의학박사 학위논문

Effects of vancomycin-induced gut microbiome alterations on the pharmacokinetics and pharmacodynamics of metformin in healthy male subjects

건강한 남성 대상자에서

반코마이신 투여에 의한 장내 미생물군집 변화가

메트포민의 약동학 및 약력학에 미치는 영향 탐색

2021 년 8 월

서울대학교 대학원

의과학과 의과학전공

김 은 우

A thesis of the Degree of Doctor of Philosophy

건강한 남성 대상자에서 반코마이신 투여에 의한 장내 미생물군집 변화가 메트포민의 약동학 및 약력학에 미치는 영향 탐색

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August 2021

Department of Biomedical Sciences

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by

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A thesis submitted to the Department of Biomedical Sciences in fulfillment of the requirements for the Degree of Doctor of Philosophy in Medicine at Seoul National University College of Medicine

July 2021

Approved by Thesis Committee:

Professor _____ Chairman

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Professor _____

Professor _____

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건강한 남성 대상자에서 반코마이신 투여에 의한 장내 미생물군집 변화가 메트포민의 약동학 및 약력학에 미치는 영향 탐색

지도교수 유 경 상

이 논문을 의학박사 학위논문으로 제출함
2021년 4월

서울대학교 대학원
의과학과 의과학전공
김 은 우

김은우의 박사 학위논문을 인준함
2021년 7월

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ABSTRACT

Effects of vancomycin-induced gut microbiome alterations on the pharmacokinetics and pharmacodynamics of metformin in healthy male subjects

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Introduction: Metformin is a most widely used treatment for type 2 diabetes. The objective of this study was to investigate the impact of vancomycin-induced gut microbiome dysbiosis on the pharmacokinetics and anti-hyperglycaemic effects of metformin.

Methods: Healthy adult male subjects aged 19-45 with no defecation abnormalities were recruited for this open-label, single-arm, four-period clinical study: baseline; post-metformin (i.e.,

multiple oral doses of 1000 mg metformin on days 1−4); post−vancomycin (i.e., multiple oral doses of 500 mg vancomycin on days 11−17 inducing gut microbiome changes); post−metformin+vancomycin (i.e., multiple oral doses of 1000 mg metformin on days 16−19). In each period, blood samples for serum glucose concentration measurement were obtained before and after an oral glucose tolerance test. In addition, faecal samples for gut microbiome composition, and safety data were obtained. Following metformin dosing at post−metformin and post−metformin+vancomycin period, plasma and urine samples for pharmacokinetics of metformin were collected.

Results: Among 9 subjects completed the entire study period, all samples were collected from a total of 8 subjects. Diversity and composition of the gut microbiome was significantly changed due to the vancomycin administration. The pharmacokinetics of metformin remained unchanged regardless of vancomycin administration ($p>0.05$). On the other hand, the anti−hyperglycaemic effect was significantly decreased after vancomycin administration ($p<0.05$), demonstrating the weak relationship between the pharmacokinetics and pharmacodynamics of metformin. Relative abundances of *Erysipelatoclostridium, Enterobacter, and Faecalibacterium* changed after vancomycin administration tended to correlate with the anti−

hyperglycaemic effects of metformin ($p<0.1$). Adverse events occurred in all subjects and were resolved without sequelae.

Conclusion: When the gut microbiome was altered due to vancomycin administration, the anti-hyperglycaemic effect of metformin was decreased, despite unchanged metformin pharmacokinetics. The anti-hyperglycaemic effect was tended to correlate with the relative abundance of several genera, suggesting that possibility of gut-mediated effects of metformin (ClinicalTrials.gov, NCT03809260).

Keyword : Metformin, gut microbiome, pharmacokinetics, anti-hyperglycaemic effects

Student Number : 2016-21977

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INTRODUCTION

Metformin is the most widely used drug for type 2 diabetes (T2D). Metformin reduces intestinal glucose absorption and hepatic glucose production via inhibition of the mitochondrial isoform of glycerophosphate dehydrogenase (mGPDH). Also, metformin enhances peripheral glucose uptake and utilization through activation of AMP-activated protein kinase (AMPK)[1, 2]. Metformin is also known to decompose free fatty acids by activating AMPK[1, 2]. Recently, some studies have reported changes in the gut microbiome after the administration of metformin, and potential for gut-mediated anti-hyperglycaemic effects of metformin[3-5]. The gut microbiome is a microbial population present in the ileum and colon that directly or indirectly affects physiological functions[6]. There are many reports that pharmacokinetics, activity, and toxicity of various drugs are affected or mediated by the gut microbiome[7]. For example, metformin is known to exhibit glucose control effects through enrichment of the short chain fatty acid (SCFA)-producing bacteria[8]. The SCFAs including butyrate produced by gut microbiome contribute to the anti-hyperglycaemic effect by binding to G protein-coupled receptors (GPCR) expressed on enteroendocrine L cells which

promotes secretion of GLP-1 and peptide YY and thereby contribute to the insulin secretion and regulation of glucose metabolism[9].

Numerous studies have supported the potential of gut-mediated effects of metformin[3, 10-13]. In one study, ^{18}F-labelled fluorodeoxyglucose accumulated markedly in the colon after metformin administration, demonstrating that the drug affects glucose handling in the colon[3]. In a clinical study, metformin was administered to human subjects alone or with pyrimethamine, a potent inhibitor of transporter that mediates renal elimination of metformin, and with the combination of pyrimethamine, systemic exposure of metformin increased significantly by approximately 2.6-fold, though the anti-hyperglycaemic effect decreased. In another clinical trial in which healthy subjects received a low or high dose of metformin, the anti-hyperglycaemic effect was found to be inverse to systemic exposure of metformin, suggesting that the partial effect of metformin occurs independently of systemic absorption[10, 11]. Furthermore, a previous study comparing intravenous metformin infusion and placebo reported no difference in acute effects on glucose control between the groups, suggesting that the chronic persistent effect is more important than is the plasma concentration or acute effect of metformin[12].

All of these findings suggest that a portion of the response to metformin is associated with an unknown action by non-absorbed portion of the drug, such as gut microbiome-mediated action[7]. Nonetheless, the relationship between systemic exposure, the anti-hyperglycaemic effect of metformin, and microbiome changes has not been established to date.

Orally administered vancomycin shows little absorption from the gastrointestinal tract[14]. At the same time, it has a profound effect on the gut microbiome[15, 16]. Therefore, the administration of oral vancomycin was conducted in this study to induce changes in the gut microbiome with little direct effect on the absorbed metformin in the body.

The objective of this study was to assess the effect of vancomycin-induced gut microbiome alterations on the pharmacokinetics and anti-hyperglycaemic effect of metformin.

METHODS

Subjects

This study aimed to enroll 10 subjects. Healthy adult male subjects who were 19–45 years old, weighed between 50.0–100.0 kg and had a body mass index of 18.0–28.0 kg/m^2 at the screening visit were included. Subjects with an active or a history of clinically significant diseases of the digestive, renal, and endocrine systems were excluded; subjects with a history of gastrointestinal disorders or surgery that might affect the absorption of investigational drugs were also excluded. Subjects with defecation less than five times a week or more than three times a day or who had excessively hard or soft stools were also excluded, as were subjects whose estimated glomerular filtration rate (eGFR) calculated by Modification of Diet in Renal Disease (MDRD) was less than 80 mL/min/1.73 m^2. The study was conducted according to Korea Good Clinical Practice and the ethical guidelines of the Declaration of Helsinki and with approval of the institutional review board of Seoul National University Bundang Hospital (B–1809–492–003) and Korea Ministry of Food and Drug Safety (ClinicalTrials.gov Identifier: NCT03809260).

Study design

The study was conducted using an open-label, single-arm design. The study consisted of four periods, which were baseline (day -1 or 1; baseline of post-metformin period), post-metformin (day 4), post-vancomycin (day 15 or 16; baseline of post-metformin+vancomycin period), and post-metformin+vancomycin (day 19), according to the treatment given in each period (Figure 1).

Subjects received 1000 mg metformin orally twice daily from day 1 (day 1, 1:30 PM and 9:00 PM; day 2 and 3, 9:00 AM and 9:00 PM) to day 4 (9:00 AM), except for the first dose which was reduced to 500 mg metformin for patient safety. After the washout period from day 5 to day 10, the subjects received 500 mg vancomycin orally twice daily (9:00 AM and 9:00 PM) from day 11 to day 17 in the morning, except for the first day (day 11) which was reduced to 250 mg vancomycin for patient safety, to cause gut microbiome change. Then, metformin was administered again from day 16 to day 19 in the same manner as on day 1 to day 4. Metformin was administered on fasting state on day 4 and day 19 for appropriate pharmacokinetic and pharmacodynamic evaluation, and other administrations were conducted in the postprandial state.

To summarize the sample collections, samples for plasma

metformin concentration measurements were collected on day 4 and 19. Blood samples for serum glucose and insulin concentration measurements were collected during an oral glucose tolerance test (OGTT) administered before the first metformin administration on day 1 (baseline) and day 16 (post−vancomycin), and after the last metformin dose on day 4 (post−metformin) and day 19 (post−metformin+vancomycin). Faecal samples for gut microbiome analysis were collected on day −1 or 1 and 15 or 16d before the first metformin administration. The optimal sample for analysis was the first faeces in the morning on days 1 and 16, but if not collected at this time, it was selected in consideration of the defecation diary among the samples obtained at the nearest time. The faecal samples were also collected after the last metformin dose on day 4 and day 19. Urine samples for urine metformin concentration measurements were collected on day −1, 4, 15, and 19 (Figure 1).

Subjects provided written consent to the prohibition of eating foods containing lactic acid bacteria, grapefruit, and caffeine during the entire study duration. Also, subjects were provided with a normal diet not containing those components and were asked to eat the full amount of the meal during the hospitalization. Any diet other than the provided meal was prohibited during the hospitalization.

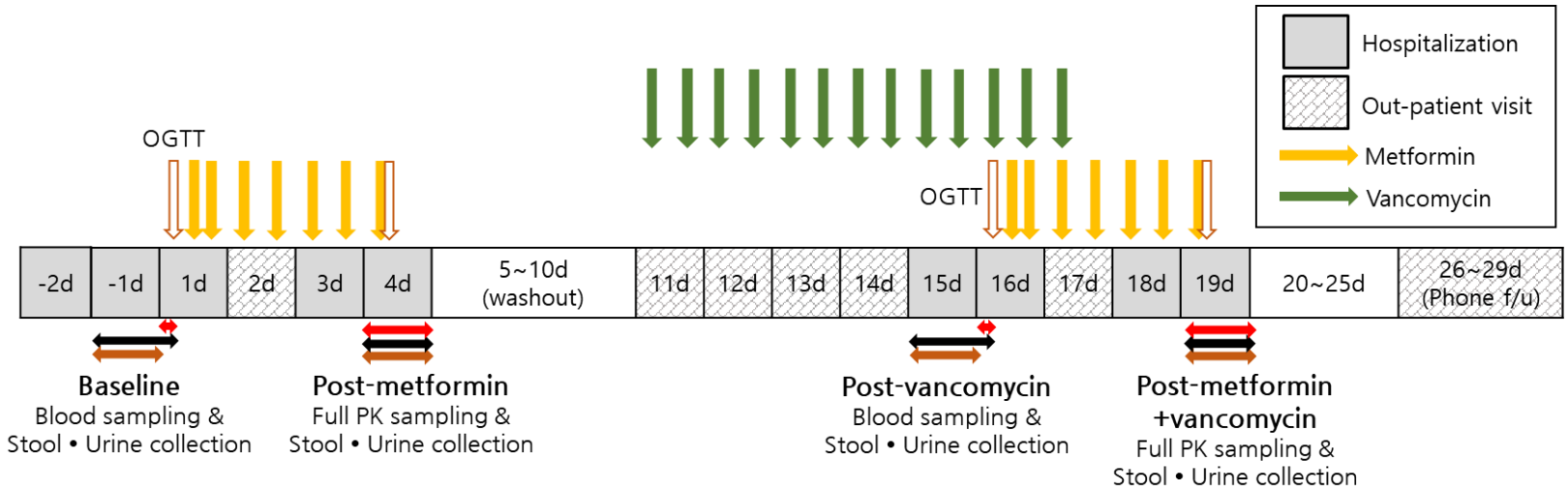


Figure 1. Study design.

Red, black and brown arrows indicate blood sampling, stool collection, and urine collection, respectively. OGTT, oral glucose tolerance test.

Pharmacokinetic and pharmacodynamic assessments of metformin

Plasma samples for pharmacokinetic evaluation were collected at 0 (pre−dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h post−metformin and post−metformin+vancomycin dose.

Blood samples collected for pharmacokinetic assessment were centrifuged at 3,000 rpm for 10 minutes at 4° C to separate plasma. Plasma and urine samples were stored frozen at −70° C until analysis. The concentration of metformin in plasma and urine was analysed by liquid chromatography−tandem mass spectrometry (LC− MS/MS, Agilent 1260 HPLC system and Agilent 6490 Mass spectrometer; Agilent Technologies, Santa Clara, CA, USA), with an internal standard of phenformin. Metformin was separated using a Kinetex column (2.6 μm, 50 x 2.1 mm, Phenomenex, USA), and positive electrospray ionization was performed. For plasma, the mean within−run accuracies were within 96.8−101.8% and the precision was ≤4.0%. The corresponding values of between−run were 92.5−97.8% and ≤6.9%, respectively. For urine, the mean within−run accuracies were within 94.0−111.7% and the precision was ≤10.6%. The corresponding values of between−run were 94.0−99.0% and ≤9.8%, respectively. The calibration curves were

linear across the range of 10−2,000 μg/L for plasma and 100−20,000 μg/L for urine.

The maximum blood concentration (C_{max}) and time to reach C_{max} (T_{max}) are presented as actual observed values. The area under the concentration-time curve from 0 to the last measurable time point (AUC_{last}) was calculated by the linear−log trapezoidal method. The AUC from time 0 to infinity (AUC_{inf}) was calculated by sum of AUC_{last} and the last observed concentration/elimination rate constant of the terminal phase (λz). The λz was estimated by linear regression of the time−log plasma concentration profile. Percentage of AUC_{inf} due to extrapolation from time of last measurable observed concentration to infinity (AUC% extrapolated) was calculated by (AUC_{inf} − AUC_{last})/AUC_{inf} · 100. The elimination half−life ($t_{1/2}$) was calculated as the ln2/λz. Urine samples were collected for 12 h at the four periods, and the amount excreted in urine (Ae) was calculated as the concentration of metformin of urine · urine volume. The fraction excreted unchanged (fe) was calculated as (Ae/dose) · 100. Renal clearance (CL_R) was calculated as Ae/AUC_{inf}.

An OGTT was performed for pharmacodynamic evaluation, and the serum insulin concentration was measured at each of the four periods. A 75 g glucose solution was administered on an empty

stomach, and samples for serum glucose concentration were collected at 0 (before 75 g glucose administration), 0.25, 0.5, 0.75, 1, 1.5, and 2 h. Insulin was measured only at 0 h.

The maximum serum glucose concentration (G_{max}) is presented as the actual observed value. The area under the glucose curve (AUGC) was calculated using the linear-linear trapezoidal method, and homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as (glucose · insulin)/405.

Both pharmacokinetic and pharmacodynamic parameters were calculated using the actual time of sampling and obtained by non-compartmental methods with Phoenix® WinNonlin® software version 8.0 (Certara USA Inc., Princeton, NJ, USA).

Glycaemic response measures (i.e., AUGC, G_{max}, HOMA-IR, fasting glucose, ⊿serum glucose at 1 h post-OGTT (PP1) and 2 h (PP2)) from the post-vancomycin period were compared to those at baseline to ascertain the status regarding glucose control before metformin dosing were similar between two periods. The ⊿serum glucose at PP1 (or PP2) was calculated as subtracting the glucose level at 0 h from that at 1 h (or 2 h) in each period.

Baseline corrected parameters, which are ⊿AUGC, ⊿G_{max}, and ⊿HOMA-IR, after metformin administration were defined by subtracting the baseline values from the post-metformin period

(i.e., AUGC at post-metformin period- AUGC at baseline), and subtracting the post-vancomycin values from the post-metformin+vancomycin period. Smaller ⊿AUGC, ⊿G_{max}, and ⊿HOMA-IR values, i.e. larger absolute values of the three parameters, were interpreted as greater effects of the metformin treatment. Differences in pharmacodynamic parameter values and changed percentages between the post-metformin period and post-metformin+vancomycin period were presented.

An exploratory measurement of the relative abundance of glucose in stool was performed. For preparation of the faecal glucose analysis, the first extraction solution, which was a mixture of acetonitrile, isopropanol, and distilled water, was spiked into the faecal sample at a ratio of 1 mL extraction solution to 50 mg faecal sample. Then, the faecal sample and extraction solution were mixed, and centrifuged with 18,945 RCF at 4° C for 10 min. The supernatant was collected, dried for concentration, and re-extracted with second extraction solution, a mixture of acetonitrile and distilled water. The extracted samples were re-dried, and derivatization was conducted. Finally, the prepared samples were injected into gas chromatography system coupled to a time-of-flight mass spectrometer (GC-TOF-MS) for analysis of relative abundance of glucose.

Statistical analyses of pharmacokinetics and pharmacodynamics

The Wilcoxon signed rank test was performed for comparison of pharmacokinetic and pharmacodynamic parameters including baseline values for glycaemic response to OGTT, with the significance level of 0.05.

The relationship between pharmacokinetics (C_{max} and AUC_{last}) and pharmacodynamics (⊿AUGC, ⊿G_{max}, and ⊿HOMA-IR) was evaluated through Spearman correlation analysis for each of post-metformin period and post-metformin+vancomycin period, respectively. The statistical analysis was performed using SAS® version 9.4 (SAS Institute, Cary, NC, USA).

Assessment of the gut microbiome

Stool samples collected at the four periods were homogenized using a 3M sample mixer, dispensed into Eppendorf tubes and stored frozen at −70° C until analysis.

For metagenomic sequencing, DNA extraction was performed using PowerSoil® DNA Isolation Kit. Amplification of 16S rRNA genes was conducted using 16S V3−V4 primers. Normalization and pooling of the final product of a subsequent limited−cycle amplification was performed using PicoGreen. The size of libraries was verified using the TapeStation DNA screentape D1000 (Agilent), and sequencing was performed with the MiSeq™ platform (Illumina, San Diego, USA). Taxonomic profiling was carried out using a module of marker data profiling of MicrobiomeAnalyst[17]. Taxonomy label was selected as QIIME, and total sum scaling was performed without data transformation. Alpha−diversity was calculated using the Shannon index, and the Kruskal−Wallis test was performed for comparison between periods. Beta−diversity was assessed using Bray−Curtis dissimilarity and represented by a principal coordinates analysis (PCoA) plot, and permutational multivariate analysis of variance (PERMANOVA) was used to compare beta−diversity between periods. Distinct bacterial taxa between periods were identified by

linear discriminant analysis (LDA) effect size (LEfSe) analysis. Data were normalized by the total sum scaling method for LEfSe. By dividing each feature count by the total library size, this yielded a relative proportional value for each feature, which eliminated the bias related to different sequencing depths[18]. The cut-offs for the false discovery rate (FDR)-adjusted p-value and log LDA score were 0.05 and 2.0, respectively. Changes in the gut microbiome caused by vancomycin administration were assessed through post-metformin vs. post-vancomycin, post-metformin vs. post-metformin+vancomycin, and baseline vs. post-vancomycin comparisons; similarly, changes in the gut microbiome by metformin administration were identified through baseline vs. post-metformin and post-vancomycin vs. post-metformin+vancomycin comparisons. Considering the washout period after metformin administration and the drug administered within the closest period of post-vancomycin, the change between baseline vs. post-vancomycin was considered to be caused by vancomycin.

Spearman correlation analysis between ⊿AUGC and the relative abundance of the microbiome was performed for genera with differences between the two periods (baseline vs. post-metformin, post-metformin vs. post-vancomycin, post-metformin vs. post-metformin+vancomycin, and baseline vs. post-

vancomycin) in LEfSe analysis using SAS® version 9.4, with a p-value cut-off of 0.1. In addition, Spearman correlation analysis using the relative abundance values of the genera at post-metformin and post-metformin+vancomycin periods paired with the ⊿AUGC of each corresponding period was performed. The exploratory correlation analysis considering the small number of subjects was conducted separately for the two periods, with a p-value cut-off of 0.1. For a negative Spearman's correlation coefficient (rho), it was interpreted that the relative abundance of genera is positively correlated with the anti-hyperglycaemic effect. For LEfSe and correlation analyses, only taxa with relative abundance ≧0.01 (1%) at least once during the compared periods are presented.

Safety

All subjects were examined for vital signs, physical examinations, and clinical laboratory tests. All symptoms and signs observed by the investigator or reported by the subject from the time of obtaining written consent to the time of completion of the clinical trial were collected as adverse events (AEs). Each AE was classified based on the first dose at each period. For example, AEs that occurred after the first administration of metformin and before the first administration of vancomycin were classified as AEs of post-metformin period. All AEs were monitored and reviewed by the investigators to determine their severity and relationship to the study drug.

In order to investigate whether there is a difference in gut microbiome according to occurrence of diarrhea, alpha-diversity and beta-diversity were compared in the post-metformin and post-metformin+vancomycin period, respectively, divided into two subject groups who developed diarrhea and those who did not. For this analysis, the diarrhea that occurred from the first metformin administration on day 1 to day 4 was considered as the diarrhea that occurred during the post-metformin period. In the same way, the diarrhea that occurred from the first metformin administration on day 16 to day 19 was considered as the diarrhea that occurred

during the post−metformin+vancomycin period.

RESULTS

Demographics

A total of 15 participants were enrolled in this study. One of them dropped out due to an adverse reaction, three withdrew their consent, and two dropped out for other reasons. Thus, 9 subjects received the study drug at least once, and faecal samples were all obtained for four periods. In a total of three subjects, metformin dose was reduced to 500 mg once in each subject due to gastrointestinal adverse events on day 17 or 18. Because all dose reductions were only once in each subject and occurred on days without pharmacokinetic, pharmacodynamic sampling including baseline, the effect of this on several evaluations was assessed to be limited. The mean age of the nine subjects was 25.8 (range: 19–33) years, with a mean body mass index of 24.3 (range: 19.2–27.6) kg/m^2. As some pharmacokinetic/pharmacodynamic samples of one subject were not collected, eight subjects completed the study. However, the analysis to detect changes in the microbiome according to the designated periods was performed on all nine subjects, including one subject whose pharmacokinetic/ pharmacodynamics samples was not complete and whose microbiome profile was similar to other 8 subjects. In contrast, the

pharmacokinetic/pharmacodynamic analysis and correlation analysis between pharmacodynamics and the microbiome were performed on eight subjects who completed the study.

Pharmacokinetics and pharmacodynamics of metformin

Overall, the pharmacokinetic profiles of post-metformin and post-metformin+vancomycin periods were similar to each other (Figure 2a), and T_{max} and $t_{1/2}$ were similar. There were no statistically significant differences in systemic exposure represented by C_{max} and AUCs between post-metformin and post-metformin+vancomycin periods. In addition, there were no statistically significant differences in Ae, Fe and CL_R (Table 1).

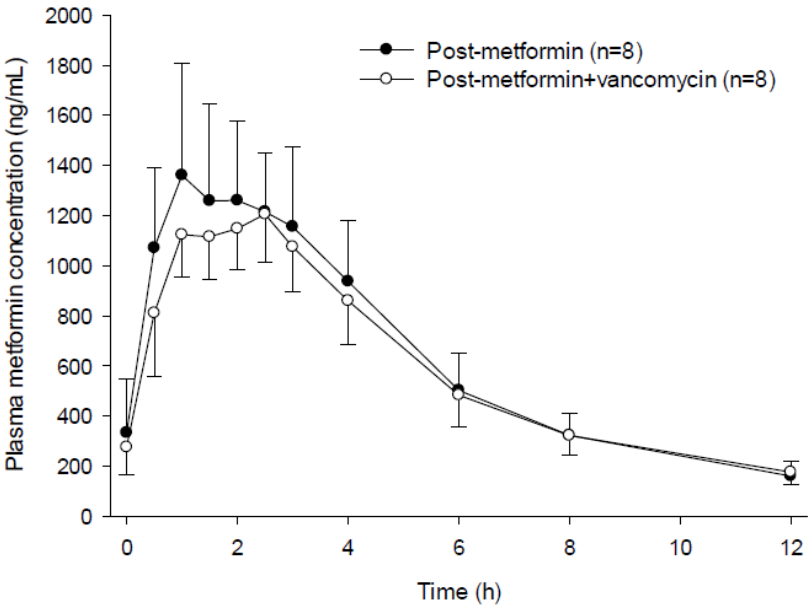
Serum glucose profiles during OGTTs and parameters at baseline and post-vancomycin periods were similar, corresponding to the baseline of post-metformin and post-metformin+vancomycin periods, respectively (Figure 2b, Table 2). The mean values of AUGC, G_{max} and HOMA-IR at baseline and post-vancomycin did not show statistically significant differences (p-value=0.25 for AUGC; 0.98 for G_{max}; 1.00 for HOMA-IR), nor did fasting glucose and Δserum glucose at 1 h (PP1) and 2 h (PP2) post-OGTT (Table 3).

The absolute value of ΔAUGC, ΔG_{max} and ΔHOMA-IR, which represent the pharmacodynamic effects of metformin, tended to be lower in the post-metformin+vancomycin period than in the

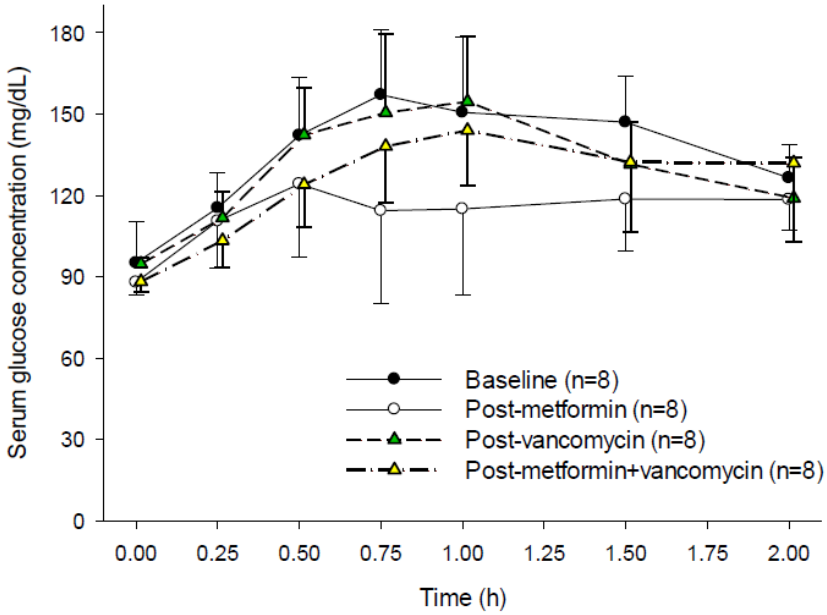
post−metformin period. Moreover, a statistically significant difference was detected for ⊿AUGC, showing a %change of −75.9% in the post−metformin+vancomycin period compared to the post−metformin period (Table 2). Furthermore, ⊿serum glucose at PP1 was significantly higher in post−metformin+vancomycin period compared to post−metformin period (p−value=0.039), which supported that the anti−hyperglycaemic effect in post−metformin+vancomycin period was relatively low (Table 3).

Although the administration of vancomycin did not influence the pharmacokinetic properties of metformin, the anti−hyperglycaemic effect was partially affected; hence, the relationship between the pharmacokinetics and pharmacodynamics was weak, as confirmed by Spearman correlation analysis (Figure 2c, Figure 3). Except for relationship between AUC_{last} and ⊿HOMA−IR, all p−values and absolute values of Spearman' s rho were >0.05 and ≤0.55, respectively. Only the results of correlation analysis for AUC_{last} and ⊿HOMA−IR during the metformin+vancomycin period showed p−values of 0.0003 and Spearman' s rho of 0.95, respectively (Figure 3).

(a)


Post-metformin (n=8)
Post-metformin+vancomycin (n=8)
Plasma metformin concentration (ng/mL)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
Time (h)

(b)


Serum glucose concentration (mg/dL)
180
150
120
90
60
30
0
Baseline (n=8)
Post-metformin (n=8)
Post-vancomycin (n=8)
Post-metformin+vancomycin (n=8)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
Time (h)

(c)

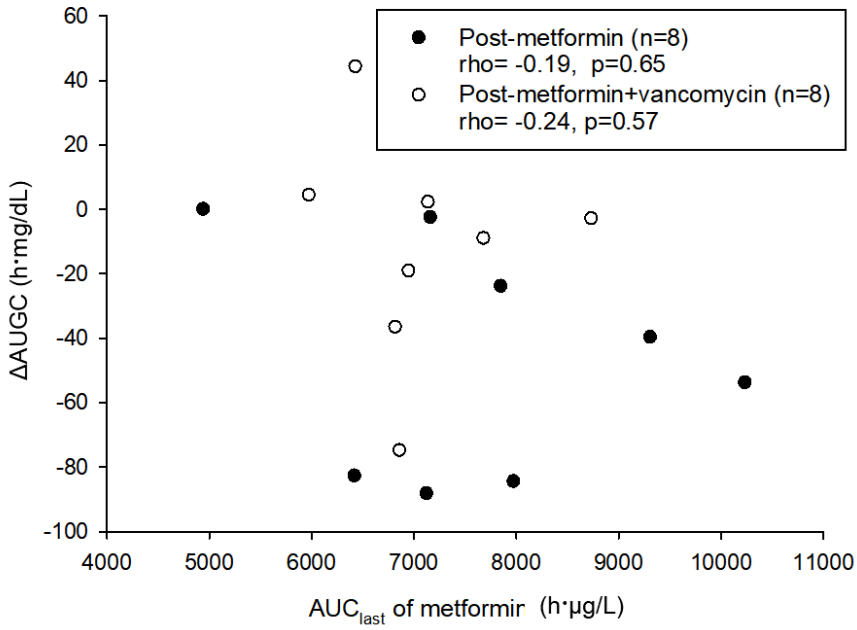


Figure 2. Pharmacokinetics of metformin and the impact of metformin and vancomycin on AUGC. Mean plasma concentration−time profiles of metformin (a), mean serum concentration−time profiles of glucose (b), and correlation between pharmacokinetic−pharmacodynamic parameters after administration of metformin and metformin+vancomycin (c).

Note: Bars represent the standard deviations in figure (a) and (b).

AUC_{last}, area under the plasma concentration curve from time 0 to last measurable time point; AUGC, area under the glucose concentration curve from time 0 to 2 h; C_{max}, maximum plasma concentration; ⊿AUGC of 'Post−metformin' obtained by subtracting the value of baseline from that of post−metformin; ⊿AUGC of 'Post−metformin+vancomycin' obtained by subtracting the value of post−vancomycin from that of post−metformin+vancomycin

Table 1. Pharmacokinetic parameters of metformin

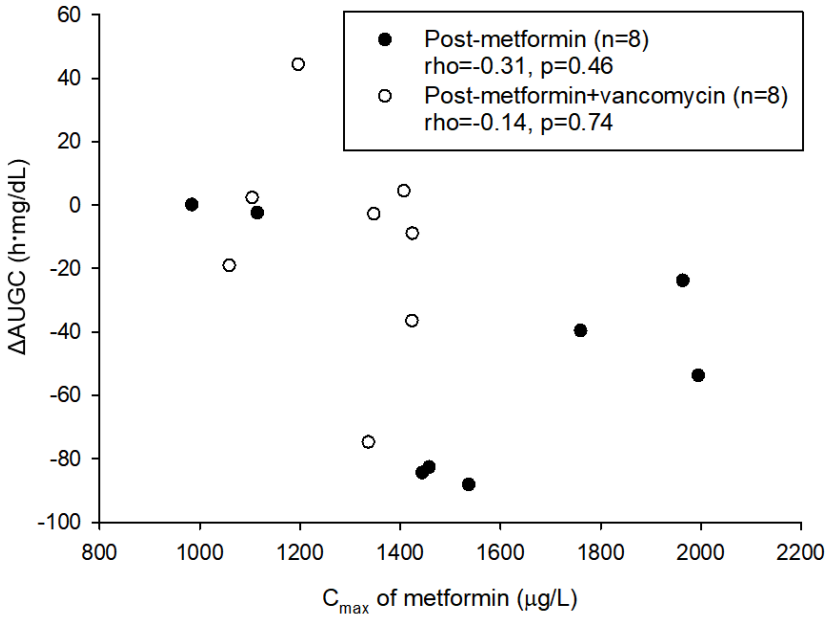
Parameters	Post−metformin (n = 8)	Post−metformin + vancomycin (n = 8)	p−value*
T_{max} (h)	1.5 [0.5 − 3.0]	2.0 [1.0 − 3.0]	−
C_{max} (μg/L)	1531.9±366.6	1287.0±147.0	0.25
AUC_{last} (h · μg/L)	7624.2±1646.1	7069.6±835.9	0.25
AUC_{inf} (h · μg/L)	8466.8±1847.5	8221.8±1242.4	0.74
AUC% extrapolated	9.8±3.7	13.6±5.3	−
$t_{1/2}$ (h)	3.5±0.4	4.4±1.2	−
Ae (mg)**	261.0±105.3	270.0±83.0	1.00
Fe (%)**	26.1±10.5	27.0±8.3	1.00
CL_R (L/h)	33.5±6.0	31.6±8.3	0.95

*p−value: Wilcoxon signed rank test

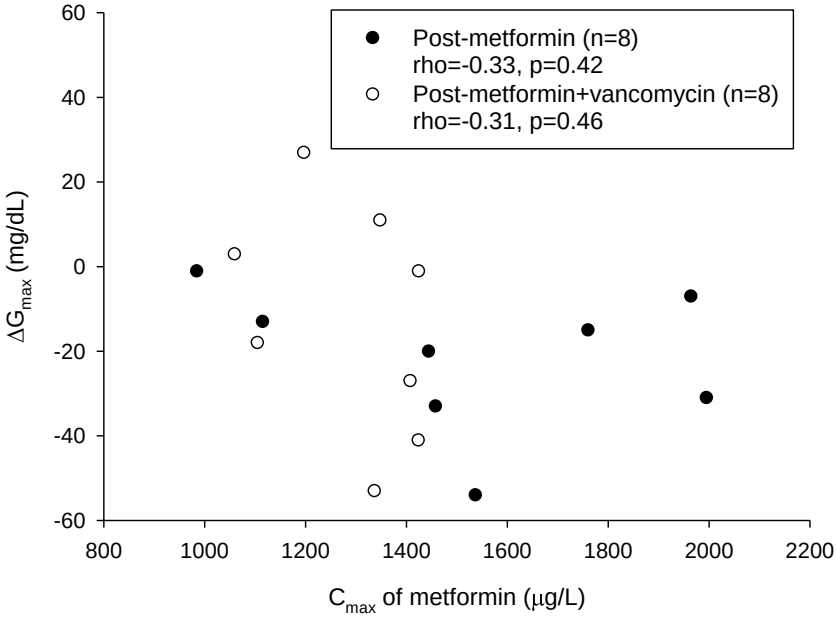
**Parameters calculated for 9 subjects, including one subject who completed urine collection but failed to complete plasma sampling, and this subject was excluded from CL_R calculation.

All data are presented as arithmetic mean ± standard deviation, except for T_{max} which is presented as median [minimum − maximum]. AUC_{inf}, area under the plasma concentration curve from time 0 to infinity; AUC_{last}, area under the plasma concentration curve from time 0 to last measurable timepoint; AUC% extrapolated, Percentage of AUC_{inf} due to extrapolation from time of last measurable observed concentration to infinity; C_{max}, maximum plasma concentration; $t_{1/2}$, elimination half−life; T_{max} , time to reach C_{max}

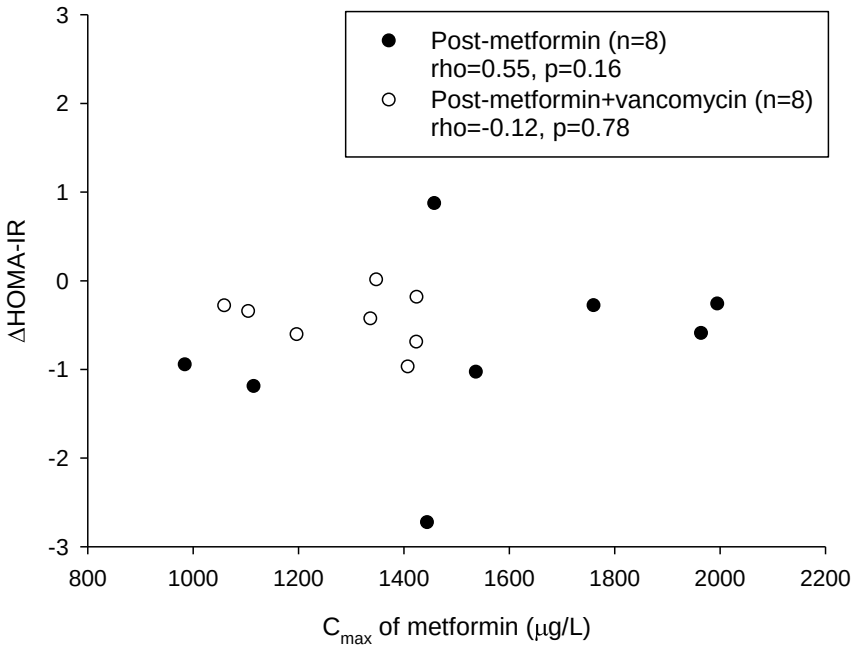
(a)


Post-metformin (n=8)
rho=-0.31, p=0.46
Post-metformin+vancomycin (n=8)
rho=-0.14, p=0.74
ΔAUGC (h·mg/dL)
60
40
20
0
-20
-40
-60
-80
-100
800
1000
1200
1400
1600
1800
2000
2200
Cmax of metformin (μg/L)

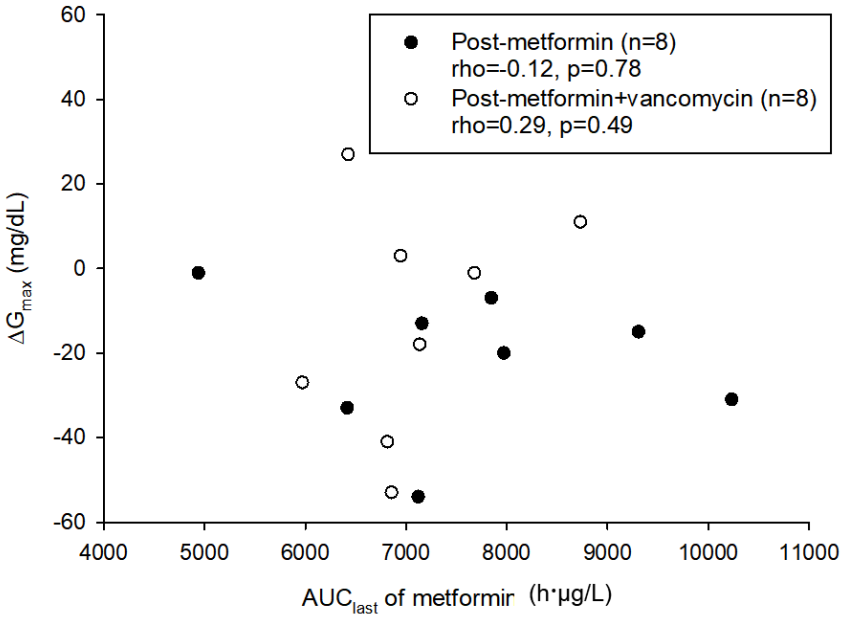
(b)


Post-metformin (n=8)
rho=-0.33, p=0.42
Post-metformin+vancomycin (n=8)
rho=-0.31, p=0.46
ΔGmax (mg/dL)
60
40
20
0
-20
-40
-60
800
1000
1200
1400
1600
1800
2000
2200
Cmax of metformin (μg/L)

(c)


Post-metformin (n=8)
rho=0.55, p=0.16
Post-metformin+vancomycin (n=8)
rho=-0.12, p=0.78
ΔHOMA-IR
3
2
1
0
-1
-2
-3
800
1000
1200
1400
1600
1800
2000
2200
Cmax of metformin (μg/L)

(d)


Post-metformin (n=8)
rho=-0.12, p=0.78
Post-metformin+vancomycin (n=8)
rho=0.29, p=0.49
ΔGmax (mg/dL)
60
40
20
0
-20
-40
-60
4000
5000
6000
7000
8000
9000
10000
11000
AUClast of metformin (h·μg/L)

(e)

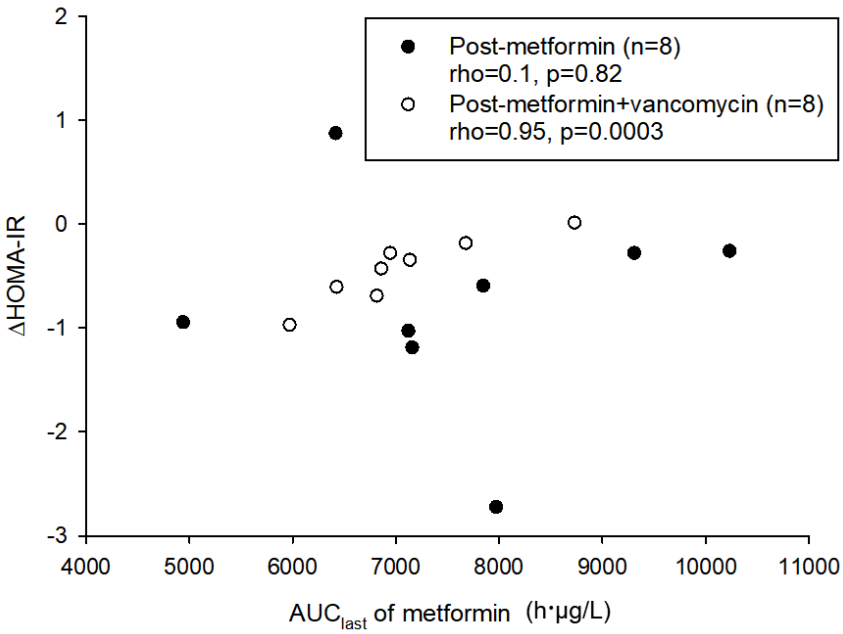


Figure 3. Correlation between pharmacokinetic−pharmacodynamic parameters after administration of metformin and metformin+vancomycin. Spearman correlation analysis between C_{max} of metformin and (a) ⊿AUGC, (b) ⊿G_{max} and (c) ⊿HOMA−IR; AUC_{last} of metformin and (d) ⊿G_{max} and (e) ⊿HOMA−IR.

AUC_{last}, area under the plasma concentration curve from time 0 to last measurable time point; AUGC, area under the glucose concentration curve from time 0 to 2 h; C_{max}, maximum plasma concentration; G_{max}, maximum glucose concentration; HOMA−IR, homeostatic model assessment of insulin resistance; Pharmacodynamic parameters of 'Post−metformin', Difference of each parameter obtained by subtracting the value of baseline from that of post−metformin; Pharmacodynamic parameters of 'Post−metformin+vancomycin', Difference of each parameter obtained by subtracting the value of post−vancomycin from that of post−metformin+vancomycin.

Table 2. Pharmacodynamic parameters of metformin

Parameters	Baseline (n=8)	Post-metformin (n=8)	Post-vancomycin (n=8)	Post-metfomin +vancomycin (n=8)	p-value*	Difference between two treatments**	%change***
AUGC (h · mg/dL)	277.2±31.5	230.3±34.0	266.5±22.4	255.2±30.7		-	-
⊿AUGC (h · mg/dL)	-	-46.8±36.3	-	-11.3±34.5	0.039	35.5±40.7	-75.9%
G_{max} (mg/dL)	162.9 ±18.2	141.1 ±18.9	163.8 ±18.7	151.4±23.0		-	-
⊿G_{max} (mg/dL)	-	-21.8±17.0	-	-12.4±27.2	0.46	9.4±29.3	-43.1%
HOMA-IR	2.2±1.0	1.5±0.3	2.0±0.2	1.6±0.2		-	-
⊿HOMA-IR	-	-0.8±1.0	-	-0.4±0.3	0.31	0.3±1.0	-42.9%

Data presented as arithmetic mean±standard deviation. AUGC, area under the glucose concentration curve from time 0 to 2 h; G_{max}, maximum glucose concentration; HOMA-IR, homeostatic model assessment of insulin resistance.

*p-value: Wilcoxon signed rank test for post-metformin vs. post-metformin+vancomycin.

**Arithmetic mean±standard deviation for 'Post-metformin+vancomycin' - 'Post-metformin'.

***%change: Each ratio of mean value of 'Difference between two treatments' compared to corresponding value of 'Post-metformin'

Table 3. Glycaemic response to oral glucose tolerance test of metformin before and after vancomycin administration

Parameters	Baseline (n=8)	Post-vancomycin (n=8)	p-value*	Post-metformin (n=8)	Post-metformin+ vancomycin (n=8)	p-value**
Fasting glucose (mg/dL)	95.1±15.0	94.8±2.4	0.37	88.0±4.7	88.3±3.8	0.80
⊿Serum glucose at PP1 (mg/dL)	55.5±25.7	59.9±22.6	0.66	27.0±31.6	55.8±22.1	0.039
⊿Serum glucose at PP2 (mg/dL)	31.4±6.4	24.3±15.4	0.27	30.5±11.8	43.8±30.0	0.37

* Baseline vs. Post-vancomycin

**Post-metformin vs. Post-metformin+vancomycin

p-values from Wilcoxon signed rank test; PP1, one-hour-post-oral glucose tolerance test; PP2, two-hour-post-oral glucose tolerance test

An exploratory analysis for glucose in stool was performed on 9 subjects whose faecal samples were all collected. The glucose levels were relatively low in post-vancomycin and post-metformin+vancomycin periods compared to baseline and post-metformin periods (Table 4). That is, the relative abundance of faecal glucose decreased to about 10 % after administration of vancomycin compared to before administration.

Table 4. Average relative abundance of glucose in faecal samples

Parameter	Baseline (n=9)	Post-metformin (n=6)[1]	Post-vancomycin (n=4)[2]	Post-metformin +vancomycin (n=5)[3]
Relative abundance of glucose	30220	24927	2896	2445

The analyzed results of relative abundance were 1000-fold of the values in the table.

[1] There were 3 missing values out of a total of 9 subjects.

[2] There were 5 missing values out of a total of 9 subjects.

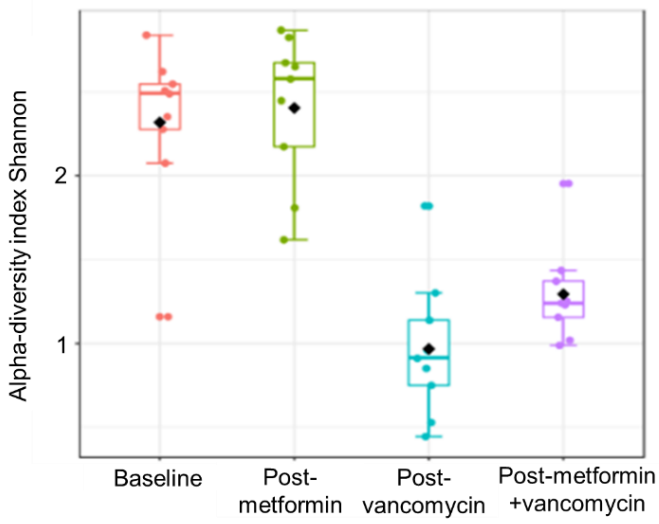
[3] There were 4 missing values out of a total of 9 subjects.

Gut microbiome

Overall, a substantial change in the diversity and composition of the microbiome was observed before and after vancomycin administration. Alpha-diversity, representing bacterial diversity, was estimated at the genus level for all periods using the Shannon index. The Shannon index was generally greater in the period before than after vancomycin administration, suggesting that vancomycin treatment decreased microbial diversity. Moreover, there was a significant difference between baseline and post-vancomycin period (p-value=0.0019) and between post-metformin and post-metformin+vancomycin periods (p-value=0.0012) (Figure 4a,Table 5).

The PCoA plot of beta-diversity, representing the difference in bacterial composition between different periods, displayed a divided pattern before and after the administration of vancomycin, suggesting that vancomycin changed the microbial composition. A significant difference between baseline and post-vancomycin period (p-value<0.001) and between post-metformin and post-metformin+vancomycin periods (p-value<0.001) at the genus level was detected (Figure 4b, Table 5).

(a)



(b)

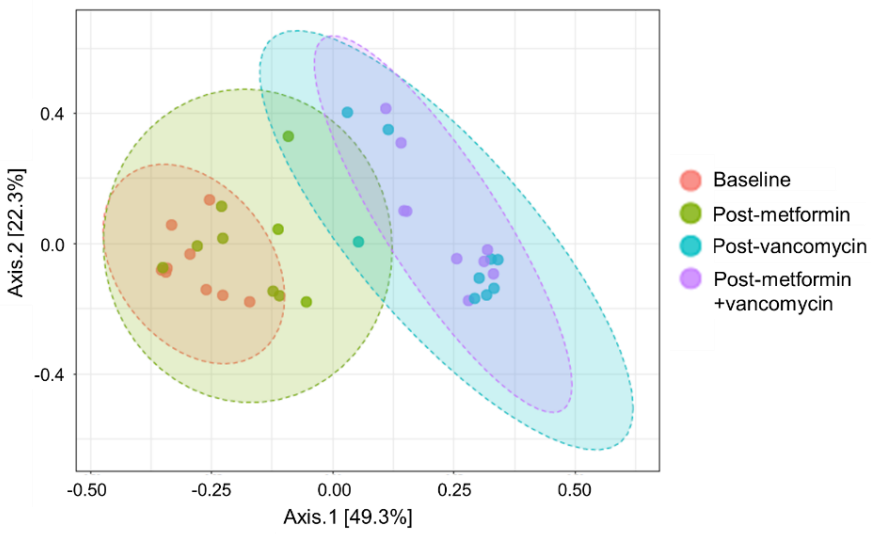


Figure 4. Changes in composition of gut microbiome represented by (a) alpha-diversity (Shannon index) and (b) beta-diversity (PCoA plot) measured at genus level.

Each axis in (b) represents the highest and second-highest percent of the variation between the samples.

Table 5. Comparison of alpha-diversity and beta-diversity evaluated between different periods

	Taxonomic level	Baseline vs. Post-metformin	Post-vancomycin vs. Post-metformin +vancomycin	Baseline vs. Post-vancomycin	Post-metformin vs. Post-metformin +vancomycin
Alpha diversity*	Phylum	p-value=0.011	p-value=0.26	p-value=1	p-value=0.30
	Genus	p-value=0.67	p-value=0.19	p-value=0.0019	p-value=0.0012
Beta diversity**	Phylum	p-value=0.004	p-value=0.47	p-value=0.002	p-value < 0.001
	Genus	p-value=0.029	p-value=0.43	p-value < 0.001	p-value < 0.001

*Alpha-diversity: Shannon, Kruskal-Wallis test.
** Beta-diversity: PCoA, Bray-Curtis, PERMANOVA

Relative abundances at the phylum level varied by individual, with the tendency of Firmicutes and Bacteroidetes predominance at baseline and post−metformin period. For post−vancomycin and post−metformin+vancomycin periods, Fusobacteria and Proteobacteria tended to increase compared to the previous two periods (Figure 5). The relative abundance at the genus level was also variable between individuals (Figure 6). A total of 50 genera were identified, 28 of which showed a relative abundance of at least 1% in at least one period.

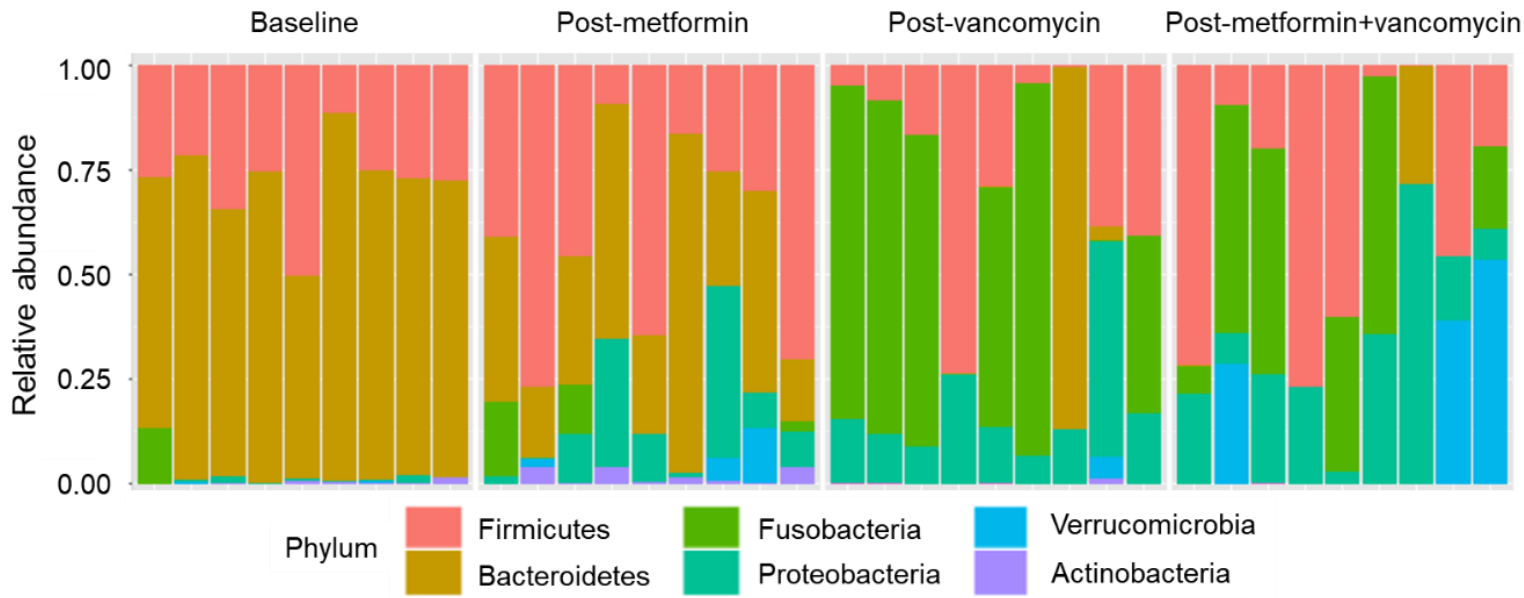


Figure 5. Relative abundance of intestinal bacterial phyla

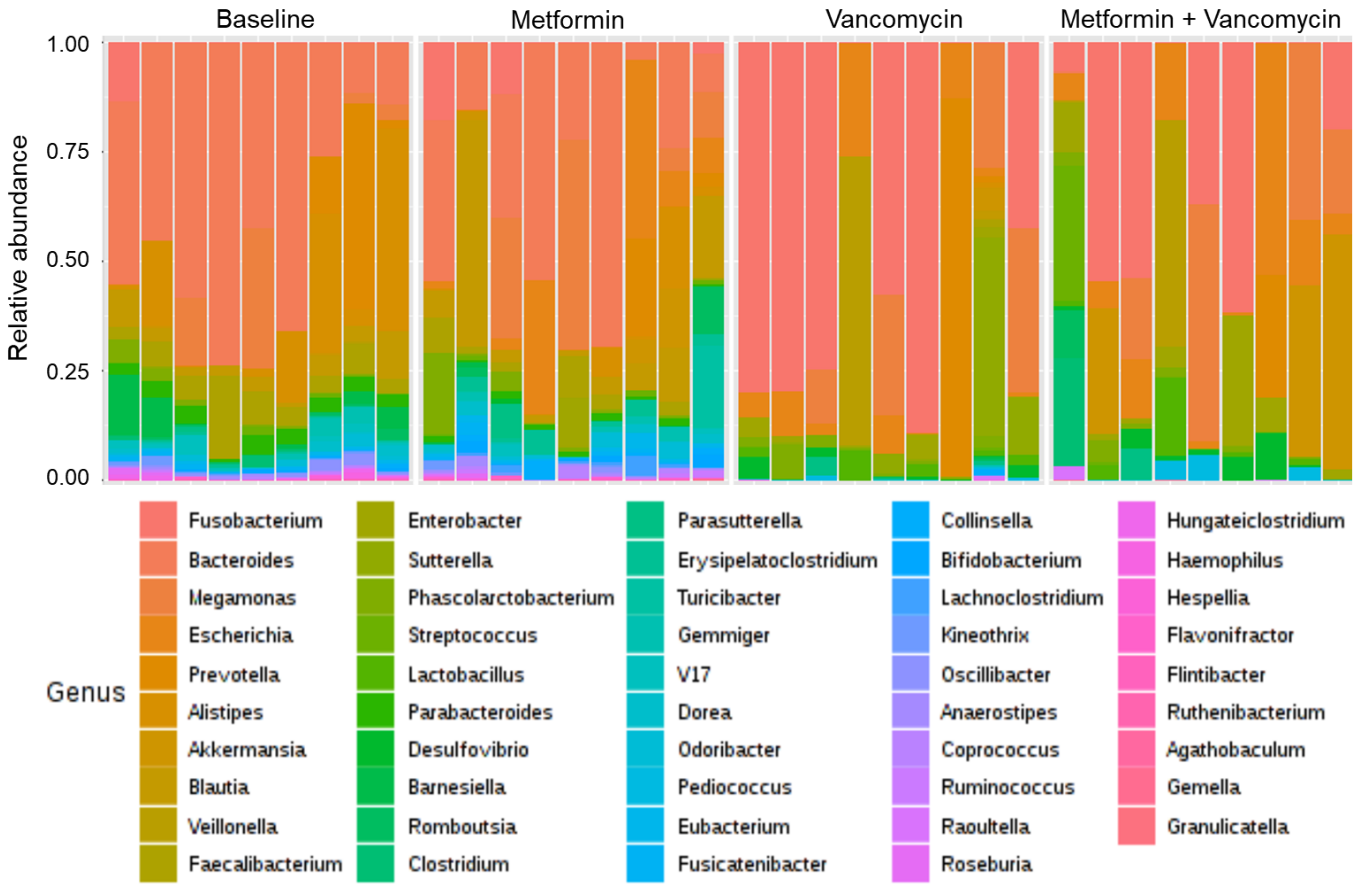


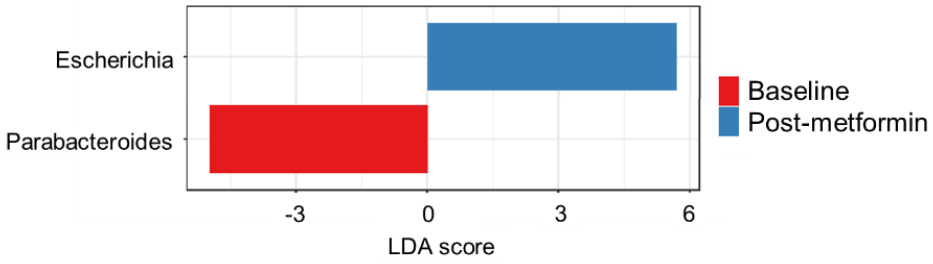
Figure 6. Relative abundance of intestinal bacterial genera.

To observe changes in gut microbiome composition more closely, we performed LEfSe analysis between different periods at the phylum and genus levels (Figure 7, Table 6). The taxa with altered relative abundance due to vancomycin administration were identified through post-metformin vs. post-vancomycin, post-metformin vs. post-metformin+vancomycin, and baseline vs. post-vancomycin comparisons, and the results were similar for all three pairs. At the phylum level, the relative abundance of Bacteroidetes and Actinobacteria was decreased by vancomycin administration, whereas that of Proteobacteria was increased at post-vancomycin compared to baseline. At the genus level, the relative abundance of *Lactobacillus* and *Enterobacter* increased, whereas that of *Bacteroides, Eubacterium, Erysipelatoclostridium, Parabacteroides, Blautia, Faecalibacterium*, and *Alistipes* decreased. Additionally, the relative abundance of *Escherichia* was increased post-vancomycin compared to baseline.

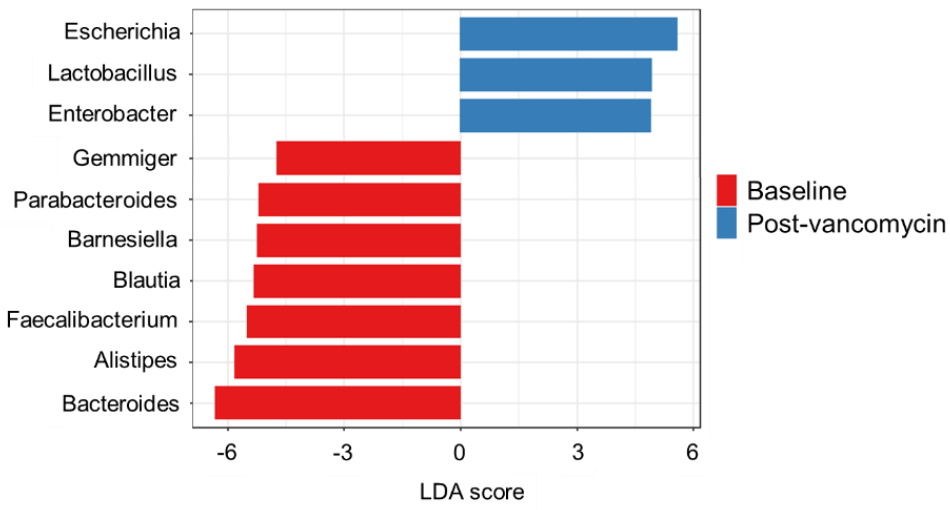
There were some differences in the taxa changed by metformin compared to vancomycin. Comparisons between baseline vs. post-metformin and post-vancomycin vs. post-metformin+vancomycin showed taxa with altered relative abundance due to metformin administration. Post-metformin, the

relative abundance of the phylum Proteobacteria was increased and Bacteroidetes decreased compared to baseline; the relative abundance of the genus *Escherichia* was increased and that of *Parabacteroides* decreased post-metformin compared to baseline. No species were changed between post-vancomycin and post-metformin+vancomycin periods at either the phylum or genus level (Figure 7, Table 6).

(a)


Escherichia
Parabacteroides
-3
0
3
6
LDA score
Baseline
Post-metformin

(b)


Escherichia
Lactobacillus
Enterobacter
Gemmiger
Parabacteroides
Barnesiella
Blautia
Faecalibacterium
Alistipes
Bacteroides
-6
-3
0
3
6
LDA score
Baseline
Post-vancomycin

(c)

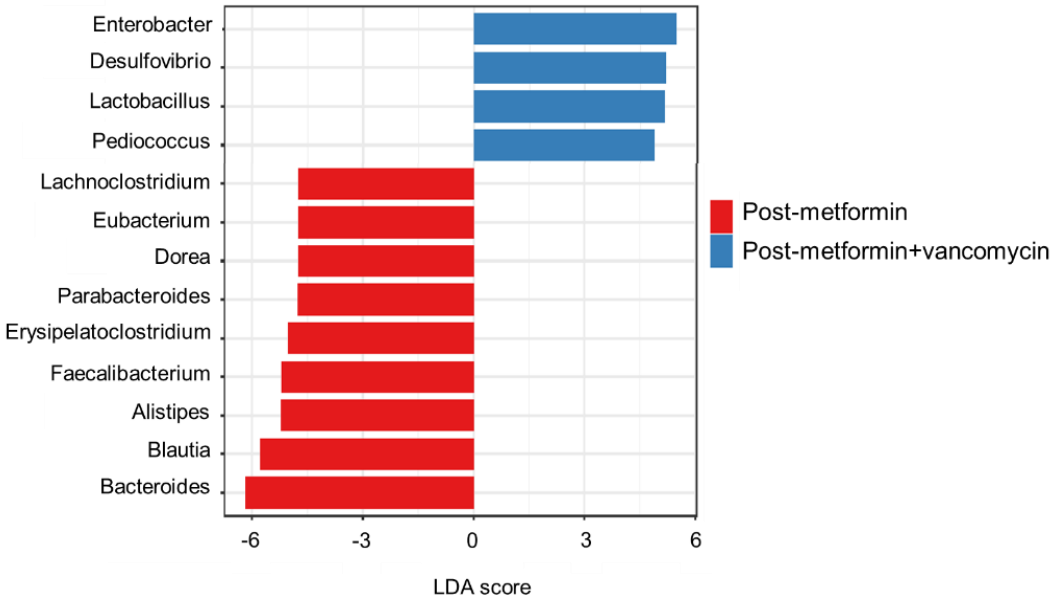


Figure 7. Linear discriminant analysis effect size (LEfSe) analysis results between (a) baseline and post-metformin, (b) baseline and post-vancomycin, and (c) post-metformin and post-metformin+vancomycin.

* Note: P-value (FDR adjusted) cutoff for significance levels was $p<0.05$, with a linear discriminant analysis (LDA) score >2.0. No species were significantly changed between post-vancomycin and post-metformin+vancomycin at genus level.

Table 6. Changes of gut microbiome in linear discriminant analysis effect size (LEfSe) analysis

Taxonomic level	Taxonomic group	Average relative abundance in each period (%)				LDA score *			
		Baseline (n=9)	Post-metformin (n=9)	Post-vancomycin (n=9)	Post-metformin+vancomycin (n=9)	Baseline vs. Post-metformin	Post-metformin vs post-vancomycin	Post-metformin vs post-metformin+vancomycin	Baseline vs post-vancomycin
Phylum	Proteobacteria	0.70	15.28	15.39	21.61	5.77			5.94
	Bacteroidetes	69.94	36.63	6.46	1.82	−6.18	−6.14	−6.25	−6.47
	Actinobacteria	0.43	1.71	0.08	0.00		−4.9	−4.95	
	Firmicutes	27.50	40.65	22.30	27.72				
	Fusobacteria	1.34	3.26	55.37	34.71				
	Verrucomicrobia	0.08	2.47	0.31	14.07				
Genus	Escherichia	0.06	12.69	7.21	9.47	5.69			5.58
	Lactobacillus	0.00	0.00	1.42	1.86		4.93	5.14	4.93
	Enterobacter	0.01	1.30	1.42	8.38		4.24	5.46	4.91
	Pediococcus	0.00	0.00	0.29	1.22			4.87	
	Desulfovibrio	0.06	0.21	1.08	2.62			5.18	

Veillonella	0.08	0.02	6.00	2.58		5.57		
Parabacteroides	3.23	1.12	0.00	0.00	−4.97	−4.75	−4.76	−5.2
Bacteroides	42.38	27.36	0.03	0.04		−6.16	−6.18	−6.32
Blautia	4.22	10.44	0.11	0.00		−5.76	−5.77	−5.32
Faecalibacterium	6.73	2.81	0.10	0.00		−5.17	−5.19	−5.5
Alistipes	12.09	3.01	0.07	0.00		−5.19	−5.21	−5.82
Gemmiger	1.02	0.95	0.00	0.00				−4.74
Barnesiella	3.57	0.16	0.02	0.00				−5.24
Erysipelatoclostridium	0.34	2.11	0.00	0.00		−4.99	−5.02	
Dorea	0.66	1.04	0.00	0.00		−4.72	−4.74	
Lachnoclostridium	0.15	1.20	0.00	0.00		−4.69	−4.74	
Eubacterium	0.56	1.11	0.01	0.00		−4.69	−4.74	

* No species were significantly changed between post−vancomycin and post−metformin+vancomycin at both phylum and genus level

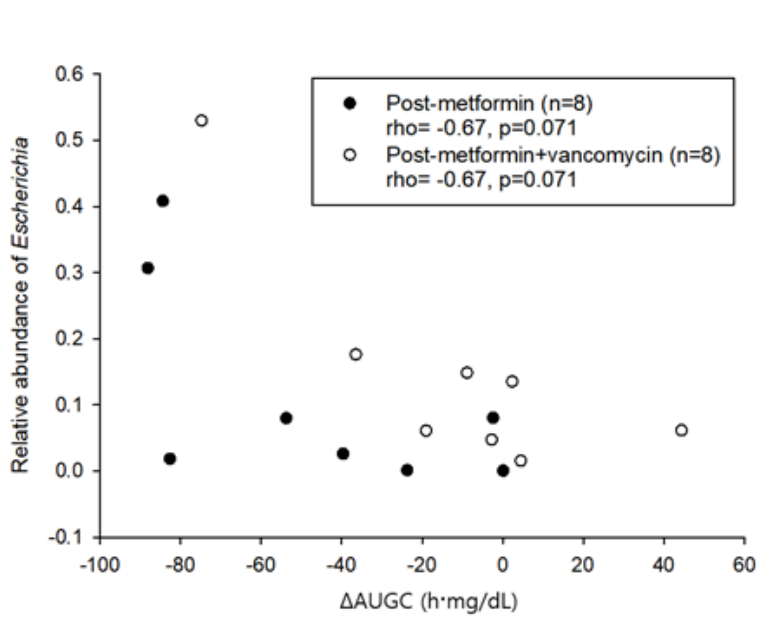
Pharmacodynamics and the gut microbiome

Because of a difference in the anti-hyperglycaemic effect before and after vancomycin administration, we investigated the relationship between this effect and genera with altered relative abundance before and after vancomycin administration. The anti-hyperglycaemic effect tended to correlate with the relative abundance of some genera (Figure 8).

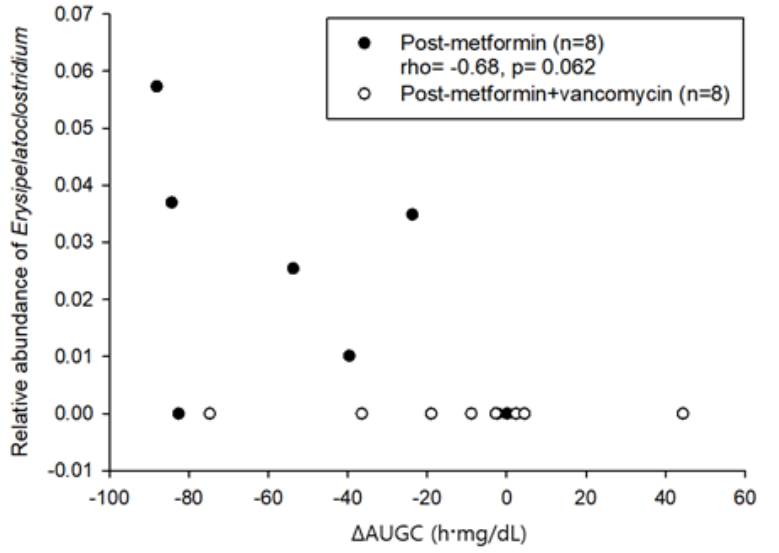
According to the exploratory Spearman correlation analysis, negative Spearman's rho, that is, positively correlated tendency between anti-hyperglycaemic effect (absolute value of ⊿AUGC) and the relative abundance of genera, was found in two genus. These were *Escherichia* (p-value=0.071, Spearman's rho= -0.67) and *Erysipelatoclostridium* (p-value=0.062, Spearman's rho= -0.68) in post-metformin. In addition, there was a positively correlated tendency between anti-hyperglycaemic effect and the relative abundance of *Escherichia* (p-value=0.071, Spearman's rho= -0.67) in post-metformin+vancomycin. And there was a negatively correlated tendency between anti-hyperglycaemic effect and the relative abundance of *Enterobacter* (p-value=0.039, Spearman's rho= 0.73) and *Faecalibacterium* (p-value=0.086, Spearman's rho= 0.64) in post-metformin. These results were

deemed exploratory, not indicating formal statistical significance.

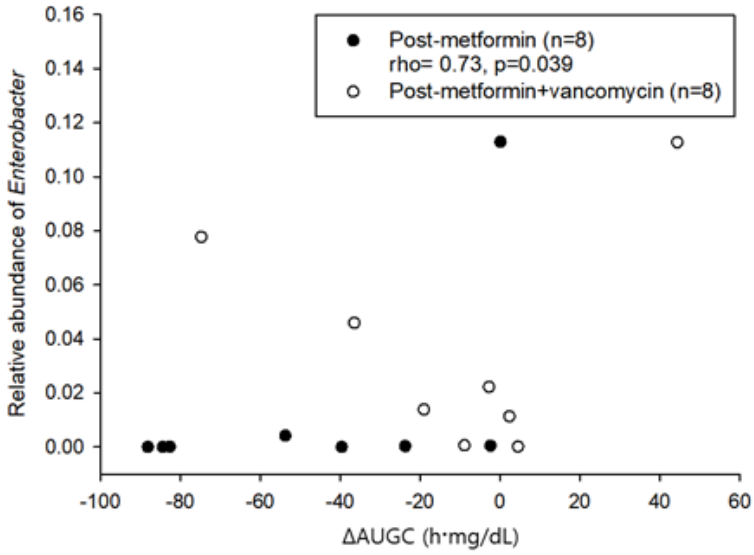
(a)


Post-metformin (n=8)
rho= -0.67, p=0.071
Post-metformin+vancomycin (n=8)
rho= -0.67, p=0.071
Relative abundance of Escherichia
ΔAUGC (h·mg/dL)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-100
-80
-60
-40
-20
0
20
40
60

(b)


Post-metformin (n=8)
rho= -0.68, p= 0.062
Post-metformin+vancomycin (n=8)
Relative abundance of Erysipelatoclostridium
ΔAUGC (h·mg/dL)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-100
-80
-60
-40
-20
0
20
40
60

(c)



(d)

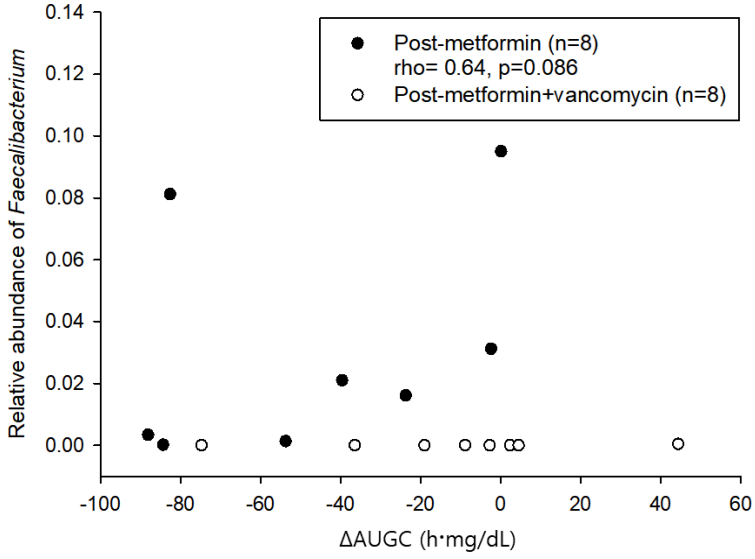


Figure 8. Correlation between ⊿AUGC and relative abundance of (a) *Escherichia*, (b) *Erysipelatoclostridium*, (c) *Enterobacter*, and (d) *Faecalibacterium*

Note: ⊿AUGC, Change of area under the glucose curve between two

periods; ⊿AUGC of 'Post-metformin' obtained by subtracting the value of baseline from that of post-metformin; ⊿AUGC of 'Post-metformin+vancomycin' obtained by subtracting the value of post-vancomycin from that of post-metformin+vancomycin; Relative abundance, relative abundance of each period (post-metformin or post-metformin+vancomycin)

Safety

Safety assessment was performed on the nine subjects who received the study drugs at least once. There were no AEs collected at baseline, though 28 AEs in 8 subjects occurred post-metformin. Of these, 16 AEs were gastrointestinal disorders. There was one case of diarrhoea and one of vomiting evaluated as moderate AEs and one case of vomiting evaluated as a severe AE. A total of 2 AEs in 2 subjects occurred post-vancomycin, all of which were assessed as mild. In addition, 20 AEs in 9 subjects occurred post-metformin+vancomycin. Of these, 15 were gastrointestinal disorders, with 1 moderate case of nausea. Of the total 50 AEs, all except 2 were revealed to have a relationship with the study drug. All AEs were resolved without sequelae.

Diarrhea, which was considered to have an effect on the gut microbiome, occurred in 4 subjects at post-metformin period and 5 subjects at post-metformin+vancomycin period, respectively. No diarrhea occurred in the baseline and post-vancomycin period. There was no difference in alpha-diversity and beta-diversity in both phylum and genus levels between subjects who developed diarrhea and those who did not during the post-metformin period. In post-metformin+vancomycin period, there was no difference in

gut microbiome diversity between subjects who had diarrhea and those who did not, except for beta-diversity at the genus level (Table 7)

Table 7. Comparison of alpha-diversity and beta-diversity evaluated between subjects with and without diarrhea

	Taxonomic level	Post-metformin	Post-metformin +vancomycin
Alpha diversity*	Phylum	p-value=0.56	p-value=0.19
	Genus	p-value=0.41	p-value=1
Beta diversity**	Phylum	p-value=0.49	p-value=0.48
	Genus	p-value=0.72	p-value=0.036

*Alpha-diversity: Shannon, Mann-Whitney U test.
** Beta-diversity: PCoA, Bray-Curtis, PERMANOVA

DISCUSSION

This study explored the effect of gut microbiome alteration on the pharmacokinetics and pharmacodynamics of metformin in healthy adult males. This study reports for the first time that the anti-hyperglycaemic effect of metformin decreased significantly after vancomycin administration, with the substantial change of gut microbiome caused by vancomycin administration. On the other hand, the systemic exposure of metformin remained unchanged regardless of gut microbiome alteration. The correlated tendency between the anti-hyperglycaemic effect and gut microbiome change, with little correlation between the pharmacokinetics and pharmacodynamics of metformin, suggest the possibility that the anti-hyperglycaemic effect of metformin is partially mediated by the gut microbiome, independent of the systemic exposure of metformin. Four genera, *Escherichia, Erysipelatoclostridium, Enterobacter* and *Faecalibacterium* showed a correlated tendency with anti-hyperglycaemic effects. Additional studies with larger subject number are needed to support the result of this study. The gut microbiome may have a key role in improving the clinical efficacy of metformin treatment in patients with T2D.

The administration of vancomycin significantly changed both

alpha-diversity and beta-diversity, as reported previously[15, 16]. The relative abundances of *Lactobacillus* and *Enterobacter* increased due to the administration of vancomycin, whereas those of *Parabacteroides, Bacteroides, Blautia, Faecalibacterium*, and *Alistipes* decreased, which was similar to previous reports (Table 6)[15, 16]. Relative abundance of *Escherichia* increased in post-metformin period compared to baseline, as in previous studies[4, 5, 19, 20]. This change appeared to persist until the post-vancomycin period, and is presumed to be indirectly affected by modified bacterium-bacterium interactions or other physiological or environmental changes[4].

Although the systemic exposure was similar in post-metformin and post-metformin+vancomycin period, the anti-hyperglycaemic effect was significantly different. There are five possible mechanisms that may alter the anti-hyperglycemic effects associated with vancomycin-induced changes in the gut microbiome.

First, metformin and butyrate can act synergistically in a way that metformin increase butyrate-producing taxa and the increased butyrate in blood circulation bind to GPCR activating downstream AMPK and leading to more butyrate production[21]. The SCFAs might also activate signaling to the brain directly or indirectly by increasing systemic circulation of GLP-1 and peptide

YY and modifying neurotransmitter levels of γ-aminobutyric acid (GABA) and serotonin[22]. The gut-brain communication by SCFAs might induce metabolic benefits such as increased insulin sensitivity and increased glucose tolerance[23]. It is inferred that butyrate-producing bacteria, *Alistipes, Bacteroides uniformis, Blautia faecis, Faecalibacterium prausnitzii, Eubacterium rectale,* and *Eubacterium hallii*, were relatively abundant and the butyrate level was higher in post-metformin period accordingly, which may have contributed to the anti-hyperglycaemic effect[24-28].

Second, the inhibitory effect of metformin on bile acid resorption and the regulatory action of bile acid on glucose may have occurred additively. Metformin suppresses intestinal bile acid resorption substantially, increasing exposure of intraluminal bile acids which potentially enhance the effect on glucose-lowering and GLP-1 secretion[29]. Secondary bile acids are generated from primary bile acid by gut microbiome such as *Parabacteroides, Bacteroides, Eubacterium* and *Blautia*, of which relative abundances were higher in post-metformin period[30-34]. Secondary bile acid acts as a major ligand of GPCR TGR5, promoting the secretion of GLP-1 by L cells[30, 33]. This results in an increase in insulin secretion and satiety, and a reduction in gastric emptying[30].

Third, the effect of vancomycin-induced gut microbiome on

anti-hyperglycaemic effect may be attributed to the increase of the relative abundance of *Desulfovibrio* in post-metformin+vancomycin period and the change in the lipopolysaccharide (LPS) level accordingly. *Desulfovibrio* has H_2S-producing activity in intestine. The elevated H_2S by *Desulfovibrio* can reduce disulfide bonds of mucous network, which increase the permeability of the intestine. Thereby, the transposition of bacterial metabolites such as LPS, a significant inflammatory factor which induces insulin resistance, may increase[35-37]. Metformin has inhibitory effects on production of LPS-induced proinflammatory cytokine and alleviates LPS-induced intestinal barrier damage by activating AMPK[38, 39]. It is presumed that the effect of metformin was attenuated by the LPS-increasing effect of *Desulfovibrio* in post-metformin+vancomycin period[40].

Forth, leucine level according to the gut microbiome change may have affected the anti-hyperglycaemic effect. Leucine is an allosteric activator of sirtuin1(SIRT1), an enzyme that improves insulin sensitivity at liver, skeletal muscle and adipose tissues when activated[41, 42]. Leucine demonstrated a synergistic effect with metformin in improving insulin sensitivity, which was presumed to appear by potentiation of SIRT1/AMPK pathway[42-44]. The relative abundance of *Dorea* and *Alistipes*, which showed a positive

correlation with leucine, was higher in the post-metformin compared to the post-metformin+vancomycin period[45, 46]. This may have influenced the greater anti-hyperglycaemic effect in post-metformin period.

And fifth, the anti-hyperglycaemic effect may be decreased by enrichment of gut microbiome with glucose uptake action by vancomycin administration. For example, *Lactobacillus* induces glucose uptake by producing sodium-glucose linked transporter (SGLT)-1 mediated metabolites[9]. In addition, relative abundance of *Lactobacillus* was shown to correlated positively with blood glucose levels[9]. Considering these points, *Lactobacillus* of which relative abundance increased after vancomycin administration may have affected the anti-hyperglycaemic effect and relative abundance of faecal glucose.

In case of AUC_{last} and ⊿HOMA-IR, an inverse relationship between the pharmacokinetics and pharmacodynamics was shown as previously reported[11]. This is presumably because the anti-hyperglycaemic effect was influenced by differences in individual gut microbiome and levels of substances such as SCFAs and bile acids. This inverse relationship could be elucidated by measuring metabolomes.

We did an exploratory investigation on the relationship

between the relative abundance of genera and anti-hyperglycaemic effects. As a result, the relative abundance of *Escherichia* and *Erysipelatoclostridium* showed a positively correlated tendency with anti-hyperglycaemic effect. Also, *Enterobacter* and *Faecalibacterium* showed a negatively correlated tendency between the two factors.

Erysipelatoclostridium tends to correlate negatively with fasting blood glucose, serum total glyceride, and body weight in mice[47]. Intestinal infusion of *Escherichia coli* protein stimulates the secretion of plasma peptide YY, which is the gut satiety hormone, and inhibits food intake in mouse and rat models, which implies a beneficial role for anti-hyperglycaemic effect[48]. In a study of dietary infection of *Enterobacter ludwigii* to fly, a diabetes-like condition such as elevated glucose level and increased amount of lipid was promoted due to the absences of production of SCFA of the bacteria[49]. These characteristics may have contributed to the positively or negatively correlated tendency between the genus and the anti-hyperglycaemic effect. However, some studies have reported characteristics of the genus opposite to the correlated tendency of each genus shown in our study. Several studies have reported that *Erysipelatoclostridium* are not beneficial[50-53]. In mice gavaged with exopolysaccharides

produced by *Enterobacter cloacae Z0206*, the hypoglycemic effect appeared possibly through AMPK-mediated effects[54]. *Faecalibacterium prausnitzii* is one of butyrate-producing genus and the abundance of *Faecalibacterium prausnitzii L2-6* was observed to be higher in the normal glucose tolerance group than in the prediabetes and T2D groups[52]. Further research is needed to clarify the which taxa are statistically significant correlated with anti-hyperglycaemic effect and the mechanism by which the gut microbiome contributes to the anti-hyperglycaemic effect and its extent.

Regarding the effects of vancomycin, an increase in stool calorie loss which indicates a decrease in nutrient absorption was observed when oral vancomycin was administered compared to placebo in healthy subjects[55]. It was accompanied by widespread change in gut microbiome with increase in relative abundance of *Akkermansia muciniphila,* implying that possible causal role for gut microbiome in nutrient absorption[55]. Considering the decrease in nutrient absorption by oral vancomycin, which can be considered contrary to decrease in anti-hyperglycaemic effect after administration of vancomycin in our study, the decrease in anti-hyperglycaemic effect in post-metformin+vancomycin period appears to be more likely due to metformin rather than vancomycin.

An exploratory analysis in this study showed that oral vancomycin administration reduced stool glucose loss to a level of nearly 10% compared to that before vancomycin administration. This suggests the possibility of increased absorption of glucose after the administration of oral vancomycin, which is aligned with the decreased anti-hyperglycaemic effect and the increased relative abundance of *Lactobacillus* having an action to induce the glucose uptake[9]. However, this result should be interpreted carefully, considering that this exploratory analysis had a high proportion of missing values and was not an analysis that yields absolute abundance. Further research including absolute quantitative analysis is needed to determine whether the oral administration of vancomycin affects stool glucose loss and glucose absorption in the body.

This study showed that the relative abundance of *Parabacteroides* decreased after metformin administration, in contrast to previous preclinical studies[56-58]. Metformin decreased the relative abundance of *Intestinibacter* and *Clostridium* in healthy individuals or T2D patients[4, 5, 19], and increased *Bifidobacterium* which increases insulin sensitivity in rodent models[4]. In the present study, however, these taxa were not significantly altered. The difference between this study and

previous studies can be due to the relatively small number of subjects in this study or difference in whether the subjects are healthy group or not.

Since the occurrence of diarrhea may affect the gut microbiome, differences in gut microbiome diversity between subjects with diarrhea and those without diarrhea were investigated for each period. As a result, there was generally no difference in gut microbiome diversity between the two subject groups at each period. Therefore, it was judged that the effect of diarrhea on the results of gut microbiome analysis was negligible.

This study showed that the anti-hyperglycaemic effect of metformin may vary depending on the microbiome composition. In other words, the anti-hyperglycaemic effect of metformin may be lower in patients taking vancomycin or other drugs, which may affect the composition of the microbiome. In T2DM patients, 40.5% of patients did not show improvement in glycemic control with metformin administration[59]. Based on the results of this study, a bacterium-based intervention can be expected as a method to improve the response to metformin. In addition, it is expected that taking probiotics that produce butyrate, secondary bile acid, or leucine with metformin can enhance the effect of metformin.

Considering that the anti-hyperglycaemic effect of

metformin and oral vancomycin-induced gut microbiome changes are a phenomenon occurring both in healthy individuals and in various patient groups[2, 15, 16], the relationship between anti-hyperglycaemic effects and the relative abundance of some gut microbiome identified in this study may be extrapolated to the use of metformin in T2D patients. However, since the underlying gut microbiome status may differ depending on the subject's condition[60], additional studies in the patient group would be necessary.

ACKNOWLEDGMENT/FUNDING

This study was funded by National Research Foundation of Korea (No. NRF-2018R1D1A1B07044406), Seoul, Republic of Korea. Pharmacokinetic samples were analyzed by APACE Inc., Seoul, Republic of Korea. Samples for assessment of anti-hyperglycaemic effect were analyzed by Seoul National University Bundang Hospital, Seongnam-si, Republic of Korea. Faecal sample for assessment of gut microbiome were analyzed by DNALINK, Inc., Seoul, Republic of Korea. Relative abundance of faecal glucose was analyzed by Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea.

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국문 초록

건강한 남성 대상자에서 반코마이신 투여에 의한 장내 미생물군집의 변화가 메트포민의 약동학 및 약력학에 미치는 영향 탐색

서론: 메트포민은 제2형 당뇨병의 가장 주요한 치료제 중 하나이다. 본 연구는 반코마이신의 경구투여를 통해 일으킨 장내 미생물군집의 변화가 메트포민의 약동학 및 항고혈당 효과에 미치는 영향을 탐색하고자 수행되었다.

방법: 본 임상시험을 위해 배변활동의 이상이 없는 19-45세의 건강한 성인 남성이 모집되었다. 본 연구는 공개형, 단일군의 임상시험으로서, 4개의 시기(period)인 baseline(기저치), post-metformin(1-4일째에 메트포민 1000mg 반복 경구투여), post-vancomycin(장내 미생물군집의 전반적인 변화를 유도하기 위해 11-17일째에 반코마이신 500mg 반복 경구투여), post-metformin+vancomycin(16-19일째에 메트포민 1000mg의 반복 경구투여) 순서로 진행되었다. 각 시기에는 혈청 포도당 농도 측정을 위하여 경구 포도당 내성 검사 전후에 혈액 검체를 수집하였다. 또한 각 시기에 장내 미생물군집의 조성 및 다양성 탐색을 위한 대변 검체를 수집하였으며, 메트포민의 약동학 분석을 위하여 post-

metformin 및 post-metformin+vancomycin 시기에 혈장 및 소변 검체를 수집하였다. 대상자의 안전성은 전체 연구기간 동안 모니터링 되었다.

결과: 총 9 명의 대상자가 전체 연구 기간을 마쳤고, 이 중 8 명에서 모든 검체가 수집되었다. 경구 반코마이신 투여에 의해 장내 미생물군집의 조성 및 다양성은 유의하게 변화하였다. 또한, 반코마이신 투여에 따른 메트포민의 약동학적 특성 변화는 나타나지 않은 반면 ($p>0.05$), 항고혈당 효과는 반코마이신 투여 후에 유의하게 감소하여 ($p<0.05$), 메트포민의 약동학과 약력학 간의 약한 관계성이 나타났다. 반코마이신 투여로 인해 상대풍부도(Relative abundance)가 변화한 여러 속(genus) 중, *Erysipelatoclostridium* 및 *Enterobacter, Faecalibacterium* 의 상대풍부도는 메트포민의 항고혈당 효과와 상관관계 경향을 보였다($p<0.1$). 이상반응은 모든 대상자에서 발생했으나, 모두 후유증없이 회복되었다.

결론: 반코마이신을 경구투여하여 장내 미생물군집을 변화시킨 후에도 메트포민의 약동학적 특성은 변화하지 않은 반면, 메트포민의 항고혈당 효과는 감소하였다. 항고혈당 효과는 특정 속의 상대풍부도와 상관관계 경향이 있었으며, 이는 메트포민 효과의 일부가 장내 미생물군집을 매개하여 나타날 수 있음을 시사한다 (ClinicalTrials.gov, NCT03809260).

* 본 내용의 일부는 *Clinical and translational science* 학술지 (Eunwoo Kim et al. *Clin Transl Sci*. 2021. doi: 10.1111/cts.13051)에 출판 완료된 내용임.

Keywords : **메트포민, 장내 미생물군집, 약동학, 약력학, 항고혈당 효과**

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