Influences of Drug Transporters on Metformin's Pharmacokinetics, Pharmacodynamics and Drug-Drug Interaction

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Influences of Drug Transporters on Metformin's Pharmacokinetics, Pharmacodynamics and Drug-Drug Interaction

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돌이켜 보면 지난 30년 동안 그리고 이 자리에 있긴 까지 감사해야 할 분들이 많습니다. 항상 저에게 진취적인 생각을 이끌어 주신 아버지 (曺永燮), 언제나 제가 하는 일을 믿고 지지해 주신 어머니 (孫惠卿), 힘들 때마다 위로를 아끼지 않은 제 동생 성화 그리고 다른 길을 가지만 항상 자극이 되고 힘들 때 위로가 되어 주는 친구들 이 모두의 사랑과 보살핌이 있었기에 오늘의 제가 있을 수 있었습니다.

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ABSTRACT

Influences of drug transporters on metformin′s pharmacokinetics, pharmacodynamics and drug-drug interaction

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The biguanide derivative, metformin, is the first-line oral hypoglycemic drug for the treatment of type 2 diabetes. Its primary action is to lower hepatic glucose production by inhibiting gluconeogenesis. Generally, the change of drug transporter activity influences its drug exposure in systemic circulation by altering drug absorption and drug excretion especially in biliary and renal excretion. Meanwhile, drug transporting activity into action sites has been known to play important roles in drug actions. Metformin is a substrate of organic cation tranasporters (OCTs) and multidrug and toxin extrusion 1 (MATE1). OCT1 is a transporter located primarily in hepatocyte sinusoidal membranes, whereas OCT2 is localized mainly in the basolateral membrane of the kidney proximal tubule. MATE1 is an H^+/organic

cation antiporter on the apical membrane of kidney tubules and on bile canaliculi. Since OCT1 is located in the drug action site, metformin may play an important role in lowering glucose. OCT2 and MATE1 may affect metformin's pharmacokinetics due to its location. There is controversy on pharmacogenetic difference of OCT2 (rs 316019) in metformin's renal clearance and plasma concentration. Because of the location of MATE1, metformin may play a more important role than OCT2 does. I investigated the role of OCT2 and MATE1 on metformin's pharmacokinetics and renal clearance through a genotype-enriched prospective clinical trial. OCT1 is related to the hepatic uptake of metformin and is associated with pharmacological action and adverse drug reaction. In this thesis, I investigated how OCT1 works with metformin to lower glucose effect by co-administering rifampin, an OCT1 inducer, and verapamil, and OCT1 inhibitor, with metformin.

My thesis is composed of three parts. To assess the influences of polymorphisms MATE1 (rs 2252281) and OCT2 (rs 316019) on metformin's pharmacokinetics and renal clearance, I conducted a genotype enriched-clinical trial in 48 subjects in Study I by balancing the MATE1 and OCT2 genotypes. In the latter two parts, I evaluated the influence of OCT1 on metformin's glucose lowering effect in Study II and III. Study II assessed the change in metformin's glucose lowering effect and metformin's pharmacokinetics before and after rifampin, an OCT1 inducer, administration. Study III evaluated the change in metformin's pharmacokinetics and metformin's glucose lowering effect with and without verapamil administration.

Metformin's glucose effect was calculated by comparing the difference of oral

glucose tolerance test (OGTT) before and after metformin administration. Three parameters were used to assess the metformin's glucose lowering effect. Maximum glucose lowering effect (G_{max}) was determined and area under the serum glucose concentration-time curve (ΔAUC_{gluc}) was calculated using the trapezoidal rule. ΔAUC_{gluc60} was defined as area under the glucose curve from 0 to 60 minutes after glucose ingestion, during which serum glucose concentration increased. Metformin's pharmacokinetic parameters were evaluated as C_{max}, T_{max}, AUC_{met} and $t_{1/2}$ for plasma pharmacokinetic parameters and renal clearance (CL_R) of metformin, Creatinine clearance (CL_{Cr}) and renal secretion of metformin ($SrCL_R$) for urine pharmacokinetic parameters.

In study I, plasma pharmacokinetic parameters (C_{max}, T_{max}, AUC_{met} and $t_{1/2}$) are not significantly different by MATE1 and OCT2 genotypes. Renal clearance and net tubular secretion of metformin were significantly different by MATE1 genotype (CL_R: 617±126 vs. 556±106 vs. 507±104 ml/min, P=0.031 and $SrCL_R$: 517±121 vs. 456±107 vs. 399±107 ml/min, P=0.017).

In study II, I found that rifampin increased G_{max} by 41.9% (*P*=0.024) and ΔAUC_{gluc60} by 54.5% (*P*=0.020). Renal clearance of metformin was increased 16% by rifampin (*P*=0.008), and systemic exposure of metformin was slightly increased (13%, *P*=0.049), possibly due to increased absorption. Rifampin increased OCT1 mRNA levels 4.1-fold in peripheral blood cells (*P*=0.001).

In study III, Verapamil treatment decreased mean G_{max} by 62.5% (16 mg/dl vs. 6 mg/dl; *P* = 0.010). The glucose lowering effect of metformin was not observed after

verapamil treatment in mean ΔAUC_{gluc60} levels (594±500 mg/dl·min vs. -6±556 mg/dl·min; $P = 0.008$) and mean ΔAUC_{gluc} levels (509±1224 mg/dl·min vs. -702±1103 mg/dl·min; $P = 0.015$). Pharmacokinetic parameters, AUC, C_{max}, CL_R and $SrCL_R$ of metformin did not change when treated with verapamil.

OCT1 plays a key role in metformin's glucose-lowering effect. OCT1 based drug-drug interaction is important in the response of metformin presumably by affecting the effective concentration of metformin in the target organ. The important drug transporter on metformin's renal clearance and secretion is not OCT2, but the MATE1 polymorphism (rs2252281).

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Key words : Metformin, OCT 1&2, MATE1, Pharmacokinetcis, Pharmacodynamics

Influences of drug transporters on metformin's pharmacokinetics, pharmacodynamics and drug-drug interaction

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I. INTRODUCTION

The biguanide derivative, metformin is the first-line oral hypoglycemic drug for the treatment of type 2 diabetes. Its primary action is to lower hepatic glucose production by inhibiting gluconeogenesis[1]. Metformin is a substrate of organic cation tranasporters (OCTs) and multidrug and toxin extrusion 1 (MATE1)[2-3]. OCT1 is located primarily in hepatocyte sinusoidal membranes[4], whereas OCT2 is localized mainly in the basolateral membrane of the kidney proximal tubule[5]. MATE1 is an H^+/organic cation antiporter on the apical membrane of the kidney tubules and on bile canaliculi[6-7]. The transporter, OCT1 related to the hepatic uptake

of metformin is associated with its pharmacological action and adverse reaction.[8-9]It is thought that co-medications with metformin influencing OCT1 expression may affect metformin's response.

Metformin is eliminated unchanged in the urine, and its renal clearance (CL_R) is greater than that of creatinine, indicating that tubular secretion is the major route of elimination.[10] The uptake process in the basolateral membrane of renal tubular secretion is mediated by OCT2.[11] The efflux into the urine in the apical membrane of proximal tubule is mediated by MATE1.[12] Pharmacokinetic results regarding OCT2 rs316019 variant is controversial between Asian and American group. Song et al. and Wand et al have reported that subjects who carry OCT2 rs 316019 variant allele showed higher blood concentration of metformin and lower renal clearance of metformin than carriers of the reference allele.[13-14] But Chen et al. has reported the opposite result. This controversial result indicates that other transporter polymorphisms related to metformin's elimination plays an important role in metformin's pharmacokinetics and pharmacodynamics.[15] Genetic variants of MATE1 have been characterized in many groups. Choi et al has associated the MATE1 promoter region rs2252281 with decreased luciferase activity.[16]The pharmacokinetic and pharmacodynamics of metfomin regarding this SNP was not studied yet.

Drug-drug interaction is important in the use of metformin. Since metformin is the first-line oral hypoglycemic agent for the treatment of type2 diabetes, lots of drugs administered concomitantly with metformin. I focused on the OCT1 mediated drug

interaction on metformin. For the induction of OCT1, Maeda et al. reported that the human preganane X receptor (PXR) agonist pregnenolone-16-carbonitrile (PCN) reduced metformin blood AUC in rats by upregulating Oct1 expression in the liver.[17]Rifampin acts as a PXR agonist in human.[18] For the inhibition of OCT1, Ahlin et al. reported that IC_{50} value of verapamil on metformin uptake was lower than the reported C_{max} of verapamil *In Vitro*.[19] This implies that co-administration of verapamil with metformin may affect reduced uptake of metformin in human hepatocytes and the response of metformin.

The first purpose of the present study is to report the effect of OCT1 inducer (rifampin) and inhibitor (verapamil) on the response of metformin and the pharmacokinetics. By assessing the OCT1 mediated drug interaction with metformin, I may suggest dose adjustment or alteration of drug prescription when it is treated with metformin. The second purpose of the study is to suggest the polymorphic effect of OCT2 and MATE1 in metformin's pharmacokinetics and pharmacodynamics. I conducted OCT2 and MATE1 balanced-prospective metformin clinical trial to evaluate the difference of metformin's glucose lowering effect and metformin's pharmacokinetics by these genotypes.

II. MATERIALS AND METHODS

1. Clinical study designs

This research consists of 3 prospective clinical trials in healthy volunteers to investigate pharmacokinetic and pharmacodynamic of metformin. Study I was designed to evaluate the genotype effect of OCT2 and MATE1 in metformin's

pharmacokinetics and pharmacodynamics. In Study I, I combined the baseline metformin's pharmacokinetics and pharmacodynamics of Study II and Study III and conducted additional clinical trial to balance OCT2 and MATE1 genotype. Study II was designed to evaluate the effect of rifampin, OCT1 inducer in the metformin's glucose lowering action and metformin's pharmacokinetics. Study III was designed to evaluate the effect of verapamil, OCT1 inhibitor in the metformin's glucose lowering action and metformin's pharmacokinetics. Study protocol of each study is same to investigate metformin's pharmacokinetics and pharmacodynamics. The following exclusion criteria are applied to all 3 studies. Exclusion criteria were anemia (hemoglobin < 12 g/dl), history of drug abuse, symptomatic coronary heart disease, significant hepatic enzyme elevation (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] > 60 IU/L), serum creatinine > 1.5 mg/dl, or presenting any one of the criteria of metabolic syndrome. Subjects consuming > 2 alcoholic drinks (at one time) twice weekly, smoking > 10 cigarettes per day, or taking any medication were also excluded. Women of childbearing age were given urine pregnancy tests to exclude pregnant women.

The study protocol was reviewed and approved by the Institutional Review Board of Severance Hospital in the Yonsei University Health System, Seoul, Korea (4-2009-0334 and 4-2010-0417). All procedures were carried out in accordance with the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use–Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki.

2. Study I: OCT2 and MATE1 genotype on metformin's pharmacokinetics and pharmacodynamics.

Forty eight healthy subjects (men, n = 44; women, n = 4; age 27±5 years; height, 174.5±6.0 cm; weight, 70.5±3.9 kg) including 28 subjects from Study II and Study III were recruited for participation in the present study. After screening 999 subjects' OCT2 and MATE1 genotype, I recruited 20 subjects to balance the OCT2 (rs 316019) and MATE1 (rs2252281) genotype in the final 48 subjects. 20 subjects conducted the same schedule of clinical trials that were carried out from Day 1 to Day 3 in Study II and Study III. The genotype distribution of OCT2 and MATE1 genotype is illustrated in the table 1.

Table 1. Genotype distribution of OCT2 (rs316019) and MATE1 (rs2252281) in Study I

SNP (rs#)		**MATE1** (rs2252281)		
	Genotype	TT	TC	CC
OCT2 (rs316019)	GG	13	7	5
	GT	8	4	5
	TT	3	1	2

3. Study II: Rifampin (OCT1 inducer) and metformin interaction study

Sixteen healthy subjects (men, n = 13; women, n = 3; age 27±4 years; height, 172.4±7.3 cm; weight, 67.4±11.2 kg) were recruited for participation in the present

study. The participants were asked to maintain normal physical activity at least 5 days before the study began. Dieticians instructed the subjects regarding the meal plan designed to maintain a carbohydrate intake of 200 to 250 g/d and the use of a food diary to record food intake for 3 days before admission. The last meal before admission was taken in the Clinical Trials Center at Severance Hospital. After an overnight fast, blood was drawn to determine OCT1 and OCT2 mRNA levels, and a 3-h oral glucose tolerance test (OGTT) (75 g glucose) was performed starting at 10 AM (Day 1). Participants received a 1000-mg oral dose of metformin (Diabex Tab; Daewoong Pharmaceutical Co., Seoul, Korea) at 8 PM. After an overnight fast, a 750-mg dose of metformin was administered at 8 AM on Day 2, followed by the second OGTT at 10 AM. Blood and urine samples were collected to determine the pharmacokinetics of metformin. After receiving a 600-mg oral dose of rifampin (Rifampin Tab; Yuhan Corp., Seoul, Korea), subjects were discharged on the morning of Day 3. Subjects continued taking rifampin (600 mg daily) through Day 12 on an outpatient basis, during which they also maintained a food diary. The subjects were admitted again to the Clinical Trials Center on Day 13 and remained through Day 15. The second pair of OGTT tests, and metformin administration and blood and urine collection were carried out according to the schedule used Day 1 to Day 3.

4. Study III: Verapamil (OCT1 inhibitor) and metformin interaction study

Twelve healthy subjects (men, n = 12; age 27±5 years; height, 174.5±6.0 cm; weight, 70.5±3.9 kg) were recruited for participation in the present study. The protocol was the same as with Study I from Day 1 to Day 3. Subjects were discharged on the morning of Day 3 after the last blood sampling. After the 11 days of wash-out period, participants started to be received a 120 mg oral dose of verapamil (Isoptin Tab; Ilsung Pharmaceutical Co., Seoul, Korea) once a day for 3 days (8 AM starting on Day 14). The subjects were admitted again to the Clinical Trials Center on Day 15 and remained through Day 17. Metformin and verapamil was administered concomitantly 8AM on Day 16. The second pair of OGTT tests and metformin administration and blood and urine collection were carried out according to the schedule used Day 1 to Day 3.

5. Blood and urine collection

For OGTT analysis, blood samples were collected before glucose ingestion and 15, 30, 45, 60, 90, 120, 150, and 180 min after glucose ingestion. To determine plasma metformin concentrations, blood samples were collected before the second dose of metformin and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, and 24 h after. After the second metformin dose, participants were asked to drink 240 ml water every 4 h to maintain urine flow. The first portion of urine was voided before urine collection during the following time intervals: 0–4, 4–8, 8–12, and 12–24 h after the second metformin dose. The volume and pH of urine were recorded before analysis. To

calculate creatinine clearance (CL_{Cr}), serum creatinine was determined from blood (3 ml) drawn before both admissions.

6. Metformin concentration analysis

Metformin concentrations in plasma and urine were determined by the highly specific and sensitive method of liquid chromatography–tandem mass spectrometry (API 3200; Applied Biosystems Sciex, Ontario, Canada). To prepare the samples for analysis, an aliquot of the plasma or urine specimen was mixed with acetonitrile in the presence or absence of the internal standard formoterol. The mixture was vortexed for 5 min and then centrifuged for 5 min at 10,000 rpm. An aliquot of the supernatant was transferred to an autosampler vial, and 1 μl was injected onto the column at 10℃. The mobile phase consisted of 75% acetonitrile, 25% double-distilled water, and 5 mM ammonium formate aqueous solution. The limit of quantification was 10ng/ml for plasma and 0.5μg/ml for urine. The intra-day and inter-day coefficients of variation were less than 10%.

7. Glucose analysis from oral glucose tolerance test

A carbohydrate-controlled diet (200–250 g/day) was followed for 3 days prior to admission. Before conducting the OGTT (10 AM), all subjects fasted for more than 14 h. Metformin lowers glucose production in diabetic patients[20] and exerts the same effect in healthy subjects if serum glucose levels are increased by glucose ingestion[8]. The OGTT was conducted four times: before and after metformin doses

prior to rifampin and verapamil administration (Day1 and Day 2) and after rifampin and verapamil administration (Day 13 and 14, Day15 and 16, respectively) in study II and study III. The OGTT was conducted twice in study I. Maximum glucose level (G_{max}) was determined and area under the serum glucose concentration-time curve (AUC_{gluc}) was calculated using the trapezoidal rule. AUC_{gluc60} was defined as area under the glucose curve from 0 to 60 min after glucose ingestion, during which serum glucose concentration increases. Metformin's glucose lowering effect was assessed as the difference of OGTT profile before and after metformin administration. (ΔAUC_{gluc60} and ΔAUC_{gluc}) The effect of rifampin, verapamil and OCT2 and MATE1 genotype effect on the glucose-lowering action of metformin was calculated.

8. Pharmacokinetics

The pharmacokinetic parameters were calculated by non-compartmental analysis using Phoenix WinNonlin 6.2 (Pharsight Corporation, Mountain View, CA, USA). Maximum metformin concentration (C_{max}) and the time of maximum concentration (T_{max}) were determined, and the area under the plasma metformin concentration-time curves (AUC_{met}) was calculated by the linear trapezoidal rule. The elimination rate constant (k_e) was estimated from the slope of the best-fit line determined by linear regression analysis of the log-transformed concentration-time curve. The elimination half-life ($t_{1/2}$) was then calculated by the equation $t_{1/2}= \ln(2)/k_e$. The renal clearance (CL_R) of metformin was calculated as the total amount of metformin

excreted in urine over 24 h divided by AUC_{met}. Creatinine clearance (CL_{Cr}) was calculated with the Cockcroft–Gault equation ([140–age]×(body weight, kg)×(0.85 if female)/ (72×serum creatinine). The renal secretion clearance of metformin was calculated by subtracting creatinine clearance (CL_{Cr}) from renal clearance of metformin (CL_R).

9. OCT2, MATE1 and MATE2-K genotyping

Genomic DNA was extracted from peripheral whole blood using a QIAamp DNA Blood Mini kit (QIAGEN GmbH, Germany). Genotyping was done using TaqMan allelic discrimination assays on an AB 7300 Real time PCR System (Applied Biosystems, Foster City, CA, USA). Ten microliters of PCR reaction mixture was prepared with 5 μl of 2× TaqMan Genotyping Master mix, 0.5 μl of 20× Drug Metabolism Genotyping Assay Mix, 3.5μl of DNase-free water, and 1 μl of genomic DNA. Genotyping for SLC22A2 (OCT2) rs316019 (Assay ID: C_3111809_20) and SLC49A1 (MATE1) rs2252281 (AH70LAY) was performed with validated TaqMan genotyping assays purchased from Applied Biosystems. PCR reactions were as follows: initial denaturation at 95℃ for 10 min, followed by 50 cycles of denaturation at 92℃ for 15 s and annealing/extension at 60℃ for 1 min. The allelic discrimination results were determined after amplification by performing an end-point read. AB Sequence Detection System 7300 sds software ver 1.3.1 (Applied Biosystems) was used for the analysis. For the analysis of SLC49A2 (MATE2-K) rs12943590, the SNaPshot assay was performed according to the manufacturer's

instructions (ABI PRISM SNaPShot Multiplex kit, Foster City, CA, USA). Analysis was carried out using Genemapper software (version 4.0; Applied Biosystems). Forward primer, reverse primer and SNP primer are designed as CAGGAAACAGCTATGACCcttctcctgctgaggccttact, TGTAAAACGACGGCCAGT cagtttctggggaaaatgtgag and TCATCCCACAAGTTGCCATGGTAGC, respectively.

10. OCT1 and OCT2 expression in Study II

Blood levels of OCT1 and OCT2 mRNA were determined by real-time PCR. Total RNA was extracted with the QIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA). Equal amounts of RNA (500 ng) from each sample were reverse transcribed with an oligo(dT) primer and RNase H- reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The cDNA was amplified with human OCT1-specific and OCT2-specific TaqMan® gene expression primer probe sets (Hs00427550_m1 and Hs01010723_m1, respectively), TaqMan® Gene Expression Master Mix, and the ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The following cycling conditions were used: 1 cycle at 50℃ for 2 min; 1 cycle at 95℃ for 10 min; and 50 cycles of 95℃ for 15 s followed by 60℃ for 1 min. A negative control using water as the template was included in every PCR experiment. Target gene mRNA levels were normalized to the endogenous control gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH: 4333764F).

11. Statistical analysis

Measurements from the same subjects before and after rifampin and verapamil treatment were compared with the Wilcoxon signed-rank test. The effect of OCT2

and MATE1 polymophism on metformin's pharmacokinetics was compared by two-way Analysis of variance (ANOVA). The data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). $P < 0.05$ was considered significant.

III. RESULTS

1. OCT2 and MATE1 allele frequency in Korean population

I investigated allele frequency of OCT2 polymorphism (rs316019) and MATE1 polymorphisms (rs2252281, rs2289669) in Korean population. I analyzed 999 healthy volunteers for rs316019 polymorphism (OCT2). Among this population, 325 healthy volunteers were genotyped to investigate the allele frequency of rs2252281, rs2289669 polymorphism (MATE1). The result is summarized in table 2.

Table 2. Allele Frequency of OCT2 and MATE1 variants in Korean population

OCT2 rs316019	Number of subjects	MATE1 rs2252281	Number of subjects
GG	777	TT	209
GT	208	TC	106
TT	14	CC	10
Minor allele frequency	0.118		0.194

2. Pharmacokinetic results by OCT2, MATE1, MATE2-K genotype (Study I)

999 Koreans were genotyped for the OCT2 polymorphism (rs316019). In the recruiting process of participants, OCT2 polymorphism (rs316019) and MATE1 polymorphism (rs2252281) were balanced to show each polymorphism's effect on metformin's pharmacokinstics. 48 subjects were recruited and the pharmacokinetic of metformin was evaluated in these participants after 1750mg metformin administration. Pharmacokinetic results for metformin by OCT2 genotype are demonstrated in figure 1 and table 3. There were no significant differences in all pharmacokinetic parameters. Pharmacokinetic results for metformin by MATE1 genotype are demonstrated in figure 2 and table 4. Renal clearance and net tubular secretion of metformin were significantly different by MATE1 genotype (CL_R: 617±126 vs. 556±106 vs. 507±104 ml/min, P=0.031 and $SrCL_R$: 517±121 vs. 456±107 vs. 399±107 ml/min, P=0.017). The renal clearance and renal secretion by MATE1 genotype is sub-grouped by OCT2 polymorphism (rs 316019). The renal clearance of metformin was significantly different by MATE1 genotype in OCT2 GG group (661±149 vs. 503±85 vs. 499±152 ml/min, P=0.026, table 5). The renal clearance of metformin was significantly different by MATE1 genotype in OCT2 GT + TT group (565±68 vs. 631±91vs. 513±65 ml/min, P=0.039). The renal secetion of metformin was significantly different by MATE1 genotype in OCT2 GG group (558±141 vs. 402±80 vs. 398±162 ml/min, P=0.023). The renal clearance of metformin was significantly different by MATE1 genotype in OCT2 GT + TT group (468±70 vs. 532±96 vs. 400±58 ml/min, P=0.019). To differentiate the OCT2's

effect on renal clearance and renal secretion, two-way analysis of variance (two-way ANOVA) between OCT2 and MATE1 was done. Two-way ANOVA on renal clearance showed significant result for MATE1 rs2252281 genotype (F=3.767 and p=0.031). No significant difference was found between OCT2 rs316019 genotype and renal clearance (F=0.209 and p=0.650). Interaction term showed significant result (F=4.165 and p=0.022). Two-way ANOVA on renal secretion showed significant result for MATE1 rs2252281 genotype (F=4.431 and p=0.018). No significant difference was found between OCT2 rs316019 genotype and renal secretion (F=0.169 and p=0.683). Interaction term showed significant result (F=4.129 and p=0.023). Considering age, weight, height, sex and CL_{Cr}, MATE1 is the only factor to affect metformin's renal clearance and renal secretion. MATE2-K rs12943590 showed a significant result on metformin's poor response[21]. I analyzed its effect on metformin's renal clearance and renal secretion. The renal clearance of metformin was not significantly different by MATE2-K genotype (556±87 vs. 569±128 vs. 635±159 ml/min, P=0.569, for those with GG (14), GA (27) and AA (7) genotypes, respectively). The renal secretion of metformin was not significantly different by MATE2-K genotype, neither (448±84 vs. 468±126 vs. 536±165 ml/min, P=0.297, for those with GG (14), GA (27) and AA (7) genotypes, respectively).

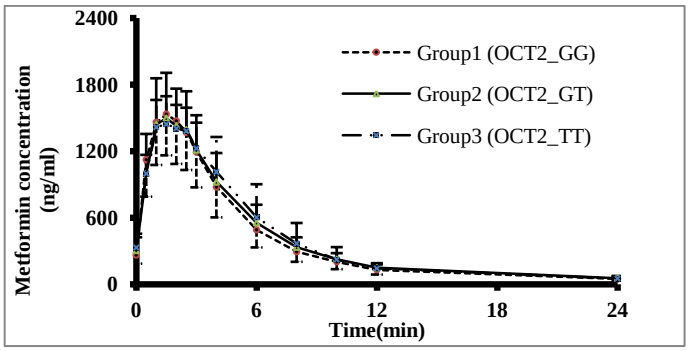

Figure 1. The plasma concentration-time curve of metformin on Day 2 by OCT2 rs316019 genotype (Group1, n=25, Group2, n=17, Group3, n=6).

Table 3. Pharmacokinetic parameters of metformin in healthy participants (N=48) by OCT2 (rs316019) genotype.

Parameters	Group1 (n=25) OCT2 (GG)	Group2 (n=17) OCT2 (GT)	Group3 (n=6) OCT2 (TT)	*P*
$AUC_{met(0-24)}$ (ng/ml·hr)	8966±2487	9114±1475	9523±1779	0.837
$t_{1/2}$ (hr)	6.26±1.62	7.21±3.11	7.14±1.99	0.386
C_{max} (ng/ml)	1546±419	1634±346	1628±293	0.739
CL_R (ml/min)	584±153	555±74	589±102	0.718
$SrCL_R$ (ml/min)	483±150	452±74	488±114	0.693
CL_{Cr} (ml/min)	102±12	103±15	101±14	0.951

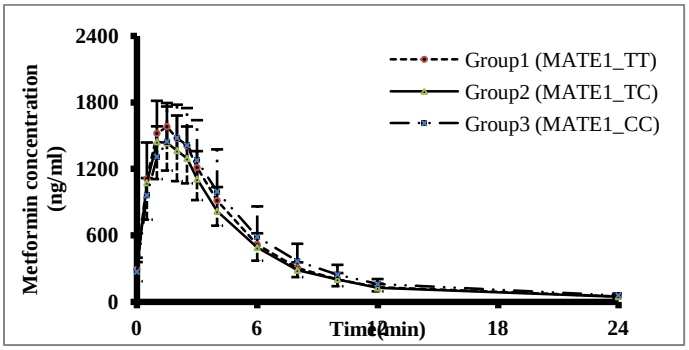

Figure 2. The plasma concentration-time curve of metformin on Day 2 by MATE1 rs2252281 genotype (Group1, n=24, Group2, n=12, Group3, n=12).

Table 4. Pharmacokinetic parameters of metformin in healthy participants (N=48) by MATE1 (rs2252281) genotype

Parameters	Group1 (n=24) MATE1 (GG)	Group2 (n=12) MATE1 (GT)	Group3 (n=12) MATE1 (TT)	*P*
$AUC_{met(0-24)}$ (ng/ml·hr)	9104±1968	8843±1696	9302±2538	0.861
$t_{1/2}$ (hr)	6.82±2.71	5.94±1.70	7.24±1.81	0.367
C_{max} (ng/ml)	1641±412	1544±285	1524±392	0.617
CL_R (ml/min)	617±126	556±106	507±104	0.031
$SrCL_R$ (ml/min)	517±121	456±107	399±107	0.017
CL_{Cr} (ml/min)	100±12	101±13	108±17	0.239

Table 5. Renal clearance of metfomin by OCT2 and MATE1 genotype

Renal clearance of metformin		**MATE1** (rs2252281)			
	Genotype	**TT**	**TC**	**CC**	**p-value**
OCT2 (rs316019)	GG	661±149 (n=13)	503±85 (n=7)	499±152 (n=5)	0.026
	GT,TT	565±68 (n=11)	631±91 (n=5)	513±65 (n=7)	0.039
	p-value	0.063	0.032	0.822	
Renal secretion of metformin		**MATE1** (rs2252281)			
	Genotype	**TT**	**TC**	**CC**	**p-value**
OCT2 (rs316019)	GG	558±141 (n=13)	402±80 (n=7)	398±162 (n=5)	0.023
	GT,TT	468±70 (n=11)	532±96 (n=5)	400±58 (n=7)	0.019
	p-value	0.067	0.029	0.983	

3. Pharmacodynamic results by OCT2, MATE1, MATE2-K genotype (Study I)

I evaluated metformin's pharmacodynamics as metformin's glucose lowering effect. The ability of metformin was assessed as to maximum blood glucose levels (G_{max}), glucose AUC during the first 60 minutes after glucose ingestion (ΔAUC_{gluc60}), and glucose AUC for the entire 180-min test (ΔAUC_{gluc}).

The G_{max} was not significantly different by OCT2 rs316019 genotype (35±15 vs. 32±17 vs. 36±8 mg/dl, P=0.783, for those with GG (25), GT (17) and TT (6) genotypes, respectively). The ΔAUC_{gluc60} was not significantly different by OCT2 genotype (861±731 vs. 628±768 vs. 1016±549 mg/dl*min, P=0.410, for those with GG (25), GT (17) and TT (6) genotypes, respectively). The ΔAUC_{gluc} was not significantly different by OCT2 genotype, neither (1228±1081 vs. 1088±1803 vs. 2006±1753 mg/dl*min, P=0.410, for those with GG (25), GT (17) and TT (6) genotypes, respectively).

The G_{max} was not significantly different by MATE1 rs2252281 genotype (34±12 vs. 35±19 vs. 33±16 mg/dl, P=0.961, for those with TT (24), TC (12) and CC (12) genotypes, respectively). The ΔAUC_{gluc60} was not significantly different by OCT2 genotype (862±732 vs. 694±709 vs. 775±768 mg/dl*min, P=0.807, for those with TT (24), TC (12) and CC (12) genotypes, respectively). The ΔAUC_{gluc} was not significantly different by OCT2 genotype, neither (1257±1441 vs. 1034±1702 vs. 1556±1277 mg/dl*min, P=0.685, for those with TT (24), TC (12) and CC (12) genotypes, respectively).

The G_{max} was significantly different by MATE2-K rs12943590 genotype (31±13

vs. 32±14 vs. 47±14 mg/dl, P=0.029, for those with GG (14), GA (27) and AA (7) genotypes, respectively). The ΔAUC_{gluc60} was not significantly different by OCT2 genotype (579±636 vs. 804±719 vs. 1212±817 mg/dl*min, P=0.169, for those with GG (14), GA (27) and AA (7) genotypes, respectively). The ΔAUC_{gluc} was not significantly different by OCT2 genotype, neither (1019±1346 vs. 1318±1591 vs. 1628±1160 mg/dl*min, P=0.658, for those with GG (14), GA (27) and AA (7) genotypes, respectively).

Table 6. Pharmacokinetic parameters of metformin in healthy participants (N=48) by MATE2-K (rs12943590) genotype

Parameter	Group1 (n=14) MATE2-K (GG)	Group2 (n=27) MATE2-K (GA)	Group3 (n=7) MATE2-K (AA)	*P*
G_{max} (mg/dl)	31±13	32±14	47±14	0.029
ΔAUC_{gluc60} (mg/dl·min)	579±636	804±719	1212±817	0.169
ΔAUC_{gluc} (mg/dl·min)	1019±1346	1318±1591	1628±1160	0.658

Data were evaluated by One-way ANOVA test and expressed as mean ± SD.

G_{max}, maximum glucose lowerin effect of metformin treatment; ΔAUC_{gluc60}, difference in partial glucose AUC (0 to 60 min after ingestion, during which glucose concentration increases) before and after metformin treatment; ΔAUC_{gluc}, difference in total AUC_{gluc} before and after metformin treatment.

4. Rifampin's effect on glucose lowering effect of metformin (Study II)

Healthy volunteers (n = 16) underwent an oral glucose tolerance test (OGTT) before and after receiving two doses of metformin on Days 1 and 2, and again on Days 13 and 14 (after a 10-day course of rifampin). Baseline serum glucose concentrations (before the first metformin dose) were similar before and after rifampin treatment; however, the effects of metformin on glucose lowering were considerably increased by the 10-day rifampin treatment (Figure 3). G_{max}, ΔAUC_{gluc60} and ΔAUC_{gluc} were compared before and after rifampin treatment (Figure 3). Rifampin treatment increased mean G_{max} by 41.9% (31 mg/dl vs. 44 mg/dl; $P = 0.024$) and mean ΔAUC_{gluc60} by 54.5% (914±510 mg/dl·min vs. 1412±555 mg/dl·min; $P = 0.020$). Mean ΔAUC_{gluc} was increased by 44% after the rifampin treatment, but the difference was not significant (Table 6).

A. B.

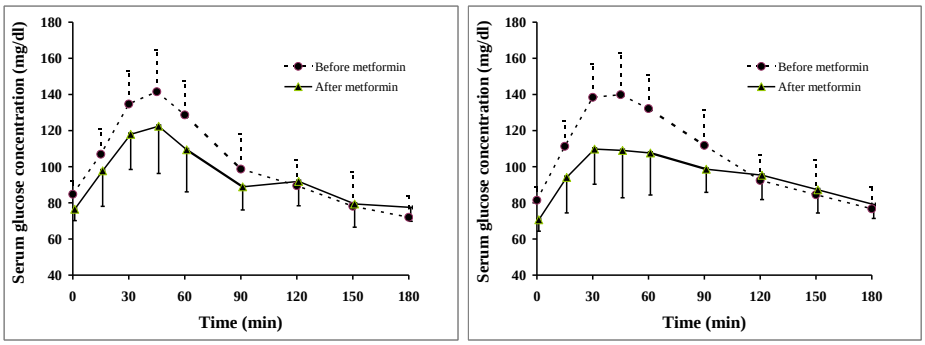

Figure 3. Serum glucose levels were determined by oral glucose tolerance test before and after metformin administration. (A) Serum glucose profile before the 10-day course of rifampin. (B) Serum glucose profile after the 10-day course of rifampin. Data are expressed as mean ± SD (n = 16).

Table 7. The glucose lowering effect parameters of metformin before and after rifampin treatment in healthy participants (n =16).

Parameter	Before rifampin	After rifampin	*P*
G_{max} (mg/dl)	31±14	44±14	0.024
ΔAUC_{gluc60} (mg/dl·min)	914±510	1,412±555	0.020
ΔAUC_{gluc} (mg/dl·min)	1,679±1,155	2,378±1,316	0.121

Data were evaluated by Wilcoxon signed-rank test and expressed as mean ± SD.

G_{max}, maximum glucose lowerin effect of metformin treatment; ΔAUC_{gluc60}, difference in partial glucose AUC (0 to 60 min after ingestion, during which glucose concentration increases) before and after metformin treatment; ΔAUC_{gluc}, difference in total AUC_{gluc} before and after metformin treatment.

5. Rifampin's effect on metformin's pharmacokinetics (Study II)

Participants received two oral doses of metformin on Days 1/2 (1750 mg total) and again on Days 13/14. The mean AUC from 0 h to 24 h ($AUC_{met(0-24)}$) and the maximum metformin concentration (C_{max}) were comparable to results of previous studies[13,15,22]. After rifampin treatment, metformin plasma concentrations were 23% increased at 0.5 h (P = 0.002) and 13% increased at 1 h (P = 0.036). The AUC for the full 24 h ($AUCmet_{(0-24)}$) increased 13% after rifampin treatment (9408±2410 ng/ml·hr vs. 10,672±3149 ng/ml·hr, P = 0.049), whereas Cmax was not significantly

different (1536±350 ng/ml vs. 1692±114 ng/ml, $P = 0.070$). Rifampin treatment did not significantly alter metformin $t_{1/2}$, but increased renal clearance (CL_R) of metformin by 16% (501±97 ml/min vs. 580±101 ml/min, $P = 0.008$); creatinine clearance (CL_{Cr}) was unchanged. In addition, net tubular secretion (Sr_{CLR}) of metformin ($CL_R - CL_{Cr}$) was increased 21% by rifampin (398±92 ml/min vs. 475±98 ml/min, $P = 0.005$). The results are summarized in figure 4 and table 7.

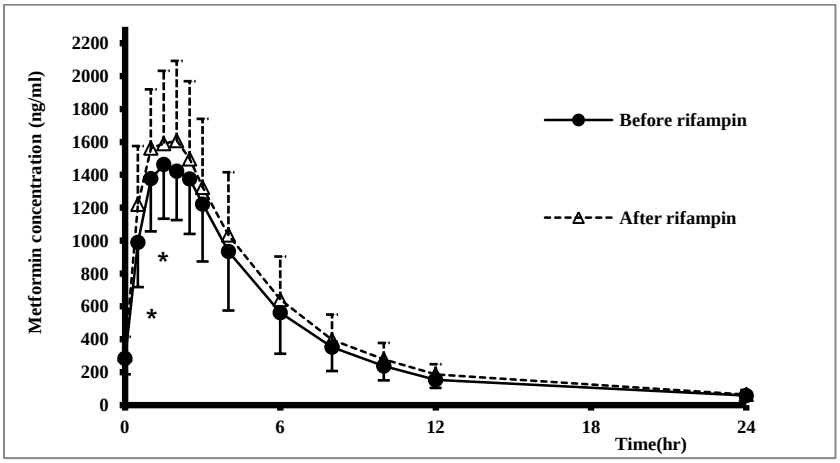

Figure 4. The plasma concentration-time curve of metformin on Day 2 (before rifampin treatment) and Day 14 (after rifampin treatment). Metformin concentrations were measured after the second dose of metformin.

Data are expressed as mean and SD (n = 16). *$P < 0.05$ (before rifampin vs. after rifampin treatment)

Table 8. Pharmacokinetic parameters of metformin in healthy participants (n = 16) before and after a 10-day course of rifampin.

	Before rifampin	**After rifampin**	***P***
$AUC_{met(0-24)}$ (ng/ml·hr)	9408±2410	10672±3149	0.049
$t_{1/2}$ (hr)	7.38±3.09	6.81±1.86	0.501
C_{max} (ng/ml)	1536±350	1692±114	0.070
T_{max} (hr)	1.63±0.53	1.59±0.64	0.881
CL_R (ml/min)	501±97	580±101	0.008
$SrCL_R$ (ml/min)	398±92	475±98	0.005
CL_{Cr} (ml/min)	109±15	105±15	0.173

Data were evaluated by Wilcoxon signed rank test and expressed as mean ± SD. $AUC_{met(a-b)}$, area under the plasma concentration-time curve from time point a to time point b; $t_{1/2}$, elimination half-life; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; CL_R, renal clearance; $SrCL_R$, renal clearance by tubular secretion; CL_{Cr}, creatinine clearance.

6. Rifampin's effect on OCT1 mRNA expression in blood (Study II)

The OCT1 and OCT2 genotypes that have been reported their functional significance and frequencies in Korean population were almost evenly distributed between wild-type and variant-type alleles for OCT2 rs316019 (GG=7, GT=7, and TT=2). To characterize the mechanism by which rifampin enhanced the glucose-lowering action of metformin, OCT1 and OCT2 mRNA levels in peripheral blood

cells were determined by real-time PCR. Metformin is transported primarily by OCT1 and OCT2 [23]; therefore, changes in OCT1 and OCT2 expression may account, at least in part, for rifampin-induced changes in metformin effects. Consistent with the rifampin-enhanced glucose tolerance, OCT1 mRNA levels were 4.1-fold higher after rifampin treatment (410±260%, $P = 0.001$) (Figure 5). However, OCT2 mRNA was not detected in the peripheral blood cells.

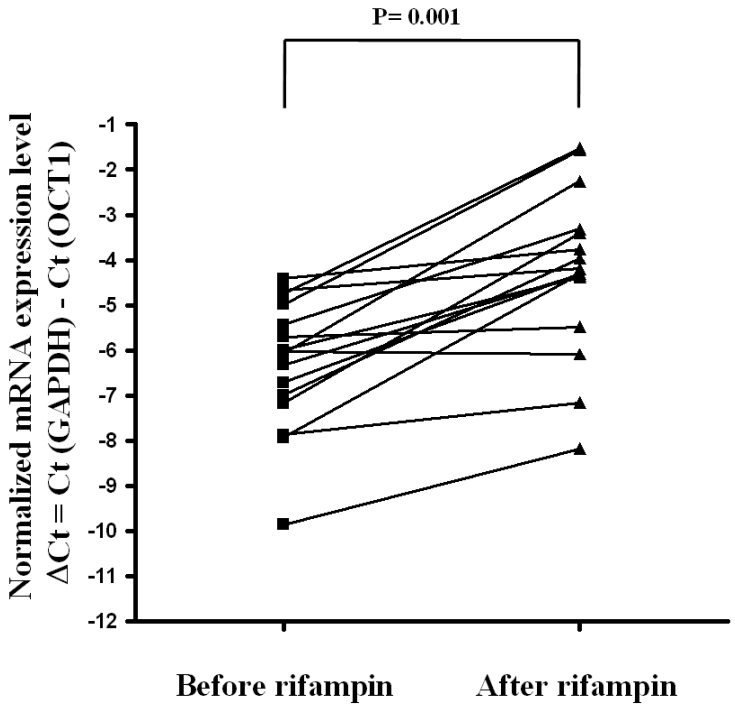

Figure 5. Rifampin treatment (10 days) increased OCT1 mRNA levels in the peripheral blood cells of 16 healthy volunteers, as determined by real-time PCR.

7. Verapamil's effect on glucose lowering effect of metformin (Study III)

Healthy volunteers (n = 12) underwent an oral glucose tolerance test (OGTT) before and after receiving two doses of metformin on Days 1 and 2, and again on Days 15 and 16 (when treatment was concomitant with verapamil). Baseline serum glucose concentrations (before the first metformin dose) were similar before and after verapamil treatment; however, the effects of metformin on glucose lowering disappeared when treatment was concomitant with verapamil (Figure 6). The ability of metformin to reduce maximum blood glucose levels (G_{max}), glucose AUC during the first 60 minutes after glucose ingestion (ΔAUC_{gluc60}), and glucose AUC for the entire 180-min test were compared with and without verapamil treatment (Table 8). Verapamil treatment decreased mean ΔG_{max} by 62.5% (16 mg/dl vs. 6 mg/dl; $P = 0.010$). The glucose lowering effect of metformin was not observed after verapamil treatment in mean ΔAUC_{gluc60} (594±500 mg/dl·min vs. -6±556 mg/dl·min; $P = 0.008$) and mean ΔAUC_{gluc} (509±1224 mg/dl·min vs. -702±1103 mg/dl·min; $P = 0.015$) (Table 8).

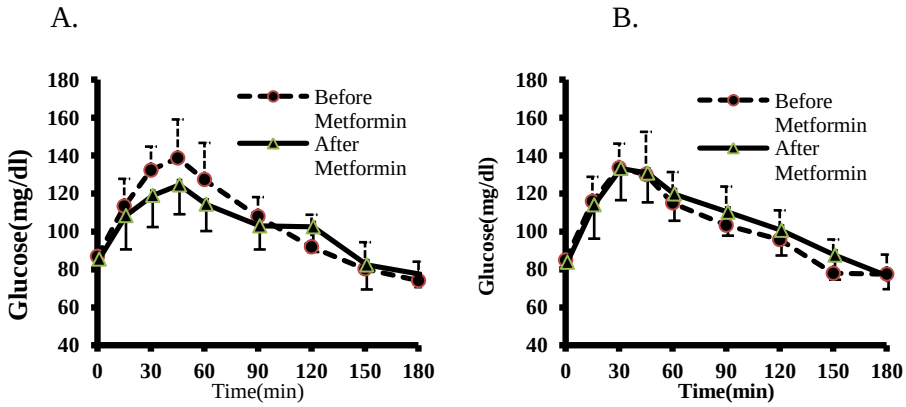

Figure 6. Serum glucose levels were determined by oral glucose tolerance test before and after metformin administration. (A) Serum glucose profile without verapamil treatment. (B) Serum glucose profile with verapamil treatment. Data are expressed as mean ± SD (n = 12).

Table 9. The glucose lowering effect parameters of metformin with and without verapamil treatment in healthy participants (n =12).

Parameter	**Without verapamil**	**With verapamil**	***P***
G_{max} (mg/dl)	16±13	6±10	0.008
ΔAUC_{gluc60} (mg/dl·min)	594±500	-6±556	0.015
ΔAUC_{gluc} (mg/dl·min)	509±1,224	-702±1,103	0.010

8. Verapamil's effect on metformin's pharmacokinetics (Study III)

Participants received two oral doses of metformin on Days 1/2 (1750 mg total) and again on Days 15/16. After verapamil treatment, the pharmacokinetic result of metformin was not changed significantly. The $AUC_{met\ (0\text{-}24)}$ and the C_{max} were not significantly different (8222±1756 ng/ml vs. 8835±2432 ng/ml, P = 0.530 and 1511±366 ng/ml vs. 1641±599 ng/ml, P = 0.754, respectively). Verapamil treatment did not significantly alter $t_{1/2}$ of metformin, either. Neither renal clearance (CL_R) of metformin, creatinine clearance, nor net tubular secretion (Sr_{CLR}) of metformin ($CL_R - CL_{Cr}$) was significantly changed after verapamil treatment. (Results are shown in Table 9.) The concentration of metformin with and without varapamil is shown in figure 7.

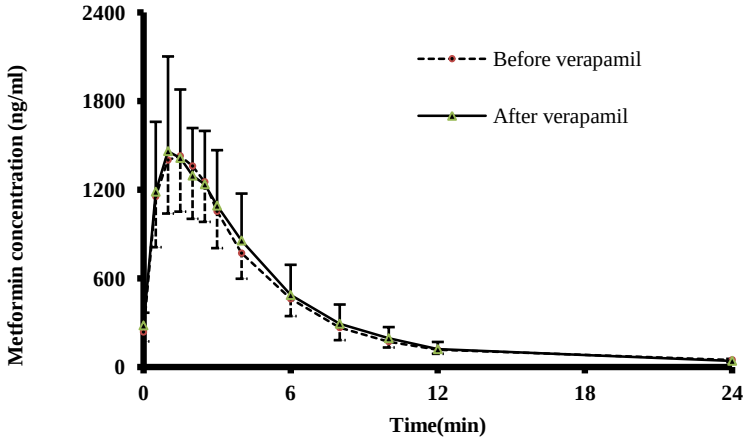

Figure 7. The plasma concentration-time curve of metformin on Day 2 (without verapamil treatment) and Day 15 (with verapamil treatment). Metformin concentrations were measured after the second dose of metformin. Data are expressed as mean and SD (n = 12).

Table 10. Pharmacokinetic parameters of metformin in healthy participants (n = 12) with and without verapamil treatment.

	Without verapamil	**With verapamil**	***P***
$AUC_{met(0-24)}$ (ng/ml·hr)	8786±1848	9250±2519	0.583
$t_{1/2}$ (hr)	7.66±2.15	6.15±2.14	0.060
C_{max} (ng/ml)	1511±366	1641±599	0.754
T_{max} (hr)	1.54±0.45	1.29±0.69	0.403
CL_R (ml/min)	662±140	681±157	0.433
$SrCL_R$ (ml/min)	563±137	585±150	0.388
CL_{Cr} (ml/min)	99±14	97±15	0.272

Data were evaluated by Wilcoxon signed rank test and expressed as mean ± SD. $AUC_{met(a-b)}$, area under the plasma concentration-time curve from time point a to time point b; $t_{1/2}$, elimination half-life; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; CL_R, renal clearance; $SrCL_R$, renal clearance by tubular secretion; CL_{Cr}, creatinine clearance.

IV. DISCUSSION

The purpose of the study was to determine the role of OCTs and MATE1 in metfomin's pharmacokinetics, pharmacodynamics and drug-drug interaction. I conducted 3 studies to show the effect.

I conducted a large prospective clinical trial to show the effect of OCT2 (rs316019) and MATE1 (rs2252281) polymorphism on metformn's pharmacokinetics. I showed

that OCT2 rs316019 did not affect metformin's pharmacokinetics and MATE1 polymorphism (rs2252281) was associated with reduced metformin CL_R and $SrCL_R$. There was opposite result of pharmacokinetic of metformin by OCT2 genotype (rs316019) in Asian and American population[13-15]. CL_R and $SrCL_R$ of metformin were decreased in subjects carry the OCT2 polymorphism in MATE1 TT group whereas CL_R and $SrCL_R$ of metformin increased in subjects carry the OCT2 polymorphism in MATE1 TC group. This can explain some of the different results by ethnics. Our study suggests that MATE1 polymorphism (rs2252281) is the main polymorphism to affect renal clearance and secretion of metfomin. It is more logical to explain the difference of renal clearance of meformin by MATE1 because MATE1 is located in the apical membrane of renal proximal tubule.

In study II and III, I showed that OCT1 plays an important role in metformin's glucose lowering effect by conducting OCT1 induction and inhibition study. I showed that rifampin increased the metformin's glucose-lowering effect through the induction mechanism of OCT1 and metformin's glucose-lowering effect was disappeared when it is treated with verapamil, OCT1 competitive inhibitor. I observed that rifampin slightly increased systemic exposure to metformin as assessed by pharmacokinetic parameters (AUCs) and verapamil did not affect the pharmacokinetics of metformin. Previous studies investigated in mice that the liver concentration of metformin in $Oct1^{-/-}$ mice showed 30 times lower than in $Oct1^{+/+}$ mice[24] and the glucose lowering effect of metfomin was not observed in $Oct1^{-/-}$ mice. The results of glucose lowering effect of metformin were consistent with our

hypothesis when the inducer or inhibitor was administered, whereas pharmacokinetic results were not consistent with the previous studies. These results may be due to volume difference between liver and plasma. The liver volume is approximately 10% of plasma volume.[25] Increased or decreased metformin transport activity into hepatocyte may affect the metformin concentration and glucose lowering effect since hepatocyte is the main action site of metformin. This change was not reflected in plasma pharmacokinetics.

CL_R and $SrCL_R$ of metformin increased after the 10-day course of rifampin in study II, but CL_{Cr} did not change, indicating that the transporter activity increased renal elimination of metformin. This is consistent with a previous study in rats reporting that the PXR ligand PCN affected the pharmacokinetics of metformin in vivo and in vitro by upregulating the expression Oct1 and Oct2[17]. OCT2 is located in the basolateral membrane of renal proximal tubule, where it plays an important in metformin elimination[26-27]. The increased metformin elimination was probably due to upregulated activity of OCT2 or another transporter.

AUC, C_{max}, CL_R and $SrCL_R$ of metformin did not change when it is treated with verapamil in Study III. In other words competitive inhibitor of OCT1 did not change the pharmacokinetics. However, the metformin's glucose lowering effect was not observed when it is treated with verapamil. Liver is the main action site of metformin.[1] This result indicates that glucose lowering effect of metformin is not shown by blocking transport mediated-uptake process completely.

Here are some limitations of our studies. First, OCT1 mRNA levels in blood may

not reflect hepatic OCT1 mRNA levels; however, it is not ethical to perform liver biopsies in healthy volunteers. Second, rifampin may regulate transporters other than OCTs that are involved in metformin pharmacokinetics and verapamil may regulate transporters other than OCTs that are involved in metformin pharmacokinetics. However, MATE1 does not appear to be affected by rifampin or PXR agonists in general [28]. On the other hand, transporters involved in absorption, such as PMAT, may be induced by rifampin. Further study is needed to investigate the change of absorption kinetics of metformin. Third, the pharmacological action of metformin is the result of multiple factors, including many that are beyond the scope of this study. The regulation of hepatic gluconeogenesis by PXR may be mediated by numerous factors including HNF4α [29], but the clinical significance of these interactions are unclear. In the present study, I observed that baseline glucose levels were not changed by rifampin, suggesting that PXR activation itself did not significantly alter serum glucose levels. It is reported that overdose of verapamil induces hyperglycemia[30]. 240mg of verapamil is not overdose and baseline glucose levels was not changed after verapamil treatment. The inhibition of metformin's seems to be solely mediated by OCT1-mediated competitive inhibition. Moreover, these prospective studies were designed to minimize the effect of confounding factors. Finally, I assessed the factors influencing the metformin's PK and PD in healthy subjects. To this knowledge, I proved that OCT1 may affect metformin's response and MATE1 may affect the metformin's disposition. It is needed to investigate the effect of OCT1 and MATE1 in diabetes patient as the aspect of drug

efficacy and adverse drug reaction.

V. CONCLUSION

In conslusion, I found that rifampin increased metformin concentration in the blood and enhanced its glucose-lowering action. In addition, rifampin increased its renal tubular secretion. OCT1 inhibitor, verapamil inhibited metformin's glucose lowering action. Because it is the competitive inhibitor of OCT1, it did not change the metformin's pharmacokinetics and renal clearance of metformin. Through the large-scaled, genotype-enriched and prospective clinical trial by OCT2 and MATE1 genotype, MATE1 is critical step in metformin's renal elimination. I found that MATE1 polymorphism affected metformin's renal clearance and tubular secretion.

OCT1 plays a key role in glucose-lowering effect of metformin. OCT1 based drug-drug interaction is important in the response of metformin even though OCT1 is a drug transporter related to the phramacokinetics. MATE polymorphism (rs2252281) affects renal excretion of metformin.

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ABSTRACT (In Korean)

메트포민의 약동, 약력학 및 약물상호 작용에 미치는 약물 수송체의 영향에 대한 연구

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조 성 권

Biguanide 유도체인 metformin은 제 2 형 당뇨병 치료를 위한 경구 혈당 강하 약물로 1차 치료제 이다. 약물 작용의 기전은 간세포 내에서의 gluconeogenesis에 의한 포도당 생산을 억제하는 것으로 이로 인해 혈액 내의 혈당이 감소한다. 일반적으로 약물 수송체의 수송능의 변화는 약물의 흡수와 배설에 영향을 미쳐 혈중 약물농도를 변화시키는 것이 중요하다고 알려져 있으나 효과 장소로의 약물 수송에 관여하는 약물 수송체는 약의 효과에 관련이 있는 것으로 알려져 있다. Metformin은 Organic Cataion Tranasporters (OCTs)과 Multidrug And Toxic Extrusion1 (MATE1)에 의해 수송된다. 이들 Drug transporter의 분표는 OCT2가 주로 renal proximal tubule의 basolateral side에 주로 분포하는 반면 OCT1은 주로 hepatocyte의 sinusoidal membrane에 위치하고 있다. MATE1은 renal proximal tubules의 apical side와 간세포에서 biliary

secretion에 관여하는 apical side에 분포하며 이의 수송은 H^+/Organic cation antiporter의 형태로 존재한다. 따라서 메트포민의 약물의 효과에는 OCT1이 영향을 미칠 것으로 생각 되고 OCT2, MATE1은 약물의 혈중농도 또는 신장의 배설에 영향을 줄 것으로 생각된다. 메트포민의 수송에 관계하는 Drug transporter의 연구는 많이 진행되었다. 특히 최근에는 Metformin의 renal excretion에 관계하는 OCT2의 polymorphism (rs316019)에 따라서 metformin renal clearance, blood concentration 연구결과가 다르다는 논란이 있다. 이에 따라 apical side에 분포하는 MATE1의 pharmacogenetic difference가 OCT2보다 더 중요한 역할을 할 수 있다. 이에 따라 본 연구에서는 genotype-enrichment clinical trial을 통해 metformin의 pharmacokinetics 및 renal clearace에 관한 OCT2와 MATE1의 polymorphism이 미치는 영향을 알아보았다. 또한 metformin의 main action site으로의 수송에 관계된 OCT1의 역할은 metformin의 약리학적 작용과 약물 부작용에 관여할 것으로 생각되므로 본 연구에서는 OCT1 유도제인 rifampin과 OCT1 억제제인 verapamil을 metformin과 동시 투여하여 metformin의 혈당강하 효과에 미치는 OCT1의 역할을 알아 보았다.

본 논문의 연구 결과는 크게 3가지로 구성된다. Study I에서는 metformin의 blood pharmacokinetics, renal clearance 그리고 metformin의 혈당 강하 효과를 MATE1 (rs 2252281) 및 OCT2 (rs

316019)에 따라 평가하였다. 뒤의 두 가지 연구에서는 메트포민의 혈당 강하 효과에 영향을 보기 위해 OCT1 inducer인 rifampine과 OCT1 inhibitor인 verapamil을 사용하여 메트포민과 약물 작용을 평가하였다. Study II, III에서는 rifampin과 verapamil 준 다음의 metformin의 pharmacokinetics와 metformin의 혈당 강하 효과를 비교 평가하였다. Metformin의 혈당 강하효과는 metformin을 주기 전과 후의 경구 혈당 검사 (OGTT)의 차이를 비교하여 계산하였다. 세 개의 변수가 metformin의 혈당 강하 효과를 평가하기 위해 사용되었다. 최대 혈당 강하 효과 (G_{max}), 혈당 농도-시간 곡선의 차이 하 면적(ΔAUC_{gluc})은 사다리꼴 규칙을 사용하여 계산되었다. ΔAUC_{gluc60}는 혈당 상승 구간에서의 차이로 포도당 섭취 후 0 ~ 60 분사이의 혈당 곡선 하 면적으로 정의되었다.

메트포민의 혈중 약동학적 파라미터의 비교에는 C_{max}, T_{max}, AUC_{met} and $t_{1/2}$가 사용되었고 메트포민의 뇨 약동학적 파라미터의 비교에는 CL_R (메트포민의 뇨 청소율), CL_{Cr} (크레아티닌 청소율), $SrCL_R$ (메트포민의 뇨 배설 청소율)이 사용되었다.

결과상 Study I에서는 혈중 메트포민의 약동학적 파라미터들은 MATE1, OCT2의 유전자형에 의해 유의한 차이를 보이지 않았으나 metformin의 renal clearance와 renal secretion의 MATE1의 유전자형에 따라 통계적인 차이를 보였다. (CL_R: 617±126 vs. 556±106 vs.

507±104 ml/min, P=0.031 and $SrCL_R$: 517±121 vs. 456±107 vs. 399±107 ml/min, P=0.017)

Study II 에서는 rifampin은 메트포민의 혈당 강하 지표인 ΔAUC_{gluc60} 54.5 % (P = 0.020), G_{max} 41.9 % 증가 것으로 나타났다. metformin의 renal clearance는 rifampin 에 의해 16 % 증가되었고 (P = 0.008), metformin의 AUC는 흡수 증가 가능성에 따라 (13 %, P = 0.049) 증가되었다. Rifampin은 말초 혈액 세포에서 OCT1 mRNA 4.1 배 (P = 0.001) 증가 시켰다. 연구 III에서는 Verapamil에 의해 메트포민의 혈당강하가 거의 나타나지 않은 것을 볼 수 있었는데 G_{max}의 경우 62.5% (16 mg/dl vs. 6 mg/dl; P = 0.010)가 감소하였다. 특히 베라파밀에 의해서 메트포민의 혈당강하가 ΔAUC_{gluc60} (594±500 mg/dl·min vs. -6±556 mg/dl·min; P = 0.008)와 ΔAUC_{gluc} (509±1224 mg/dl·min vs. -702±1103 mg/dl·min; P = 0.015) 두 파라미터를 통해서 거의 나타나지 않은 것을 알 수 있었다. 하지만 베라파밀에 의해서 약동학적 수치인 AUC, C_{max}, CL_R and $SrCL_R$ 들은 변화하지 않은 것을 알 수 있었다.

이상의 연구들을 통해 OCT1은 metformin의 혈당강하 효과에 핵심적인 역할을 담당하는 것을 알 수 있었다. 특히 약물의 수송에 관계되어 약동학적 수송에 더 영향을 미칠 것으로 생각되는 OCT1이 약력학적인 측면에 더 관계가 있다는 것은 약의 효과가 나타나는 곳으로의 수송이 약력학적인 측면에서 중요하다는 것을 의미한다. 이를 통해 Drug

transporter인 OCT1 기반의 약물-약물 상호 작용이 메트포민의 약물 반응에 중요하다는 사실을 알 수 있다. 그리고 신장으로 주로 배설되는 메트포민의 renal clearance와 secretion에 영향을 미치는 것으로는 기존에 알려진 OCT2보다는 MATE1의 polymorphism (rs2252281)이 중요한 역할을 한다는 사실을 알 수 있었다.

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핵심되는 말 : 메트포민, OCT 1&2, MATE1, 약동학, 약력학

PUBLICATION LIST

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