의학박사 학위논문

A clinical study to investigate the effect of multidrug and toxin extrusion transporter inhibitor on pharmacokinetic characteristics and anti-hyperglycaemic activity of metformin

Multidrug and toxin extrusion 수송체 저해제가 메트포민의 약동학 특성 및 혈당 강하 효과에 미치는 영향 평가 연구

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A thesis of the Degree of Doctor of Philosophy

Multidrug and toxin extrusion 수송체 저해제가 메트포민의 약동학 특성 및 혈당 강하 효과에 미치는 영향 평가 연구

A clinical study to investigate the effect of multidrug and toxin extrusion transporter inhibitor on pharmacokinetic characteristics and anti-hyperglycaemic activity of metformin

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ABSTRACT

Introduction: Renal tubular secretion of metformin is mediated mainly via Multidrug And Toxin Extrusion (MATE) transporters at the apical membrane. We hypothesized that the anti-hyperglycaemic activity of metformin would change with inhibition of the MATE transporter.

Methods: Twenty healthy male subjects received 500 mg of metformin with and without 50 mg of pyrimethamine (a potent MATE inhibitor) twice daily, with 1 week of washout in between. The anti-hyperglycaemic activity of metformin were assessed for each period by administering the oral glucose tolerance test (OGTT) before and after the metformin dose; the differences in mean, maximal and 2-hour-post OGTT glucose levels (ΔG_{mean}, ΔG_{max} and ΔPP2) were calculated. Metformin concentrations in plasma and urine were determined using liquid chromatography-electrospray ionization-tandem mass spectrometry, and pharmacokinetic (PK) parameters were calculated using a noncompartmental method. A general linear mixed effects model was used to compare metformin's PK and PD parameters between treatment periods.

Results: When metformin was co-administered with

pyrimethamine, its renal clearance was reduced by 72% (P < 0.05), and its area under the concentration–time curve from 0 to 12 hours (AUC_{0-12h}) was increased by 101% (P < 0.05), where the anti-hyperglycaemic effects of metformin were decreased. The mean differences (90% CI) in ΔG_{mean}, ΔG_{max} and ΔPP2 were −0.6 (−1, −0.2), −0.9 (−1.6, −0.3) and −0.5 (−1.1, 0.1) mmol/L, respectively.

Conclusions: These findings indicate that the response to metformin is not only related with the plasma exposure of metformin but also related with other factors such as inhibition of uptake transporters and gastrointestinal based pharmacology of metformin.

* Part of this work has been published in *diabetes, obesity and metabolism* (Oh J, et al. *Diabetes Obes Metab.* 2016 Jan 18. doi: 10.1111/dom.12577).

Keywords: Metformin, drug transporter, MATE, pharmacokinetics, pharmacodynamics

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AUC_{0-12h}	Area under the concentration–time curve from 0 to 12 hours
$AUC_{infinite}$	Area under the concentration–time curve from 0 to infinity
AUG_{0-3h}	Area under the glucose concentration–time curve for 3 hours
BMI	Body mass index
CI	Confidence interval
CL/F	Total apparent clearance
CL_{Cr}	Creatinine clearance
CL_R	Renal clearance
C_{max}	Maximum concentration of metformin
G_{max}	Maximal glucose level during OGTT
G_{mean}	Mean glucose level during OGTT
GMR	Geometric mean ratio
IR	Immediate release formulation
Ki	Inhibition constant
MATE	Multidrug and toxic compound extrusion protein
OCT	Organic cation transporter

OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PK	Pharmacokinetic
PP2	2-hour-post OGTT glucose level
SLC47A1	Solute carrier family 47 member 1
SNP	Single nucleotide polymorphism
SST	Serum separating tube
$t_{1/2}$	Terminal half-life
T2DM	Type 2 diabetes mellitus
T_{max}	Time to reach maximum concentration of metformin
V_z/F	Apparent volume of distribution
λ_z	Terminal elimination rate constant

INTRODUCTION

Metformin is an oral antidiabetic medication of the biguanide class and is the most frequently used drug for the treatment of hyperglycaemia in patients with type 2 diabetes mellitus (T2DM) [1]. Its precise mode of action remains unclear, but the reduction of gluconeogenesis in the liver is believed to be the primary mechanism by which metformin exerts its effects [2-4]. Metformin exhibits saturable intestinal absorption, and the absorbed metformin is not metabolized and does not bind to plasma proteins. Metformin is primarily eliminated through the renal system, and active secretion in the renal tubule is important for the elimination of metformin in humans [2, 5-7].

Many drug transporters including organic cation transporters (OCT1, OCT2 and OCT3), the multidrug and toxin extrusion (MATE) transporters, and plasma membrane monoamine transporter (PMAT) are involved in the disposition of metformin and can affect its pharmacokinetic (PK) and pharmacodynamic (PD) properties [2, 8-12]. OCT1 and OCT3 mediate the hepatic uptake of metformin from the blood, and OCT3 may also contribute to the muscular uptake of metformin [2, 12, 13]. OCT2 also plays an important role in the renal

elimination of metformin [2, 9]. The multidrug and toxin extrusion (MATE) transporters MATE1 and MATE2-K are thought to mediate the renal and biliary elimination of metformin [2, 14].

The contribution of MATE transporter function to inter-individual variability in the response to metformin therapy has recently been investigated in various *in vivo* studies [15-19]. MATE1 is encoded by the *SLC47A1* gene and is expressed in the liver, kidneys and skeletal muscles. When metformin was administered to *SLC47A1* knock-out mice, the metformin concentrations in these organs were higher than the concentrations found in wild-type mice [15, 16]. T2DM patients with the homozygous rs2289669 c.922-158G>A SNP variant showed higher systemic exposure and responses to metformin than both those with the heterozygous variant and the wild-type patients [17-19]. However, the corresponding polymorphism is located in the non-coding region of the *SLC47A1* gene, and the mechanism whereby that genetic polymorphism affects metformin's PKs and efficacy has not yet been clearly explained.

The role of MATE transporters in the PKs and PDs of metformin can be investigated via drug–drug interaction studies using MATE inhibitors. Pyrimethamine is an antiparasitic compound used for the treatment of toxoplasmosis and malaria, and it is a potent and specific inhibitor of MATE1 and MATE2-K. In an earlier study, 50 mg of pyrimethamine significantly reduced the renal elimination of metformin, thereby increasing plasma exposure to metformin in healthy subjects [20, 21]. However, the effect of MATE inhibition on the PDs of metformin has not been evaluated.

The functional change of MATE transporters by pyrimethamine can be assessed by creatinine clearance. Creatinine is an endogenous substance that is known to undergo OCT2–, MATE1– and MATE2–K–mediated secretion in the renal tubules, and previous studies have shown that pyrimethamine decreased the renal creatinine clearance without affecting the glomerular filtration rate (GFR) [22–24].

Genetic polymorphism of drug transporters can also affect PKs and PDs of metformin. An intronic SNP of MATE1 (c.922–158G>A, rs2289669), two promotor variants of MATE2–K (c.–396C>T, rs34834489 and c.–130C>T, rs12943590) and

one missense variant of OCT2 (c.808G>T, rs316019) are known to be associated with metformin's renal elimination [11, 14, 17-19, 25]. The subject's genetic status need to be considered in drug-drug interaction studies, because the degree of MATE inhibition by pyrimethamine can be differed among the subjects according to those genetic variants.

Based on these findings, we hypothesized that MATE inhibition would increase plasma concentrations of and intracellular exposure to metformin and alter its glucose-lowering effect. To this end, metformin PK and oral glucose tolerance test (OGTT) results were compared in healthy male volunteers before and after co-administration of pyrimethamine while also considering the genetic polymorphism.

METHODS

Subjects

This clinical study (NCT01973933) was approved by the Institutional Review Board of Seoul National University Bundang Hospital, Seongnam, Korea (B-1305/201-003) and was conducted in accordance with the principles of the Declaration of Helsinki and ICH Good Clinical Practice. All of the subjects who participated were informed of the study protocol and every possible adverse event before written consent was obtained. A total of 20 healthy Korean male subjects (age 23.3 ± 2.5 years; height 174.2 ± 5.7 cm; weight 66.9 ± 7.2 kg; BMI 22.1 ± 2.3 kg/m^2; fasting glucose 96 ± 6 mg/dL) were enrolled, and all of the subjects completed the trial as planned.

Clinical study design

An open-label, 2-period, single-sequence clinical study was conducted (**Figure 1**). Subjects maintained a carbohydrate intake of 200 to 250 g/d and restricted alcohol intake for 3 days prior to admission. The subjects were admitted to the Clinical

Trials Center at Seoul National University Bundang Hospital one day before metformin administration in period 1. After an overnight fast, a 3-hour OGTT using 75 g of glucose was conducted. Two hours after the evening meal, the subjects received 750 mg of metformin IR (Diabex Tab; Daewoong Pharmaceutical Co., Seoul, Korea) with 240 mL of water and continued fasting until the next day. Twelve hours after the first metformin dose, 500 mg of metformin IR was orally administered with 240 mL of water. Serial blood and urine samples were collected to analyse metformin PKs and creatinine levels over 12 hours. An OGTT was repeated 2 hours after the second metformin dose. The subjects were discharged from the hospital after every procedure. Five days after the day of discharge (period 2), the subjects underwent the same in-hospital procedures that were performed during period 1, except that 50 mg of oral pyrimethamine was administered together with the evening metformin dose and 1 hour before the morning metformin dose. Genotypes known to be associated with metformin's renal elimination were retrospectively analysed in the subjects.

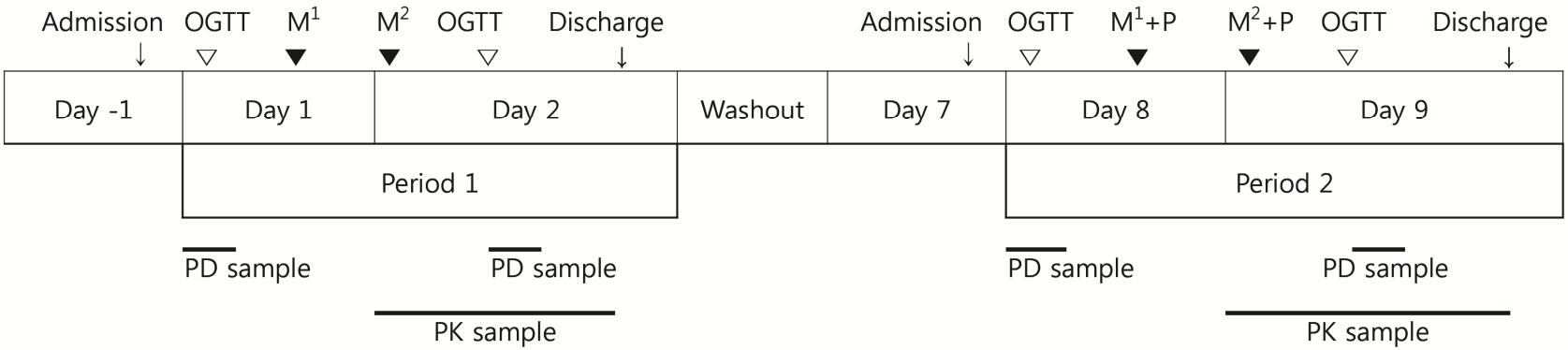


Figure 1. Study design. M^1: Metformin 750 mg, M^2: Metformin 500 mg, P: Pyrimethamine 50 mg.

Blood and urine collection

To analyse the plasma concentration of metformin, serial blood samples were collected using a heparinized tube at 0 (i.e., pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours after the morning metformin dose. To analyse blood glucose, blood samples were collected using a serum-separating tube (SST) at 0 (i.e., pre-OGTT), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 hours after the OGTT. To analyse serum creatinine levels, blood samples were collected using an SST at 0 (i.e., pre-dose), 3, 6, 8 and 12 hours after the morning metformin dose. To analyse the urine concentration of metformin and creatinine, urine was collected at 0-6 and 6-12 hours after the morning metformin dose.

Determination of metformin concentrations

Metformin concentrations in plasma and urine were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (Agilent 1260 HPLC system and Agilent 6490 Triple Quadrupole; Agilent Technologies, Santa Clara, CA, USA). To prepare the samples for analysis, an aliquot of either plasma or urine specimen was mixed with acetonitrile in the

presence of an internal standard (phenformin; Sigma-Aldrich, Yongin, Kyounggi, Korea). The mixture was vortexed for 5 minutes and then centrifuged for 5 minutes at 10,000 rpm. An aliquot of the supernatant was transferred to an autosampler vial, and 1 μL was injected onto the column (Kinetex HILIC, 50 × 2.1 mm, 5 μm; Phenomenex, Torrance, CA, USA) at 10° C. The aqueous mobile phase consisted of 100% acetonitrile and 5 mM ammonium formate. Detection of the precursor to product ion transition was achieved by employing electrospray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The precursor-to-product ion pairs at mass-to-charge ratio (m/z) were 130.1 to 71.1 for metformin and 206.2 to 60.1 for phenformin. The calibration curves were linear over the range of 10 - 5,000 μg/L for the plasma samples and 100 - 25000 μg/L for the urine samples.

Genotyping of MATE1, MATE2-K and OCT2

An intronic SNP of MATE1 (c.922-158G>A, rs2289669), two promotor variants of MATE2-K (c.-396C>T, rs34834489 and c.-130C>T, rs12943590) and one missense variant of OCT2 (c.808G>T, rs316019) were genotyped because they are

known to be associated with metformin's renal elimination [11, 14, 17-19, 25]. Genomic DNA was extracted from 200 μl of peripheral whole blood from each volunteer using a QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Germany). A total of 10 μl of PCR reaction mixture was prepared with 5 μl of 2X TaqMan Genotyping Master Mix, 0.25 μl of 40X Drug Metabolism Genotyping Assay Mix, 3.75 μl of DNase-free water, and 1 μl of genomic DNA. PCR reactions were carried out as follows: initial denaturation at 95° C for 10 min, 50 cycles of denaturation at 92° C for 15 s, and annealing/extension at 60° C for 1 min. The allelic discrimination results were determined after the amplification by performing an end-point read. AB 7500 Real-Time PCR System software version 2.0.6 (Applied Biosystems, Foster City, CA, USA) was used for the analysis.

Pharmacodynamics data analysis from the OGTT

Metformin reduces glucose production in diabetic patients [3, 4, 26] and exerts the same effect in non-diabetic subjects if their serum glucose concentrations are increased by glucose ingestion [10]. Based on earlier studies, the OGTT was

performed to evaluate the PDs of metformin [10, 27-29]. Serum glucose concentrations and plasma metformin concentrations were analysed by noncompartmental analysis using Phoenix® WinNonlin® software version 1.3 (Certara, St. Louis, MO, USA). The area under the blood glucose concentration-time curve for 3 hours (AUG_{0-3h}) after the OGTT was calculated using the linear trapezoidal rule, and mean serum glucose concentration during OGTT (G_{mean}) was calculated as $G_{mean} = AUG_{0-3h}/3$. The observed concentrations were used to estimate the maximal and 2-hour-post-OGTT glucose concentrations (G_{max} and PP2). The glucose-lowering activity of metformin in each period was calculated as the differences in the G_{mean}, G_{max} and PP2 values between the samples taken before and after the second metformin dose (ΔG_{mean}, ΔG_{max} and ΔPP2).

Pharmacokinetics data analysis

The area under the concentration-time curve from 0 to 12 hours (AUC_{0-12h}) after metformin administration was calculated using the linear up, log down trapezoidal method. The observed concentrations and times were used to estimate the maximum

concentration of metformin (C_{max}) and the time to reach the C_{max} (T_{max}). The terminal elimination rate constant (λ_z) was estimated from a regression of the log-transformed plasma concentration of metformin versus time over the terminal log-linear portion of the concentration-time profile, and the terminal half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2 / \lambda_z$. The area under the concentration-time curve from 0 to infinity ($AUC_{infinite}$) was calculated as the sum of AUC_{0-12h} and the ratio of the last measurable plasma concentration to the elimination rate constant ($AUC_{infinite} = AUC_{0-12h} + C_{last} / \lambda_z$). The total apparent clearance (CL/F) was calculated as the administered dose of metformin divided by the AUC_{0-12h}, and the apparent volume of distribution (Vz/F) was calculated as the CL/F divided by the λ_z. The renal clearance (CL_R) of metformin was calculated as the total amount of metformin excreted through the urine during the 12-hour period divided by the AUC_{0-12h} and the administered dose of metformin. The net renal clearance by secretion (CL_{SR}) of metformin was calculated as CL_R – Creatinine clearance (CL_{Cr}).

Serum and urine creatinine data analysis

Serum and urine creatinine levels were determined by the kinetic Jaffe method using a Toshiba TBA 200FR and 120FR Chemistry Analyzer (Diamond Diagnostics, MA, USA) [30]. CL_{Cr} was calculated using the following formula:

$$CL_{Cr}\ (mL/min) = \left(\frac{\text{creatinine excreted through the urine over 12 hours}}{\text{mean serum creatinine over 12 hours} \cdot 12 \cdot 60}\right)$$

Statistical analysis

All of the individual PK and PD parameters are presented as the arithmetic mean and standard deviation. A general linear mixed effects model was used to compare AUC_{0-12h}, $AUC_{infinite}$, C_{max}, $t_{1/2}$, CL/F, CL_R, V_Z/F, CL_{Cr}, ΔG_{mean}, ΔG_{max} and $\Delta PP2$ between the two treatment periods. The geometric mean ratio (GMR) and its 90% confidence interval (CI) were estimated for the PK parameters, and the mean difference and 90% CI were estimated for the PD parameters. A signed rank test was used to compare T_{max} between the periods. Kruskal–Wallis and Mann–Whitney U tests were used to compare the PK and PD parameters that were measured for each period between the genotype groups. Statistical significance was determined when

the *P*-value was less than 0.05. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Effects of MATE inhibition on systemic exposure to metformin

Although the PD parameters were decreased after co-administration of pyrimethamine, plasma exposure to metformin during period 2 was increased by 2.02- to 2.68-fold over the exposure during period 1, and this increase was statistically significant (**Figure 2, Table 1**). The GMR (90% CI) for the AUC_{0-12h} of metformin after pyrimethamine administration compared to baseline was 2.58 (2.30, 2.89), and the C_{max} of metformin was 2.02 (1.79, 2.29). The T_{max} of metformin was prolonged after pyrimethamine administration (change of median value from 1.7 to 2.5 hours, $P = 0.010$). The CL/F, CL_R and V_Z/F of metformin were significantly reduced by 62-72% during period 2 compared to period 1 ($P < 0.001$) (**Figure 3, Table 1**).

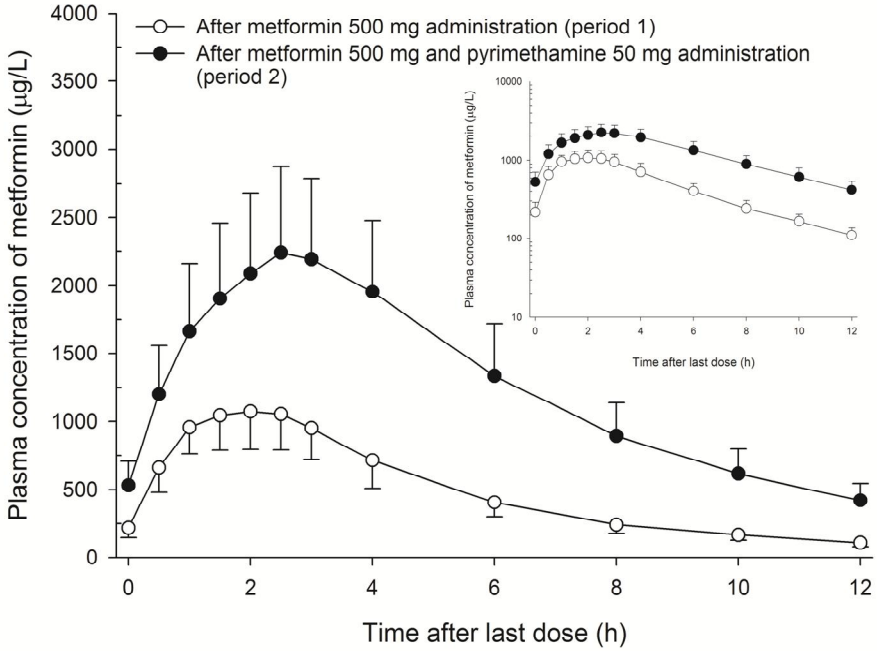


Figure 2. The plasma concentration profile of metformin before and after pyrimethamine co-administration

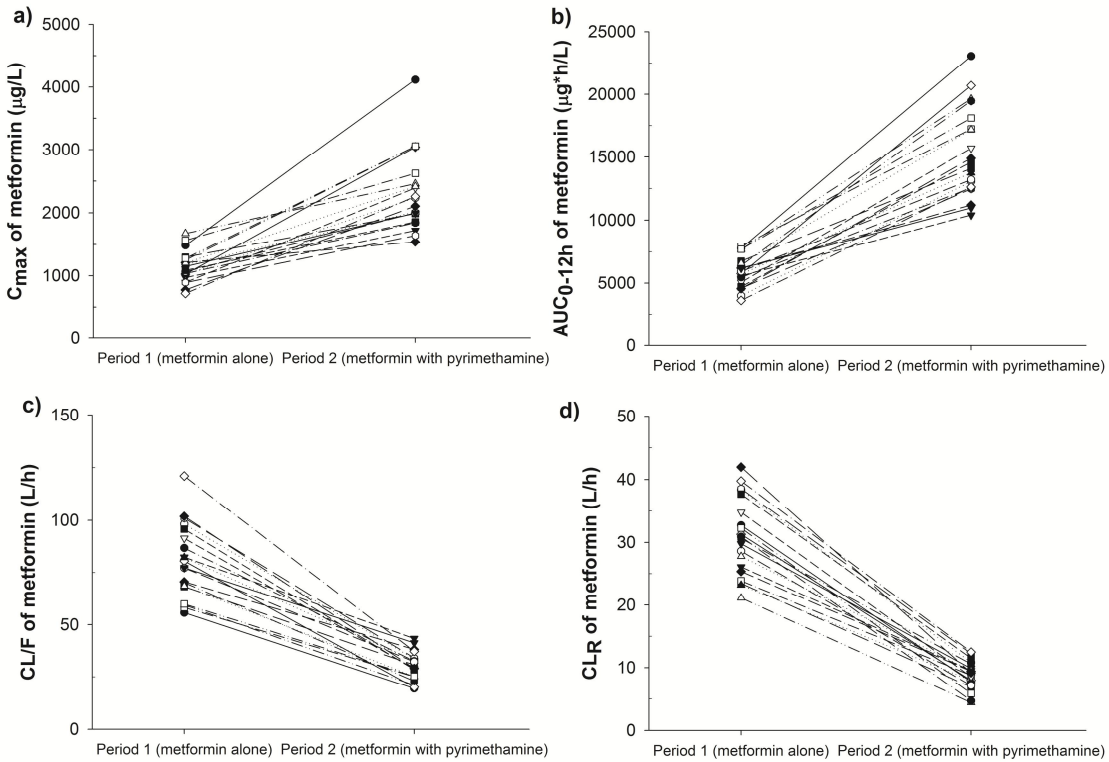


Figure 3. Changes in metformin' s pharmacokinetic characteristics after pyrimethamine co-administration; AUC_{0-12h}, area under the concentration-time curve from 0 to 12 h; CL/F, total apparent clearance; CL_R, renal clearance; C_{max}, maximum concentration of metformin.

Table 1. The metformin's pharmacokinetic parameters before and after pyrimethamine co-administration; Period 1: 500 mg of metformin was administered alone; period 2: 500 mg of metformin was administered with 50 mg of pyrimethamine

Pharmacokinetic parameters	Period 1 (metformin alone) (N = 20)	Period 2 (metformin with pyrimethamine) (N = 20)	Geometric Mean Ratio[1] (90% Confidence Interval)
AUC_{0-12h} (μg·h/L)	5908 ± 1267.6	15240.2 ± 3531.9	2.58 (2.30 – 2.9)
$AUC_{infinite}$ (μg·h/L)	6502.9 ± 1374.4	17531.3 ± 4167.7	2.68 (2.40 – 3.00)
C_{max} (μg/L)	1137.7 ± 247.3	2317.5 ± 618.8	2.02 (1.79 – 2.29)
T_{max} (h)	1.7 [0.5 – 3]	2.5 [1 – 6]	0.010[2, †]
CL/F (mL/min)	1338.2 ± 289.5	500.3 ± 113.9	0.37 (0.33 – 0.42)
CL_R (mL/min)	515.1 ± 102	145.9 ± 37.6	0.28 (0.25 – 0.31)
CL_{SR} (mL/min)	384.5 ± 102.5	56.2 ± 34.3	0.12 (0.09 – 0.16)
Creatinine clearance (mL/min)	130.6 ± 37.6	89.7 ± 24.4	0.69 (0.62 – 0.77)
V_Z/F (L)	432.5 ± 186	159.1 ± 36.7	0.38 (0.33 – 0.45)
$t_{1/2}$ (h)	3.7 ± 1.1	3.7 ± 0.5	1.03 (0.92 – 1.15)

Data are presented as the arithmetic mean ± standard deviation unless indicated.
T_{max} is presented as the median [min – max].
[1] Geometric mean ratio of period 2 to period 1 for the pharmacokinetic parameters; [2] Signed rank test; † $P < 0.05$

Effect of MATE inhibition on the glucose-lowering effect of metformin

When 50 mg of pyrimethamine was co-administered with metformin (period 2), the ability of metformin to lower glucose concentrations was significantly decreased compared to baseline. The ΔG_{mean}, ΔG_{max} and ΔPP2 decreased during period 2 compared to period 1; the mean differences in these parameters were −0.6, −0.9 and −0.5mmol/l, respectively **(Figure 4, Table 2)**.

The G_{mean}, G_{max} and PP2 measured after metformin administration were similar for both periods, but the values of G_{mean}, G_{max} prior to metformin administration were significantly higher during period 1 compared to period 2 (i.e., pyrimethamine co-administration) (P = 0.038 and P = 0.004, respectively) **(Figure 5, Table 3)**.

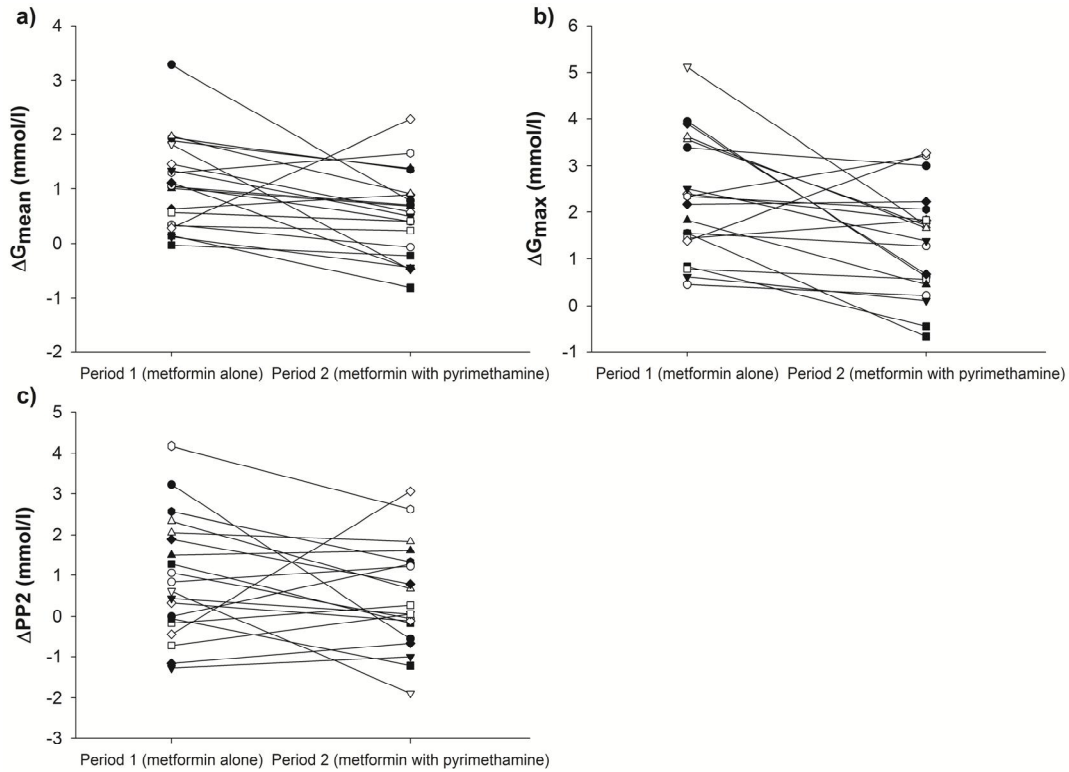


Figure 4. Changes in metformin' s anti-hyperglycaemic activity after pyrimethamine co-administration; ΔG_{mean}, differences in mean serum glucose concentration; ΔG_{max}, differences in maximal serum glucose concentration; Δ PP2, differences in 2-hour-post-OGTT serum glucose concentration

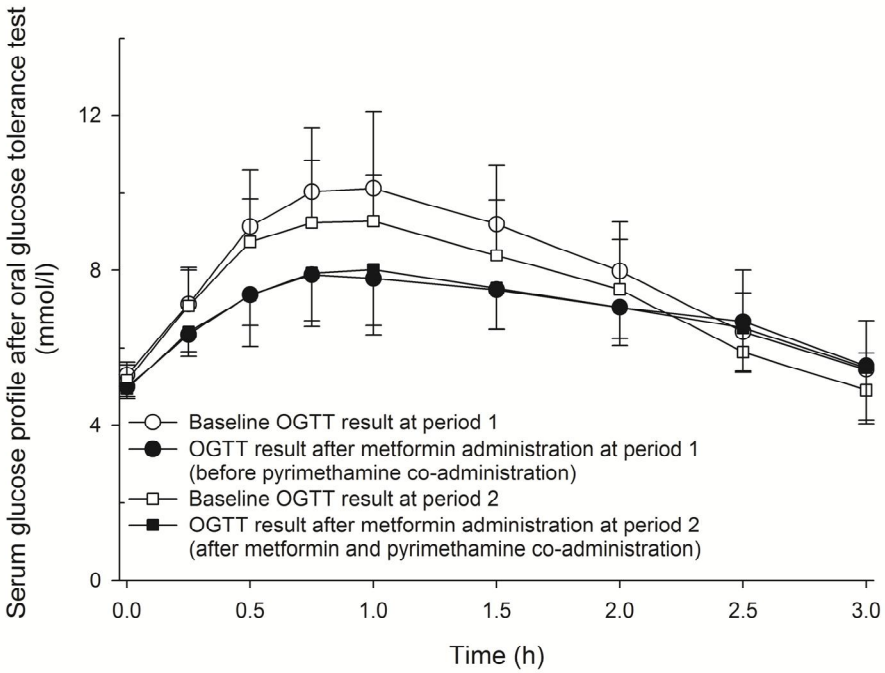


Figure 5. Serum glucose profiles after oral glucose tolerance test during period 1 (before and after metformin administration) and during period 2 (before and after metformin and pyrimethamine co-administration.

Table 2. The metformin's anti-hyperglycaemic activity before and after pyrimethamine co-administration; Period 1: 500 mg of metformin was administered alone; period 2: 500 mg of metformin was administered with 50 mg of pyrimethamine

Parameters	Period 1 (metformin alone) (N = 20)	Period 2 (metformin with pyrimethamine) (N = 20)	Mean Difference[1] (90% Confidence Interval)
ΔG_{mean} (mmol/l)	1.1 ± 0.8	0.5 ± 0.8	−0.6 (−1, −0.2)
ΔG_{max} (mmol/l)	2.3 ± 1.3	1.3 ± 1.1	−0.9 (−1.6, −0.3)
⊿Serum glucose at PP2 (mmol/l)	0.9 ± 1.5	0.5 ± 1.3	−0.5 (−1.1, 0.1)

Parameters are presented as the arithmetic mean ± standard deviation.

[1] Mean (marginal mean) difference of period 2 to period 1 for the parameters

Table 3. The results of a 75 g oral glucose tolerance before and after pyrimethamine co-administration; Period 1: 500 mg of metformin was administered alone; period 2: 500 mg of metformin was administered with 50 mg of pyrimethamine

Parameters	Period 1 (metformin alone) (N = 20)		Period 2 (metformin with pyrimethamine) (N = 20)	
	Pre-metformin	Post-metformin	Pre-metformin	Post-metformin
G_{mean} (mmol/l)	8.1 ± 3.3	7 ± 1.8	7.5 ± 2.2	7 ± 1.7
G_{max} (mmol/l)	10.7 ± 1.7	8.4 ± 1.2	9.9 ± 1.3	8.5 ± 1.2
Serum glucose at PP2 (mmol/l)	8.0 ± 1.3	7.1 ± 1.0	7.5 ± 1.3	7.1 ± 0.8

Parameters are presented as the arithmetic mean ± standard deviation.

Effects of MATE inhibition on the serum creatinine and creatinine clearance

The serum creatinine level was significantly increased, and the creatinine clearance, based on the creatinine levels in a 12-hour urine specimen and a serum specimen, was significantly lowered after 50 mg of pyrimethamine was administered ($P < 0.001$ for both parameters) (**Figure 6**). The serum creatinine and creatinine clearance (mean ± SD) at period 1 were 0.9 ± 0.1 mg/dL and 130.6 ± 37.6 mL/min, respectively, and the same values at period 2 were 1.1 ± 0.1 mg/dL and 89.7 ± 24.4 mL/min, respectively.

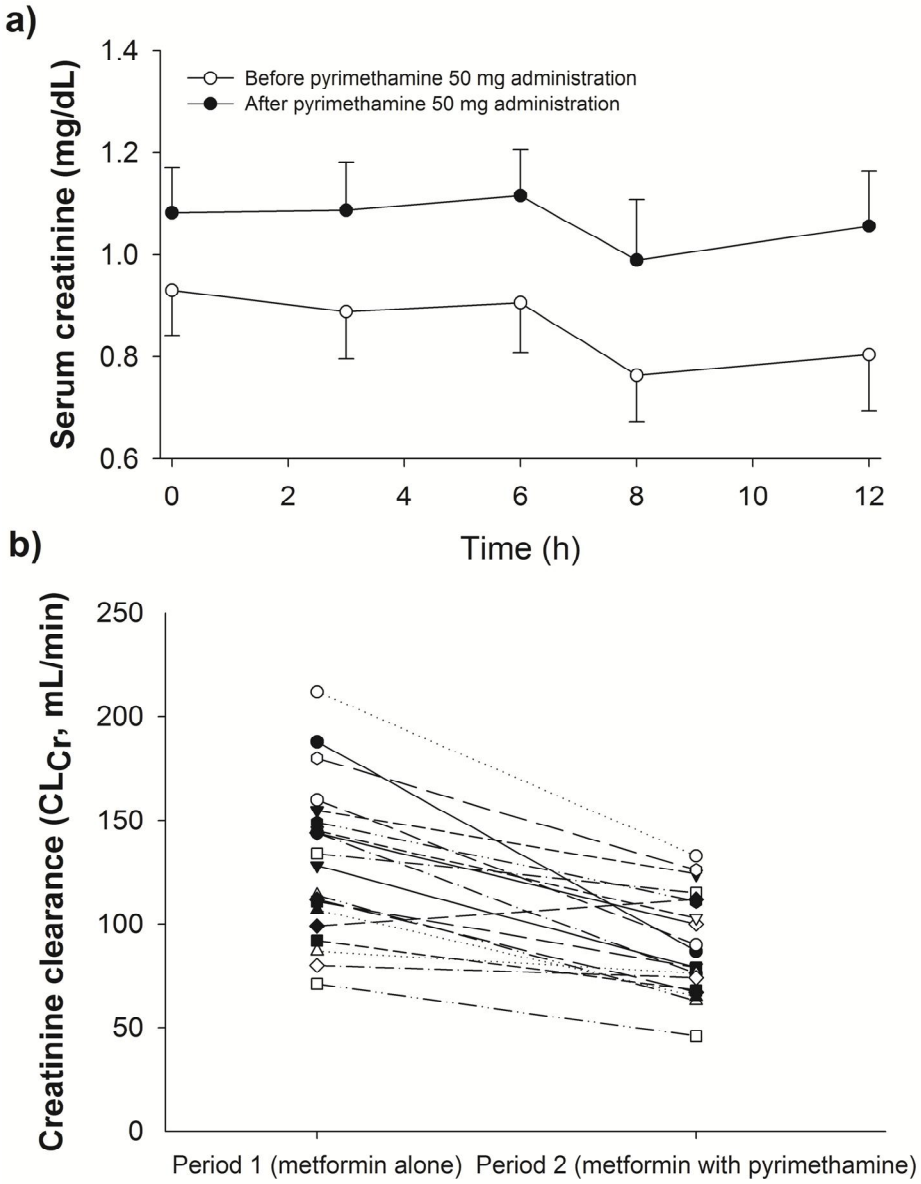
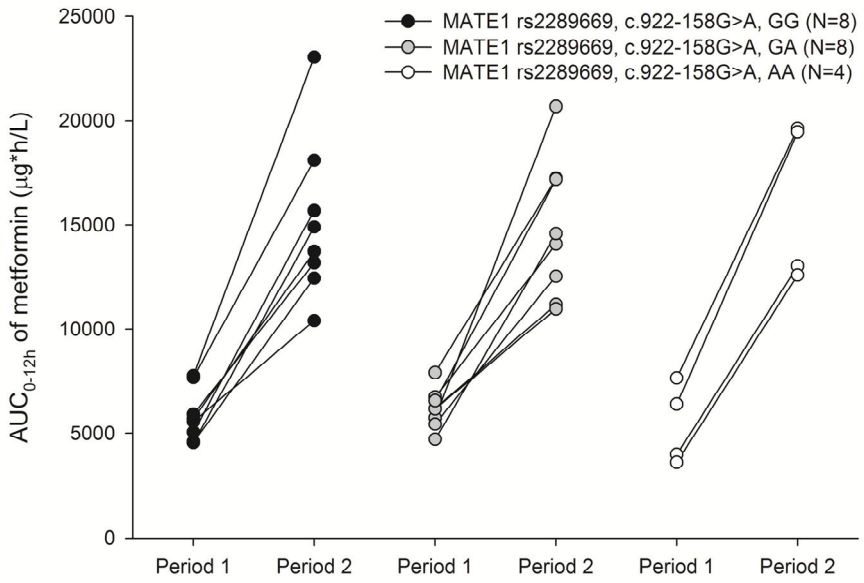


Figure 6. The plasma creatinine profile and creatinine clearance (calculated from the creatinine measured in a 24-hour urine specimen and a serum specimen) before and after the oral administration of 50 mg of pyrimethamine; a) plasma creatinine profile; b) change of creatinine clearance.

Effects of MATE1, MATE2-K and OCT2 genotype on the drug-drug interaction between the pyrimethamine and the metformin

The trend of a decrease in the ΔG_{mean} of metformin after pyrimethamine administration was similar in all of the MATE1, MATE2-K and OCT2 genotype groups (**Figure 7 to 10**). There were no statistically significant differences in the PK parameters between the MATE1, MATE2-K and OCT2 genotype groups during either period (**Table 4 to 7**).

a)



b)

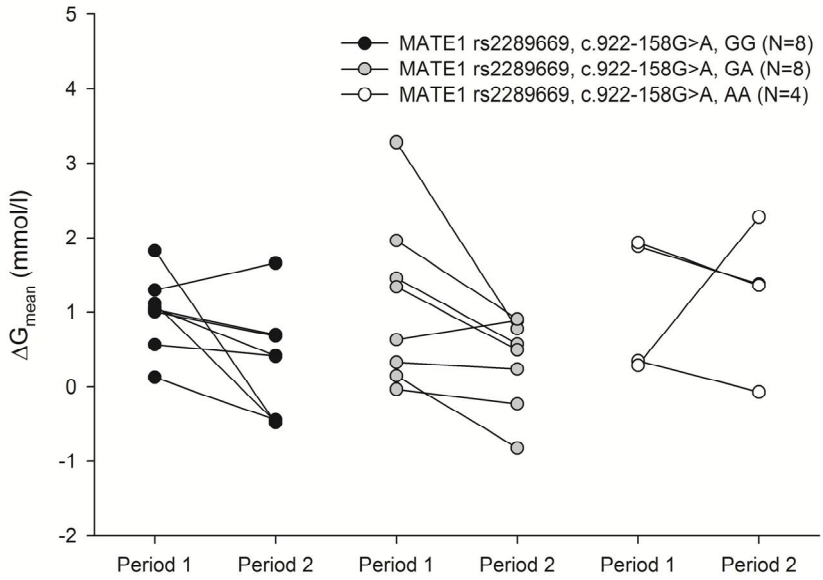
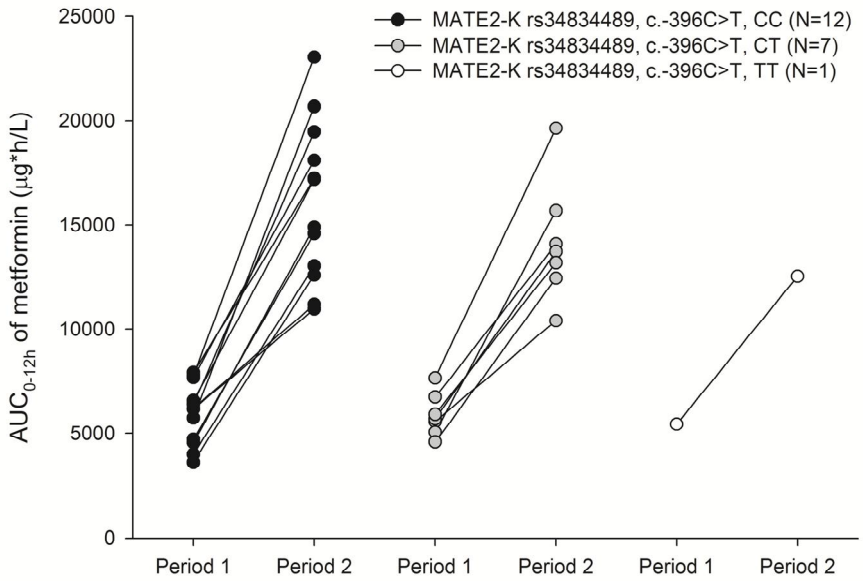


Figure 7. Changes in metformin exposure and its glucose lowering effects after pyrimethamine co-administration, categorized by c.922-158G>A (rs2289669) genotypes

a)



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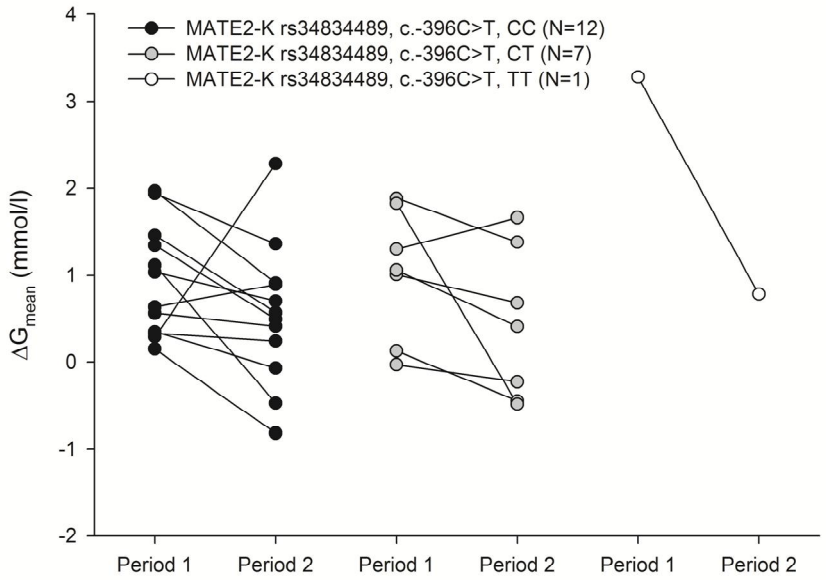
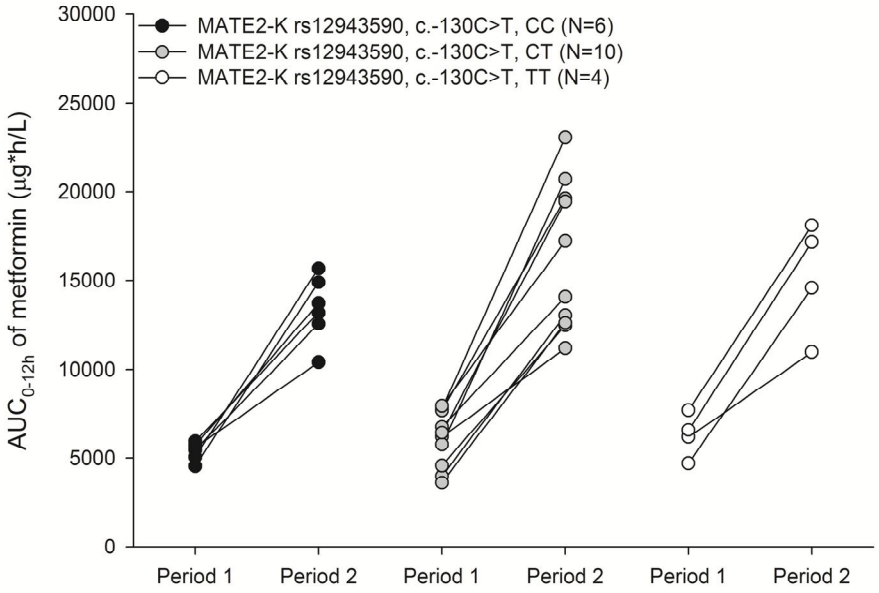


Figure 8. Changes in metformin exposure and its glucose lowering effects after pyrimethamine co-administration, categorized by c.-396C>T (rs34834489) genotypes

a)



b)

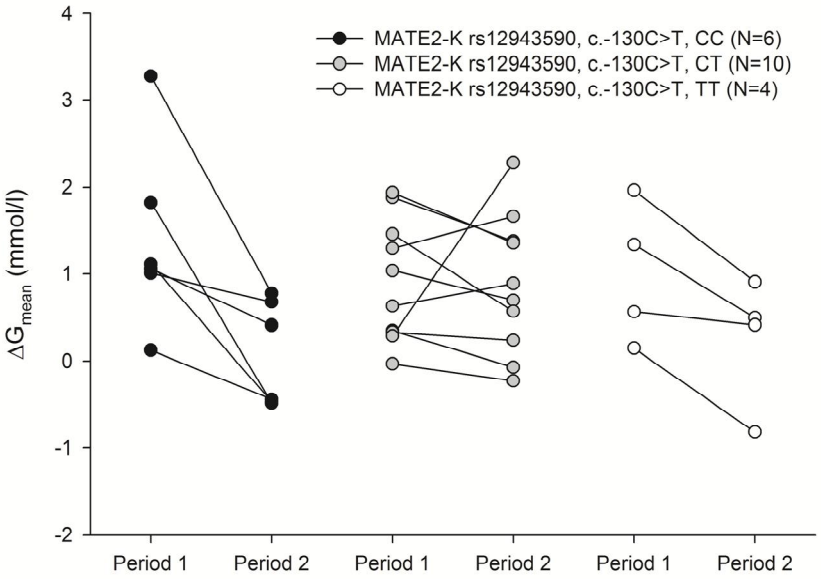
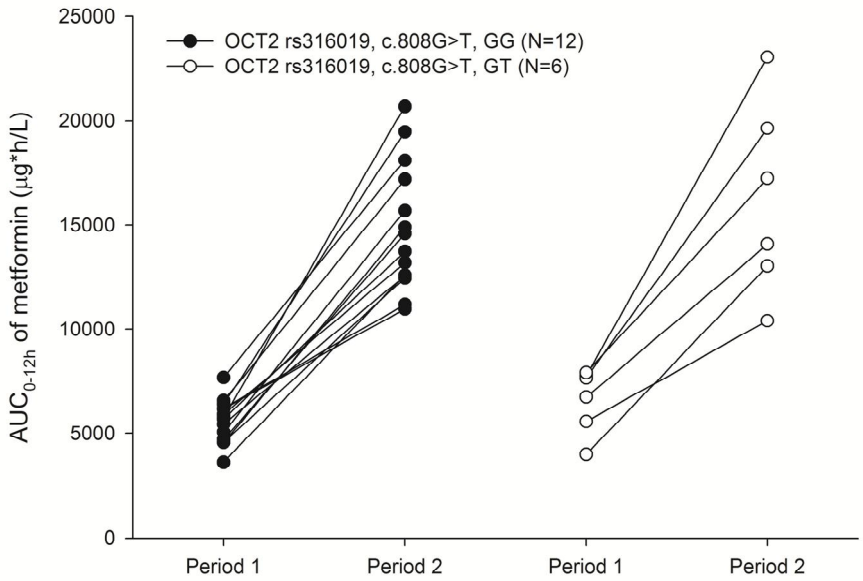


Figure 9. Changes in metformin exposure and its glucose lowering effects after pyrimethamine co-administration, categorized by c.-130C>T (rs12943590) genotypes

a)



b)

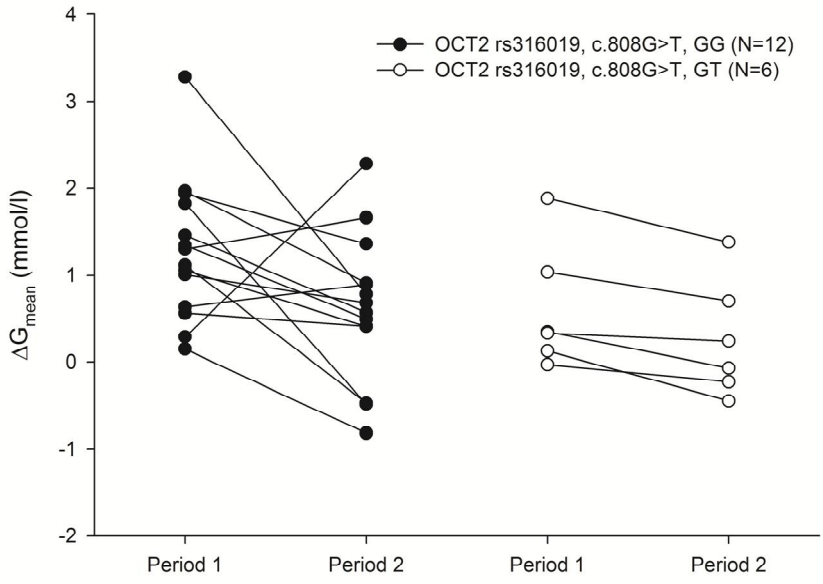


Figure 10. Changes in metformin exposure and its glucose lowering effects after pyrimethamine co-administration, categorized by c.808G>T (rs316019) genotypes

Table 4. The metformin's pharmacokinetic and pharmacodynamic parameters and creatinine clearance categorized by c.922−158 G>A (rs2289669) genotype before and after pyrimethamine co−administration.

Period 1 (metformin alone)				
c.922−158G>A (rs2289669)	GG (N=8)	GA (N=8)	AA (N=4)	*P*−value[1]
ΔG_{mean} (mmol/l)	1 ± 0.5	1.1 ± 1.1	1.1 ± 0.9	0.939
ΔG_{max} (mmol/l)	2.6 ± 1.5	2.2 ± 1.1	1.9 ± 1.3	0.668
⊿Serum glucose at PP2 (mmol/l)	0.5 ± 1.8	1.1 ± 1.2	1.4 ± 1.4	0.434
AUC_{0-12h} (μg*h/L)	5861.3 ± 1261.2	6195.1 ± 957.8	5427.2 ± 1946.9	0.526
$AUC_{infinite}$ (μg*h/L)	6412.8 ± 1506	6747.4 ± 1055.5	6193.9 ± 1940	0.648
C_{max} (μg/L)	1065.8 ± 235.3	1196.1 ± 175.1	1164.8 ± 403.2	0.448
T_{max} (h)	2.2 [1 − 3]	1.5 [0.5 − 3]	1.3 [1 − 2]	0.301
CL/F (mL/min)	1357.2 ± 285.1	1261.8 ± 198	1452.8 ± 464.5	0.648
CL_R (mL/min)	537.8 ± 104.3	479.4 ± 78.8	541.2 ± 142.9	0.422
Creatinine clearance (mL/min)	139.8 ± 40	117.4 ± 23.2	138.8 ± 56.4	0.331
V_Z/F (L)	389.8 ± 73.6	372.6 ± 99.9	638.1 ± 335.3	0.315
$t_{1/2}$ (h)	3.4 ± 0.7	3.4 ± 0.7	4.9 ± 1.7	0.059

Period 2 (metformin with pyrimethamine)				
c.922−158G>A (rs2289669)	GG (N=8)	GA (N=8)	AA (N=4)	*P*−value[1]
ΔG_{mean} (mmol/l)	0.3 ± 0.8	0.4 ± 0.6	1.2 ± 1	0.186
ΔG_{max} (mmol/l)	1.2 ± 1	1.2 ± 1.3	1.8 ± 1.3	0.610
⊿Serum glucose at PP2 (mmol/l)	0.4 ± 1.5	0.1 ± 0.9	1.3 ± 1.3	0.374
AUC_{0-12h} (μg*h/L)	15194.2 ± 3911.8	14811.4 ± 3371.1	16190 ± 3883.8	0.839
$AUC_{infinite}$ (μg*h/L)	17173.2 ± 4240.4	17415.6 ± 4172.8	18479.1 ± 5084.1	0.939
C_{max} (μg/L)	2380.6 ± 827.9	2162.5 ± 490.6	2501.1 ± 380.4	0.350
T_{max} (h)	2.5 [1 − 4]	2.5 [1.9 − 6]	2.8 [1.5 − 3]	0.923
CL/F (mL/min)	509.3 ± 116.3	502.5 ± 117.8	478.1 ± 131.9	0.939
CL_R (mL/min)	142 ± 31.7	142.9 ± 34.7	159.6 ± 59.1	0.481
Creatinine clearance (mL/min)	88.5 ± 28.5	88.1 ± 18.1	95.3 ± 32.5	0.988
V_Z/F (L)	157 ± 37.8	170 ± 36.7	141.3 ± 35.8	0.341
$t_{1/2}$ (h)	3.6 ± 0.4	4 ± 0.5	3.5 ± 0.7	0.338

Data are presented as the arithmetic mean ± standard deviation unless indicated. T_{max} is presented as the median [min − max]. [1]Parameters between genotype groups were compared using Kruskal−Wallis Test

Table 5. The metformin's pharmacokinetic and pharmacodynamic parameters and creatinine clearance categorized by c.−396C>T (rs34834489) genotype before and after pyrimethamine co−administration.

Period 1 (metformin alone)				
c.−396C>T (rs34834489)	CC (N=7)	CT (N=12)	TT (N=1)	*P*−value[1]
ΔG_{mean} (mmol/l)	0.9 ± 0.6	1.0 ± 0.8	3.3	0.257
ΔG_{max} (mmol/l)	2.2 ± 1.2	2.3 ± 1.6	3.4	0.729
⊿Serum glucose at PP2 (mmol/l)	0.5 ± 1.2	1.4 ± 1.7	3.2	0.153
AUC_{0-12h} (μg*h/L)	5951.1 ± 1471.9	5898.1 ± 1038.6	5459.5	0.794
$AUC_{infinite}$ (μg*h/L)	6623.3 ± 1566.4	6401.4 ± 1162.8	5767.6	0.811
C_{max} (μg/L)	1146.5 ± 248.8	1137.3 ± 279.7	1035.5	0.855
T_{max} (h)	2 [0.5 − 3]	1.5 [1 − 3]	1	0.568
CL/F (mL/min)	1329.8 ± 338.6	1337.2 ± 230.9	1444.8	0.811
CL_R (mL/min)	543.7 ± 95.9	466.2 ± 108.1	515.1	0.263
Creatinine clearance (mL/min)	124.7 ± 43.6	138.9 ± 28.4	144	0.489
V_Z/F (L)	473.2 ± 230.8	376.9 ± 58.4	334	0.528
$t_{1/2}$ (h)	4 ± 1.3	3.3 ± 0.3	2.7	0.169

Period 2 (metformin with pyrimethamine)				
c.−396C>T (rs34834489)	CC (N=7)	CT (N=12)	TT (N=1)	*P*−value[1]
⊿G_{mean} (mmol/l)	0.5 ± 0.8	0.4 ± 0.9	0.8	0.785
⊿G_{max} (mmol/l)	1.3 ± 1.1	1.2 ± 1.2	3.0	0.353
⊿Serum glucose at PP2 (mmol/l)	0.6 ± 1.2	0.4 ± 1.6	−0.6	0.609
AUC_{0-12h} (μg*h/L)	16086.6 ± 3847.8	14172.9 ± 2897.4	12555.6	0.382
$AUC_{infinite}$ (μg*h/L)	18387.6 ± 4501.4	16372.3 ± 3747.4	15369	0.531
C_{max} (μg/L)	2524.1 ± 700.7	2031 ± 306.2	1842.4	0.147
T_{max} (h)	2.5 [1.5 − 6]	2.5 [1 − 4]	2	0.555
CL/F (mL/min)	479.6 ± 120.6	529.9 ± 110.8	542.2	0.531
CL_R (mL/min)	157.9 ± 29.9	135 ± 40.6	78.1	0.110
Creatinine clearance (mL/min)	89 ± 25.3	92.9 ± 25.9	76	0.850
V_Z/F (L)	149.2 ± 35.4	166.4 ± 31.1	225.3	0.162
$t_{1/2}$ (h)	3.6 ± 0.5	3.7 ± 0.4	4.8	0.257

Data are presented as the arithmetic mean ± standard deviation unless indicated. T_{max} is presented as the median [min − max]. [1]Parameters between genotype groups were compared using Kruskal−Wallis Test

Table 6. The metformin's pharmacokinetic and pharmacodynamic parameters and creatinine clearance categorized by c.−130C>T (rs12943590) genotype before and after pyrimethamine co−administration.

Period 1 (metformin alone)				
c.−130C>T (rs12943590)	CC (N=6)	CT (N=10)	TT (N=4)	*P*−value[1]
ΔG_{mean} (mmol/l)	1.4 ± 1.1	0.9 ± 0.7	1.0 ± 0.8	0.809
ΔG_{max} (mmol/l)	2.7 ± 1.7	2.0 ± 1.2	2.3 ± 1.0	0.692
⊿Serum glucose at PP2 (mmol/l)	0.6 ± 1.7	1.3 ± 1.5	0.4 ± 1.2	0.552
AUC_{0-12h} (μg*h/L)	5381.7 ± 495.1	6070.9 ± 1571.4	6290.1 ± 1234.1	0.283
$AUC_{infinite}$ (μg*h/L)	5797.4 ± 551.3	6792.3 ± 1661.2	6837.5 ± 1324.5	0.292
C_{max} (μg/L)	984.9 ± 139.8	1213.3 ± 305.6	1178.1 ± 81.1	0.164
T_{max} (h)	2.2 [1 − 3]	1.3 [0.5 − 2.5]	2.5 [1.5 − 3]	0.067
CL/F (mL/min)	1448.9 ± 145.7	1304.7 ± 361.8	1255.5 ± 255.8	0.292
CL_R (mL/min)	515.4 ± 111.9	508.5 ± 114.7	531.3 ± 71.5	0.961
Creatinine clearance (mL/min)	137.2 ± 22.3	141.1 ± 42.2	94.5 ± 24.1	0.076
V_Z/F (L)	385 ± 57.1	488.9 ± 240.2	363 ± 138.1	0.399
$t_{1/2}$ (h)	3.1 ± 0.3	4.2 ± 1.3	3.3 ± 0.7	0.021*

Period 2 (metformin with pyrimethamine)				
c.−130C>T (rs12943590)	CC (N=6)	CT (N=10)	TT (N=4)	*P*−value[1]
ΔG_{mean} (mmol/l)	0.1 ± 0.6	0.9 ± 0.8	0.2 ± 0.7	0.129
ΔG_{max} (mmol/l)	1.2 ± 1.1	1.5 ± 1.3	1.1 ± 1.2	0.616
⊿Serum glucose at PP2 (mmol/l)	−0.2 ± 1.4	1.0 ± 1.1	0.2 ± 1.3	0.205
AUC_{0-12h} (μg*h/L)	13414.5 ± 1860.2	16347.8 ± 4175.5	15210 ± 3190.1	0.441
$AUC_{infinite}$ (μg*h/L)	15394 ± 2096.2	18884.1 ± 4968.5	17355.3 ± 3653.7	0.453
C_{max} (μg/L)	2012.6 ± 230.9	2495.3 ± 764.9	2330 ± 538.1	0.414
T_{max} (h)	2.2 [1 − 3]	2.5 [1.5 − 4]	2.5 [2 − 6]	0.696
CL/F (mL/min)	551.3 ± 88.4	469.8 ± 122	500.2 ± 127.5	0.453
CL_R (mL/min)	130.6 ± 29.4	152.2 ± 41.9	153.2 ± 39.8	0.395
Creatinine clearance (mL/min)	87.5 ± 23	100 ± 23.4	67.3 ± 14.9	0.119
V_Z/F (L)	179.8 ± 35.4	149.8 ± 35.5	151 ± 37.3	0.276
$t_{1/2}$ (h)	3.8 ± 0.6	3.7 ± 0.5	3.5 ± 0.4	0.707

Data are presented as the arithmetic mean ± standard deviation unless indicated. T_{max} is presented as the median [min − max]. [1]Parameters between genotype groups were compared using Kruskal−Wallis Test

Table 7. The metformin' s pharmacokinetic and pharmacodynamic parameters and creatinine clearance categorized by c.808G>T (rs316019) genotype before and after pyrimethamine co-administration.

Period 1 (metformin alone)			
c.808G>T (rs316019)	CC (N=14)	CT (N=6)	*P*-value[1]
ΔG_{mean} (mmol/l)	1.1 ± 0.9	0.9 ± 0.6	0.776
ΔG_{max} (mmol/l)	2.2 ± 1.3	2.4 ± 1.4	0.903
ΔSerum glucose at PP2 (mmol/l)	1.1 ± 1.6	0.5 ± 0.9	0.492
AUC_{0-12h} (μg*h/L)	5885.2 ± 1387.2	5961.3 ± 1047.5	0.903
$AUC_{infinite}$ (μg*h/L)	6497.8 ± 1504.1	6514.6 ± 1138.5	0.968
C_{max} (μg/L)	1148.5 ± 286.5	1112.5 ± 133.6	0.968
T_{max} (h)	1.7 [0.5 - 2.5]	2 [1 - 3]	0.457
CL/F (mL/min)	1349.8 ± 321.4	1310.9 ± 220.8	0.968
CL_R (mL/min)	511.5 ± 114.3	523.5 ± 74.1	0.839
Creatinine clearance (mL/min)	136.9 ± 38.4	115.8 ± 34.1	0.355
V_Z/F (L)	448.9 ± 210.7	394.3 ± 116.3	0.776
$t_{1/2}$ (h)	3.8 ± 1.2	3.5 ± 0.8	0.544

Period 2 (metformin with pyrimethamine)			
c.808G>T (rs316019)	CC (N=14)	CT (N=6)	*P*-value[1]
ΔG_{mean} (mmol/l)	0.7 ± 0.8	0.2 ± 0.7	0.165
ΔG_{max} (mmol/l)	1.4 ± 1.2	1.3 ± 1.0	0.968
Δ Serum glucose at PP2 (mmol/l)	0.7 ± 1.3	−0.2 ± 1.2	0.279
AUC_{0-12h} (μg*h/L)	15789.6 ± 3775	13958.4 ± 2744.9	0.443
$AUC_{infinite}$ (μg*h/L)	18223 ± 4516.6	15917.3 ± 2915.5	0.443
C_{max} (μg/L)	2393.7 ± 657.8	2139.6 ± 525.4	0.443
T_{max} (h)	2.5 [1.5 – 4]	2.2 [1 – 6]	0.340
CL/F (mL/min)	483.7 ± 117.9	539.1 ± 103	0.443
CL_R (mL/min)	146.1 ± 40.4	145.4 ± 33.7	0.968
Creatinine clearance (mL/min)	92.6 ± 24.9	83 ± 24.1	0.684
V_Z/F (L)	154.7 ± 35.4	169.2 ± 40.8	0.397
$t_{1/2}$ (h)	3.7 ± 0.6	3.6 ± 0.5	0.715

Data are presented as the arithmetic mean ± standard deviation unless indicated. T_{max} is presented as the median [min – max]. [1]Parameters between genotype groups were compared using Kruskal–Wallis Test

DISCUSSION

In the present study, the plasma concentration of metformin increased by 2.02- to 2.68-fold after the administration of 50 mg of pyrimethamine, but the increased drug exposure did not subsequently increase the glucose-lowering effect of metformin. Pyrimethamine is known to inhibit MATE1 in the liver as well as MATE1 and MATE2-K in the kidney [2, 20, 21], and the hepatocellular concentration and the glucose-lowering effect of metformin were expected to increase due to MATE inhibition by pyrimethamine. These results were quite unexpected and challenging, and two potential reasons for our observations are discussed below.

First, the increase in plasma metformin concentrations following co-administration of pyrimethamine may not lead to an increase in the intracellular concentration of metformin at its sites of action. Pyrimethamine was shown to inhibit OCT1 in an *in vitro* study, and the corresponding inhibition constant (Ki) was 3.8 ± 0.3 μM [21]. Following daily administration of 50 mg of pyrimethamine to HIV patients for 3 weeks, the C_{max} of pyrimethamine was 8.3 μM (2.1 μg/mL) [31]. In addition, pyrimethamine was shown to localize to the liver after systemic

administration, and the concentration of pyrimethamine in the portal vein was shown to be higher than the systemic plasma concentration [32, 33]. The maximal total and unbound pyrimethamine concentrations in the portal vein were estimated to be 20.8 μM and 2.7 μM, respectively, considering the protein binding of pyrimethamine (~13%) [34, 35]. Although we did not measure pyrimethamine concentrations in our study, its concentration in the portal vein might have been sufficient to inhibit the OCT1- and/or OCT3-mediated hepatic uptake of metformin. Second, the alteration of gastrointestinal environment by pyrimethamine can lead to decreased pharmacological effect of metformin observed in our study. The anti-hyperglycaemic activity of metformin is not only related with the plasma exposure of metformin but also related with the gastrointestinal pharmacology of metformin [36-38]. Pyrimethamine antagonizes the folic acid metabolism of the microbiome in the gastrointestinal tract which can affect the gastrointestinal based pharmacology of metformin [36-39]. Third, pyrimethamine might be able to alter glucose metabolism through its own pharmacological actions. Pyrimethamine antagonizes folic acid metabolism and thereby exhibits an

antimicrobial effect [39]. A disturbance in folic acid metabolism can lead to an increase in the levels of homocysteine and homocysteine thiolactone (a primary metabolite of homocysteine) and can inhibit the activity of insulin through a mechanism involving oxidative stress [40, 41]. However, no previous studies have examined the effect of pyrimethamine on glucose metabolism.

Several confounding factors might have affected the results of our study. The AUG and G_{max} measured prior to the metformin administration were somewhat lower during period 2 compared to period 1 (**Figure 5, Table 3**), which may have been caused by a carryover effect of the metformin administered during period 1. Because the half-life of metformin is approximately 4 to 8.7 hours, 1 week was thought to be sufficient to exclude a carryover effect of metformin and we did not observe a carryover effect in earlier studies that used a similar design [27, 28, 42]. Although it remains possible that there was a carryover effect in this study, the higher systemic metformin exposure observed during period 2 did not result in increased PD effects, and this result cannot be explained by the potential confounding factors. Additionally, genetic

polymorphisms of transporters that affect metformin' s tissue distribution to hepatocytes and its renal elimination could confound the effect of pyrimethamine on metformin. However, similar effects were observed in the MATE1, MATE2-K and OCT2 genotype groups (**Figure 7 to 10, Table 4 to 7**). Although other genetic polymorphisms that were not examined in our study might have affected metformin' s PKs and PDs, such an effect seems likely to be limited due to the similar effect of pyrimethamine in the majority of the subjects. In the last subject of our study, the C_{max} and AUC_{0-12h} of metformin were increased by 3.1- and 3.5 folds along with marked increases in the PD parameters were observed after pyrimethamine co-administration; contrary to the observations from other subjects. Because all subjects received same study procedures, the result of that subject is considered to be an interindividual variation in the responses to pyrimethamine.

The glucose-lowering effect of metformin may have been saturated in our study. The plasma glucose profile after metformin administration, with or without pyrimethamine, was similar between period 1 and 2, and this observation could be explained by a saturated PD effect (**Figure 5, Table 3**).

However, when we used a higher dose of metformin (1000 and 750 mg) in the earlier study, the plasma glucose level was even lower than in the present study, indicating that the PD effect of metformin might not be saturated with the dose that was used in this study [27].

Systemic exposure to metformin increased from 102% to 168% in period 2, and the CL/F and Vz/F of metformin decreased by 63% and 62%, respectively, after pyrimethamine co-administration (**Figure 3, Table 1**). We hypothesize that the decrease in the CL_R that resulted from the inhibition of MATE1 caused the change in the CL/F (**Figure 3, Table 1**). The metformin's net renal clearance by secretion was decreased by 88% after pyrimethamine co-administration (**Table 1**). This result suggests that the elimination of metformin by renal tubular secretion was almost completely inhibited by pyrimethamine.

The T_{max} of metformin significantly prolonged after pyrimethamine co-administration while the $t_{1/2}$ did not changed. It suggest decreased absorption rate of metformin. Taken together with change of CL/F and Vz/F of metformin, the systemic absorption of metformin might be changed by

pyrimethamine, and the inhibition of hepatic uptake transporters such as OCT1 or OCT3 would be one of the reasons for it [2, 12, 13, 42]. Although the uptake transporter OCT1, which is located in the basolateral membrane of intestinal cells, can be inhibited by pyrimethamine, oral absorption of metformin is thought to be primarily mediated by a saturable paracellular process in the intestine, and the inhibition of intestinal OCT1 would not significantly affect the absorption of metformin [7].

The present study had some limitations. First, our study was conducted in healthy subjects to minimize confounding factors, although type II diabetic patients differ from healthy subjects with respect to insulin sensitivity and glucagon regulation [43–45]. However, metformin' s primary site of action is the same in these two groups, and we can assume that the effect of pyrimethamine on the PKs and PDs of metformin would vary minimally between the subject groups as long as the transporter was expressed at similar levels. Further studies with diabetic patients will be needed to evaluate the clinical significance of this drug interaction. Second, we did not measure plasma concentrations of pyrimethamine because Because of pyrimethamine's long half-life (more than 100

hour), we expected that the plasma concentration of pyrimethamine would be to sufficient to competitively inhibit MATE during the study and the significant inhibition of MATE caused by the administration of 50 mg of pyrimethamine has already been investigated in humans [20]. Furthemore, our primary objective was to investigate the changes in the PDs of metformin that were expected to result from the MATE inhibition. Additional studies are required to expand the results of our study. Finally, this study was performed using a one-way crossover design to avoid the potential carryover effect of pyrimethamine. However, the G_{mean} and G_{max} measured prior to the metformin administration were somewhat lower during period 2 compared to period 1, which may have been caused by a carryover effect of the metformin administered during period 1 or by a prolonged diet modification at period 2. Although a randomized two-way crossover design may have been more effective in balancing the effects of confounds, we knew that pyrimethamine increases metformin exposure, and our design was suitable to investigate whether the increased exposure led to increased metformin activity.

In conclusion, pyrimethamine, a potent MATE1 inhibitor, did not increase the glucose-lowering effect of metformin, although it did increase the systemic exposure to metformin by decreasing its renal clearance. Our study result indicates that the response to metformin is not only related with the plasma exposure of metformin but also related with other factors such as inhibition of uptake transporters and gastrointestinal based pharmacology of metformin. Further study with a small group of type II diabetic patients may be helpful for evaluating the clinical significance of this drug interaction.

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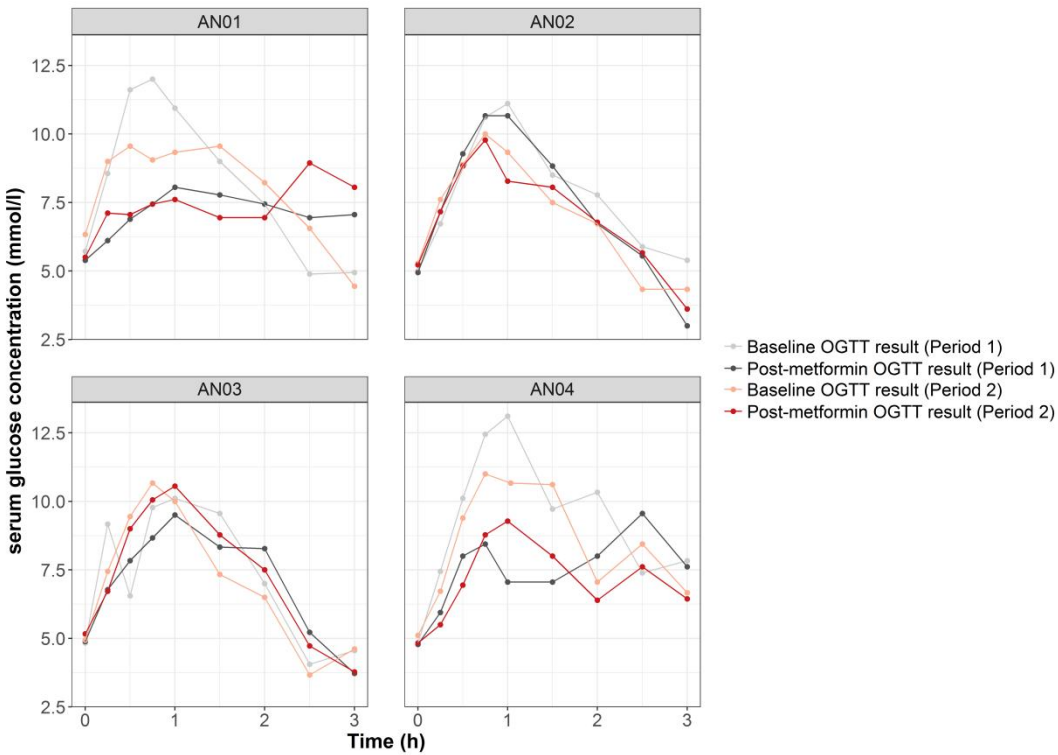
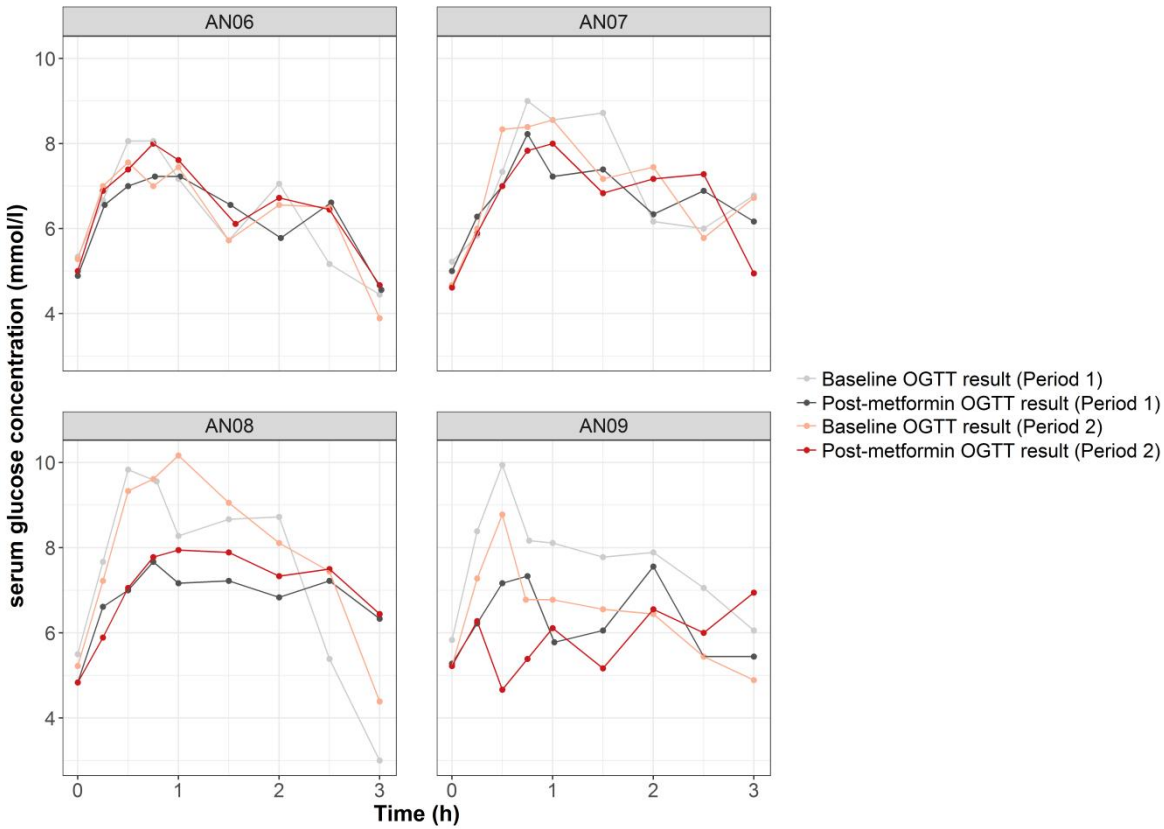
APPENDICES

Individual serum glucose profiles after oral glucose tolerance test (OGTT)



Individual serum glucose profiles after oral glucose tolerance test (OGTT)



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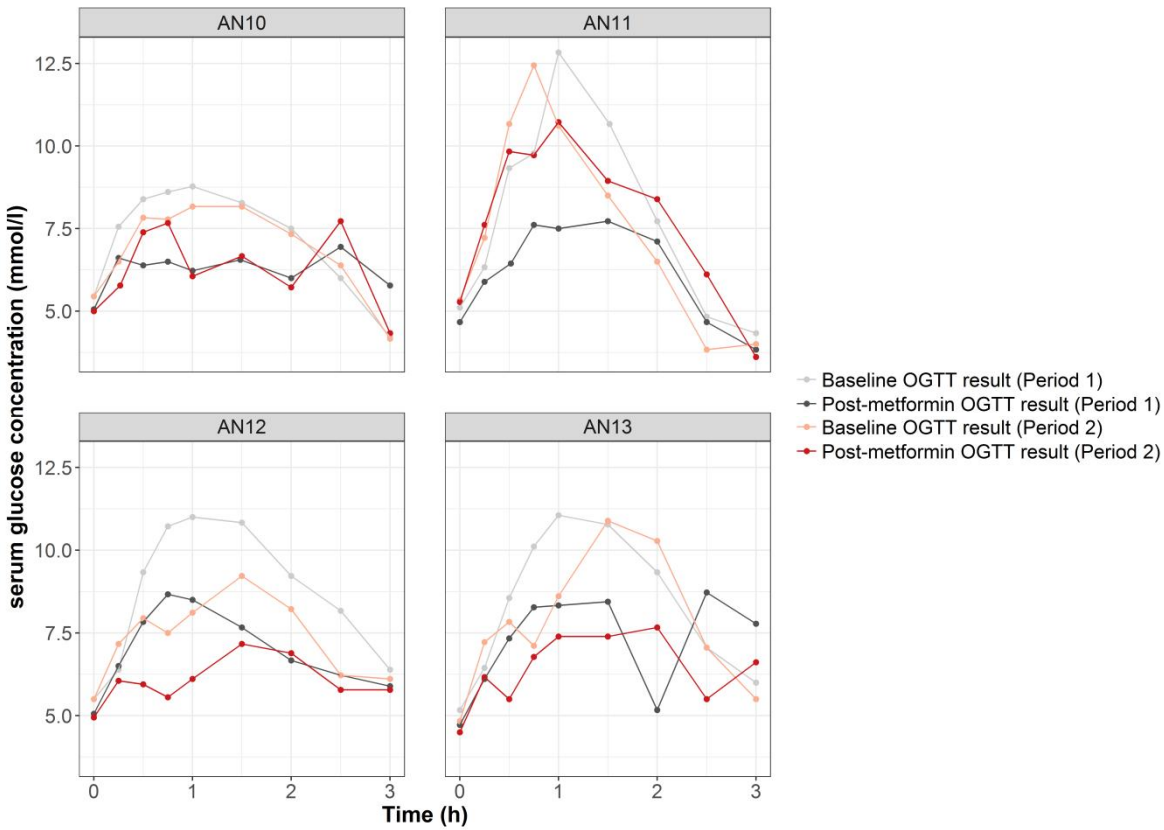

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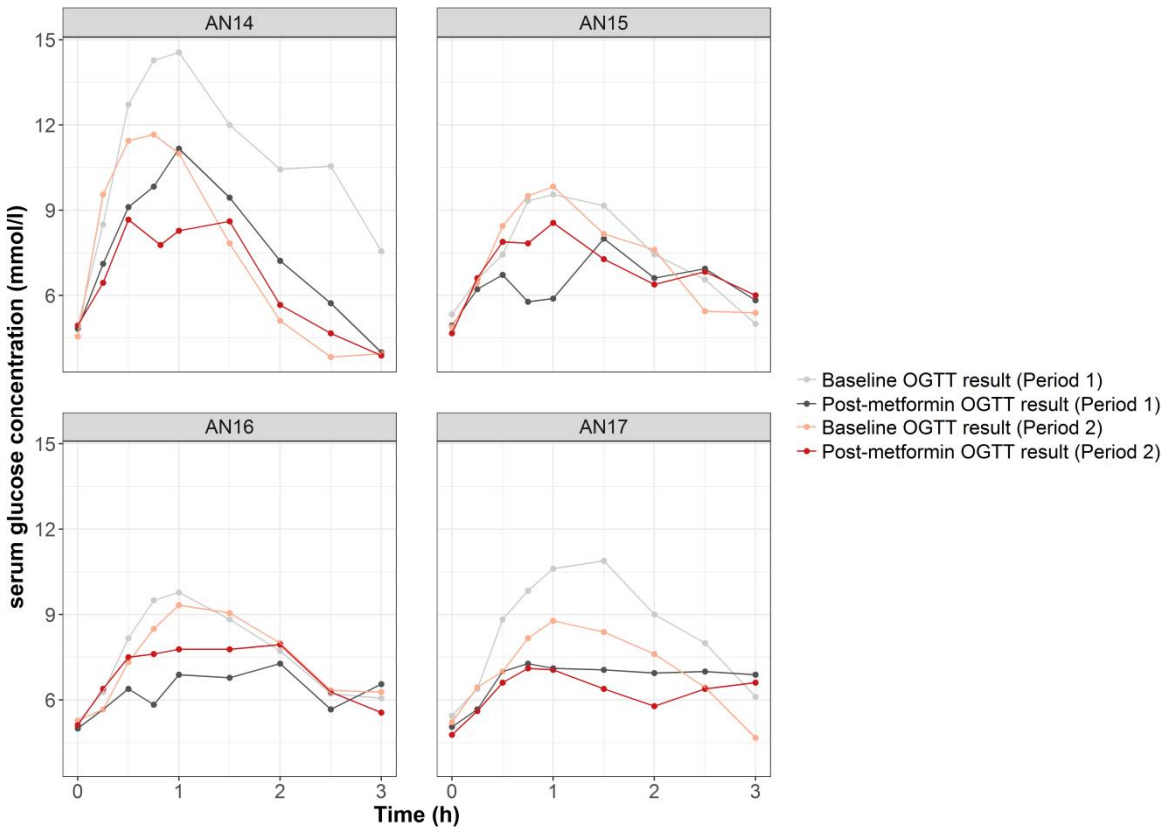
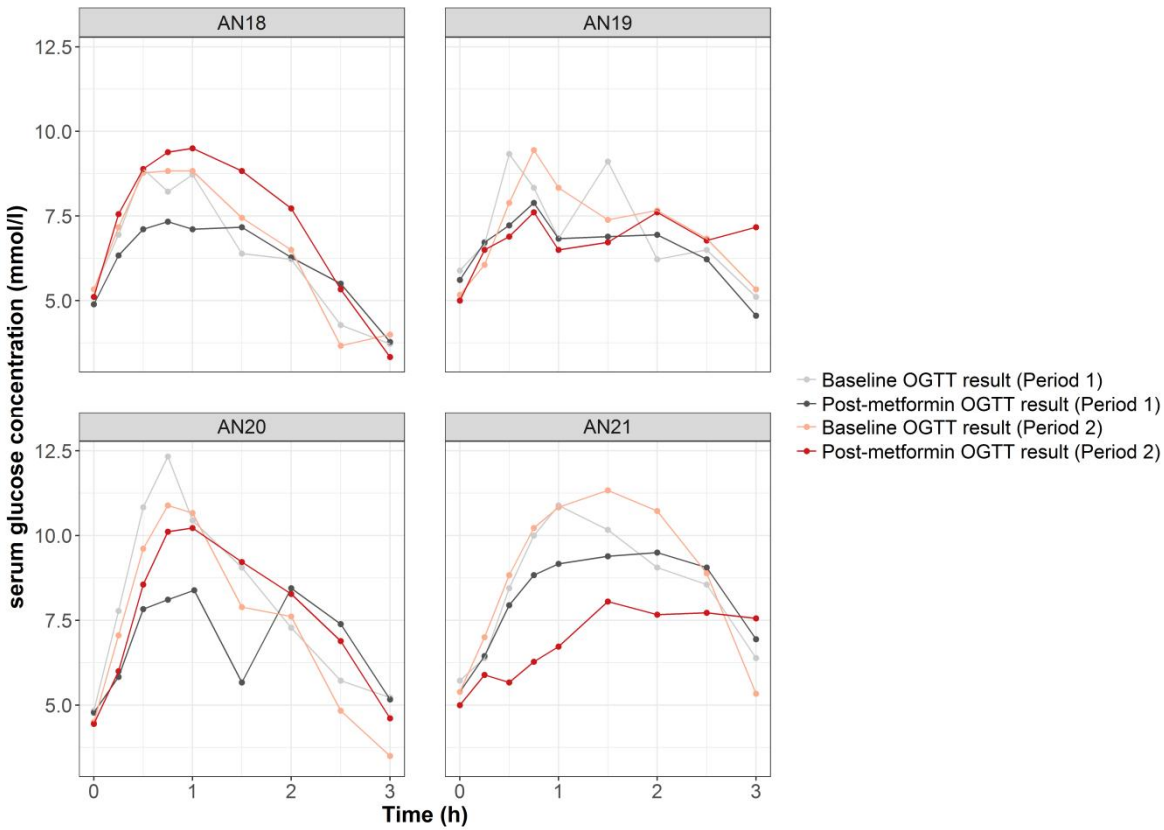


Individual serum glucose profiles after oral glucose tolerance test (OGTT)

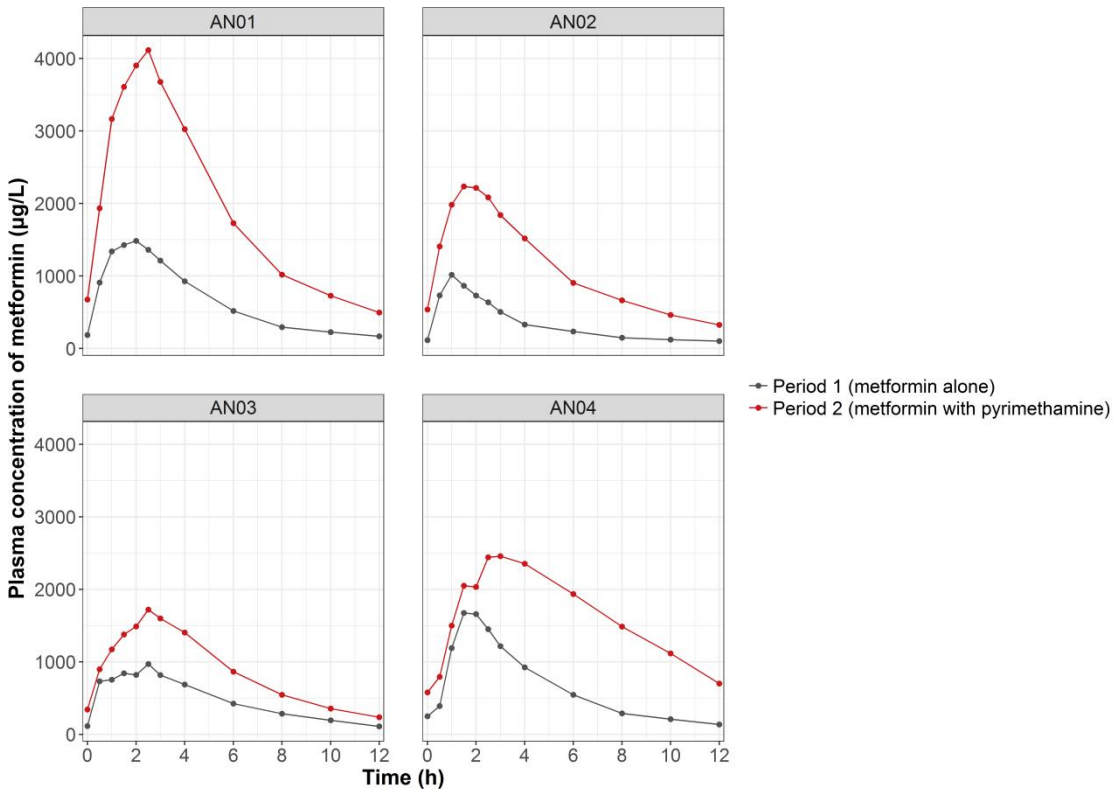


Individual pharmacodynamic parameters before and after pyrimethamine co-administration; Period 1: 500 mg of metformin was administered alone; period 2: 500 mg of metformin was administered with 50 mg of pyrimethamine

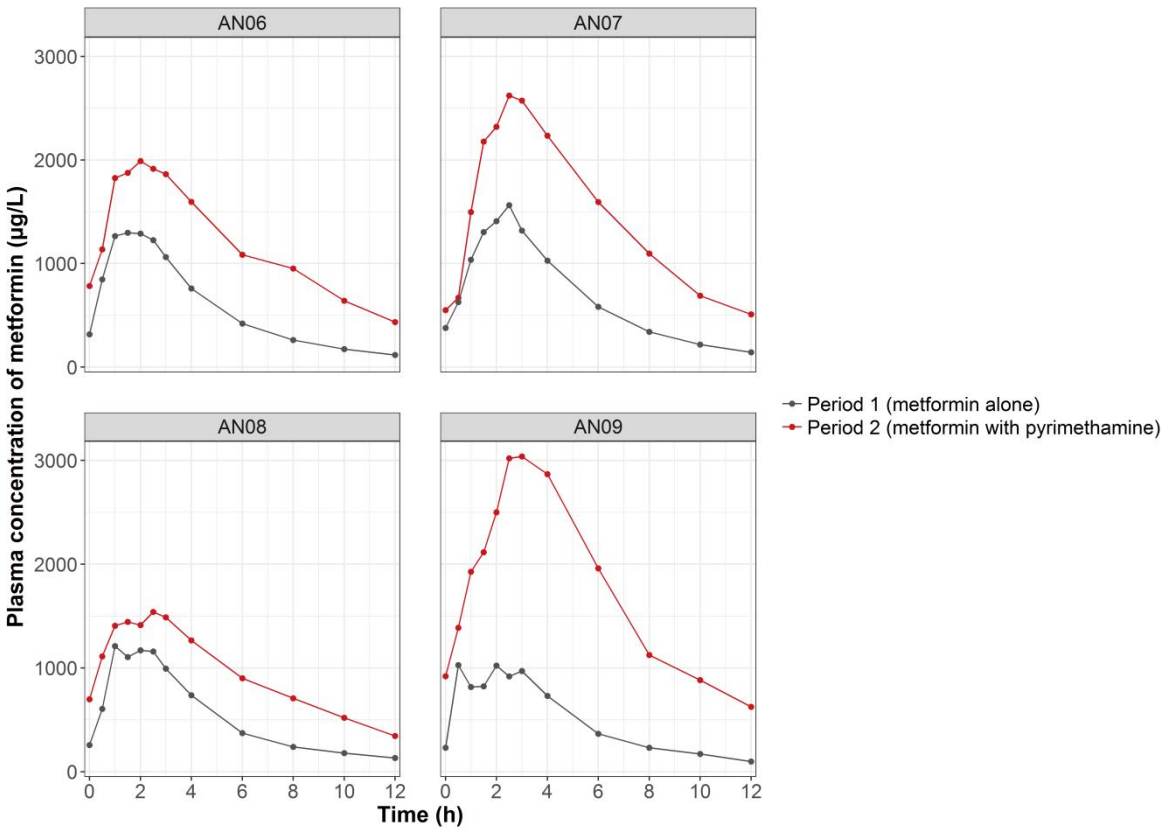
Subject	Period	⊿G_{mean} (mmol/l)	⊿G_{max} (mmol/l)	⊿Serum glucose at PP2 (mmol/l)
AN01	1	3.1	3.9	0.0
AN01	2	2.1	0.6	1.3
AN02	1	1.0	0.4	1.1
AN02	2	−0.2	0.2	−0.1
AN03	1	0.4	0.6	−1.3
AN03	2	−1.3	0.1	−1.0
AN04	1	5.7	3.6	2.3
AN04	2	4.1	1.7	0.7
AN06	1	−0.1	0.8	1.3
AN06	2	−0.7	−0.4	−0.2
AN07	1	1.0	0.8	−0.2
AN07	2	0.7	0.6	0.3
AN08	1	1.9	2.2	1.9
AN08	2	2.7	2.2	0.8
AN09	1	4.4	2.4	0.3
AN09	2	1.7	1.8	−0.1
AN10	1	3.0	1.8	1.5
AN10	2	2.0	0.4	1.6
AN11	1	5.5	5.1	0.6
AN11	2	−1.5	1.7	−1.9
AN12	1	5.8	2.3	2.6
AN12	2	4.1	2.1	1.3
AN13	1	3.9	2.3	4.2
AN13	2	5.0	3.2	2.6
AN14	1	9.8	3.4	3.2
AN14	2	2.3	3.0	−0.6
AN15	1	3.2	1.6	0.8

Subject	Period	⊿G_{mean} (mmol/l)	⊿G_{max} (mmol/l)	⊿Serum glucose at PP2 (mmol/l)
AN15	2	1.2	1.3	1.2
AN16	1	4.0	2.5	0.4
AN16	2	1.5	1.4	0.1
AN17	1	5.9	3.6	2.1
AN17	2	2.7	1.7	1.8
AN18	1	0.4	1.6	−0.1
AN18	2	−2.5	−0.7	−1.2
AN19	1	1.7	1.4	−0.7
AN19	2	1.2	1.8	0.1
AN20	1	3.3	3.9	−1.2
AN20	2	−1.4	0.7	−0.7
AN21	1	0.9	1.4	−0.4
AN21	2	6.8	3.3	3.1

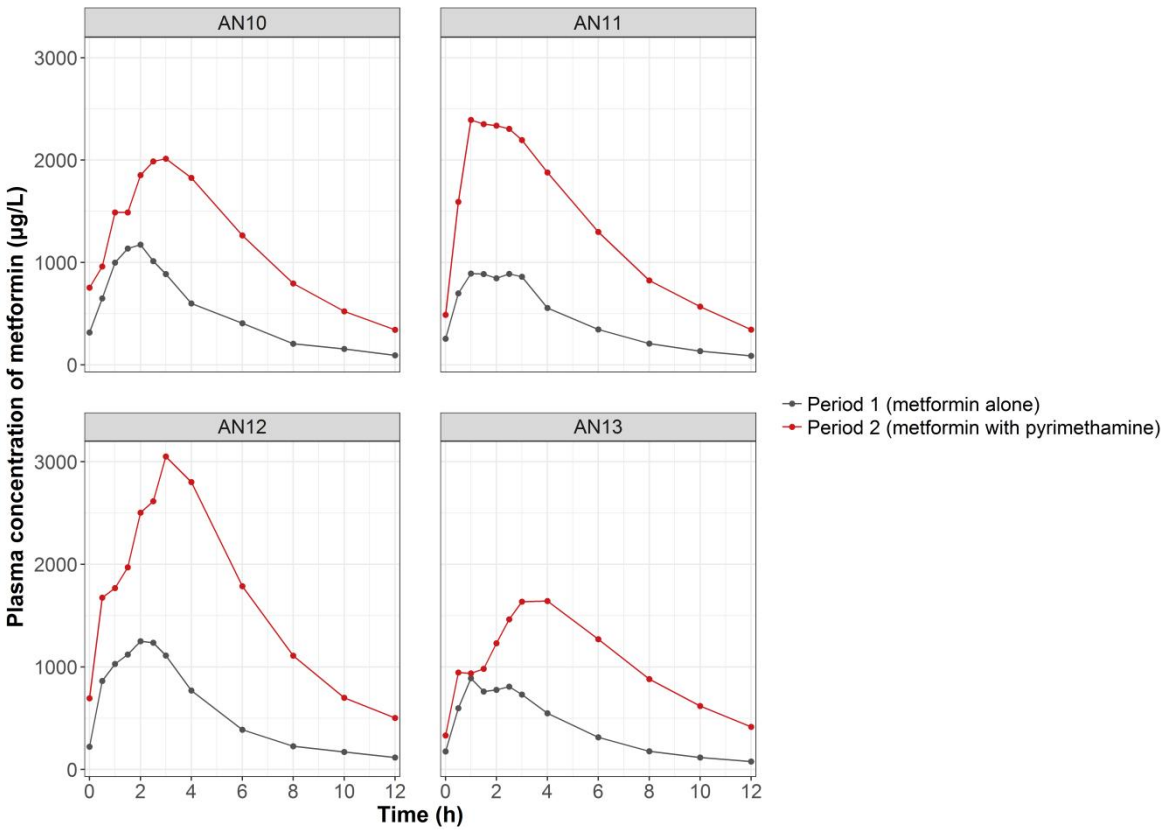
Individual plasma concentration profile of metformin before and after pyrimethamine co-administration



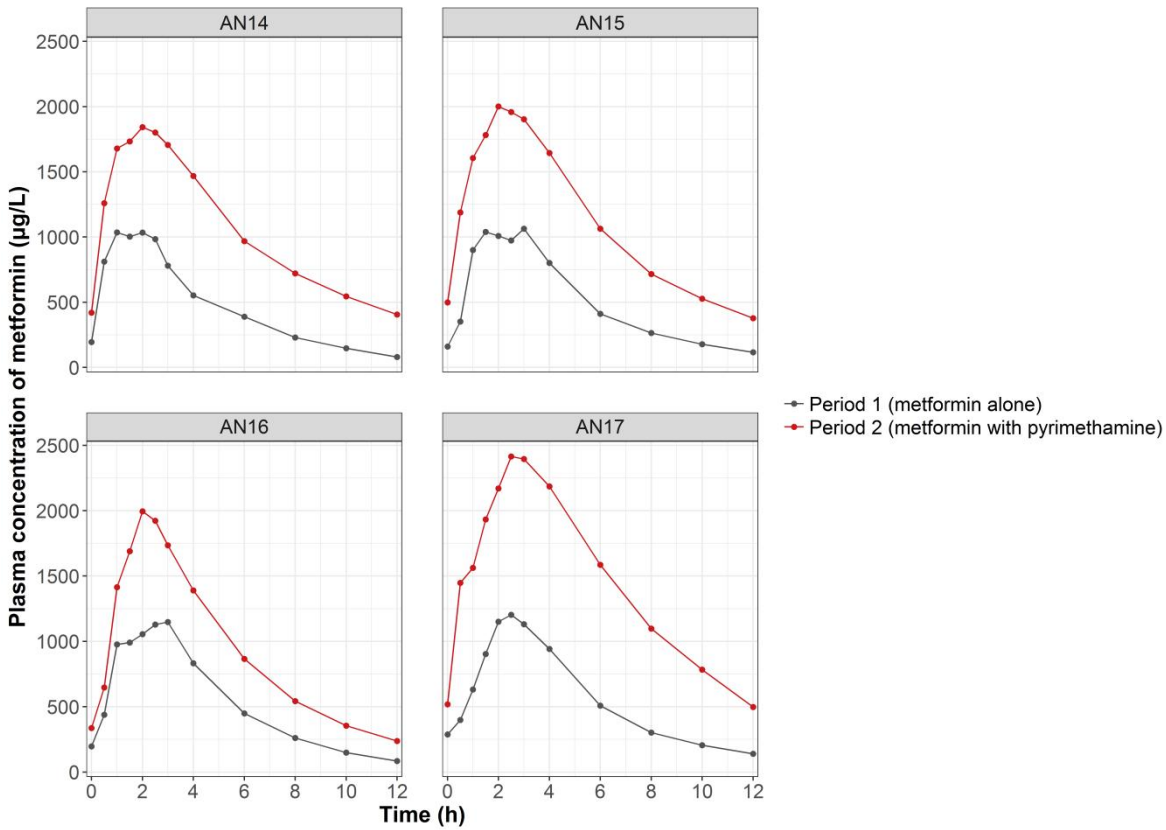
Individual plasma concentration profile of metformin before and after pyrimethamine co-administration



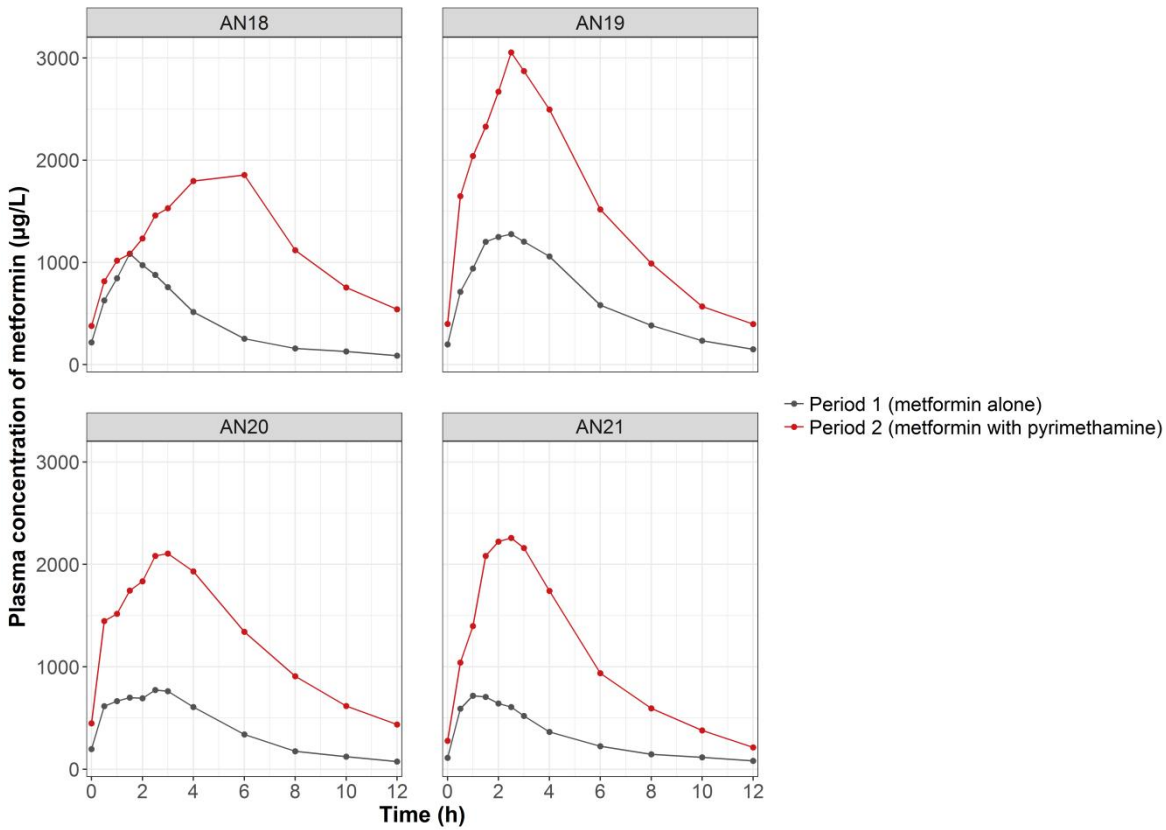
Individual plasma concentration profile of metformin before and after pyrimethamine co-administration



Individual plasma concentration profile of metformin before and after pyrimethamine co-administration



Individual plasma concentration profile of metformin before and after pyrimethamine co-administration



Individual pharmacokinetic parameters before and after pyrimethamine co-administration; Period 1: 500 mg of metformin was administered alone; period 2: 500 mg of metformin was administered with 50 mg of pyrimethamine

Subject	Period	AUC_{0-12h} (μg·h/L)	$AUC_{infinite}$ (μg·h/L)	C_{max} (μg/L)	T_{max} (h)	CL/F (mL/min)	CL_R (mL/min)	V_Z/F (L)	$t_{1/2}$ (h)
AN01	1	7780.3	8978.0	1485.8	1.95	928.2	545.4	396.9	4.9
AN01	2	23059.0	25831.6	4115.4	2.5	322.6	129.1	108.0	3.9
AN02	1	3988.9	5096.3	1017.1	1	1635.2	639.7	1061.3	7.5
AN02	2	13050.0	14885.1	2235.8	1.5	559.8	180.2	188.8	3.9
AN03	1	5577.5	6074.2	970.2	2.5	1371.9	434.8	374.3	3.2
AN03	2	10410.7	11539.0	1721.6	2.5	722.2	158.6	207.1	3.3
AN04	1	7681.4	8419.1	1673.9	1.5	989.8	351.9	319.1	3.7
AN04	2	19633.3	23823.8	2457.7	3	349.8	73.6	125.7	4.2
AN06	1	6749.8	7330.4	1296.8	1.52	1136.8	391.0	339.1	3.4
AN06	2	14077.9	16301.1	1989.8	1.93	511.2	114.6	156.7	3.5
AN07	1	7936.0	8590.3	1563.7	2.5	970.1	397.9	267.0	3.2
AN07	2	17226.7	19982.2	2622.9	2.5	417.0	146.1	135.2	3.7
AN08	1	6197.2	7085.4	1210.2	1	1176.1	422.5	474.5	4.7
AN08	2	11193.8	13116.2	1540.5	2.5	635.3	156.9	212.2	3.9
AN09	1	5755.1	6212.7	1026.8	0.5	1341.3	521.4	377.2	3.2

Subject	Period	AUC_{0-12h} (μg·h/L)	$AUC_{infinite}$ (μg·h/L)	C_{max} (μg/L)	T_{max} (h)	CL/F (mL/min)	CL_R (mL/min)	V_Z/F (L)	$t_{1/2}$ (h)
AN09	2	20698.8	24980.3	3038.7	3	333.6	132.7	137.1	4.7
AN10	1	5709.9	6086.8	1174.2	1.97	1369.1	386.6	329.8	2.8
AN10	2	13732.3	15370.5	2014.0	3	542.2	132.2	155.4	3.3
AN11	1	5063.6	5473.1	892.3	1	1522.6	579.6	425.3	3.2
AN11	2	15695.5	17324.9	2392.2	1	481.0	142.7	136.7	3.3
AN12	1	6413.6	7129.9	1250.0	1.95	1168.8	510.5	426.2	4.2
AN12	2	19465.4	21758.6	3051.7	3	383.0	176.3	105.0	3.2
AN13	1	4582.2	4950.3	890.2	1	1683.4	642.5	482.1	3.3
AN13	2	12461.9	14713.5	1641.8	4	566.4	204.3	184.0	3.8
AN14	1	5459.5	5767.6	1035.5	1	1444.8	515.1	334.0	2.7
AN14	2	12555.6	15369.0	1842.4	1.95	542.2	78.1	225.3	4.8
AN15	1	5922.6	6475.7	1063.4	3	1286.9	476.7	368.4	3.3
AN15	2	13198.3	15533.3	2000.2	1.95	536.5	118.8	199.4	4.3
AN16	1	6175.8	6473.2	1147.5	3	1287.4	496.5	273.3	2.5
AN16	2	10978.3	12123.2	1995.0	1.95	687.4	164.5	199.3	3.3
AN17	1	6576.3	7296.6	1202.9	2.5	1142.1	462.8	354.4	3.6
AN17	2	17177.1	19891.5	2415.1	2.5	418.9	158.7	137.2	3.8
AN18	1	4711.0	5222.5	1085.7	1.5	1595.7	628.2	560.9	4.1

Subject	Period	AUC_{0-12h} (μg·h/L)	$AUC_{infinite}$ (μg·h/L)	C_{max} (μg/L)	T_{max} (h)	CL/F (mL/min)	CL_R (mL/min)	V_Z/F (L)	$t_{1/2}$ (h)
AN18	2	14583.2	17561.0	1855.8	6	474.5	191.9	156.7	3.8
AN19	1	7697.5	8357.7	1276.2	2.5	997.1	537.7	263.3	3.1
AN19	2	18101.4	19845.3	3054.2	2.5	419.9	97.6	110.7	3.0
AN20	1	4557.1	4907.0	774.2	2.5	1698.3	699.5	478.0	3.3
AN20	2	14894.3	17227.4	2105.3	3	483.7	153.0	154.9	3.7
AN21	1	3624.9	4130.4	718.1	1	2017.6	662.7	745.8	4.3
AN21	2	12611.3	13448.7	2259.1	2.5	619.6	208.3	145.7	2.7

MATE1, MATE2-K and OCT2 genotype result

Subject	OCT2 c.808T>G	MATE1 c.922-158G>A	MATE2-K c.396G>a	MATE2-K c.130G>A
AN01	GG	GG	GG	GA
AN02	GG	AA	GG	GA
AN03	GG	GG	GA	GG
AN04	GG	AA	GA	GA
AN06	GG	GA	GA	GA
AN07	GG	GA	GG	GA
AN08	GT	GA	GG	GA
AN09	GG	GA	GG	GA
AN10	GG	GG	GA	GG
AN11	GT	GG	GA	GG
AN12	GG	AA	GG	GA
AN13	GG	GG	GA	GA
AN14	GG	GA	AA	GG
AN15	GT	GG	GA	GG
AN16	GT	GA	GG	AA
AN17	GG	GA	GG	AA
AN18	GT	GA	GG	AA
AN19	GT	GG	GG	AA
AN20	GG	GG	GG	GG
AN21	GG	AA	GG	GA

국문 초록

서론: 제 2 형 당뇨병의 1 차 치료제인 메트포민은 인체에 투여 후 주로 신장을 통한 여과와 분비를 통해 체내 제거된다. 신장 정단막(apical membrane)의 Multidrug And Toxin Extrusion (MATE) 수송체는 메트포민의 신장 세뇨관을 통한 분비에 중요한 역할을 담당한다. 본 연구는 MATE 수송체가 메트포민의 혈당 강하 효과에 미치는 영향을 평가하고자 하였다.

방법: 스무명의 건강한 성인 남성 대상자에게 메트포민을 투여 후 혈당 강하 효과와 메트포민의 약동학 특성을 평가하였다. 첫 번째 평가기간(period 1)에는 메트포민을 단독 투여하였으며 일주일의 휴약기 이후 두 번째 평가기간(period 2)에는 강력한 MATE 수송체 억제제인 피리메타민(pyrimethamine)과 함께 메트포민을 투여하였다. 각 평가기간에 메트포민 750 mg 와 500 mg 이 대상자들에게 12 시간 간격으로 2 회 투여되었으며, 혈당 강하 효과 평가를 위해 메트포민 투여 전과 후에 경구당부하검사를 수행한 후 평균 혈당 강하량(ΔG_{mean}), 최대 혈당 강하량(ΔG_{max}), 경구당부하검사 2 시간 후 혈당강하량 ($\Delta PP2$)을 산출하였다. 약동학 평가를 위해 혈장과 소변 내 메트포민 약물 농도를 액체크로마토그래피 질량분석기로 측정하였으며, 비구획분석법을 통해 약동학 파라미터를 산출하였다. 각 평가기간의 혈당 강하 효과 및 약동학 파라미터의 차이는

혼합 모형(general linear mixed effects model)을 이용하여 비교 분석하였다.

결과: 메트포민을 피리메타민과 함께 병용 투여한 결과 메트포민의 신장 청소율이 72%감소하였으며($P < 0.05$), 투여 후 12시간 동안 혈장 내 메트포민 약물농도-시간 곡선 하 면적(AUC_{0-12h})은 101% 상승하였다($P < 0.05$). 그러나 메트포민의 혈당 강하 효과는 피리메타민과 함께 병용 투여하였을 때 오히려 감소하였으며 ΔG_{mean}, ΔG_{max}, ΔPP2 의 평균과 90% 신뢰구간은 각각 −0.6 (−1, −0.2), −0.9 (−1.6, −0.3), −0.5 (−1.1, 0.1) mmol/L이었다.

결론: 피리메타민을 통한 MATE 수송체 억제 시 신장 청소율 감소로 인해 메트포민의 체내 약물 농도는 상승하였지만 메트포민의 혈당 강하 효과는 함께 증가하지 않았다. 이와 같은 결과는 메트포민에 의한 혈당 강하 효과가 체내 약물 수송체의 기능과 장에서의 메트포민 약리 작용 등 혈중 농도 외 다른 요소들과도 관련이 있음을 시사한다. 본 연구에서 관찰된 MATE 수송체를 통한 메트포민의 약물상호작용의 임상적 의의를 평가하기 위해 소규모의 당뇨환자 대상 임상 연구가 유용할 것으로 판단된다.

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주요어 : 메트포민, 약물 수송체, MATE, 약동학, 약력학

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