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ARTICLE

Effects of vancomycin-induced gut microbiome alteration on the pharmacodynamics of metformin in healthy male subjects

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Abstract

Metformin is a major treatment for type 2 diabetes. This study was conducted to investigate the impact of gut microbiome dysbiosis on the pharmacokinetics and antihyperglycemic effects of metformin. Healthy adult males aged 19–45 years with no defecation abnormalities were recruited for this 4-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); and post-metformin + vancomycin (i.e., multiple oral doses of 1000 mg metformin on days 16–19). In each period, serum glucose and insulin concentrations following an oral glucose tolerance test, fecal samples for gut microbiome composition, and safety data were obtained. Following metformin dosing, plasma and urine samples for pharmacokinetics were collected. Nine subjects completed the study. The pharmacokinetics of metformin remained unchanged, and the antihyperglycemic effect was significantly decreased after vancomycin administration (p value = 0.039), demonstrating the weak relationship between the pharmacokinetics and pharmacodynamics of metformin. Relative abundances of some genus were changed after vancomycin administration, and tended to correlate with the antihyperglycemic effects of metformin (p value = 0.062 for *Erysipelatoclostridium*; p value = 0.039 for *Enterobacter*; and p value = 0.086 for *Faecalibacterium*). Adverse events occurred in all subjects and were resolved without sequelae. In conclusion, a decrease in the antihyperglycemic effect of metformin was observed after concomitant administration with vancomycin, without changes in metformin pharmacokinetics. The antihyperglycemic effect was tended to correlate with the relative abundance of several genus, suggesting that the effect of metformin is partly attributable to the gut microbiome (ClinicalTrials.gov, NCT03809260).

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The relationship between the systemic exposure and antihyperglycemic effect of metformin is weak. The possibility of gut-mediated effects of metformin has recently emerged.

WHAT QUESTION DID THIS STUDY ADDRESS?

Is there a relationship between the antihyperglycemic effect of metformin and the gut microbiome?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

When the gut microbiome was altered in healthy male subjects due to oral vancomycin administration, the antihyperglycemic effect of metformin was less pronounced, despite similar metformin pharmacokinetics. The antihyperglycemic effect of metformin tended to correlate with the relative abundance of some genera.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The results of this study showed that the antihyperglycemic effect of metformin is not related to its systemic exposure, and is likely to be associated with the relative abundance of some gut microbiome, suggesting the possibility that the effect of metformin is partly attributable to the gut microbiome, which may be extrapolated to the use of metformin in patients with type 2 diabetes.

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) and enhances peripheral glucose uptake and utilization through activation of AMP-activated protein kinase (AMPK). Metformin is also known to decompose free fatty acids by activating AMPK.[1,2] Recent studies have reported that metformin has a potential intestine-mediated effect.[3–7] In one study, ^{18}F-labeled fluorodeoxyglucose accumulated markedly in the colon after metformin administration, demonstrating that the drug affects glucose handling in the colon.[3] In another 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 ~ 2.6-fold, although the antihyperglycemic effect decreased.[4] In a clinical trial in which healthy subjects received a low or high dose of metformin, the antihyperglycemic effect was found to be inverse to systemic exposure, suggesting that the effect of metformin occurs through a nonabsorbed portion as well as systemic absorption.[5] 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.[6]

All of these findings suggest that a portion of the response to metformin is associated with an unknown action by the nonabsorbed portion of the drug, such as gut microbiome-mediated action. The gut microbiome is a microbial population present in the ileum and colon that directly or indirectly affects physiological functions.[8] The gut microbiome affects reactions, such as hydrolysis and reductive metabolism of various drugs, thereby affecting pharmacokinetics, activity, and toxicity.[9] Some studies have observed changes in the gut microbiome after the administration of metformin,[3,10,11] and there is growing evidence that these changes are partly related to antihyperglycemic effects. Nonetheless, the relationship between systemic exposure, the antihyperglycemic effect of metformin, and microbiome changes has not been established to date.

Orally administered vancomycin shows little absorption from the gastrointestinal tract.[12] At the same time, it has a profound effect on the gut microbiome.[13,14] 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 antihyperglycemic 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 and 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 S1).

Subjects received 1000 mg metformin (Diabex Tab, Daewoong Pharmaceutical Co., Ltd., South Korea) orally twice daily from day 1 (day 1, 1:30 p.m. and 9:00 p.m. and days 2 and 3, 9:00 a.m. and 9:00 p.m.) to day 4 (9:00 a.m.), 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 a.m. and 9:00 p.m.) 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 days 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). Fecal samples for gut microbiome analysis were collected on days −1 or 1 and 15 or 16 days before the first metformin administration. Urine samples for urine metformin concentration measurements were collected on days −1, 4, 15, and 19 (Figure S1).

Subjects provided written consent to the prohibition of eating foods containing lactic acid bacteria, grapefruit, and caffeine during the entire study duration. In addition, 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.

Pharmacokinetic and pharmacodynamic assessments of metformin

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

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 the time of the last measurable observed concentration to infinity (AUC% extrapolated) was calculated by $(AUC_{inf} - AUC_{last})/AUC_{inf}$ 100. The elimination terminal half-life ($t_{1/2}$) was calculated as the ln2/λz. Urine samples were collected for 12 h at the 4 periods, and the amount excreted in urine (Ae) was calculated as the concentration of metformin of 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 noncompartmental methods with Phoenix WinNonlin software version 8.0 (Certara USA).

Glycemic response measures (i.e., AUGC, G_{max}, HOMA-IR, fasting glucose, Δserum 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 Δserum 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 ΔAUGC, ΔG_{max}, and ΔHOMA-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 ΔAUGC, ΔG_{max}, and ΔHOMA-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.

Statistical analyses of pharmacokinetics and pharmacodynamics

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

The relationship between pharmacokinetics (C_{max} and AUC_{last}) and pharmacodynamics (ΔAUGC, ΔG_{max}, and ΔHOMA-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).

Assessment of the gut microbiome

Taxonomic profiling was carried out using a module of marker data profiling of MicrobiomeAnalyst.[15] 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.[16] The cutoffs 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 versus post-vancomycin, post-metformin versus post-metformin + vancomycin, and baseline versus post-vancomycin comparisons; similarly, changes in the gut microbiome by metformin administration were identified through baseline versus post-metformin and post-vancomycin versus 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 versus post-vancomycin was considered to be caused by vancomycin.

Spearman correlation analysis between ΔAUGC 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 cutoff 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 ΔAUGC 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 cutoff 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 antihyperglycemic effect. For LEfSe and correlation analyses, only taxa with relative abundance greater than or equal to 0.01 (1%) at least once during the compared periods are presented. The datasets generated and analyzed during the current study are available from Supplementary Material (dataset).

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.

RESULTS

Demographics

A total of 15 participants were enrolled in this study. One of them dropped out due to an adverse reaction, 3 withdrew their

consent, and 2 dropped out for other reasons. Thus, nine subjects received the study drug at least once, and fecal samples were all obtained for 4 periods. In a total of 3 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 9 subjects was 25.8 years (range: 19–33 years), with a mean body mass index of 24.3 kg/m^2 (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 eight 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 1), 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 1, 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 S1).

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 ΔAUGC, showing a percentage change of −75.9% in the post-metformin + vancomycin period compared to the post-metformin period (Table 2). Furthermore, Δserum glucose at PP1 was significantly higher in post-metformin + vancomycin period compared to post-metformin period (p value = 0.039), which supported that the antihyperglycemic effect in post-metformin + vancomycin period was relatively low (Table S1).

Although the administration of vancomycin did not influence the pharmacokinetic properties of metformin, the antihyperglycemic effect was partially affected; hence, the relationship between the pharmacokinetics and pharmacodynamics was weak, as confirmed by Spearman correlation analysis (Figure 1, Figure S2). Except for one case, all p values and absolute values of Spearman's rho were greater than 0.05 and less than or equal to 0.55, respectively. Only the results of correlation analysis for AUC_{last} and ΔHOMA-IR during the metformin + vancomycin period showed p values of 0.0003 and Spearman's rho of 0.95, respectively (Figure S2).

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 2a, Table S2).

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 2b, Table S2).

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 3). The relative abundance at the genus level was also variable between individuals (Figure S3). A total of 50 genera were identified, 28 of which showed a relative abundance of at least 1% in at least one period.

To observe changes in gut microbiome composition more closely, we performed LEfSe analysis between different periods at the phylum and genus levels (Table 3).

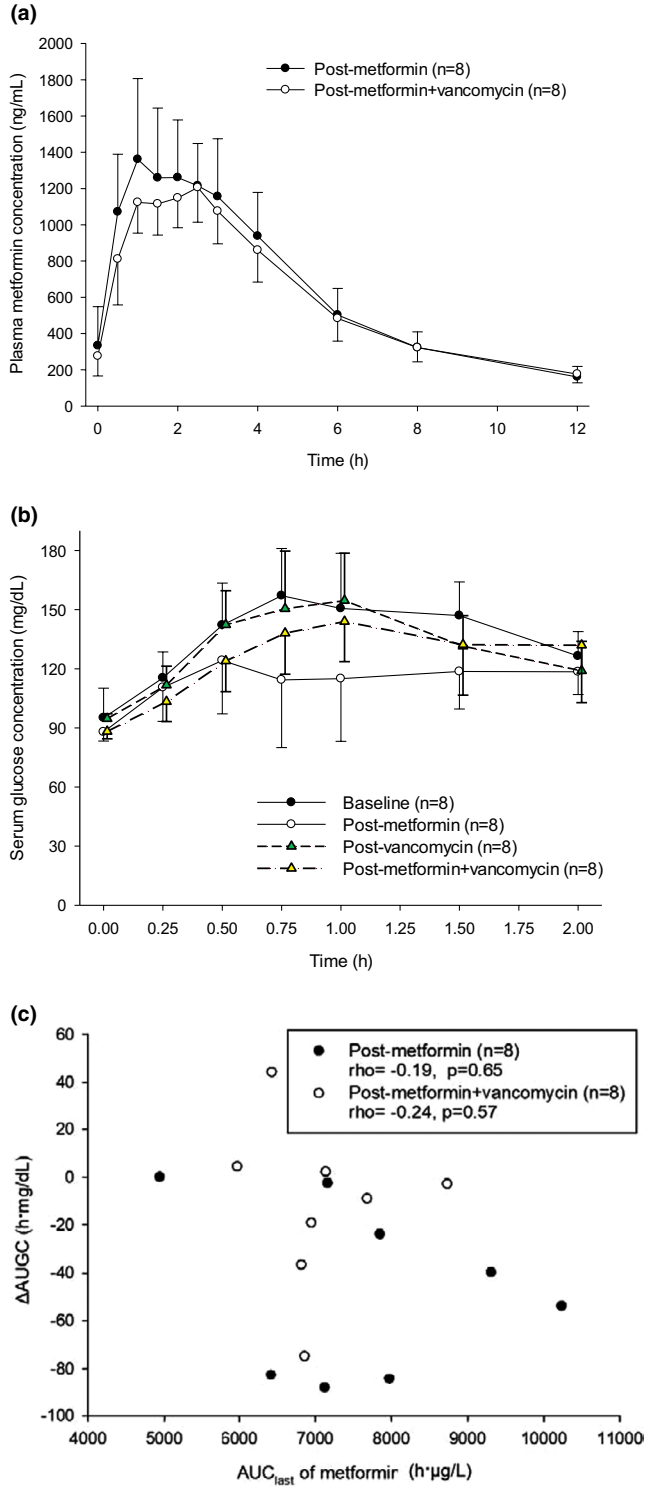

FIGURE 1 Pharmacokinetics of metformin and the impact of metformin and vancomycin on AUGC. Mean plasma concentration-time profiles of metformin (a), mean serum concentration-time profiles of glucose (b), and correlation between pharmacokinetic-pharmacodynamic parameters after administration of metformin and metformin + vancomycin (c). Note: Bars represent the SDs in (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; ΔAUGC of post-metformin obtained by subtracting the value of baseline from that of post-metformin; ΔAUGC of post-metformin + vancomycin obtained by subtracting the value of post-vancomycin from that of post-metformin + vancomycin

The taxa with altered relative abundance due to vancomycin administration were identified through post-metformin versus post-vancomycin, post-metformin versus post-metformin + vancomycin, and baseline versus 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 versus post-metformin and post-vancomycin versus 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 (Table 3).

Pharmacodynamics and the gut microbiome

Because of a difference in the antihyperglycemic 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 antihyperglycemic effect tended to correlate with the relative abundance of some genera (Figure 4).

According to the exploratory Spearman correlation analysis, negative Spearman's rho, that is, positively correlated tendency between antihyperglycemic effect (absolute value of ΔAUGC) 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 antihyperglycemic effect and the relative abundance of *Escherichia* (p value = 0.071, Spearman's rho = –0.67)

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

Note: All data are presented as arithmetic mean ± SD, 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; CL_R, renal clearance; C_{max}, maximum plasma concentration; $t_{1/2}$, elimination terminal half-life; T_{max}, time to reach C_{max}.

*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.

in post-metformin + vancomycin. In addition, there was a negatively correlated tendency between antihyperglycemic 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.

Safety

Safety assessment was performed on the nine subjects who received the study drugs at least once. There were no AEs collected at baseline, although 28 AEs in 8 subjects occurred post-metformin. Of these, 16 AEs were gastrointestinal disorders. There was one case of diarrhea 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.

DISCUSSION

This study explored the effect of gut microbiome alteration on the pharmacokinetics and pharmacodynamics of metformin in healthy adult men. This study reports for the first time that the antihyperglycemic 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 antihyperglycemic effect and gut microbiome change, with little correlation between the pharmacokinetics and pharmacodynamics of metformin, suggest the possibility that the antihyperglycemic 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 antihyperglycemic effects. Additional studies with larger subject number are needed to support the results of this study. The gut microbiome may have a key role in improving the clinical efficacy of metformin treatment in patients with T2D.

There was no significant correlation between the pharmacokinetics and pharmacodynamics of metformin as shown in previous studies.[5] In case of AUC_{last} and ΔHOMA-IR, the correlation coefficient was positive, demonstrating an inverse relationship between the pharmacokinetics and pharmacodynamics, as previous reported.[5]

The administration of vancomycin significantly changed both alpha-diversity and beta-diversity, as reported previously.[13,14] The relative abundances of *Lactobacillus* and *Enterobacter* increased due to the administration of

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[a]	Percentage change[b]
AUGC (h mg/dl)	277.2 ± 31.5	230.3 ± 34.0	266.5 ± 22.4	255.2 ± 30.7		-	-
ΔAUGC (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		-	-
ΔG_{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		-	-
ΔHOMA-IR	-	−0.8 ± 1.0	-	−0.4 ± 0.3	0.31	0.3 ± 1.0	−42.9%

Note: 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 versus post-metformin + vancomycin.

aArithmetic mean ± SD for post-metformin + vancomycin - post-metformin.

bA percentage change: Each ratio of mean value of difference between two treatments compared to corresponding value of post-metformin.

vancomycin, whereas those of *Parabacteroides*, *Bacteroides, Blautia, Faecalibacterium*, and *Alistipes* decreased, which was similar to previous reports (Table 3).[13,14] Relative abundance of *Escherichia* increased in post-metformin period compared to baseline, as in previous studies.[10,11,17,18] 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.[10]

We did an exploratory investigation on the relationship between the relative abundance of genera and antihyperglycemic effects. As a result, the relative abundance of *Escherichia* and *Erysipelatoclostridium* showed a positively correlated tendency with antihyperglycemic effect. In addition, *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.[19] Intestinal infusion of *Escherichia coli* protein stimulates the secretion of plasma peptide YY (PYY), which is the gut satiety hormone, and inhibits food intake in mouse and rat models, which implies a beneficial role for antihyperglycemic effect.[20] 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 short-chain fatty acid (SCFA) of the bacteria.[21] These characteristics may have contributed to the positively or negatively correlated tendency between the genus and the antihyperglycemic effect. On the other hand, several studies have reported that *Erysipelatoclostridium* are not beneficial.[22–25] In mice gavaged with exopolysaccharides produced by *Enterobacter cloacae* Z0206, the hypoglycemic effect appeared possibly through AMPK-mediated effects.[26] 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.[24] Metformin is known to exhibit glucose control effects through inhibition of mGPDH, activation of AMPK, and enrichment of the SCFA-producing bacteria..[1,2,27] Further research is needed to clarify which taxa are statistically significant correlated with antihyperglycemic effect and the mechanism by which the gut microbiome contributes to the antihyperglycemic 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.[28] It was accompanied by widespread change in gut microbiome with increase in relative abundance of *Akkermansia muciniphila*, implying that it is a possible causal role for gut microbiome in nutrient absorption.[28] Considering the decrease in nutrient absorption by oral vancomycin, which can be considered contrary to decrease in antihyperglycemic effect after administration of

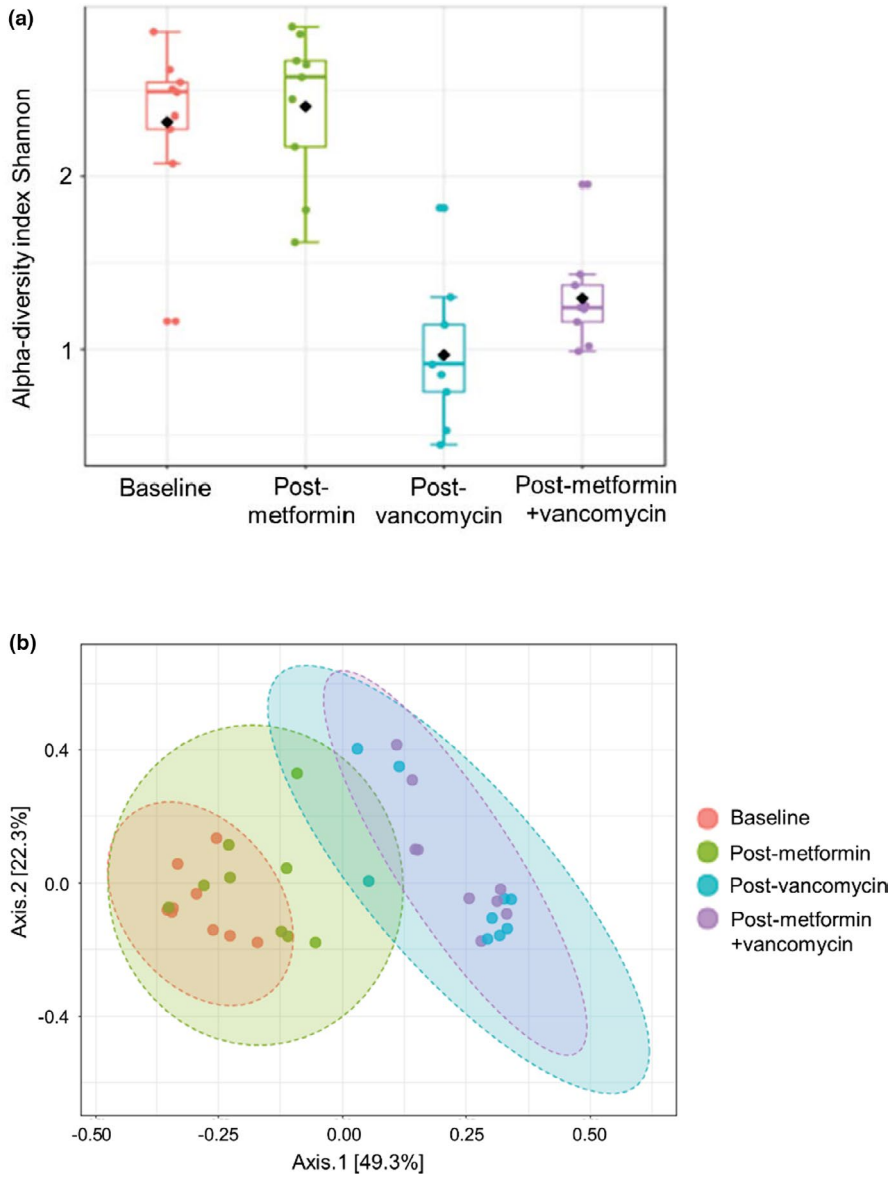

FIGURE 2 Changes in composition of gut microbiome represented by (a) alpha diversity (Shannon index) and (b) beta diversity (principal coordinates analysis plot) measured at genus level. Each axis in (b) represents the highest and second-highest percent of the variation between the samples

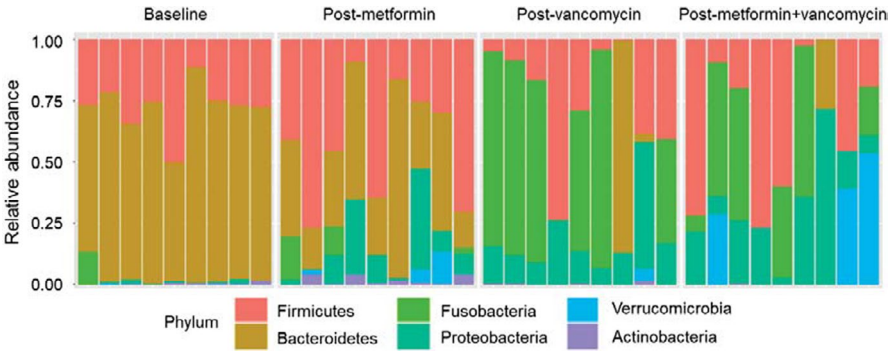

FIGURE 3 Relative abundance of intestinal bacterial phyla

vancomycin in our study, the decrease in antihyperglycemic effect in post-metformin + vancomycin period appears to be more likely due to metformin rather than vancomycin.

This study showed that the relative abundance of *Parabacteroides* decreased after metformin administration, in contrast to previous preclinical studies.[29–31] Metformin decreased the relative abundance of *Intestinibacter* and *Clostridium* in healthy individuals or patients with T2D,[10,11,17] and increased *Bifidobacterium*, which increases insulin sensitivity in rodent models.[10] In the present study, however, these taxa were not significantly altered. The difference between this study and previous studies can be due to

TABLE 3 Changes of gut microbiome in LEfSe analysis

Taxonomic level	Taxonomic group	Average relative abundance in each period (%)				LDA score[a]			
		Baseline ($n = 9$)	Post-metformin ($n = 9$)	Post-vancomycin ($n = 9$)	Post-metformin + vancomycin ($n = 9$)	Baseline versus Post-metformin	Post-metformin versus post-vancomycin	Post-metformin versus post-metformin + vancomycin	Baseline versus 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	

Abbreviations: LDA, linear discriminant analysis; LEfSE, linear discriminant analysis effect size.

aNo species were significantly changed between post-vancomycin and post-metformin + vancomycin at both phylum and genus level.

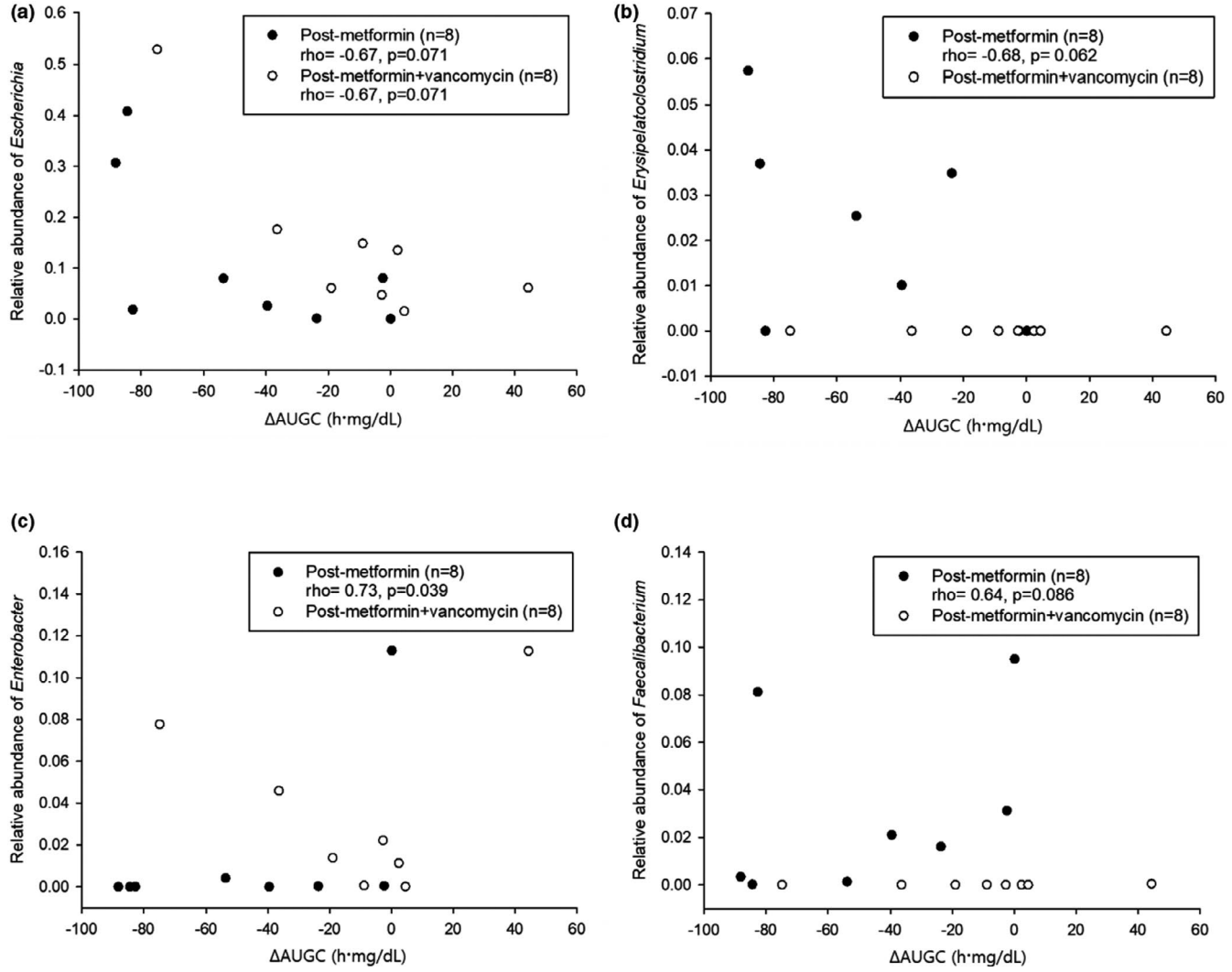

FIGURE 4 Correlation between ΔAUGC and relative abundance of (a) *Escherichia*, (b) *Erysipelatoclostridium*, (c) *Enterobacter*, and (d) *Faecalibacterium*. Note: ΔAUGC, change of area under the glucose curve between two periods; ΔAUGC of post-metformin obtained by subtracting the value of baseline from that of post-metformin; ΔAUGC 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)

the relatively small number of subjects in this study or difference in whether the subjects are in the healthy group or not.

This study showed that the antihyperglycemic effect of metformin may vary depending on the microbiome composition. In other words, the antihyperglycemic effect of metformin may be lower in patients taking vancomycin or other drugs, which may affect the composition of the microbiome. This finding suggests the need for bacterium-based intervention.

Considering that the antihyperglycemic effect of metformin and oral vancomycin-induced gut microbiome changes are a phenomenon occurring both in healthy individuals and in various patient groups,[2,13,14] the correlated tendency between antihyperglycemic effects and the relative abundance of some gut microbiome identified in this study may be extrapolated to the use of metformin in patients with T2D. However, because the underlying gut microbiome status may differ depending on the subject's condition,[32] additional studies in the patient group would be necessary.

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CONFLICT OF INTEREST

All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

E.K., A.H.K., Y.L., K.-S.Y., and J.-Y. Chung wrote the manuscript. E.K., A.H.K., and J.-Y. Chung designed the research. E.K., A.H.K., Y.L., S.C.J., and J.-Y. Cho performed the research. E.K., A.H.K., and J.-Y. Chung analyzed the data. A.H.K., Y.L., and S.C.J. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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