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RESEARCH ARTICLE

Effect of the potent CYP2D6 inhibitor sarpogrelate on the pharmacokinetics and pharmacodynamics of metoprolol in healthy male Korean volunteers

Doo-Yeoun Cho[1,2]*, Soo Hyeon Bae[3]*, Joeng Kee Lee[3], Jung Bae Park[3], Yang-Weon Kim[4], Sukhyang Lee[5], Euichaul Oh[3], Bom-Taeck Kim[1], and Soo Kyung Bae[3]

[1]Department of Family Practice and Community Health, Ajou University School of Medicine, Yeongtong-gu, Suwon, South Korea, [2]Clinical Trial Centre, Ajou University Medical Centre, Suwon, South Korea, [3]College of Pharmacy and Integrated Research Institute of Pharmaceutical Sciences, The Catholic University of Korea, Wonmi-gu, Bucheon, South Korea, [4]Department of Emergency Medicine, Inje University College of Medicine, Busan, South Korea, and [5]College of Pharmacy, Ajou University, Suwon, South Korea

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Abstract

1. Recently, we demonstrated that sarpogrelate is a potent and selective CYP2D6 inhibitor *in vitro*. Here, we evaluated the effect of sarpogrelate on the pharmacokinetics and pharmacodynamics of metoprolol in healthy subjects.
2. Nine healthy male subjects genotyped for *CYP2D6**1/*1 or *1/*2 were included in an open-label, randomized, three treatment-period and 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 and treatment B), or after pretreatment of sarpogrelate for three days (100 mg tid.; treatment C). Plasma levels of metoprolol and α-hydroxymetoprolol were determined using a validated LC-MS/MS method. Changes in heart rate and blood pressure were monitored as pharmacodynamic responses to metoprolol.
3. Metoprolol was well tolerated in the three treatment groups. In treatment B and C groups, the AUC_t of metoprolol increased by 53% (GMR, 1.53; 90% CI, 1.17–2.31) and by 51% (1.51; 1.17–2.31), respectively. Similar patterns were observed for the increase in C_{max} of metoprolol by sarpogrelate. However, the pharmacodynamics of metoprolol did not differ significantly among the three treatment groups.
4. Greater systemic exposure to metoprolol after co-administration or pretreatment with sarpogrelate did not result in clinically relevant effects. Co-administration of both agents is well tolerated and can be employed without the need for dose adjustments.

Keywords

CYP2D6 inhibition, drug–drug interaction, metoprolol, pharmacodynamics, pharmacokinetics, sarpogrelate

History

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Introduction

Sarpogrelate ((*R*,*S*)-1-{2-[2-(3-methoxyphenyl)ethyl]phenoxy}-3-(dimethylamino)-2-propyl hydrogen succinate chloride) is a highly specific 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptor antagonist. It was approved in Japan for the treatment of peripheral arterial disease in 1993 (Furukawa et al., 1991; 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 (Nishihira et al., 2006; Rashid et al., 2003). Additionally, it has beneficial effects against restenosis after coronary stenting (Fujita et al., 2003; Saini et al., 2004), pulmonary hypertension (Saini et al., 2004), angina pectoris (Kinugawa et al., 2002) and diabetes mellitus (Ogawa et al., 1999; Pietraszek et al., 1993). Sarpogrelate is metabolized to (±)-1-{2-[2-(3-methoxyphenil)ethyl]-phenoxy}-3-(dimethylamino)-2-propanol hydrochloride (M-1) upon hydrolysis (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 & Elz, 1995). In general, one 100 mg tablet of sarpogrelate is taken three times per day after meals (Kim et al., 2014). 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 μM at 0.7 h, and was eliminated rapidly from plasma with a half-life ($t_{1/2}$) of 0.8 h (Kim et al., 2014).

*These authors equally contributed to this work.

Address for correspondence: Soo Kyung Bae, PhD, College of Pharmacy and Integrated Research Institute of Pharmaceutical Sciences, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon 420-743, South Korea. Tel: +82 2 2164 4054. Fax: +82 2 2164 4096. E-mail: baesk@catholic.ac.kr

Bom-Taeck Kim, MD, PhD, Department of Family Practice & Community Health, Ajou University School of Medicine, 206, World cup-ro, Yeongtong-gu, Suwon 433-749, South Korea. E-mail: lovesong@ajou.ac.kr

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The active metabolite M-1 reached a C_{max} of 0.137 μM at 0.9 h and exhibited slower elimination than that shown by sarpogrelate, with a $t_{1/2}$ of 4.4 h (Kim et al., 2014).

Recently, we reported that sarpogrelate competitively inhibited CYP2D6-mediated dextromethorphan *O*-demethylation potently and selectively *in vitro* (inhibition constant [K_i], 1.24 μM). M-1 also inhibited CYP2D6 activity markedly *in vitro*; its inhibitory effect (K_i, 0.120 μM) was more potent than that of sarpogrelate (Cho et al., 2014). [I] represents the mean steady-state C_{max} value of an inhibitor exposed to the active site of an enzyme. Hence, based on the $1+[I]/K_i$ ratio, the potency of *in vivo* inhibition of sarpogrelate against completely CYP2D6-cleared drug (which represents the fold increase of the area under the curve [AUC] in the presence of the inhibitor [AUC_i]-to-AUC ratio) has been estimated to be 1.17−11.5 (Cho et al., 2014). This estimate of the magnitude of drug–drug interactions for a CYP2D6-cleared drug is largely attributable to whether the concentrations of unbound sarpogrelate or total sarpogrelate in plasma or the portal vein are most relevant to enzyme inhibition *in vivo* (Cho et al., 2014). If the ratio calculated above ≥1.1, then the clinical evaluation with a sensitive probe substrate is recommended (US Food and Drug Administration, 2012).

Metoprolol (1-(isopropylamino)-3[*p*-(β-methoxyethyl)-phenoxy]-2-propanol) is a selective β_1-receptor blocker. It is used widely for the treatment of angina pectoris, hypertension and coronary artery disease (Olsson et al., 1985; Regardh et al., 1983; Wikstrand et al., 1991). It undergoes significant first-pass metabolism, with approximately 85% of the dose converted mostly into an inactive metabolite, α-hydroxymetoprolol, via CYP2D6 (Lennard et al., 1986; McGourty et al., 1985; Tucker et al., 2001; Wang et al., 2008). Metoprolol is used as a CYP2D6 probe substrate for clinical studies of drug–drug interactions. With regard to sarpogrelate use in patients taking metoprolol, the metoprolol concentration could be increased owing to inhibition of CYP2D6-mediated metoprolol metabolism by sarpogrelate and M-1. However, the potential for clinical drug–drug interactions between sarpogrelate and metoprolol has not been evaluated.

The purpose of the present study was to evaluate the effect of sarpogrelate on the pharmacokinetics and pharmacodynamics of metoprolol (a typical CYP2D6 substrate *in vivo*) in healthy Korean volunteers. Genetic variants of the *CYP2D6* gene are known to play a major part in CYP2D6 activities. 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 (Damy et al., 2004; Feld et al., 2013; Hamelin et al., 2000; Lessard et al., 2001). Thus, to evaluate the inhibitory effects of sarpogrelate on CYP2D6 activity, subjects with *CYP2D6**1/*1 or *1/*2 genotype who were phenotyped as extensive metabolizers of CYP2D6 were enrolled in the present study.

Materials and methods

Subjects

Healthy male Korean volunteers who fulfilled the following criteria were eligible for the study: age, 19–55 years; weight, ≥55 kg and body weight within ± 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 (haematology, 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 three 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, haematological, 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 three months before administration of the study drug; donation of whole blood within two months or any blood products within one month before administration of the study drug; use of drugs that are inducers of CYP activity (e.g. phenobarbital) or inhibitors of CYPs within one month before administration of the study drug and use of prescription drugs or herbal remedies within two weeks or use of over-the-counter medication within one week before administration of the study drug.

The study protocol was approved by the Ethics Review Board of Ajou University Medical Centre (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.

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 (Kim et al., 2010; Lee et al., 2009; 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® SNaPshot™ Multiplex kit (Applied Biosystems, Foster City, CA) (Lee et al., 2009). Analyses were carried out using GeneMapper® v4.0 (Applied Biosystems). 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. Duplicate samples and

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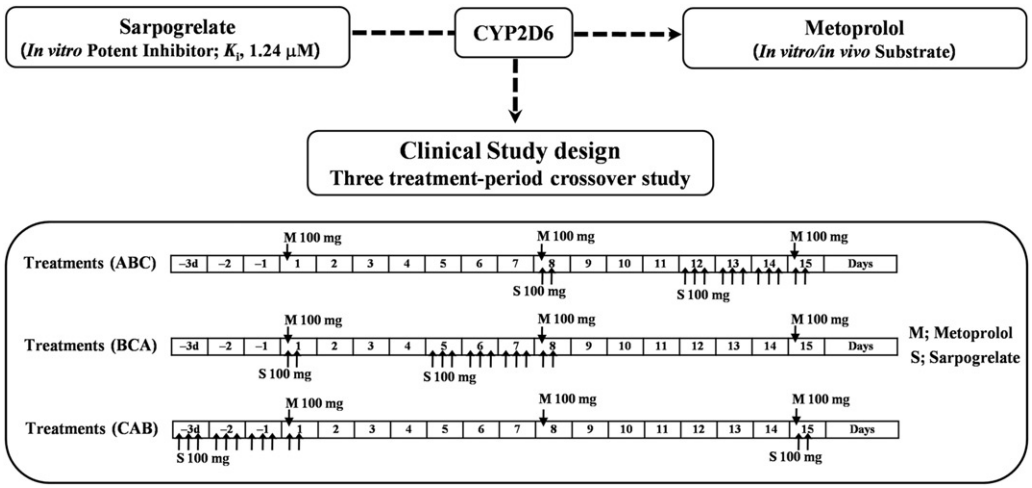

Figure 1. Clinical trial design.

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negative controls were included to ensure the accuracy of genotyping.

Study design

This randomized, open-label, three-period, crossover and single-centre, study was conducted at Ajou University Medical Centre Clinical Trial Centre (Suwon, South Korea). Nine healthy volunteers 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; Figure 1) according to a Latin-square design. The treatments administered were as follows: metoprolol 100 mg (Betaroc®; Yuhan Corporation, Yongin, South Korea) alone at 09:00 am (treatment A; metoprolol alone); metoprolol 100 mg at 09:00 am, with sarpogrelate 100 mg (Anplag®; Yuhan Corporation, Yongin, South 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 three days at 6-h intervals (treatment C; pretreatment of sarpogrelate for three days). There was a washout period of seven days between treatments. The subjects fasted overnight before metoprolol administration, and were allowed water *ad libitum* 2 h after dosing. Each dose was administered with 240 mL of water. All subjects received a standard meal at 4 h and 10 h after each 09:00 am dose. Following each dose, the subjects remained in the study centre for 24 h (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.

Sample collection

Samples of venous blood (3 mL) were drawn from a venous catheter in the forearm and collected into lithium heparin tubes just before drug administration and 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 24 h after metoprolol dosing. After centrifugation of blood samples, plasma samples (1.5 mL) were transferred immediately to polyethylene tubes and stored at −80 °C until analyses. In addition, heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded after 5-min rest in the sitting position pre-dose as well as 1, 2, 3, 4, 5, 6, 8, 10 and 12 h 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-min intervals. The average value was used for analyses and given as the change from the individual pre-dose value.

Bioanalytical methods

Plasma concentrations of metoprolol and α-hydroxymetoprolol were analyzed using liquid chromatography–tandem mass spectrometry according to the method of Bae et al. (2014). The system comprised a 1260 high-performance liquid chromatography (HPLC) setup (Agilent Technologies, Wilmington, DE) coupled with an API 3200 Triple Quadrupole Mass Spectrometer (AB Sciex, Foster City, CA). Briefly, metoprolol, α-hydroxymetoprolol and the internal standard (chlorpropamide) were extracted from plasma (50 μL) 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/min. The total run time was 3.0 min 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 → 116.2 for metoprolol, m/z 284.0 → 116.0 for α-hydroxymetoprolol and m/z 277.0→111.0 for chlorpropamide. The optimised 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 α-hydroxymetoprolol and 60 V, 45 V and 5.5 V for chlorpropamide, respectively. Nitrogen gas was used for the nebuliser, curtain and collision-activated dissociation gas at pressures of 20, 10 and 6 psi, respectively. The linear ranges of

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concentration for metoprolol and α-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. The intra- and inter-day precision ($n = 5$) of the assay ranged from 3.94% to 10.6%, and the intra- and inter-day accuracies ($n = 5$) ranged from 89.0 to 109%. No relevant cross-talk or matrix effect was observed. All analytes were stable under various conditions of storage and handling, and relevant crosstalk and matrix effects were not observed.

Pharmacokinetic/pharmacodynamic assessments

The pharmacokinetic parameters of metoprolol and α-hydroxymetoprolol were calculated by non-compartmental analytical methods using WinNonlin Professional v5.2 (Pharsight Corporation, Mountain View, CA). 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 $AUC_{0-\infty} = AUC_t + C_{12\,h}/k_e$, where $C_{12\,h}$ was the plasma concentration measured 12 h after metoprolol administration. As a pharmacodynamic test, the area under the effect curve from 0 h to 12 h ($AUEC_{0-12\,h}$) for HR, SBP and DBP was calculated using the trapezoidal rule.

Tolerability assessment

Safety was evaluated throughout the study based on adverse events (AEs), vital signs (blood pressure, pulse rate and body temperature) and results of physical examination, laboratory tests (haematology, blood biochemistry and urinalyses) and 12-lead ECG at predetermined time points. The causal relationships for all AEs were categorized by the investigator as probable, possible or not related.

Statistical analysis

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 (Karonen et al., 2011; Misaka et al., 2013; Stout et al., 2010).

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 α-hydroxymetoprolol by using the general linear mixed-effects model in SAS v9.1.3 (SAS Institute, Cary, NC). 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 three 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_{max} was assessed using a non-parametric analysis with the Wilcoxon signed rank test; $p < 0.05$ was considered significant. Statistical analyses were carried out using SAS v9.1.3. Pharmacokinetic data are the mean ± 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 $AUEC_{0-12\,h}$ 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.

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

Results

Subjects

In the 50 subjects evaluated, the allele frequencies of *CYP2D6**1, *2, *10, *41 and *5 were 36.0%, 8.0%, 52.0%, 2.0% and 2.0%, respectively. This result was similar to 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 (Choi et al., 2012; Jin et al., 2008; Lim et al., 2008; Wu et al., 2014; Yoo et al., 2011 and references therein). 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.

Pharmacokinetic changes of metoprolol and α-hydroxymetoprolol

Mean plasma concentrations of metoprolol and α-hydroxymetoprolol with and without (metoprolol alone: treatment A) co-administration of sarpogrelate (treatment B or treatment

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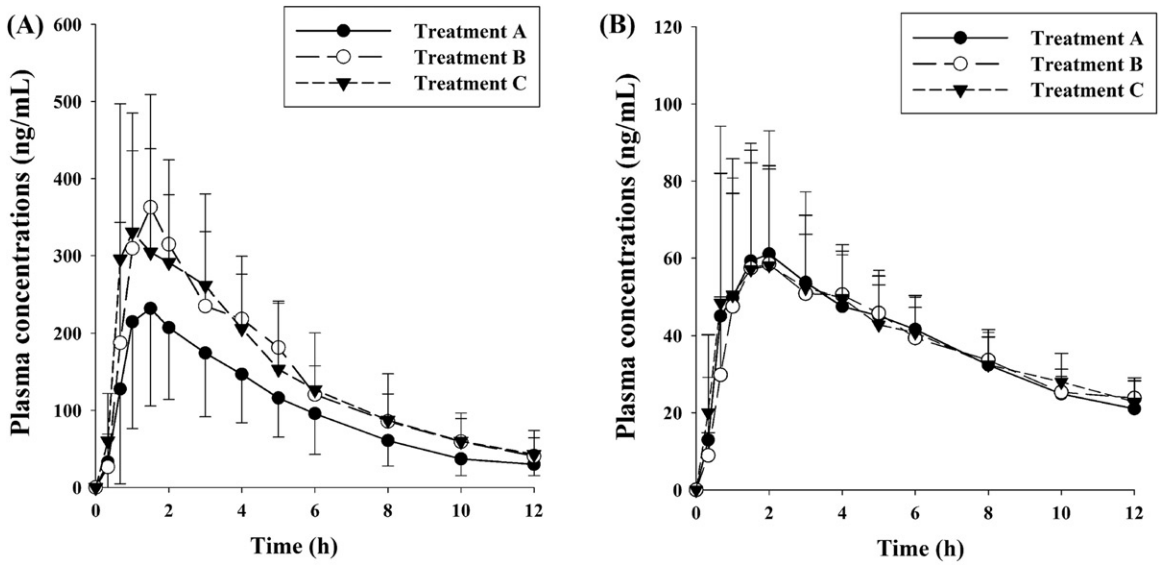

Figure 2. Mean plasma concentrations of metoprolol (A) and α-hydroxymetoprolol (B) after oral administration of 100 mg metoprolol alone (●, treatment A) and co-administered with sarpogrelate on the day (○, treatment B) or pretreatment with sarpogrelate for three days (▼, treatment C) in nine healthy male subjects. Bars represent standard deviation.

Table 1. Pharmacokinetic parameters (mean ± SD) of metoprolol and α-hydroxymetoprolol after oral administration of 100 mg metoprolol alone (treatment A), co-administered with sarpogrelate (100 mg bid.; a total dose of 200 mg and treatment B) or pretreatment of sarpogrelate for three days (100 mg tid.; treatment C) in nine healthy volunteers.

	Treatment A ($n = 9$) Mean ± SD	Treatment B ($n = 9$) Mean ± SD	GMR[a] (90% CI[b])	Treatment C ($n = 9$) Mean ± SD	GMR[a] (90% CI[b])
Metoprolol					
AUC_t (ng h/mL)[c]	1210 ± 560	1580 ± 537	1.53 (1.09–2.32)	1520 ± 748	1.51 (1.04–2.30)
C_{max} (ng/mL)[d]	279 ± 116	404 ± 119	1.62 (1.16–2.58)	415 ± 89.4	1.67 (1.23–2.55)
t_{max} (h)[e]	1.50 (0.67–5.0)	1.50 (0.67–4.0)		1.0 (0.67–3.0)	
Terminal $t_{1/2}$ (h)	3.28 ± 0.440	3.43 ± 0.809		3.50 ± 0.560	
α-hydroxymetoprolol					
AUC_t (ng h/mL)[c]	456 ± 122	425 ± 120	0.971 (0.780–1.21)	442 ± 76.3	0.989 (0.797–1.23)
C_{max} (ng/mL)[d]	66.7 ± 39.9	56.7 ± 24.1	0.884 (0.593–1.32)	64.8 ± 26.9	0.891 (0.588–1.35)
t_{max} (h)[e]	1.50 (0.67–5.0)	1.50 (1.5–4.0)		1.50 (0.67–6.0)	
Terminal $t_{1/2}$ (h)	5.30 ± 1.53	6.90 ± 2.06		7.01 ± 0.945	

[a]Geometric mean ratio treatment B or treatment C to treatment A.
[b]Ninenty percent confidence interval.
[c]Total area under the plasma concentration–time curve from time zero to time last sampling time.
[d]Peak plasma concentration.
[e]Time to reach C_{max}; median (ranges).

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C) are shown in Figure 2(A) and (B). The relevant pharmacokinetic parameters of metoprolol and α-hydroxymetoprolol as well as GMR and 90% CI are listed in Table 1.

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 variations were noted) (Figure 2A). There were no apparent differences among the three treatment groups with regard to the plasma concentrations of α-hydroxymetoprolol (Figure 2B). In treatment B, the AUC_t and C_{max} of metoprolol increased by 53% and 64% compared with those in the treatment A (metoprolol alone)-based GMR of 1.53 (90% CI, 1.09–2.32) and 1.64 (90% CI, 1.16–2.58), respectively (Table 1). The C_{max} of α-hydroxymetoprolol decreased slightly by 11.6% in treatment B; GMR of 0.884 (90% CI, 0.593–1.32), but the AUC_t did not decrease; GMR of 0.971 (90% CI, 0.780–1.21) (Table 1). The terminal $t_{1/2}$ and t_{max} of metoprolol and α-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% (1.23–2.55) compared with metoprolol alone (Table 1). The GMRs (90% CI) for α-hydroxymetoprolol were C_{max}, 0.891 (0.588–1.35) and AUC_t, 0.989 (0797–1.23). In treatment C, multiple dosing of sarpogrelate did not affect the terminal $t_{1/2}$ and t_{max} of metoprolol or α-hydroxymetoprolol; they were not significantly different from those in treatment A (Table 1). Increased exposures of metoprolol by co-administration with sarpogrelate alone (treatment B) were similar to those observed upon pretreatment with sarpogrelate for three days (treatment C). Intra-subject changes in the respective AUC_t and C_{max} of metoprolol among the three treatments are shown in Figure 3.

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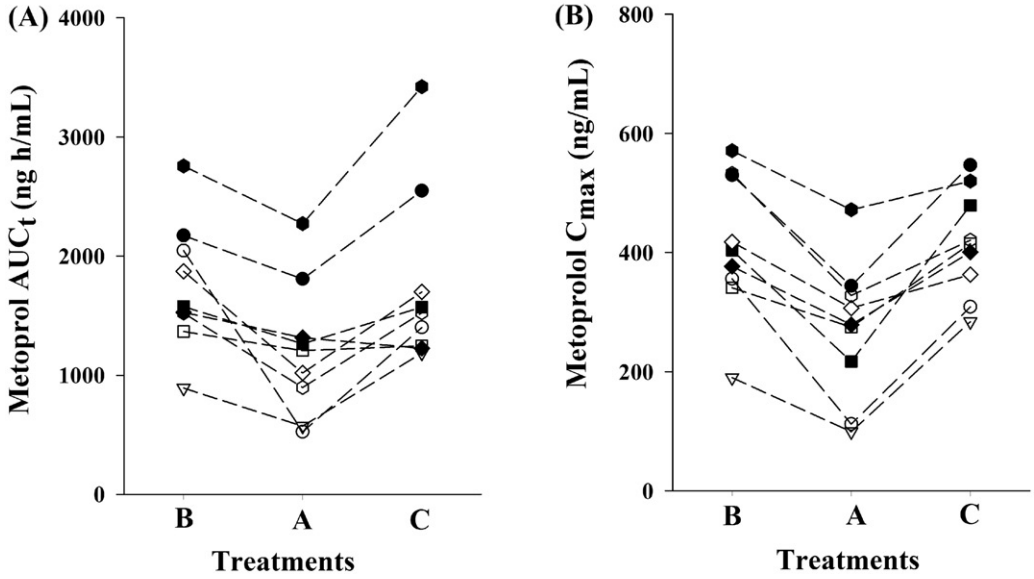

Figure 3. Intra-individual changes in the respective AUC_t (A) and C_{max} (B) of metoprolol among the three treatment groups.

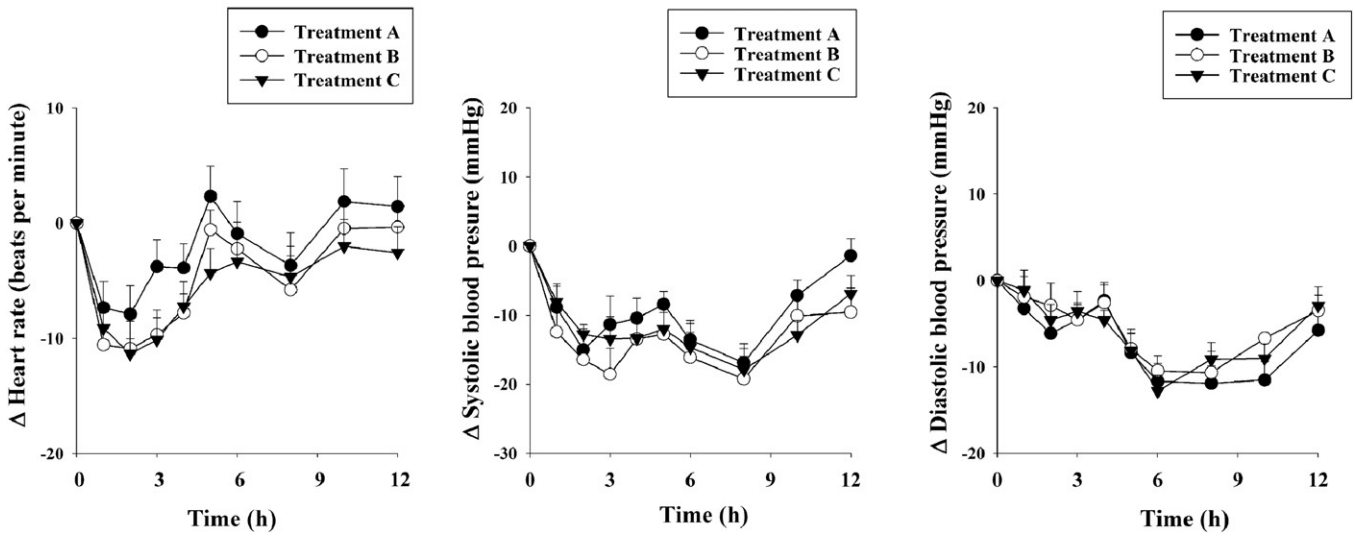

Figure 4. Pharmacodynamic responses to metoprolol. The differences from baseline value of heart rate (A), systolic blood pressure (B) and diastolic blood pressure (C) after oral administration of 100 mg metoprolol alone ($n = 9$; ●, treatment A) and co-administered with sarpogrelate on the day (○, treatment B) or pretreatment with sarpogrelate for three days (▼, treatment C). Bars represent standard error.

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Pharmacodynamic changes 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 ± 8.81 beats/min, 116 ± 9.54 mmHg and 66.4 ± 7.31 mmHg, respectively. Baseline values of HR, SBP and DBP among the three treatment groups were similar (Figure 4). Changes in HR, SBP and DBP >12 h after metoprolol administration are expressed as the difference from the baseline value in Figure 3. 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 pre-dose levels ≈ 12 h 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 (data not shown). Hence, co-administration of sarpogrelate or pretreatment with sarpogrelate was considered not to have an important effect on the pharmacodynamics of metoprolol.

Tolerability

Nine subjects completed the study with no serious AEs and no clinically significant changes in vital signs, laboratory values or 12-lead ECG.

Discussion

In the present study, metoprolol was dosed at 100 mg and sarpogrelate 100 mg up to three times daily to best reflect the

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clinical treatment of hypertensive subjects. We wished to evaluate the effect of sarpogrelate (a potent CYP2D6 inhibitor *in vitro*) on the pharmacokinetics and pharmacodynamics of metoprolol (a sensitive CYP2D6 substrate *in vivo*). Furthermore, the probability of co-administration of sarpogrelate and metoprolol in the clinic is high. In addition, evaluation of the *in vivo* interactions between metoprolol with sarpogrelate may provide useful information for safe and efficacious therapy.

Several studies have shown that patients without 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 result in further reduction in CYP2D6 activity; thus, no meaningful changes in the overall exposure to metoprolol would be observed (Damy et al., 2004; Feld et al., 2013; Hamelin et al., 2000; Lessard et al., 2001). Hence, subjects with *CYP2D6**1/*1 or *1/*2 genotypes were enrolled prospectively in this 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 (1.16–2.58), respectively, after co-administration with sarpogrelate on the day (treatment B, Table 1). Similar results were obtained after pretreatment of sarpogrelate for three days (treatment C): AUC_t, 1.51-fold (90% CI, 1.04–2.30) and C_{max}, 1.67-fold (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 three 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 three 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. These 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 5-fold increase in substrate AUC upon inhibition is deemed to be a ''strong'' interaction; an increase between 2-fold and 5-fold is a ''moderate'' interaction; and a less than 2-fold increase is a ''weak interaction'' (US Food and Drug Administration, 2012). In the present study, sarpogrelate was shown to exhibit weak inhibition of CYP2D6 (less than 2-fold). Decreases in HR, mean SBP and mean 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 μM) and M-1 (0.120 μM) for CYP2D6, weak clinical inhibition was observed. These results might be attributable to their considerably short $t_{1/2}$ (sarpogrelate, 0.64 h; M-1, 4.98 h) and t_{max} (0.9 h; 1.08 h). 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 (Hiemke & Härtter, 2000; Preskorn et al., 1994).

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. However, there are limitations to our conclusions. We determined the pharmacodynamic effects (i.e. HR, SBP and DBP) at rest in healthy subjects. It has been reported that haemodynamic parameters such as HR during exercise are good markers of β-blocker activity in non-hypertensive subjects (Hemeryck et al., 2000). In addition, our results were limited by the relatively small sample size ($n = 9$) and because all participants were healthy volunteers.

Conclusion

Sarpogrelate weakly inhibited a sensitive CYP2D6 substrate, metoprolol, by increasing metoprolol exposure by less than 2-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 our results to clinical practice suggests that no special monitoring is necessary if administering sarpogrelate with sensitive CYP2D6 substrates.

Declaration of interest

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