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Original Article

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Common *ABCB1* SNP, C3435T could affect systemic exposure of dapagliflozin in healthy subject

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ABSTRACT

P-glycoprotein (P-gp) is a transporter that plays an excretory role in epithelial cells. It is encoded by *ABCB1*, and single nucleotide polymorphisms (SNPs) in this gene can affect systemic drug exposure. Dapagliflozin and sitagliptin, used in type 2 diabetes treatment, are P-gp substrates. Here, we aimed to investigate whether *ABCB1* polymorphisms affect dapagliflozin and sitagliptin pharmacokinetics (PK) in healthy Korean subjects.
The study population consisted of 100 healthy Korean subjects (94 men and 6 women) who participated in four different clinical trials and received dapagliflozin and sitagliptin doses of 10 and 100 mg, respectively. We determined *ABCB1* genotypes for the C3435T, C1236T, and G2677T/A SNPs. The relationship between the genotypes and dapagliflozin PKs was examined. Dapagliflozin and sitagliptin PK parameters were not statistically significantly affected by *ABCB1* SNP genotypes. However, homozygous 3435TT subjects showed higher dapagliflozin PK parameters than CT and CC subjects. In subjects with the 3435TT and those with 3435CC and 3435CT genotypes, mean C_{max}, AUC_{inf}, and $AUC_{0\text{-}t}$ values of dapagliflozin were 223.06 ng/mL and 194.81 ng /mL ($p = 0.2767$), 673.58 ng*h/mL and 573.96 ng*h/mL ($p = 0.0492$), and 128.53 ng*h/mL and 104.61 ng*h/mL ($p = 0.2678$), respectively.
In summary, dapagliflozin and sitagliptin PK parameters were not significantly different between individuals with C1236T and C2677T/A ABCB1 genetic polymorphisms. Dapagliflozin exhibited higher systemic exposure in 3435TT subjects than in CC/CT subjects.

Keywords: Single Nucleotide Polymorphism; ATP-Binding Cassette Transporter; P-glycoprotein; Sodium-Glucose Transporter 2 Inhibitors; Dipeptidyl-Peptidase IV Inhibitors

INTRODUCTION

The concentrations and clinical effects of most drugs on the market differ between individuals due to several factors. To provide more effective treatment and prevention strategies against diseases, the concept of personalized drug therapy i.e., precision medicine, has emerged. To achieve the goals of precision medicine, it is essential to identify disease- and drug metabolism-related genetic or genomic factors [1,2].

Funding

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Data Availability Statement

Data supporting the findings of this study are available from the corresponding author, Min Kyu Park, upon reasonable request.

Principle Investigator Statement

The authors confirm that the principal investigator for this paper is Min Kyu Park, and that he had direct clinical responsibility for the subjects.

Conflict of Interest

- Authors: Nothing to declare
- Reviewers: Nothing to declare
- Editors: Nothing to declare

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Author Contributions

Conceptualization: Hwang JG, Lee S, Park MK; Data curation: Hwang JG; Investigation: Hwang JG; Methodology: Hwang JG, Lee Y; Project administration: Park MK; Software: Jung SI, Kim YK, Lee Y; Validation: Ji SC; Visualization: Jung SI; Writing - original draft: Hwang JG, Jung SI; Writing - review & editing: Kim YK, Lee S, Park MK.

According to the US FDA early phase clinical trial guidelines, genetic differences between individuals can affect factors associated with several disease and its treatment, including the rate of disease occurrence and the risk of disease progression or recurrence. Therefore, blood and/or urine DNA sample collection from healthy subjects, as well as the collection of information on metabolic or transporter gene polymorphisms that influence drug exposure and response, are recommended [3]. ICH E17 guidelines suggest that the impact of genetic differences, such as polymorphisms in drug-metabolizing enzymes or drug molecular targets, on the effects of treatments, could be explored in an early phase clinical trials. Furthermore, the definition of terms used in pharmacogenomics, as well as sample handling methods, are described in the E15 and E18 guidelines [4,5].

Sitagliptin was the first dipeptidyl peptidase 4 (DPP4) inhibitor to be approved by US FDA. DPP4 inhibitors prolong the action of incretin hormones, such as glucagon-like peptide 1 and glucose-dependent insulinotropic pepttide, which stimulate insulin secretion and suppress glucagon secretion, respectively [6,7]. Thus, DPP4 inhibitors are widely used as monotherapies or in combination with other antihyperglycemic drugs for type 2 diabetes mellitus (T2DM) treatment [8].

Dapagliflozin is a potent and selective sodium-glucose co-transporter 2 (SGLT2) inhibitor which improves glycemic control in T2DM patients [9,10]. As dapagliflozin has been shown to lower the risk of cardiovascular disease that is a major T2DM complication, and improves hyperglycemia independently of insulin, it is an attractive option for T2DM treatment in combination with DPP4 inhibitors [11]. Until present, several studies have demonstrated the effectiveness of the combined use of SGLT2 and DPP4 inhibitors for T2DM treatment [12,13].

Dapagliflozin is mainly metabolized via direct UGT1A9-mediated glucuronide conjugation; however, sitagliptin is barely metabolized and over 80% of the drug is eliminated as unchanged compound in the urine [14,15]. Therefore, there is no PK interaction between dapagliflozin and sitagliptin [16]. Despite differences in metabolism, both these drugs are P-glycoprotein (P-gp) substrates [14,17].

P-gp, also called ATP-binding cassette transporter B1 (ABCB1) or multidrug resistance protein 1, is a transporter protein expressed in the membranes of several tissues, including colonic, small intestinal, pancreatic and bile ductile, blood-brain barrier, and kidney proximal tubular tissues. On the apical surfaces of intestinal or bile duct enterocytes and renal tubular cells, P-gp plays an excretory role [18,19].

Single-nucleotide polymorphisms (SNPs) of *ABCB1*, which encodes P-gp, have been widely studied as they affect the systemic concentrations of some drugs. Among these SNPs, the most well-studied include C1236T (rs1128503) on exon 12, G2677T/A (rs2032582) on exon 21, and C3435T (rs1045642) on exon 26. G2677T/A is a non-synonymous SNP that directly influences amino acid substitutions. Although C1236T and C3435T are considered silent SNPs, some studies have reported that mutations in their sequences are associated with changes in the characteristics of their corresponding proteins [20-22].

This study therefore aimed to investigate the relationship between dapagliflozin/sitagliptin pharmacokinetics (PK) and *ABCB1* SNPs in healthy Korean subjects who received single oral dapagliflozin and sitagliptin doses.

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METHODS

Subjects

The study population consisted of 100 healthy Korean subjects (94 men and 6 women) who participated in 4 different clinical trials performed at Clinical Trial Center of Chungbuk National University Hospital. All studies were approved by the Korean Ministry of Food and Drug Safety, as well as the Institutional Review Board of Chungbuk National University Hospital (IRB No.: H2020-07-016, H2021-06-012, H2021-09-031, H2022-04,010). In addition, the studies were conducted in accordance with the Declaration of Helsinki and within the tenets of Korean Good Clinical Practices. Participants provided written informed consent during the screening period before any study-related procedures were performed.

Included in this study were subjects aged 19 years or more, with body mass indices (BMI) ranging from 18.0–30.0 kg/m^2. Subjects were excluded from the study if they had a clinically significant history or were suffering from any disease that could affect dapagliflozin and sitagliptin safety or PK. In addition, subjects with estimated glomerular filtration rates less than 60 mL/min/1.73 m^2, as determined using the CKD-EPI equation, as well as those with AST, ALT, GGT, or total bilirubin levels greater than 2 times the upper normal limit were excluded from the study.

Study design

All 4 trials had a randomized, single-dose, two-sequence, two-period, crossover design. Subjects were orally administered 10 mg of dapagliflozin (Forxiga Tab 10 mg) and 100 mg of sitagliptin (Januvia Tab 100 mg) with 150 mL of water at a period determined based on the randomization method. Washout periods were at least 7 days.

PK evaluation

Plasma dapagliflozin concentrations were determined before drug administration and at 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours following drug administration. In two of the clinical trials, blood was collected at 15-min intervals rather than 20-min intervals, and additional samples were collected 5 and 7 hours following drug administration. Plasma sitagliptin concentrations were evaluated from blood samples collected before drug administration and at 15, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 48 hours after administration. In two of the clinical trials, additional blood samples were collected 10 minutes and 72 hours following drug administration. At each blood sampling point, 5–8 mL of blood was collected in a sodium heparin tube and centrifuged at 3,000 rpm for 10 minutes at 4°C. Then, the supernatant was collected in Eppendorf tubes and stored at −70°C until analysis. In each analysis in a separate study, the concentration of the plasma dapagliflozin and sitagliptin was measured using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) in negative-ion and positive-ion electrospray mode, respectively. The entire analytical procedure was validated in terms of linearity, accuracy, precision, limit of detection and limit of quantification, interference, carryover, selectivity and matrix effect on its performance, recovery, dilution integrity, and stability.

Single-dose dapagliflozin (10 mg) and sitagliptin (100 mg) PK parameters were determined through a non-compartmental method using Phoenix® WinNonlin® 8.1 (Certara, L.P., St. Louis, MO, USA). The PK parameters evaluated included the maximum plasma concentration (C_{max}), which was directly determined from the individual drug plasma concentration-time profiles. The areas under the concentration-time curve from time 0 extrapolated to infinite,

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from time 0 to 1, and from time 0 to 2 (AUC_{inf}, AUC_{0-1}, and AUC_{0-2}) were calculated using linear-up log-down trapezoidal rules.

Genotyping

Blood samples (3 mL) were collected from each subject before drug administration. The DNA for ABCB1 genotyping was isolated from 100 μL of peripheral whole blood using a Maxwell® CSC Blood DNA Kit and Maxwell® CSC Instrument (Promega, Madison, WI, USA). The genotyping was conducted using TaqMan allelic discrimination assays on a real time-polymerase chain reaction (PCR) System (Applied Biosystems®, Foster City, CA, USA). The PCR reaction mixture comprised 5 μL of 2X TaqMan Universal Master Mix II, 0.5 μL of 20X Drug Metabolism Genotyping Assay Mix, 1 μL of DNA, and 3.5 μL of DNase-free water. The genotyping for the ABCB1 SNPs rs1128503 (1236C>T, assay ID: C___7586662_10), rs2032582 (2677G>T/A, assay ID: C__11711720C_30, C__11711720D_40), and rs1045642 (3435C>T, assay ID: C___7586657_20) were performed with validated TaqMan Genotyping Assays purchased from Applied Biosystems. The PCR reactions were carried out as follows: initial denaturation at 95°C for 10 minutes, 40 cycles for denaturation at 95°C for 15 seconds, and anneal/extension at 60°C for 1 minute. After the amplification, the allelic discrimination results were obtained by performing an end-point read using a QuantStudio™ 3 Real-Time PCR Systems software version 1.5.2 (Applied Biosystems).

Statistical analysis

Statistical analyses were performed using the SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA). All descriptive data are presented as mean ± standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. To compare the demographic and PK parameters of each ABCB1 mutation, one-way analysis of variance (ANOVA) or the Kruskal-Wallis test was used, depending on the normality, as determined using the Shapiro-Wilk and Kolmogorov–Smirnov tests.

RESULTS

ABCB1 genotype distribution in Korean subjects

The genotype and allele frequencies for the C1236T, G2677T/A, and C3435T SNPs in the 100 Korean subjects are shown in **Table 1**. No statistically significant genotype distributions were observed ($p = 0.4924$, 0.4289, and 1.0000 for C1236T, G2677T/A, and C3435T, respectively), and the population distribution for all three SNPs was in Hardy-Weinberg equilibrium. The C1236T, G2677T, G2677A, and C3435T mutations had variant allele frequencies of 54.5%,

Table 1. *ABCB1* SNP genotype and allele frequencies in healthy Korean subjects

SNP	Exon	Genotype	No. (%)	Allele	No. (%)
C1236T	12	CC	19 (19.0)	C	91 (45.5)
		CT	53 (53.0)	T	109 (54.5)
		TT	28 (28.0)		
G2677T/A	21	GG	25 (25.0)	G	97 (48.5)
		GT	27 (27.0)	T	62 (31.0)
		GA	20 (20.0)	A	41 (20.5)
		TT	9 (9.0)		
		TA	17 (17.0)		
		AA	2 (2.0)		
C3435T	26	CC	49 (49.0)	C	140 (70.0)
		CT	42 (42.0)	T	60 (30.0)
		TT	9 (9.0)		

SNP, single-nucleotide polymorphisms.

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31.0%, 20.5%, and 30.0%, respectively. The distributions for each genotype were similar to those previously published in Japanese and Chinese populations [23,24]. The mean (SD) age, height, weight, and BMI of the subjects were 25.42 (6.90) years, 172.76 (5.96) cm, 71.83 (9.55) kg, and 24.02 (2.54) kg/m^2, respectively, and none of these factors was found to affect the genotype distribution (**Supplementary Table 1**).

Influence of individual SNPs on dapagliflozin PK

Subjects were grouped according to SNP genotype, and the influence of SNPs on dapagliflozin PK was evaluated. Following the administration of a single 10-mg oral dose of dapagliflozin, there was a significant difference in AUC_{inf} between subjects of the 3435CC and 3435CT mutations (n = 91) and those of 3435TT mutation (n = 9), with observed mean (SD) AUC_{inf} values of 573.96 (154.80) ng*h/mL and 673.58 (118.21) ng*h/mL, respectively (p = 0.0492) (**Fig. 1**, **Table 2**). However, although both C_{max} and AUC_{inf} of plasma dapagliflozin were found to be higher for the 3435TT genotype than for the 3435CC and 3435CT genotypes, with their mean (SD) C_{max} and AUC_{inf} values being 223.06 (75.94) ng/mL, 197.79 (74.71) ng/mL, and 191.34 (71.79) ng/mL (p-value = 0.5039), and 673.58 (118.21) ng*h/mL, 578.91 (157.73) ng*h/mL, and 568.17 (153.00) ng*h/mL (p-value = 0.1715) for the 3435TT, 3435CC, and 3435CT genotypes, respectively, (**Table 3**), there was no significant difference in its PK with respect to ABCB1 genetic polymorphism (**Figs. 2** and **3**). Their $AUC_{0\text{-}1}$ values representing characteristics of absorption phase were 128.53 (62.87) ng*h/mL, 103.47 (56.20) ng*h/mL, and 105.95 (51.31) ng*h/mL, respectively.

For C1236T mutations, mean (SD) of C_{max}, AUC_{inf}, and $AUC_{0\text{-}1}$ for the 1236CC, 1236CT, and 1236TT genotypes were 217.69 (84.87) ng/mL, 184.93 (71.14) ng/mL, and 207.07 (67.03) ng/mL, (p = 0.1770), 631.29 (176.67) ng*h/mL, 561.59 (147.94) ng*h/mL, and 590.47 (146.51) ng*h/mL (p = 0.2299), and 112.84 (60.70) ng*h/mL, 101.43 (51.04) ng*h/mL, and 112.74 (58.06) ng*h/mL (p = 0.6065), respectively. We did not evaluate the 2677AA SNP due to its low frequency; in addition, no significant trends were observed for the 2677T/A SNP.

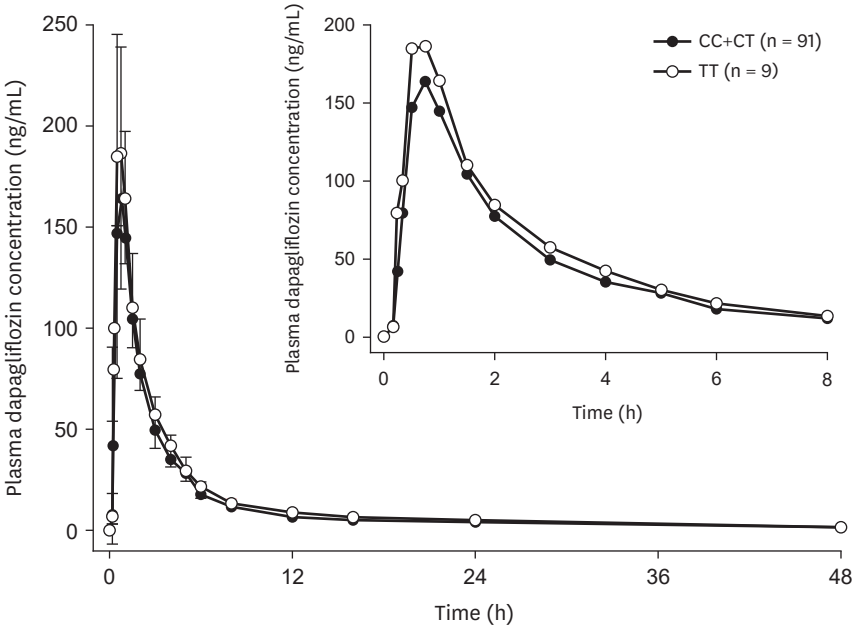

Figure 1. Comparison of dapagliflozin plasma concentration time-profiles between groups with different genotypes at position 3435 of *ABCB1* following the administration of a single 10-mg oral dose. Values are presented as mean ± standard deviation. (●): 3435CC and 3435CT; (○): 3435TT.

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Table 2. Effects of alleles types on dapagliflozin and sitagliptin pharmacokinetic parameters in healthy Korean subjects

SNP	Genotype	No.	Dapagliflozin						Sitagliptin					
			C_{max} (ng/mL)		AUC_{inf} (ng*h/mL)		AUC_{0-1} (ng*h/mL)		C_{max} (ng/mL)		AUC_{inf} (ng*h/mL)		AUC_{0-2} (ng*h/mL)	
			Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value
C1236T	CC	19	217.69 ± 84.87	0.2579[A]	631.29 ± 176.67	0.1501[A]	112.84 ± 60.70	0.6068[A]	506.87 ± 117.07	0.0821[A]	3,707.20 ± 739.53	0.1521[K]	496.44 ± 157.17	0.2072[A]
	CT+TT	81	192.58 ± 70.13		571.58 ± 147.18		105.34 ± 53.48		456.01 ± 123.74		3,436.61 ± 584.59		466.21 ± 202.20	
	TT	28	207.07 ± 67.03	0.2386[K]	590.47 ± 146.51	0.6723[A]	112.74 ± 58.06	0.4224[K]	473.77 ± 144.98	0.8544[A]	3,444.94 ± 534.23	0.3549[K]	453.58 ± 229.61	0.3555[A]
	CC+CT	72	193.57 ± 75.79		579.99 ± 157.80		104.44 ± 53.55		462.52 ± 115.15		3,504.78 ± 655.77		479.10 ± 179.71	
G2677T/A	GG	25	202.29 ± 81.00	0.7121[A]	587.70 ± 165.83	0.9203[A]	109.18 ± 66.63	0.9542[A]	467.26 ± 109.77	0.8357[A]	3,360.93 ± 506.44	0.1238[A]	451.55 ± 196.50	0.7935[A]
	GA+GT	47	192.43 ± 64.71		583.89 ± 159.82		102.07 ± 47.93		467.77 ± 140.92		3,628.80 ± 723.63		467.12 ± 193.94	
	TT	9	215.62 ± 85.98	0.5673[A]	631.79 ± 151.87	0.1989[A]	124.04 ± 68.38	0.5993[A]	465.73 ± 111.86	0.7434[A]	3,477.17 ± 596.14	0.6441[K]	507.45 ± 262.86	0.9524[A]
	AT+GT	44	194.80 ± 69.92		562.35 ± 134.88		107.34 ± 49.43		457.31 ± 142.79		3,475.54 ± 576.82		477.65 ± 192.07	
	AA*	2	183.49	0.8735[K]	600.61	0.8735[K]	71.06	0.3900[K]	584.75	0.1710[K]	3,481.41	0.9239[K]	633.05	0.2388[K]
	AT+GA	37	192.58 ± 71.86		574.00 ± 160.98		104.23 ± 45.40		457.90 ± 98.21		3,501.79 ± 714.08		464.22 ± 162.43	
C3435T	CC	49	197.79 ± 74.71	0.9535[A]	578.91 ± 157.73	0.7583[A]	103.47 ± 56.20	0.4589[A]	474.14 ± 116.38	0.3878[A]	3,530.20 ± 669.78	0.5444[A]	471.06 ± 195.51	0.7886[A]
	CT+TT	51	196.94 ± 72.79		586.77 ± 151.92		109.93 ± 53.54		457.53 ± 130.73		3,447.51 ± 576.31		472.82 ± 194.67	
	TT	9	223.06 ± 75.94	0.2767[A]	673.58 ± 118.21	0.0492[K]	128.53 ± 62.87	0.2678[K]	472.81 ± 102.95	0.7438[A]	3,528.50 ± 564.05	0.8756[K]	511.11 ± 259.84	0.8114[A]
	CC+CT	91	194.81 ± 73.04		573.96 ± 154.80		104.61 ± 53.72		464.97 ± 125.88		3,484.02 ± 630.25		468.08 ± 187.81	

p-values are obtained from Kruskal-Wallis test.
Data are expressed as arithmetic mean ± SD, unless otherwise stated.
SNP, single-nucleotide polymorphisms; C_{max}, peak serum concentration; AUC, area under the serum concentration-time curve; SD, standard deviation.
*Data are presented as means.

Table 3. Effects of individual SNPs on dapagliflozin and sitagliptin pharmacokinetic parameters in healthy Korean subjects

SNP	Genotype	No.	Dapagliflozin						Sitagliptin					
			C_{max} (ng/mL)		AUC_{inf} (ng*h/mL)		AUC_{0-1} (ng*h/mL)		C_{max} (ng/mL)		AUC_{inf} (ng*h/mL)		AUC_{0-2} (ng*h/mL)	
			Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value
C1236T	CC	19	217.69 ± 84.87	0.1770[A]	631.29 ± 176.67	0.2299[A]	112.84 ± 60.70	0.6065[K]	506.87 ± 117.07	0.1752[A]	3,707.20 ± 739.53	0.3114[K]	496.44 ± 157.17	0.4972[K]
	CT	53	184.93 ± 71.14		561.59 ± 147.94		101.43 ± 51.04		446.62 ± 111.28		3,432.22 ± 614.42		472.88 ± 188.14	
	TT	28	207.07 ± 67.03		590.47 ± 146.51		112.74 ± 58.06		473.77 ± 144.98		3,444.94 ± 534.23		453.58 ± 229.61	
G2677T/A	GG	25	202.29 ± 81.00	0.9653[K]	587.70 ± 165.83	0.7782[K]	109.18 ± 66.63	0.8359[K]	467.26 ± 109.77	0.6930[K]	3,360.93 ± 506.44	0.6211[K]	451.55 ± 196.50	0.8457[K]
	GT	27	194.25 ± 64.61		573.12 ± 141.07		104.88 ± 51.36		466.01 ± 166.64		3,590.95 ± 626.30		477.69 ± 213.27	
	GA	20	189.96 ± 66.46		598.44 ± 184.98		98.27 ± 43.88		470.15 ± 100.35		3,679.91 ± 852.18		452.85 ± 168.66	
	TT	9	215.62 ± 85.98		631.79 ± 151.87		124.04 ± 68.38		465.73 ± 111.86		3,477.17 ± 596.14		507.45 ± 262.86	
	TA	17	195.67 ± 79.73		545.26 ± 126.69		111.25 ± 47.48		443.49 ± 96.62		3,292.25 ± 445.67		477.60 ± 158.86	
	AA*	2	183.49		600.61		71.06 ± 59.41		584.75		3,481.41		633.05 ± 202.44	
C3435T	CC	49	197.79 ± 74.71	0.5039[A]	578.91 ± 157.73	0.1715[A]	103.47 ± 56.20	0.4968[K]	474.14 ± 116.38	0.7379[A]	3,530.20 ± 669.78	0.7499[K]	471.06 ± 195.51	0.9520[K]
	CT	42	191.34 ± 71.79		568.17 ± 153.00		105.95 ± 51.31		454.26 ± 136.79		3,430.15 ± 584.13		464.62 ± 180.70	
	TT	9	223.06 ± 75.94		673.58 ± 118.21		128.53 ± 62.87		472.81 ± 102.95		3,528.50 ± 564.05		511.11 ± 259.84	

Data are expressed as arithmetic mean ± SD, unless otherwise stated.
SNP, single-nucleotide polymorphisms; C_{max}, peak serum concentration; AUC, area under the serum concentration-time curve; SD, standard deviation; A, analysis of variance; K, Kruskal-Wallis test.
*Data are presented as means.

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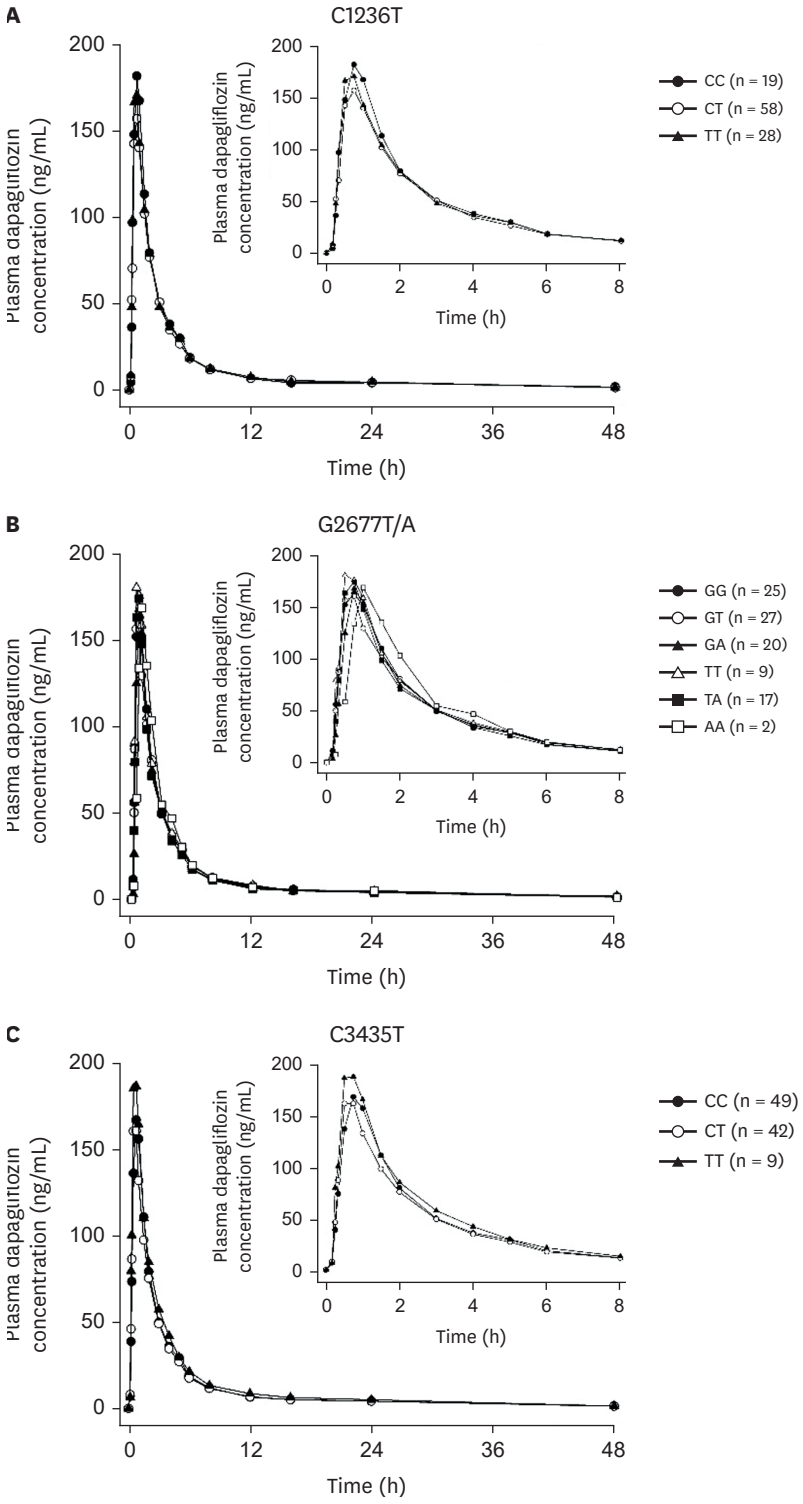

Figure 2. Comparison of dapagliflozin plasma concentration-time profiles between groups with the (A) C1236T, (B) G2677T/A, and (C) C3435T *ABCB1* genotypes following the administration of a single oral 10-mg dose. Values are presented as means.

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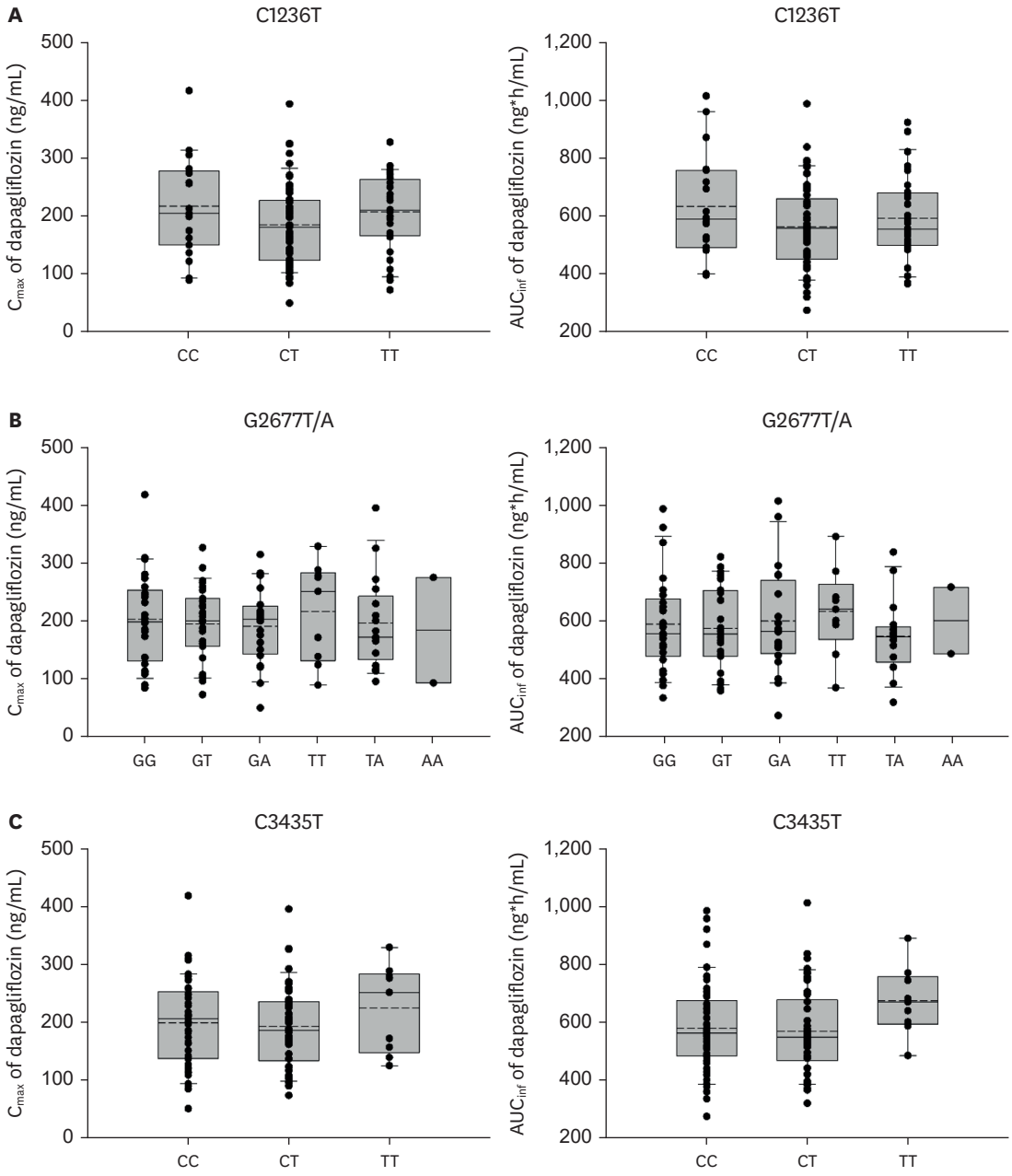

Figure 3. Box plots for the comparison of dapagliflozin C_{max} (left) and AUC_{inf} (right) between groups with the (A) C1236T, (B) G2677T/A, and (C) C3435T *ABCB1* genotypes following the administration of a single oral 10-mg dose. The box marks the 25th and 75th percentiles, and the solid and dashed lines between them represent the median and mean, respectively. The whiskers end at the maximum and minimum points unless there are outliers (open circle) beyond 1.5 times the H-spread (the distance between the hinges) from the hinges.

Influence of individual SNPs on sitagliptin PK

Subjects were grouped by SNP genotype, and the effects of the three SNPs on sitagliptin PK were evaluated. The 2677AA SNP was detected but not included in the investigation due to its low frequency. No significant differences in sitagliptin PK were observed between subjects with mutations at positions of 1236, 2677 and 3435, respectively, and their dominant/recessive models (**Tables 2** and **3**, **Fig. 4**). However, its mean (SD) C_{max} in subjects with the 1236CT and 1236TT genotypes were 446.62 (111.28) ng/mL and 473.77 (144.98) ng*h/mL, respectively, and these values were lower than that in subjects with the 1236CC mutation, in whom a mean

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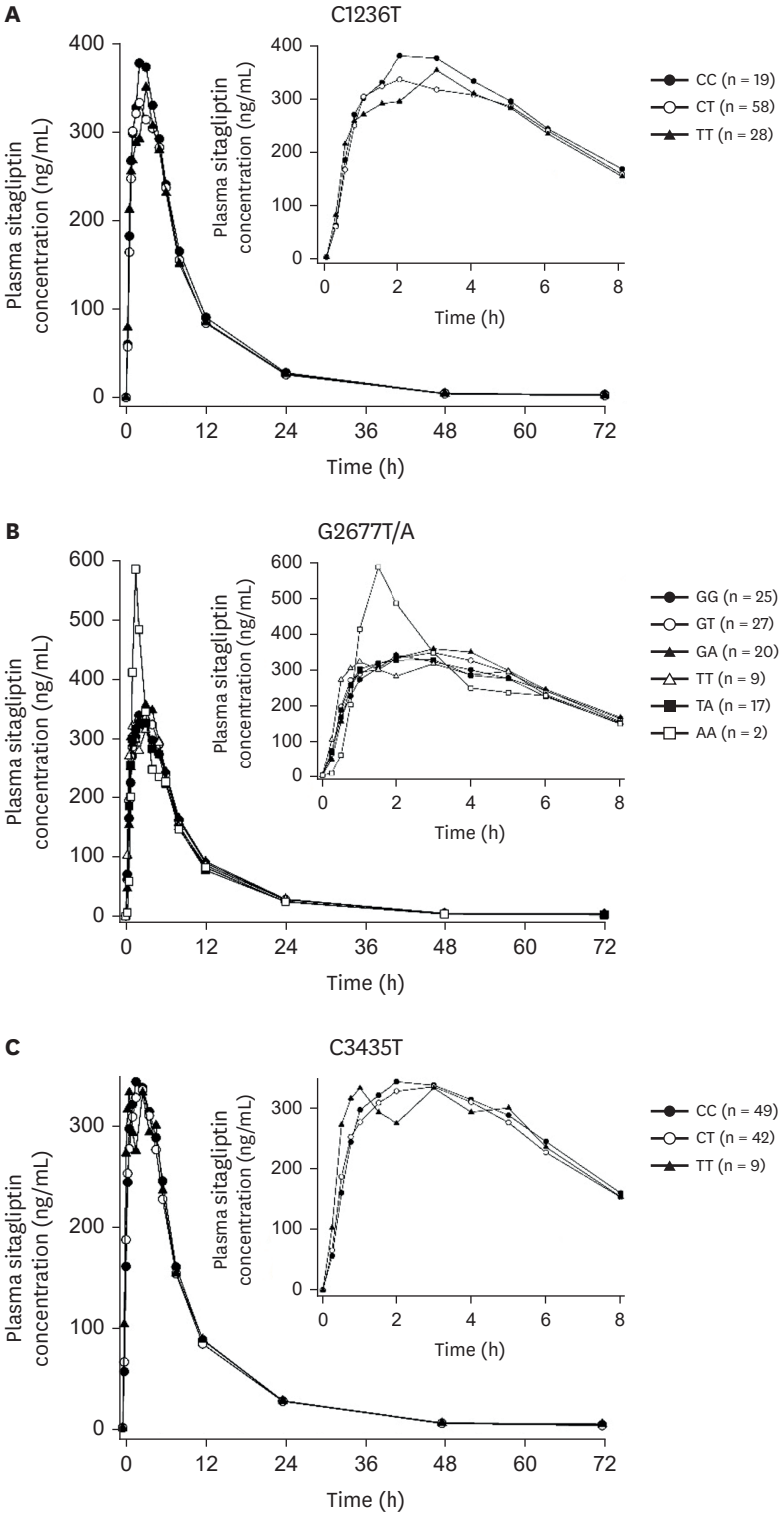

Figure 4. Comparison of sitagliptin plasma concentration time-profiles between groups with the (A) C1236T, (B) G2677T/A, and (C) C3435T *ABCB1* genotypes following the administration of a single 100-mg oral dose. Values are presented as means.

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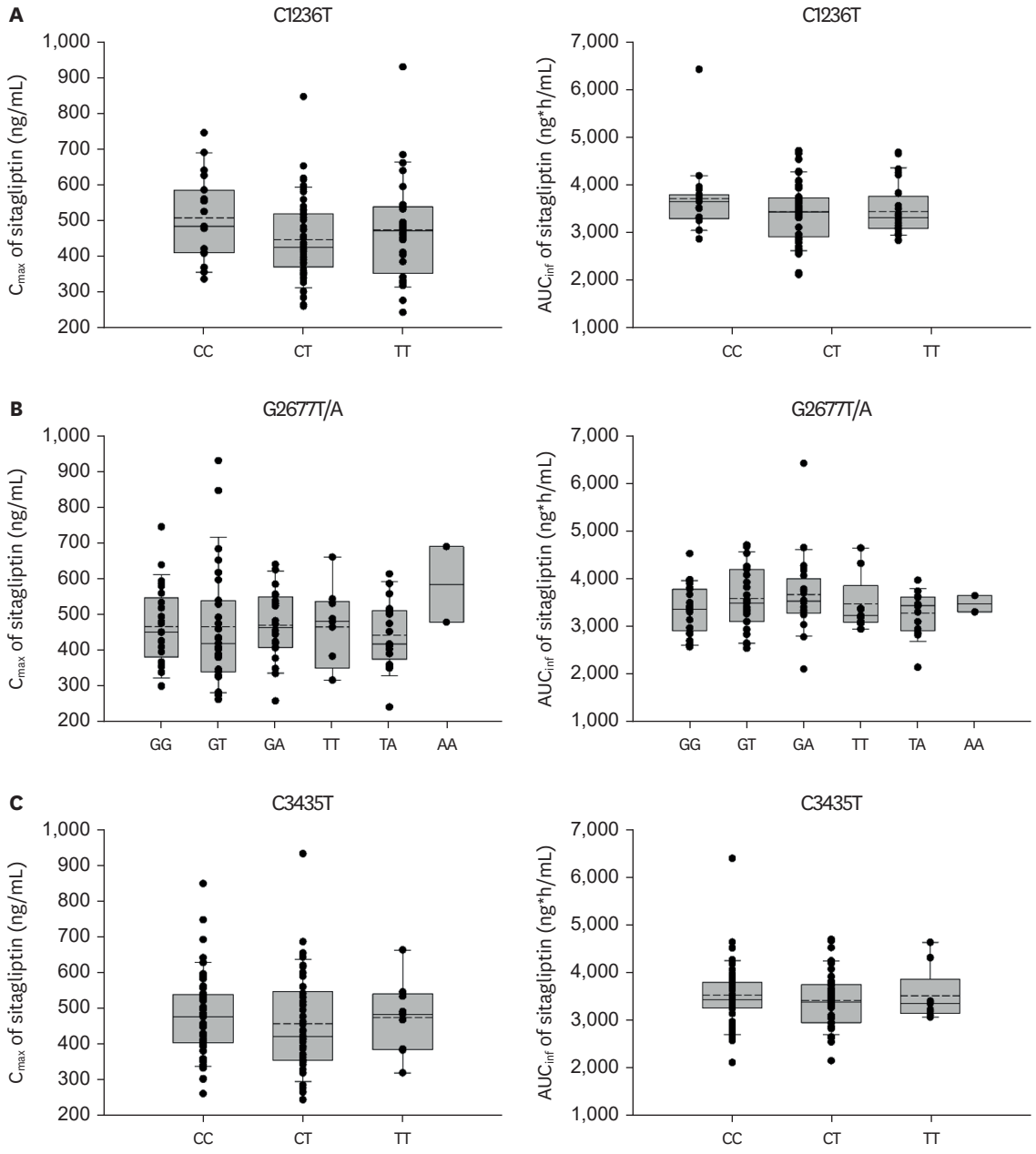

Figure 5. Box plots for the comparison of sitagliptin C_{max} (left) and AUC_{inf} (right) between groups with the (A) C1236T, (B) G2677T/A, and (C) C3435T of *ABCB1* genotypes following the administration of a single oral 100-mg dose. The box marks the 25th and 75th percentiles, and the solid and dashed lines between them represent the median and mean, respectively. The whiskers end at the maximum and minimum points unless there are outliers (open circle) beyond 1.5 times the H-spread (the distance between the hinges) from the hinges.

(SD) C_{max} of 506.87 (117.07) ng/mL was observed ($p = 0.1762$) (**Table 3**, **Fig. 5**). For C3435T polymorphisms, no trends were observed for mean C_{max}, AUC_{inf}, and $AUC_{0\text{-}2}$.

DISCUSSION

This is the first study to explore the effect of ABCB1 SNP on PK in dapagliflozin. Since dapagliflozin and sitagliptin are P-gp substrates, we expected *ABCB1* polymorphisms to

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influence their PK. However, we found no statistically significant relationship between *ABCB1* SNPs and PK parameters of sitagliptin and dapagliflozin. That notwithstanding, lower dapagliflozin systemic exposure was observed in subjects with the 3435TT genotype as compared to those with the CC + CT genotypes.

Based on the known mode of action of P-gp, diverse studies have investigated whether *ABCB1* SNPs can induce a clinically significant change in drug systemic concentrations and effects. Several studies have investigated the functional significance of the C3435T SNP in the disposition of digoxin, a well-known P-gp substrate. In a study involving 34 Caucasian subjects, digoxin concentrations were found to be 20% higher in 3435TT homozygous subjects than in CC and CT subjects [25]. Similarly, using a population PK approach, digoxin clearance was found to be reduced by 26.6% in subjects with TT alleles as compared to CC and CT subjects [26]. However, Becquemont et al. found no relationship between the C3435T SNP and digoxin concentrations following the administration of a single digoxin dose [27]. Contrary to these findings, a Japanese study involving 114 subjects reported a 20% lower AUC for digoxin in subjects with the 3435 TT genotype than in subjects with the CC and CT genotypes [28]. As seen in the case of digoxin, controversial findings have been reported even for the same drug as concerns the relationship between *ABCB1* SNPs and its concentrations [29,30]. Therefore, the current state of knowledge does not permit reliable predictions as concerns *ABCB1* SNP-induced changes in systemic drug concentrations and effectiveness.

Determining the effects of *ABCB1* SNPs on drug PK is challenging. First, most drugs pass through multiple pathways during the disposition process. Dapagliflozin is mainly metabolized by UGT1A9, but other CYP enzymes and OAT3 also contribute to its excretion process [14]. Sitagliptin metabolism and excretion involve CYP2C8 and transporters such as OAT3 and OATP4C1 [15]. Therefore, these other pathways could be confounding factors that interfere with the analysis of the relationship between P-gp and drug PK [31]. In addition, previous study has found P-gp expression levels to be 1.5–2 times lower in subjects with the 3435TT genotype than in those with the CC and CT genotypes [32]. As only a single dose was administered in this study, it may have been difficult to observe a clear difference in PK due to insufficient amount of substrate for P-gp saturation in the intestinal lumen or renal proximal tubule [33].

This study had some limitations. First, P-gp expression may vary depending on sex and race. In a study that involved the administration of cyclosporin to Caucasians, significant differences in both ABCB1 expression and blood cyclosporin concentrations were observed between male and female subjects [34]. In our study, gender-specific PK differences were not observed for dapagliflozin and sitagliptin (**Supplementary Table 2**). Since this study involved 94 men and only 6 women, the number of female subjects were not sufficient to verify possible sex-related differences. Second, the frequencies of the homozygous 2677AA and 3435TT genotypes, which are associated with differences in drug PK and efficacy, were relatively low. Therefore, it was difficult to evaluate differences in drug PK with respect to SNPs. However, the participants of this study were considered to represent the standard population as they satisfied the Hardy-Weinberg equilibrium requirement and SNP frequency distribution in this population was not significantly different from that reported in other Asian populations [23,24]. Third, pharmacodynamic biomarkers, such as blood glucose, were not assessed in this study, and the relationship between *ABCB1* SNPs and PD or clinical outcomes was not evaluated. Further prospective studies are necessary to solve these problems.

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In summary, dapagliflozin showed higher systemic exposure in subjects with the 3435TT genotype as compared to those with the CC or CT genotypes. But C1236T and C2677T/A SNP did not affect PK parameters of dapagliflozin and sitagliptin.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Demographic characteristics of the study participants with respect to *ABCB1* SNP genotype

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Supplementary Table 2

Effects of sex on dapagliflozin and sitagliptin pharmacokinetic parameters in healthy Korean subjects

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