B), CYP2B6 for bupropion 6-hydroxylase (C), CYP2C8 for rosiglitazone *p*-hydroxylase (D), CYP2C9 for tolbutamide 4-hydroxylase (E), CYP2C19 for *S*-mephenytoin 4-hydroxylase (F), CYP2D6 for dextromethorphan *O*-demethylase (G), CYP2E1 for chlorzoxazone 6-hydroxylase (H), and CYP3A4/5 for midazolam 1'-hydroxylase (I). Data are the mean  $\pm$  SD of triplicate determinations. The dashed lines represent the best fit to the data using non-linear regression.

**Table 1. Values of IC<sub>50</sub> (μM) of sarpogrelate and M-1 for each CYP isoforms in human liver microsomes.**

CYPs	Reactions	IC <sub>50</sub> (μM)	
		Sarpogrelate	M-1
CYP1A2	Phenacetin <i>O</i> -deethylation	>50	>50
CYP2A6	Coumarin 7-hydroxylation	>50	>50
CYP2B6	Bupropion 6-hydroxylation	>50	>50
CYP2C8	Rosiglitazone <i>p</i> -hydroxylation	>50	>50
CYP2C9	Tolbutamide 4-hydroxylation	>50	>50
CYP2C19	<i>S</i> -Mephenytoin 4-hydroxylation	>50	>50
CYP2D6	Dextromethorphan <i>O</i> -demethylation	3.05	0.201
CYP2E1	Chlorzoxazone 6-hydroxylation	>50	>50
CYP3A4/5	Midazolam 1'-hydroxylation	>50	>50

Data are expressed as the mean of triplicate determinations.

To characterize the type of reversible inhibition of CYP2D6 by sarpogrelate or M-1 based on the  $IC_{50}$  values, enzyme kinetic assays were conducted with varying concentrations of sarpogrelate or M-1 and dextromethorphan. Identical parallel incubation samples containing a known potent inhibitor of CYP2D6, quinidine, were included. The  $K_i$  values of sarpogrelate, M-1 and quinidine are listed in Table 2. Representative Dixon plots for the inhibition of CYP2D6 by sarpogrelate, M-1 and quinidine in human liver microsomes are shown in Fig. 5. Sarpogrelate and M-1 strongly and selectively inhibited CYP2D6 with  $K_i$  values of 1.24  $\mu$ M and 0.120  $\mu$ M, respectively. Specifically, inhibition of CYP2D6 by M-1 was more potent than that of sarpogrelate, and was similarly potent as quinidine ( $K_i$ , 0.129  $\mu$ M) (Table 2). Visual inspection of the Dixon plots and further analysis of the enzyme inhibition modes suggested that the inhibition data of sarpogrelate, M-1 and quinidine all fit well to a competitive inhibition type.

A shift in the inhibition curve to a lower  $IC_{50}$  value by 30 minutes preincubation in the presence of NADPH is an indicator of time-dependent inhibition. After 30 minutes preincubation of sarpogrelate or M-1 with human liver microsomes in the presence of NADPH, no obvious shift in  $IC_{50}$  was observed for inhibition of the nine CYPs. Representative  $IC_{50}$  shift plots for CYP2D6 activity by sarpogrelate or M-1 are shown in Fig. 6. These suggest that sarpogrelate and M-1 are not time-dependent inhibitors.



Table 2.  $K_i$  values of the inhibition for CYP2D6 by sarpogrelate, M-1, and quinidine at microsomal protein concentrations of 0.25 mg/mL.

CYPs	$K_i$ ( $\mu\text{M}$ )		
	Sarpogrelate	M-1	Quinidine
CYP2D6	1.24 <sup>a</sup>	0.120 <sup>a</sup>	0.129 <sup>a</sup>

Concentrations of sarpogrelate, M-1, and quinidine were as following; 0 - 10  $\mu\text{M}$  for sarpogrelate and M-1, and 0 - 1  $\mu\text{M}$  for quinidine, respectively.

<sup>a</sup> Inhibition type was determined by the best fit to competitive mode based on Akaike Information Criterion (AIC).

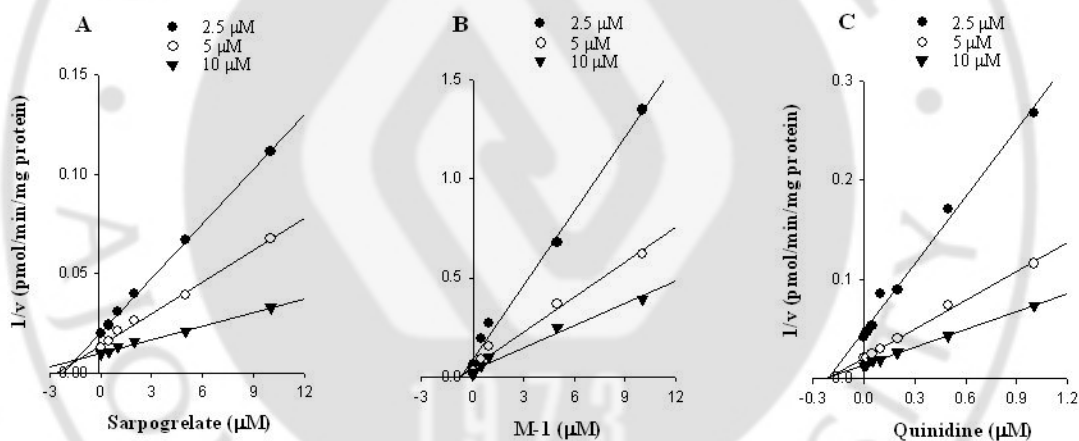
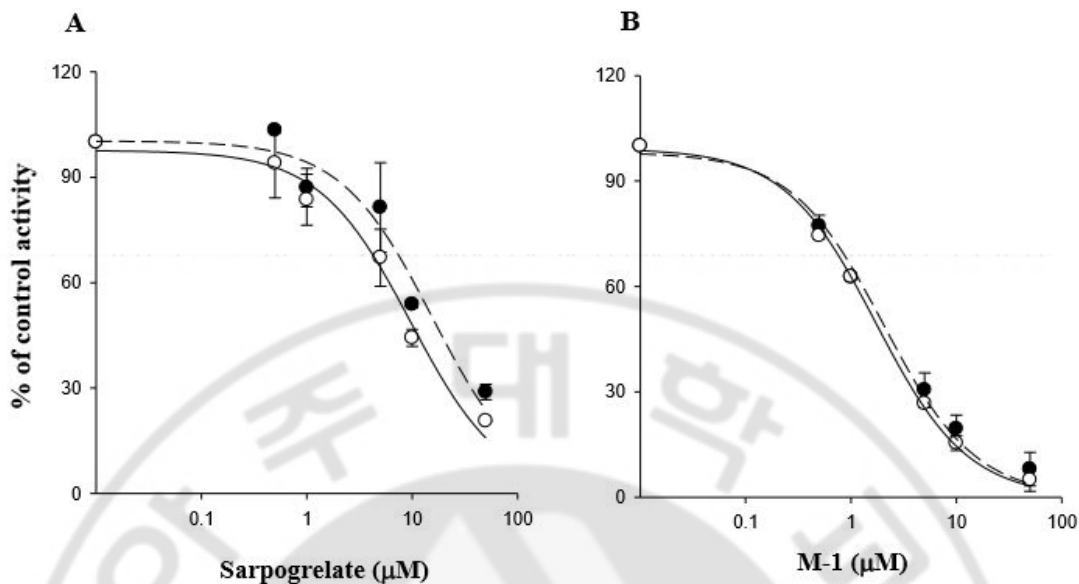


Fig. 5. Dixon plots to determine  $K_i$  values for CYP2D6 of sarpogrelate (A), M-1 (B), and quinidine (C). The concentrations of dextromethorphan were determined 2.5 ( $\bullet$ ), 5 ( $\circ$ ), and 10 ( $\blacktriangledown$ )  $\mu\text{M}$ , respectively.  $V$  represents formation rate of dextrophan (pmol/min/mg protein). Data are the mean values of triplicate determinations. The solid lines of sarpogrelate, M-1 and quinidine fit well to all competitive inhibition types.



**Fig. 6. Representative IC<sub>50</sub> shift plots for CYP2D6 by sarpogrelate (A) and M-1 (B) with human liver microsomes.** In the presence (●, the dashed lines) and absence (○, the solid lines) of NADPH for 30 minutes pre-incubation. Data are the mean ± SD of triplicate determinations. The dashed lines and solid lines represent the best fit to the data using non-linear regression.

## B. Pharmacokinetics of metoprolol associated with CYP2D6 genotypes

The plasma concentration-time profiles of metoprolol and  $\alpha$ -hydroxymetoprolol are shown in Fig. 7, and the relevant pharmacokinetic parameters are summarized in Table 3. Seven healthy subjects were divided into two groups based on genotype: *CYP2D6\*1/\*1* and *CYP2D6\*10/\*10*. The  $AUC_t$  and the  $C_{max}$  of metoprolol in the *CYP2D6\*10/\*10* group were significantly greater than those in the *CYP2D6\*1/\*1* group, whereas, the  $AUC_t$  and the  $C_{max}$  of  $\alpha$ -hydroxymetoprolol in the *CYP2D6\*10/\*10* group were significantly lower than in the *CP2D6\*1/\*1* group ( $p < 0.01$ ). There were no significant differences in  $T_{max}$  of metoprolol or  $\alpha$ -hydroxymetoprolol between the two groups. The  $t_{1/2}$  of metoprolol and  $\alpha$ -hydroxymetoprolol were significantly longer in the *CYP2D6\*10/\*10* group than in the *CYP2D6\*1/\*1* group ( $p < 0.05$ ) (Table 3). Thus, the metabolic ratio calculated by  $AUC_{t,metoprolol}/AUC_{t,\alpha\text{-hydroxymetoprolol}}$  was markedly higher ( $0.931 \pm 0.178$  versus  $0.0724 \pm 0.0443$ ) in the *CYP2D6\*10/\*10* group than in the *CYP2D6\*1/\*1* (Table 3).

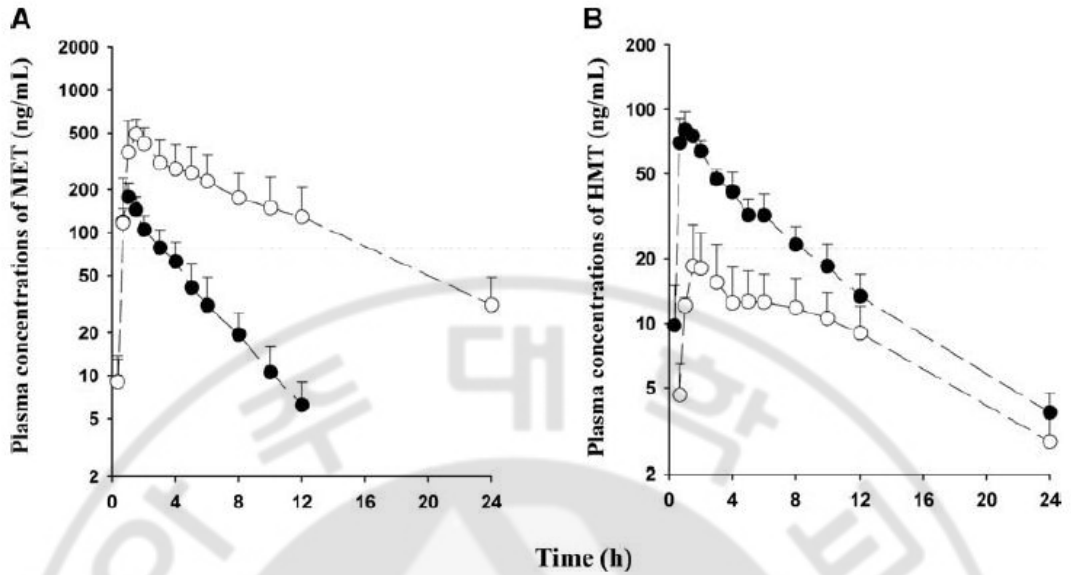


Fig. 7. Mean plasma concentrations of metoprolol (A) and α-hydroxymetoprolol (B) after oral administration of 100 mg metoprolol tablet in the two groups of CYP2D6 genotypes (●, *CYP2D6*\*1/\*1, *n*=4; ○, *CYP2D6*\*10/\*10, *n*=3). MET, metoprolol; HMT, α-hydroxymetoprolol.

**Table 3. Pharmacokinetic parameters of metoprolol and  $\alpha$ -hydroxymetoprolol after oral administration of 100 mg metoprolol in the two groups by CYP2D6 genotypes**

Parameters	<i>CYP2D6</i> *1/*1 ( <i>n</i> =4)	<i>CYP2D6</i> *10/*10 ( <i>n</i> =3)	<i>p</i> -value
<b>Metoprolol</b>			
AUC <sub>t</sub> (ng*h/mL)	556 ± 173	3500 ± 1700	<0.01
C <sub>max</sub> (ng/mL)	178 ± 44.9	507 ± 155	<0.01
t <sub>1/2</sub> (h)	2.49 ± 0.446	5.94 ± 0.736	<0.05
T <sub>max</sub> (h)	1 (0.67 - 1)	1.5 (1 - 1.5)	>0.05
<b><math>\alpha</math>-hydroxymetoprolol</b>			
AUC <sub>t</sub> (ng*h/mL)	499 ± 93.4	207 ± 76.1	<0.01
C <sub>max</sub> (ng/mL)	82.8 ± 12.5	19.3 ± 9.80	<0.01
t <sub>1/2</sub> (h)	6.08 ± 0.643	7.85 ± 0.884	<0.05
T <sub>max</sub> (h)	1 (0.67 - 1.5)	1 (0.67 - 2)	>0.05
Metabolic ratio <sup>a</sup>	0.931 ± 0.178	0.0724 ± 0.0443	<0.01

Values are mean ± SD or median (range).

<sup>a</sup> Metabolic ratio as expressed by AUC<sub>t,metoprolol</sub>/AUC<sub>t, $\alpha$ -hydroxymetoprolol</sub>

## C. Effect of Sarpogrelate on the pharmacokinetics and pharmacodynamics of metoprolol

### 1. Subjects

In the 50 subjects evaluated, the allele frequencies of *CYP2D6*\*1, \*2, \*10, \*41, and \*5 were 36%, 8%, 52%, 2%, and 2%, respectively. This result was similar to the data obtained by other scholars (Lee et al, 2009; Yoo et al, 2011). Approximately 7-10% of Caucasians and 1% of Asians are homozygous for non-functional CYP2D6 alleles. Despite the low frequency of poor metabolizers of CYP2D6 substrates in Asian populations, these individuals have been found to carry 51% of the 100C>T (exon 1) polymorphism (*CYP2D6*\*10), which causes an amino-acid substitution (Pro34Ser) that leads to an unstable enzyme with lower metabolic activity (Bertilsson et al, 1992; Johansson et al, 1994). Indeed, the *CYP2D6*\*10 reduces the CYP2D6-dependent metabolism of many substrates, resulting in their increased exposure in Asians (Jin et al, 2008; Lim et al, 2008; Yoo et al, 2011; Choi et al, 2012; Wu et al, 2014). It has been reported that the AUC of metoprolol increases approximately 2.23-fold and 5.73-fold in Korean subjects with *CYP2D6*\*1/\*10 and \*10/\*10 genotypes, respectively, compared to that in individuals with *CYP2D6*\*1/\*1 genotype (Jin et al, 2008). To evaluate the inhibitory effects of sarpogrelate on CYP2D6 activity, after genotyping of CYP2D6, subjects with *CYP2D6*\*1/\*1 ( $n=8$ ) or \*1/\*2 ( $n=1$ ) genotypes were enrolled in this study. The mean age was 24.1 (range: 22-27) years, mean weight was 69.9 (58.4-85.9) kg, and mean height was 175 (166-189) cm.

## 2. Pharmacokinetics of metoprolol and $\alpha$ -hydroxymetoprolol

Mean plasma concentrations of metoprolol and  $\alpha$ -hydroxymetoprolol with and without (metoprolol alone: treatment A) co-administration of sarpogrelate (treatment B or treatment C) are shown in Fig. 8. The relevant pharmacokinetic parameters of metoprolol and  $\alpha$ -hydroxymetoprolol as well as GMR and 90% CI are listed in Table 4.

Subjects following single (treatment B) or multiple (treatment C) sarpogrelate dosing had greater plasma exposure of metoprolol in comparison to that in subjects administered metoprolol alone (although large inter-subject variabilities were noted) (Fig. 8(A)). There were no apparent differences among the three treatment groups with regard to the plasma concentrations of  $\alpha$ -hydroxymetoprolol (Fig. 8(B)). In treatment B, the  $AUC_t$  and  $C_{max}$  of metoprolol increased by 53% and 62% compared with those in the treatment A (metoprolol alone)-based GMR of 1.53 (90% CI, 1.09-2.32) and 1.62 (90% CI, 1.16-2.58), respectively (Table 4). The  $C_{max}$  of  $\alpha$ -hydroxymetoprolol decreased slightly by 12% in treatment B; GMR of 0.88 (90% CI, 0.59-1.32), but the  $AUC_t$  did not decrease; GMR of 0.97 (90% CI, 0.78-1.21) (Table 4). The  $T_{max}$  and  $t_{1/2}$  of metoprolol and  $\alpha$ -hydroxymetoprolol were not significantly different from those in treatment A (metoprolol alone).

After multiple dosing of sarpogrelate (treatment C), the  $AUC_t$  of metoprolol increased by 51% (90% CI, 1.04-2.30) and the  $C_{max}$  of metoprolol increased by 67% (90% CI, 1.23-2.55) compared with metoprolol alone (Table 4). The GMRs (90% CI) for  $\alpha$ -hydroxymetoprolol were  $C_{max}$ , 0.89 (0.59-1.35) and  $AUC_t$ , 0.99 (0.80-1.23). In treatment C, multiple dosing of sarpogrelate also did not affect the  $T_{max}$  and  $t_{1/2}$  of metoprolol or  $\alpha$ -hydroxymetoprolol; they were not significantly different from those in treatment A (Table 4).

Increased exposures of metoprolol by co-administration with sarpogrelate alone (treatment B) were similar to those observed upon pretreatment with sarpogrelate for 3 days (treatment C). Intra-subject changes in the respective  $AUC_t$  and  $C_{max}$  of metoprolol among the three treatments are shown in Fig. 9.

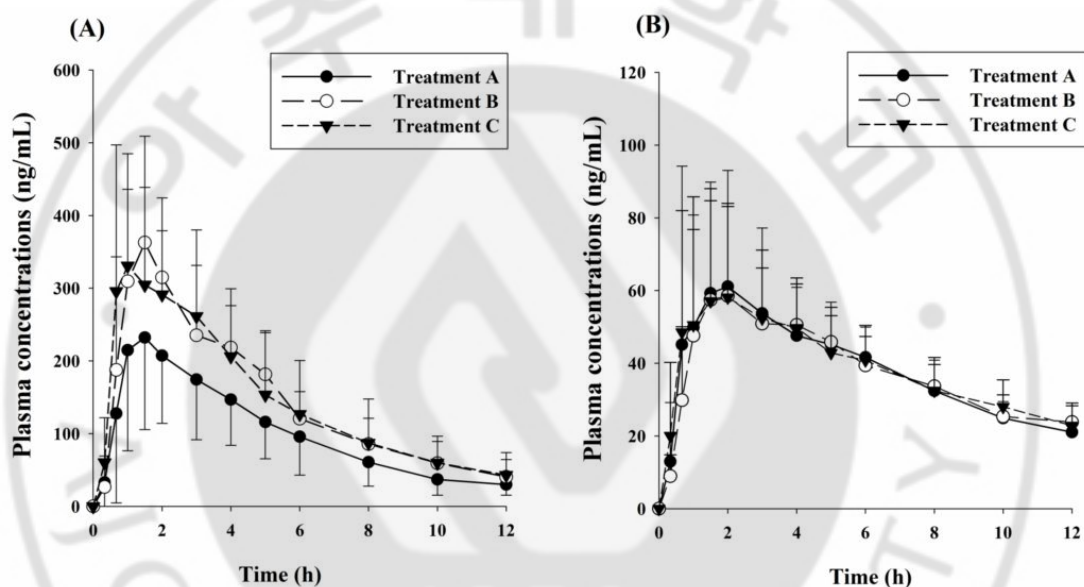


Fig. 8. Mean plasma concentrations of metoprolol (A) and  $\alpha$ -hydroxymetoprolol (B) after oral administration of 100 mg metoprolol alone (●, treatment A) and co-administered with sarpogrelate on the day (○, treatment B) or pretreatment with sarpogrelate for 3 days (▼, treatment C) in nine healthy male subjects. Bars represent SD.



Table 4. Mean ( $\pm$ SD) pharmacokinetic parameters of metoprolol and  $\alpha$ -hydroxymetoprolol after oral administration of 100 mg metoprolol alone (treatment A), co-administered with sarpogrelate on the day (treatment B) or pretreatment of sarpogrelate for 3 days (treatment C) in nine subjects.

Parameter	Treatment A (n=9)	Treatment B (n=9)		Treatment C (n=9)	
	Mean $\pm$ SD	Mean $\pm$ SD	GMR <sup>a</sup> (90%CI <sup>b</sup> )	Mean $\pm$ SD	GMR <sup>a</sup> (90%CI <sup>b</sup> )
<b>Metoprolol</b>					
AUC <sub>t</sub> (ng*h/mL)	1210 $\pm$ 560	1580 $\pm$ 537	1.53 (1.09-2.32)	1520 $\pm$ 748	1.51 (1.04-2.30)
C <sub>max</sub> (ng/mL)	279 $\pm$ 116	404 $\pm$ 119	1.62 (1.16-2.58)	415 $\pm$ 89.4	1.67 (1.23-2.55)
t <sub>1/2</sub> (h)	3.28 $\pm$ 0.44	3.43 $\pm$ 0.81		3.50 $\pm$ 0.560	
T <sub>max</sub> (h)	1.50 (0.67-5.0)	1.50 (0.67-4.0)		1.0 (0.67-3.0)	
<b><math>\alpha</math>-hydroxymetoprolol</b>					
AUC <sub>t</sub> (ng*h/mL)	456 $\pm$ 122	425 $\pm$ 120	0.97 (0.78-1.21)	442 $\pm$ 76.3	0.99 (0.80-1.23)
C <sub>max</sub> (ng/mL)	66.7 $\pm$ 39.9	56.7 $\pm$ 24.1	0.88 (0.59-1.32)	64.8 $\pm$ 26.9	0.89 (0.59-1.35)
t <sub>1/2</sub> (h)	5.30 $\pm$ 1.53	6.90 $\pm$ 2.06		7.01 $\pm$ 0.945	
T <sub>max</sub> (h)	1.50 (0.67-5.0)	1.50 (1.5-4.0)		1.50 (0.67-6.0)	

Values are mean  $\pm$  standard deviation or median (range).

<sup>a</sup> Geometric mean ratio of treatment B or treatment C to treatment A.

<sup>b</sup> 90% confidence interval

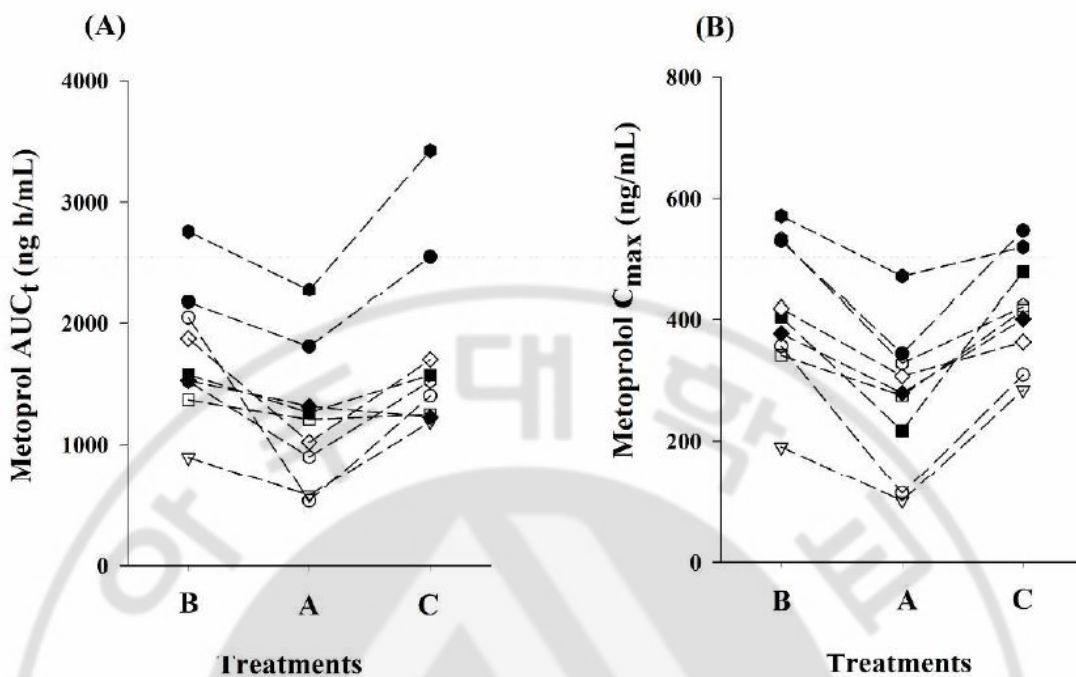
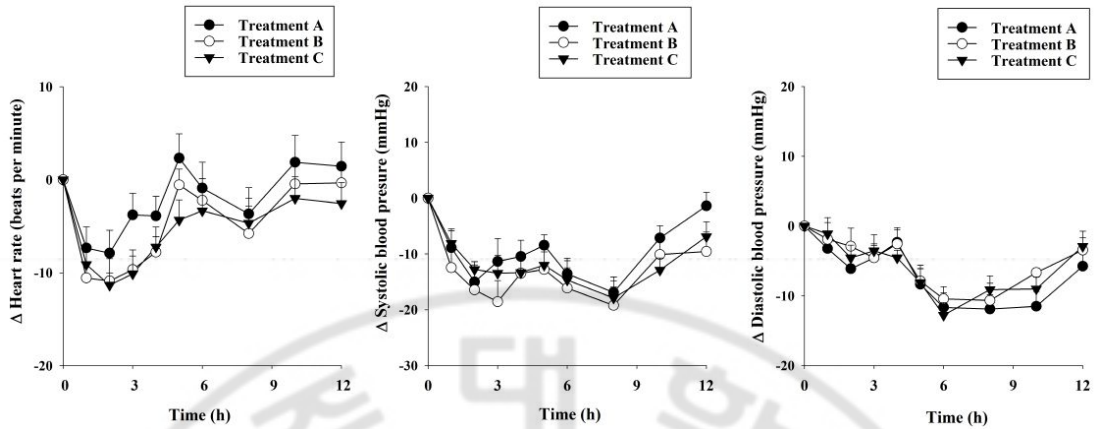


Fig. 9. Intra-individual changes in the respective AUC<sub>t</sub> (A) and C<sub>max</sub> (B) of metoprolol after oral administration of 100 mg metoprolol alone (treatment A), co-administered with sarpogrelate on the day (treatment B) or pretreatment of sarpogrelate for 3 days (treatment C).

### 3. Pharmacodynamics of metoprolol

To assess the pharmacodynamic responses to metoprolol, HR and blood pressure were recorded periodically after metoprolol administration. The mean baseline (pre-dose) values of HR, SBP, and DBP were  $62.1 \pm 8.81$  beats/minute,  $116 \pm 9.54$  mmHg, and  $66.4 \pm 7.31$  mmHg, respectively. Baseline values of HR, SBP, and DBP among the three treatment groups were similar (Fig. 10). Changes in HR, SBP, and DBP after metoprolol administration are expressed as the difference from the baseline value in Fig. 10. The overall shapes of the changes in HR, SBP, and DBP *versus* time curves were similar among the three treatment groups. HR, SBP, and DBP decreased with metoprolol treatment and recovered to the pre-dose levels about 12 hours after metoprolol treatment was discontinued. When comparing each treatment using repeated-measures ANOVA, all data were contained in the no-effect interval of 0.8-1.25. Hence, co-administration of sarpogrelate or pretreatment with sarpogrelate was considered not to have an important effect on the pharmacodynamics of metoprolol.



**Fig. 10.** Pharmacodynamic responses to metoprolol. The differences from baseline value of heart rate, systolic blood pressure, and diastolic blood pressure after oral administration of 100 mg metoprolol alone ( $n = 9$ ; ●, treatment A) and co-administered with sarpogrelate on the day (○, treatment B) or pretreatment with sarpogrelate for 3 days (▼, treatment C). Bars represent the standard error of the mean.

## IV. DISCUSSION

*In vitro* study showed that sarpogrelate is a potent and selective competitive inhibitor of CYP2D6. Additionally, M-1, an active metabolite of sarpogrelate, significantly inhibited CYP2D6 activities; its inhibitory effects with an  $IC_{50}$  ( $K_i$ ) value of 0.201  $\mu$ M (0.120  $\mu$ M) was more potent than those of sarpogrelate, with an  $IC_{50}$  ( $K_i$ ) value of 3.05  $\mu$ M (1.24  $\mu$ M). However, sarpogrelate and M-1 showed no apparent inhibition of the other eight CYPs: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP2E1. Preincubation of sarpogrelate or M-1 with human liver microsomes and an NADPH-generating system did not alter the inhibition potencies against the nine CYPs, suggesting that sarpogrelate or M-1 are not time-dependent inhibitors.

Genetic variants of the CYP2D6 gene are known to play a major part in CYP2D6 activities. The metabolic ratio calculated by  $AUC_{t,metoprolol}/AUC_{t,\alpha\text{-hydroxymetoprolol}}$  was markedly higher ( $0.931 \pm 0.178$  versus  $0.0724 \pm 0.0443$ ) in the *CYP2D6*\*10/\*10 group than in the *CYP2D6*\*1/\*1 after oral administration of 100 mg metoprolol. In addition, several reports have shown that patients lacking CYP2D6 genes or who are poor metabolizers of CYP2D6 substrates have little or no CYP2D6 activity, and that further enzyme inhibition from a CYP2D6 inhibitor does not affect exposure to a sensitive CYP2D6 substrate (Hamelin et al, 2000; Lessard et al, 2001; Damy et al, 2004; Feld et al, 2013). Thus, to evaluate the inhibitory effects of sarpogrelate on CYP2D6 activity, subjects with *CYP2D6*\*1/\*1 or \*1/\*2 genotype were enrolled in the drug-drug interaction study.

In the drug-drug interaction study, only modest inhibition was observed in subjects with an increase in the  $AUC_t$  and  $C_{max}$  of metoprolol by an average 1.53-fold (90% CI, 1.09 - 2.32) and 1.62-fold (90% CI, 1.16 - 2.58), respectively, after co-administration with sarpogrelate on the day (treatment B). Similar results were obtained after pretreatment of sarpogrelate for 3 days (treatment C):  $AUC_t$ , 1.51-fold (90% CI, 1.04 - 2.30) and  $C_{max}$ , 1.67-fold (90% CI, 1.23 - 2.55). The  $t_{max}$  of metoprolol was not affected by co-administration with sarpogrelate on the day (treatment B) or after pretreatment with sarpogrelate for 3 days (treatment C). These findings suggest that the increase in systemic exposure to metoprolol was unlikely to be due to increased oral absorption and was, therefore, likely to be due to inhibition of the metabolism of metoprolol in the presence of sarpogrelate. Increased exposures of metoprolol by co-administration with sarpogrelate on the day were similar to those observed upon pretreatment of sarpogrelate for 3 days. After multiple dosing of sarpogrelate, minimal accumulation of sarpogrelate and M-1 might be expected owing to the relatively short  $t_{1/2}$  of sarpogrelate and M-1.

Pharmacokinetic interaction results were not in the default "no-effect boundaries" of 0.8 - 1.25, but the observed drug-drug interaction can be classified as "weak". There is a consensus on the risk category of drug interactions based on the observed magnitude of the resulting AUC change (e.g., guidance provided by the US Food and Drug Administration). That is, a more than five-fold increase in substrate AUC upon inhibition is deemed to be a "strong" interaction; an increase between two-fold and five-fold is a "moderate" interaction; and a less than two-fold increase is a "weak interaction" (US Food and Drug Administration, 2012). In this study,

sarpogrelate was shown to exhibit weak inhibition of CYP2D6 (less than two-fold).

Decreases in HR, SBP, and DBP were observed after metoprolol administration. However, the intervals of change did not differ significantly among the three treatment groups. These findings suggest that co-administration with sarpogrelate or pretreatment with sarpogrelate do not have clinical importance with regard to the pharmacodynamics of metoprolol.

Despite the high *in vitro* inhibitory potencies of sarpogrelate ( $K_i$ , 1.24  $\mu$ M) and M-1 (0.120  $\mu$ M) for CYP2D6, weak clinical inhibition was observed. These results might be attributable to their considerably short  $t_{1/2}$  (sarpogrelate, 0.64 hour; M-1, 4.98 hour) and  $t_{max}$ . These short  $t_{1/2}$  values would be expected to shorten the duration of the inhibitory effects of sarpogrelate and M-1. The magnitude of CYP2D6 inhibition correlates with its plasma concentrations and dose (Preskorn et al, 1994; Hiemke and Härtter, 2000).

Taken together, although single or multiple co-administration of sarpogrelate showed a weak inhibitory effect on the pharmacokinetics of metoprolol, it had no clinically relevant effect on the pharmacodynamics of metoprolol.

## V. CONCLUSION

Sarpogrelate and M-1 were potent and selective competitive inhibitors of CYP2D6 *in vitro*. Especially, inhibition of CYP2D6 by M-1 was ten-fold more potent than that of sarpogrelate, and was similarly potent as quinidine, a well-known typical CYP2D6 inhibitor. Sarpogrelate weakly inhibited a sensitive CYP2D6 substrate, metoprolol, by increasing metoprolol exposure by less than two-fold, but sarpogrelate had few effects on the pharmacodynamics of metoprolol. Higher systemic exposure to metoprolol if co-administered with sarpogrelate is not expected to be clinically meaningful. Extrapolation of these results to clinical practice suggests that no special monitoring is necessary if administering sarpogrelate with sensitive CYP2D6 substrates.



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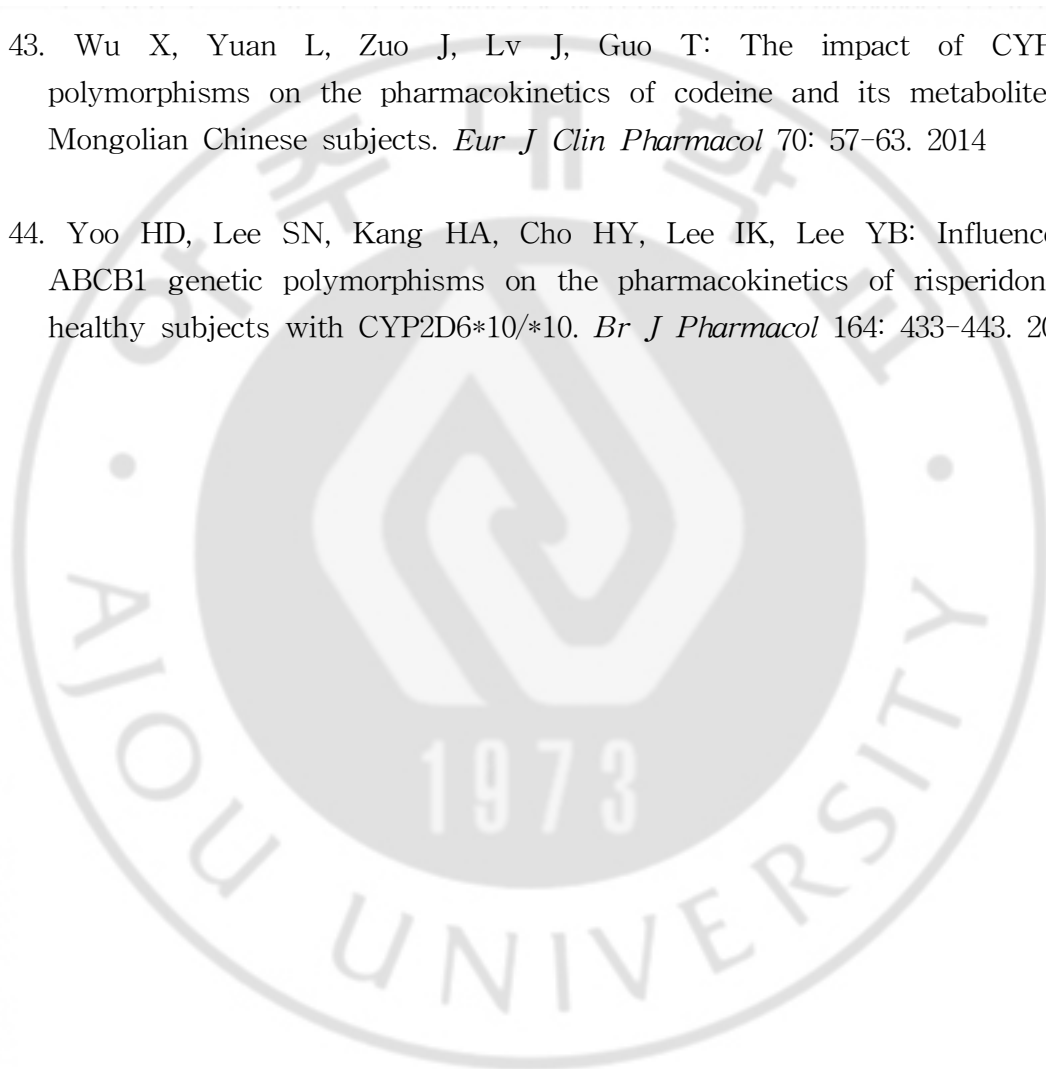
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## Sarpogrelate hydrochloride의 CYP2D6 억제 효과와 약물-약물 상호작용

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Sarpogrelate는 5-HT<sub>2A</sub> 수용체를 통한 혈소판 응집, 혈전 형성, 혈관수축, 혈관 평활근 세포 증식 등을 억제하는 효과가 있어 말초동맥질환에 의한 허혈 증상을 완화시킨다. Sarpogrelate가 널리 사용되고 우수한 약리작용을 가지고 있으나 아직까지 sarpogrelate와 M-1 (sarpogrelate의 활성대사체)의 CYP 동종효소에 대한 억제나 약물-약물 상호작용에 대한 정보는 없다.

사람 간 마이크로솜에서 sarpogrelate와 M-1이 아홉 가지 CYP 동종효소에 미치는 영향을 각테일 시험을 통해 측정하였다. CYP2D6\*1/\*1 또는 \*1/\*2 유전형을 가진 아홉 명의 건강한 남성을 대상으로 공개, 무작위배정, 3군 3기 교차 설계 임상시험을 실시하였다. 치료군은 metoprolol 100 mg 단독 투여 (치료군 A), sarpogrelate 100 mg 1일 2회와 metoprolol 100 mg 병용 투여 (치료군 B) 및 sarpogrelate 100 mg 1일 3회, 3일간 사전투여와 metoprolol 100 mg 병용 투여 (치료군 C)의 세군을 두었다. 혈장 중 metoprolol과  $\alpha$ -hydroxymetoprolol의 농도는 액체크로마토그래피-질량분석법 (LC-MS/MS)을 사용하여 정량하였다. Metoprolol에 대한 약력학적 반응으로 심박수와 혈압을 측정하였다.

Sarpogrelate는 선택적, 경쟁적으로 CYP2D6에 의한 dextromethorphan O-demethylation을 억제하였다 (IC<sub>50</sub> 3.05  $\mu$ M, K<sub>i</sub> 1.24  $\mu$ M). M-1 또한 CYP2D6



억제능을 보였는데, 그 억제효과는 sarpogrelate보다 강했다 ( $IC_{50}$  0.201  $\mu$ M,  $K_i$  0.120  $\mu$ M). Sarpogrelate의 병용 투여는 metoprolol의 혈장농도-시간곡선하면적 ( $AUC_t$ )을 각각 53% (치료군 B, 기하 평균 비율 1.53; 90% 신뢰구간, 1.09 - 2.32), 51% (치료군 C, 기하 평균 비율 1.51; 90% 신뢰구간, 1.04-2.30) 증가시켰다. Sarpogrelate에 의한 metoprolol의 최고혈장농도 ( $C_{max}$ )도 유사하게 증가하였다. 그러나 metoprolol에 의한 심박수와 혈압의 변화는 세 치료군 간에 유의한 차이를 보이지 않았다.

생체 외 (*in vitro*) 실험에서 sarpogrelate와 M-1은 CYP2D6의 선택적, 경쟁적 억제제였다. 특히 M-1에 의한 CYP2D6 억제는 sarpogrelate보다 10배 이상 강했다. Sarpogrelate는 CYP2D6 기질약물인 metoprolol의 혈장농도-시간곡선하면적을 2배미만으로 증가시킴으로써 약동학적으로 metoprolol의 대사를 약하게 억제하였으나, metoprolol의 약력학적 특성에 영향을 미치지 않았다. Sarpogrelate의 병용 투여로 인한 metoprolol 농도 증가의 임상적인 의미는 없는 것으로 판단되었다. 따라서 sarpogrelate와 CYP2D6 기질약물 병용 투여 시 특별한 모니터링이 필요하지는 않을 것으로 기대된다.

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핵심어: Sarpogrelate, CYP2D6 억제, 약물-약물 상호작용, Metoprolol, 약동학, 약력학