7-1-2020

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Citation: Clin Transl Sci (2020) 13, 752–760; doi:10.1111/cts.12761

ARTICLE

Impact of Vancomycin-Induced Changes in the Intestinal Microbiota on the Pharmacokinetics of Simvastatin

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The pharmacokinetic (PK) properties of drugs are affected in several ways by interactions with microbiota. The aim of this study was to investigate the effects of oral vancomycin on the gut microbiota and, consequently, on the PKs of simvastatin. An open-label, single arm, sequential crossover study was conducted in six healthy Korean male subjects. After 6 days on a control diet, simvastatin 40 mg was orally administered to the subjects before and after 1 week of oral vancomycin treatment. Blood samples for PK analysis and fecal samples for metagenomic and metabolomic analyses were collected. After vancomycin treatment, the richness of microbiota considerably decreased, and the composition was altered. In particular, the relative abundance of Bacteroidetes decreased, whereas that of proteobacteria increased. In addition, changes in fecal metabolites, including D-glucuronic acid, were observed. However, systemic exposure of simvastatin was not changed whereas that of hydroxysimvastatin showed a tendency to increase. The relationship between the change of PKs of simvastatin and the change of gut microbiota and fecal metabolites were not clearly observed.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Drug-microbiota interaction occurs through numerous ways, and it can alter the pharmacokinetic properties of drugs.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ In this study, we investigated the effect of oral vancomycin on changes in gut microbiota and the effect of these changes on the pharmacokinetics of simvastatin. Furthermore, the mechanism underlying gut microbiota alteration was explored through fecal metabolomics approach.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The gut microbiota composition was changed by oral vancomycin, resulting in the alteration in fecal metabolites. However, these changes did not alter systemic exposure of simvastatin. Moreover, 6 days of diet control among subjects reduced the intersubject variability of gut microbiota.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Six-day controlled diet reduces interindividual variability of gut microbiota composition and oral vancomycin changes gut microbiota significantly. During the clinical study related with gut microbiota, food, and concomitant antibiotics should be strictly controlled for accurate clinical evaluation.

Human gut microbiota is a community of microorganisms living in the human intestinal ecosystems, including commensal, symbiotic, and pathogenic microorganisms.[1] The number of microbial cells in the human gut is 10 times higher than that of the host cells, and the gene set of the gut microbiota is 100 times more diverse than that of the host.[2] Therefore, the gut microbiota is expected to have strong interactions with the human body, affecting its normal physiology and disease pathophysiology. Thus, the concept of host–microbiota interactions has emerged, and various diseases, including dyslipidemia, inflammatory bowel disease, and allergic diseases, as well as type 2 diabetes mellitus and obesity have been revealed to be associated with the gut microbiota.[3–6] It has also been suggested that interactions between microbiota and the human immune system may influence the outcomes of cancer immunotherapy.[7]

Drug–microbiota interactions occur through different mechanisms and may alter pharmacokinetic (PK) properties of drugs. The gut microbiota can directly affect the PK of prodrugs that are transformed into active metabolites in the gastrointestinal tract (e.g., sulfasalazine and lovastatin),[8] and drugs or metabolites that are inactivated or eliminated in the gastrointestinal tract (e.g., digoxin and irinotecan).[9] In addition, the gut microbiota can indirectly affect hepatic metabolism through metabolites produced by microorganisms (e.g., acetaminophen).[10] Moreover, the gut microbiota may

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Received: September 9, 2019; accepted: December 19, 2019. doi:10.1111/cts.12761

affect the PK of various drugs excreted in the bile because 98% of excreted bile acids are reabsorbed through enterohepatic recirculation, wherein the gut microbiota plays a significant role.[11]

Numerous factors, including the diet and concomitant drugs, can change the human intestinal microbiota and may consequently affect drug responses. Antibiotics induce significant changes in the gut microbiota. Systemic exposure to lovastatin decreased when ampicillin was orally co-administered to rats.[12] The bioavailability of digoxin increased when erythromycin was co-administered, leading to the destruction of the gut microbiota.[13] These changes are thought to be due to alterations in the gut microbiota. Therefore, when a drug is administered in combination with an antibiotic, antibiotic-mediated microbiota–drug interactions should be carefully considered.[12]

Statins are widely used to treat dyslipidemia and have been proven to be safe and effective.[14] Simvastatin, one of the representative statins, is a lactone prodrug that is hydrolyzed to the active hydroxyl acid. Simvastatin and simvastatin acid are transported to hepatocytes by solute carrier organic anion transporters and metabolized by cytochrome P450 (CYP) enzymes and uridine 5'-diphosphoglucuronosyltransferase.[15] The PK properties of simvastatin varies highly, depending on genetic polymorphism of these enzymes and transporters.[15] Hepatic metabolites of simvastatin, including glucuronides, are mainly excreted into the bile. However, simvastatin and simvastatin acid only account for a small fraction in feces because simvastatin acids are reabsorbed through enterohepatic circulation.[16–19] As the gut microbiota is known to play an important role in enterohepatic circulation, it is suggested that the changes in the gut microbiota may alter the PK of simvastatin.

Intravenous vancomycin is a widely used antibiotic for methicillin-resistant *Staphylococcus aureus*, whereas oral vancomycin is indicated for intestinal infection of *Clostridium difficile*. Antibiotics, including vancomycin, can have an effect not only on pathogenic microorganisms but also on normal gut microbiota, resulting in gut dysbiosis.[20] The diversity of human gut microbiota has been shown to significantly decrease and the absolute number of gram-positive bacteria to reduce after 7-day oral vancomycin treatment.[21] In addition, oral vancomycin increased the proportion of proteobacteria and bacilli, decreased the proportion of bacteroidetes and clostridium in the intestinal microflora, and changed the composition of bile acids and short chain fatty acids in patients with obesity.[22] The dysbiosis of gut microbiota, induced by oral vancomycin, is expected to alter the PK of drugs undergoing enterohepatic circulation.

In this study, we aimed to investigate the effects of oral vancomycin on the gut microbiota and the effect of potential microbiota changes on the PK of simvastatin. In addition, the underlying mechanism of alteration in the gut microbiota was explored through a fecal metabolomics approach.

METHODS

Subjects and study design

An open-label, single arm, sequential crossover study was conducted in six healthy Korean male subjects aged 19–50 years. All the subjects voluntarily signed the informed consent form and participated in this study. Only those with the *SLCO1B1* *1/*1 genotype were enrolled. The exclusion criteria were as follows: subjects who had a history of clinically significant hypersensitivity to statins, antibiotics, aspirin, or specific foods; those with the renal glomerular filtration rate < 60 mL/min/1.73 m^2 as calculated using the Modification of Diet in Renal Disease; those with the total cholesterol, low-density lipoprotein, or triglyceride level exceeding the normal upper limit by 1.5 times; those with high-density lipoprotein of < 35 mg/dL; and those who were taking strong CYP3A4 inhibitors or inducers.

The overall study design is shown in **Figure 1**. The subjects were admitted to the Seoul National University Hospital Clinical Trials Center at 6 days prior to the first administration of simvastatin 40 mg (day -6) as a run-in period to control their diet. During the study period, all subjects were provided a diet that did not contain lactic acid bacteria. The subjects were required to eat the whole meal as a principle, and other diets were forbidden. On the first day of simvastatin dosing (day 1), simvastatin 40 mg was orally administered once under fasted conditions. After 24 hours, vancomycin 500 mg was orally administered once a day for 7 days (from days 2–8). Three days after the last dosing of vancomycin (day 11), simvastatin 40 mg was orally administered once again. The subjects were discharged on day 12 after completing the schedule, and safety evaluation was performed by telephone interview at 2–5 days after discharge. The reason for setting the washout period as 2 days was, after 2 days, oral vancomycin did not remain in the intestine, and, therefore, it was expected that there would be no interaction between simvastatin and oral vancomycin.

During the study, fecal samples were collected for metagenome and metabolite analyses over three intervals: (i) from immediately after admission (day -6) to 2 days prior to the first simvastatin dosing (day -2); (ii) from day -2 to just before

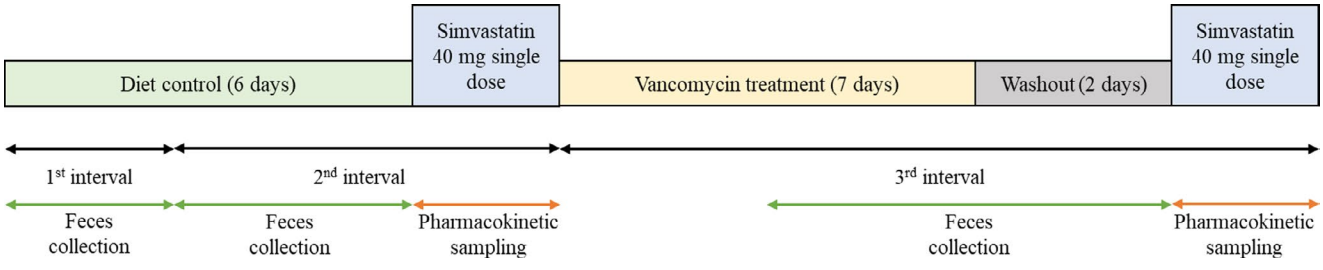

Figure 1 Study design.

the first simvastatin dosing (day 1); and (iii) from the fifth vancomycin dosing (day 6) to just before the second simvastatin dosing (day 11). A feces diary was distributed to all subjects to collect all information about feces. For the PK analysis of simvastatin and hydroxysimvastatin, serial blood samples were collected at predose and 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 hours after each simvastatin dosing (days 1 and 11). Safety and tolerability were assessed based on the occurrence of adverse events (AEs), physical examination, vital signs, 12-lead electrocardiogram, and clinical laboratory tests.

This clinical trial was approved by the Ministry of Food and Drug Safety and the Institutional Review Board of Seoul National University Hospital (Numbers: 1709-013-881 and NCT03403972). This study was based on the principles of Declaration of Helsinki and was conducted in accordance with Korean Good Clinical Practice guideline.

Stool preparation

To collect fecal samples, special stool collection tools were provided to the subjects to prevent mixing the feces with water. After collection, feces weight was measured, and the samples were homogenized using a sample mixer. The homogenized fecal samples (approximately the size of the thumb nail) were placed in a small black tube and frozen at –70°C until analysis.

Metagenome analysis of stool microbiota

The total DNA from human stool samples was extracted using the PowerMax Soil DNA Isolation Kit (MoBio, Carlsbad, CA) following the standard protocol. The quality and quantity of DNA were measured using the PicoGreen assay kit (Invitrogen, Eugene, OR) and NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). Illumina 16S Metagenomic Sequencing Library protocols to amplify the V3 and V4 regions were used for bacterial metagenomics analysis. Ten nanograms of the extracted DNA was used as a template for PCR amplification with 16S_Amplicon_PCR Forward_Primer (5′-TCGTCGGCAGCG TCAGATGTGTATAAGAGACAGCCTACGGGN GGCWGCAG-3′) and 16S_Amplicon_PCR Reverse_Primer (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC-3′). The final purified products were quantified using quantitative polymerase chain reaction according to the quantitative polymerase chain reaction Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and qualified using the LabChip GX HT DNA High Sensitivity Kit (PerkinElmer, Waltham, MA). Paired-end (2 × 300 bp) sequencing was performed using the MiSeq platform (Illumina, San Diego, CA). Taxonomic assignment was performed using UCLUST (version 1.2.22) and QIIME (version 1.8.0) against the 16S rRNA gene sequence database in Silva (release 123).

Determination of simvastatin and hydroxysimvastatin plasma concentrations

The plasma concentration of simvastatin and hydroxysimvastatin was measured by liquid chromatography-tandem mass spectrometry.[23,24] The analysis matrix was plasma. The samples were frozen about 1 month at –70°C immediately after plasma collection until batch analysis. The precision and accuracy were determined at four concentrations (lower limit of quantification (LLOQ), low, medium, and high) during inter-batch and intra-batch analyses. For simvastatin, precision during the intra-batch analysis ranged from 100.7–108.6% (LLOQ: 114.0%) and accuracy ranged from 1.509–8.127%. For hydroxysimvastatin, precision during the intra-batch analysis ranged from 97.04–105.0% (LLOQ: 106.0%) and accuracy ranged from 2.001–7.018%. For simvastatin, precision during the inter-batch analysis ranged from 100.7–103.0% (LLOQ: 96.86%) and accuracy ranged from 6.622–13.267%. For hydroxysimvastatin, precision during the inter-batch analysis ranged from 102.3–103.2% (LLOQ: 98.32%) and accuracy ranged from 2.708–11.338%. The LLOQ of simvastatin and hydroxysimvastatin was 0.2 and 0.5 ng/mL, respectively. A standard curve was generated using linear regression with $1/x^2$ weighting, where x is the spiked concentration of simvastatin (or hydroxysimvastatin) and y is the ratio of the peak area of simvastatin (or hydroxysimvastatin) to that of the internal standard.

PK and statistical analysis

The PK parameters of simvastatin and hydroxysimvastatin were calculated using a noncompartmental method with Phoenix WinNonlin software version 8.1 (Certara, St. Louis, MO). The maximum plasma concentration (C_{max}) and area under the concentration–time curve from 0 to the last measurable time (AUC_{last}) were calculated and compared with the point estimates of geometric mean ratio of simvastatin for simvastatin alone and for the combination of vancomycin with simvastatin. AUC_{last} was computed using the linear up/log down method. The time to reach C_{max} (T_{max}) was determined from the measured value. The terminal half-life was calculated for subjects whose terminal constant rate (λ_z) was calculated. When λ_z was not calculated, terminal half-life was recorded as missing. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) at a significance level of 0.05.

Untargeted metabolomics analysis of fecal samples

The fecal samples collected from the second and third intervals were prepared using a previously described method,[25] with minor modifications, for untargeted metabolomics analysis. Metabolites from ≥ 50 mg of homogenized fecal samples were extracted using 50 mg/mL of degassed extraction solution (3:3:2, acetonitrile:isopropanol:H_2O). The extracted solution (100 µL) from all samples was pooled to prepare the quality control samples. A nitrogen (N_2) evaporator was used to evaporate sample extractions. Then, second extraction with 400 µL of extraction solution (1:1, acetonitrile:H_2O) was performed as a clean-up step. The extracted samples were dried using an N_2 evaporator, and the dried samples were subjected to derivatization with 10 µL of 20 mg/mL MeOX in pyridine solution at 30°C for 90 minutes in a shaking incubator and cooled at room temperature. The final derivatization was carried out using 90 µL of mixture solution (5% FAME in MSTFA) at 70°C for 45 minutes in a shaking incubator and cooled to room temperature. The

derivatized samples were transferred into gas chromatography (GC) injection vials for further analysis.

The prepared fecal samples were analyzed using a gas chromatography-time of flight mass spectrometry (GC-TOF-MS) system (LECO, St. Joseph, MI). Each prepared sample (1 μL) was injected into the GC system with a front inlet split ratio of 20. After injection, the metabolites from the plasma samples were separated using Rtx-5MS columns (Restek, Bellefonte, PA). The initial GC oven temperature was 50°C, which was increased at a rate of 20°C/minute to separate the metabolites. Mass spectrometry (TOF-MS) method was used to detect metabolites in the range of 50–800 m/z, with acquisition voltage and rate of 1750 V and 20 spectra/s, respectively. The transfer line and ion source temperatures were set at 280 and 250, respectively.

The analyzed data were processed using Chroma TOF 4.6 (LECO). Multivariate analysis using MetaboAnalyst 4.0[26] was performed for the metabolic profiles of fecal samples from the second and third intervals. Principal component analysis was performed to observe unsupervised metabolome profiles from each sample. To select the markers, both volcano plot (fold change below or above 2.0, raw P value < 0.01, and significant count threshold 100%) and partial least square discriminant analysis (PLS-DA; variable importance in projection score > 1.0) were analyzed between the second and third intervals. Then, overlapping markers with peaks detected in all samples between the volcano plot and PLS-DA were selected.

Identification of fecal metabolic markers

To identify markers, commercially available reference standards were analyzed to compare the spectrum and retention time of the markers with libraries. In addition, the retention time of the metabolites detected from the samples and the reference standard was compared by calculating the relative retention index.

$$\text{Relative retention index} = \text{Retention index of octadecanoate/Retention index of markers from the samples or reference standards}$$

where, the retention index of all markers was calculated using the retention time of octadecenoate, which was added to the sample during sample preparation as the internal standard according to Fiehn.[25] Unidentifiable metabolites were excluded from the results of this study.

Spearman's rank-order correlation analysis was carried out between metabolomics (normalized peak area) and metagenomics (operational taxonomic unit (OTU)) data. Changes in OTU of six major phyla before and after administration of vancomycin were subjected to Spearman's rank-order correlation analysis with selected markers.

RESULTS

Study population

Six subjects were enrolled and completed the study. The mean ± SD (range) of age, height, weight, and body mass index was 36.9 ± 9.1 (21–47) years, 1.77 ± 0.04 (1.71–1.83) m, 70.4 ± 4.1 (64.9–75.4) kg, and 22.5 ± 1.3 (19.7–23.7) kg/m^2, respectively.

Effects of diet and vancomycin on the intestinal microbiota

After 6 days on control diet, the composition of the intestinal microbiota was similar among the subjects, with decreased variability in the relative abundance of all phyla. The relative abundance of Bacteroidetes changed from 0.587 ± 0.193 to 0.645 ± 0.065. The corresponding values were altered from 0.289 ± 0.157 to 0.299 ± 0.063 for Firmicutes, from 0.080 ± 0.104 to 0.034 ± 0.043 for Proteobacteria, and from 0.021 ± 0.025 to 0.004 ± 0.004 for Actinobacteria (**Figure S1**).

After vancomycin treatment, the richness of microbiota, which was evaluated as the number of OTUs, considerably decreased (**Figure 2a**), and the diversity of microbiota, which was evaluated using the Shannon diversity index, was significantly reduced (**Figure 2b**). In addition, the relative abundance of bacterial phyla changed in the gut microbiota (**Figure 2c**). The relative abundance of Bacteroidetes significantly decreased, whereas that of Proteobacteria significantly increased. Thus, the dominant phyla changed from Bacteroidetes and Firmicutes to Proteobacteria and Firmicutes following vancomycin treatment, except in one subject. In this subject (M112), unlike that in the other subjects, Fusobacteria was one of the major phyla prior to treatment, whereas Fusobacteria and Firmicutes dominated the gut microbiota after treatment.

PKs of simvastatin and hydroxysimvastatin

The concentration–time profiles of simvastatin were not clearly altered in each subject before and after oral vancomycin treatment. However, the systemic exposure, measured as C_{max} and AUC_{last} of hydroxysimvastatin, increased in all subjects, but the difference in PK parameters was not significant (**Table 1**, **Figure 3**).

Among the six subjects, the concentration–time profiles of simvastatin and hydroxysimvastatin showed atypical elimination patterns in one subject (M105), which was judged as an outlier through statistical testing. When this subject was excluded, the mean systemic exposure to simvastatin and hydroxysimvastatin increased after vancomycin treatment compared with that before treatment. However, the difference was not significant (**Table 1**, **Figure 3**).

Fecal metabolic markers related to changes in the gut microbiota

Untargeted metabolomics. A total of 1,284 metabolites were detected by GC-TOF-MS-based untargeted metabolomics analysis of 12 fecal samples collected before and after the administration of vancomycin. The principal component analysis using unpaired data set showed tightly clustered quality control samples and a clear separation before and after vancomycin treatment. Seventy-five and 106 metabolic markers were selected using PLS-DA and volcano plot, respectively (data not shown). Among them, five markers overlapped between the two methods.

Among the five overlapping markers, four markers, namely D-glucuronic acid, L-phenylalanine, nicotinic acid, and D-arabinose, were identified. The normalized peak area of D-glucuronic acid and L-phenylalanine was significantly higher, whereas that of nicotinic acid and D-arabinose was

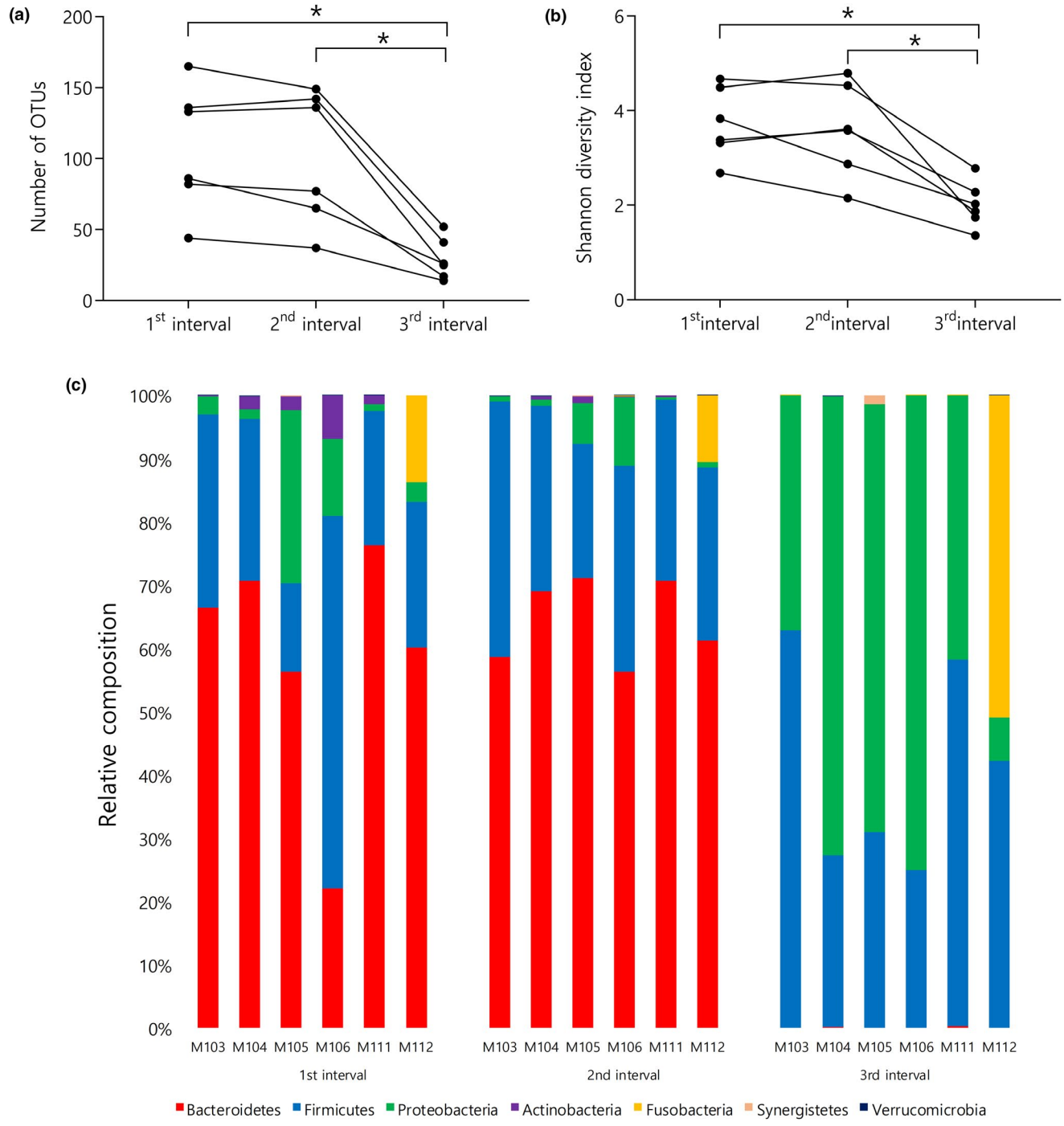

Figure 2 Changes in intestinal microbiota composition during the study. Number of operational taxonomic units (OTU) (**a**) and Shannon diversity index (**b**) were significantly reduced by vancomycin administration. The relative abundance of intestinal bacterial phyla (**c**) changed considerably after vancomycin treatment (*$P < 0.01$).

significantly lower after vancomycin treatment than those before treatment (**Figure 3**). In Spearman's rank-order correlation analysis, the change in markers was positively or negatively correlated with the change in the intestinal microbiota. The change in D-glucuronic acid and L-phenylalanine was positively correlated with the change in Firmicutes and Proteobacteria, but negatively correlated with the change in Bacteroidetes. In contrast, the change in nicotinic acid and D-arabinose was positively correlated with the change in Bacteroidetes, but negatively correlated with the change in Firmicutes and Proteobacteria (**Figures 4 and 5**).

Safety and tolerability. Six AEs, including loose stools and localized itching, were reported in three subjects. All AEs were mild and disappeared without any sequelae. The frequency and severity of AEs were not different before and

Table 1 Summary of simvastatin and hydroxysimvastatin pharmacokinetic parameters

	Simvastatin			Hydroxysimvastatin		
	Day 1	**Day 11**	***P* value***	**Day 1**	**Day 11**	***P* value***
T_{max}, hour	1.4 [0.5–6]	2 [0.8–6]	–	4 [4–8]	4 [4–6]	–
C_{max}, ng/mL	6.7 ± 3.0 (44.8)	8.7 ± 4.7 (53.9)	0.5887	2.0 ± 0.9 (45.2)	3.7 ± 1.6 (43.1)	0.0411
AUC_{last}, h ng/mL	44.2 ± 36.0 (81.5)	38.4 ± 17.9 (46.5)	1.0000	17.1 ± 7.6 (44.4)	24.4 ± 9.3 (38.3)	0.2403
$t_{1/2}$, hour	9.1 ± 9.2 (100.9)	4.9 ± 2.5 (51.0)	–	7.2 ± 4.3 (59.2)	5.5 ± 1.7 (31.0)	–
Metabolic ratio	–	–	–	0.6 ± 0.4 (71.0)	0.7 ± 0.2 (26.3)	0.2403
T_{max},hour[a]	0.8 [0.5–6]	2 [0.8–6]	–	4 [4–8]	4 [4–6]	–
C_{max}, ng/mL[a]	6.5 ± 3.3 (51.0)	7.2 ± 3.3 (45.9)	0.8413	2.1 ± 1.0 (45.4)	3.2 ± 1.0 (31.8)	0.1508
AUC_{last}, h ng/mL[a]	31.4 ± 20.0 (63.7)	36.1 ± 19.0 (52.5)	0.6905	15.4 ± 7.1 (46.3)	23.3 ± 10.0 (43.0)	0.3905
$t_{1/2}$, hour[a]	5.7 ± 4.2 (73.7)	5.2 ± 2.7 (51.4)	–	5.5 ± 1.4 (25.9)	5.8 ± 1.7 (28.4)	–
Metabolic ratio[a,b]	–	–	–	0.6 ± 0.4 (65.1)	0.7 ± 0.2 (28.1)	0.4206

AUC_{last}, area under the concentration–time curve from 0 to the last measurable time; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; T_{max}, time to reach maximum plasma concentration.
[a]Exclusion of M105 subject. [b]AUC_{last} of hydroxysimvastatin/AUC_{last} of simvastatin. *Exact Wilcoxon Two-sample test.

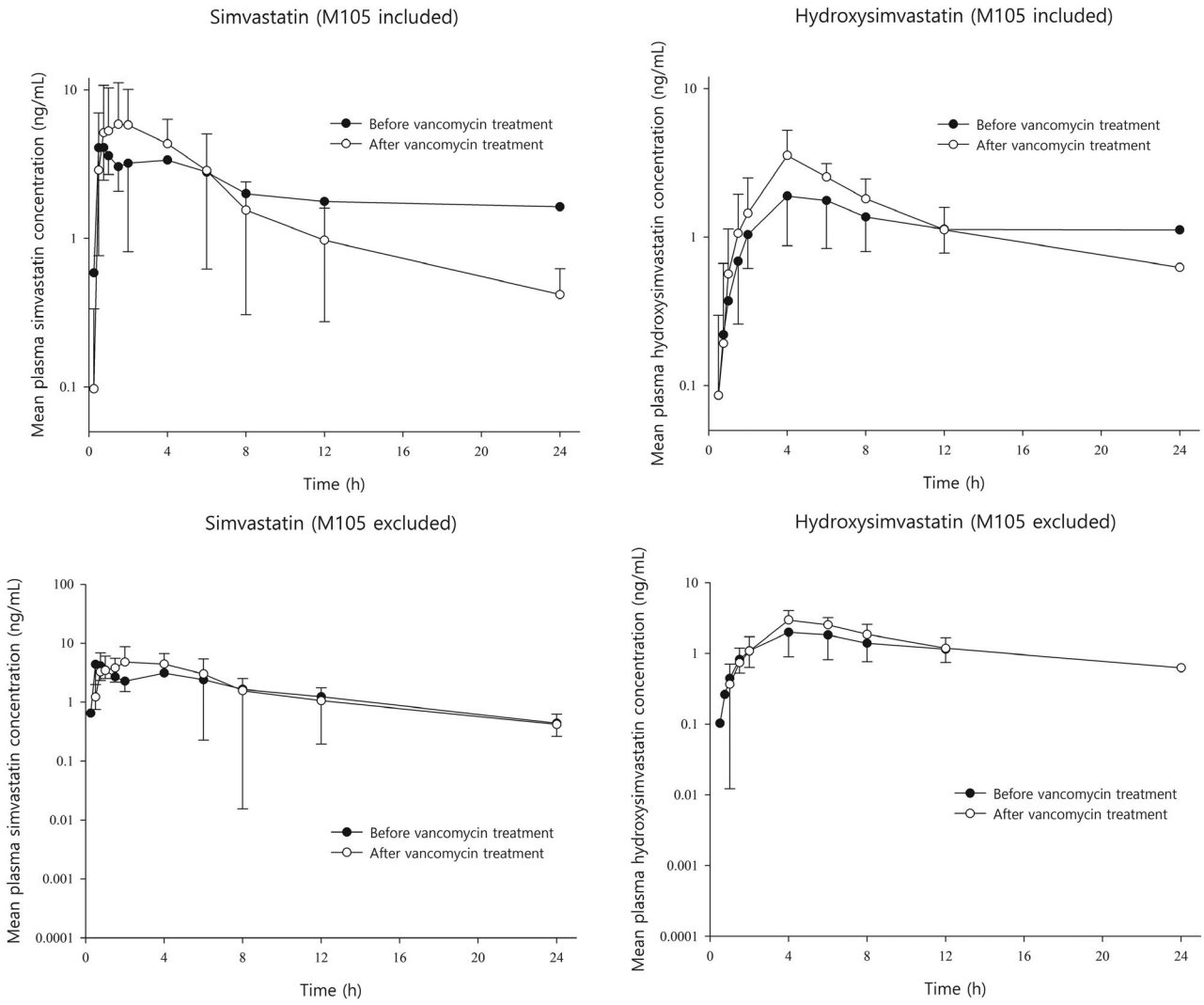

Figure 3 Mean concentration–time graph of simvastatin and hydroxysimvastatin. Black circle indicates the concentration after the first simvastatin administration and white circle indicates the concentration after the second simvastatin administration (after vancomycin treatment period). The upper figures show data including M105, and the lower figures show data excluding M105.

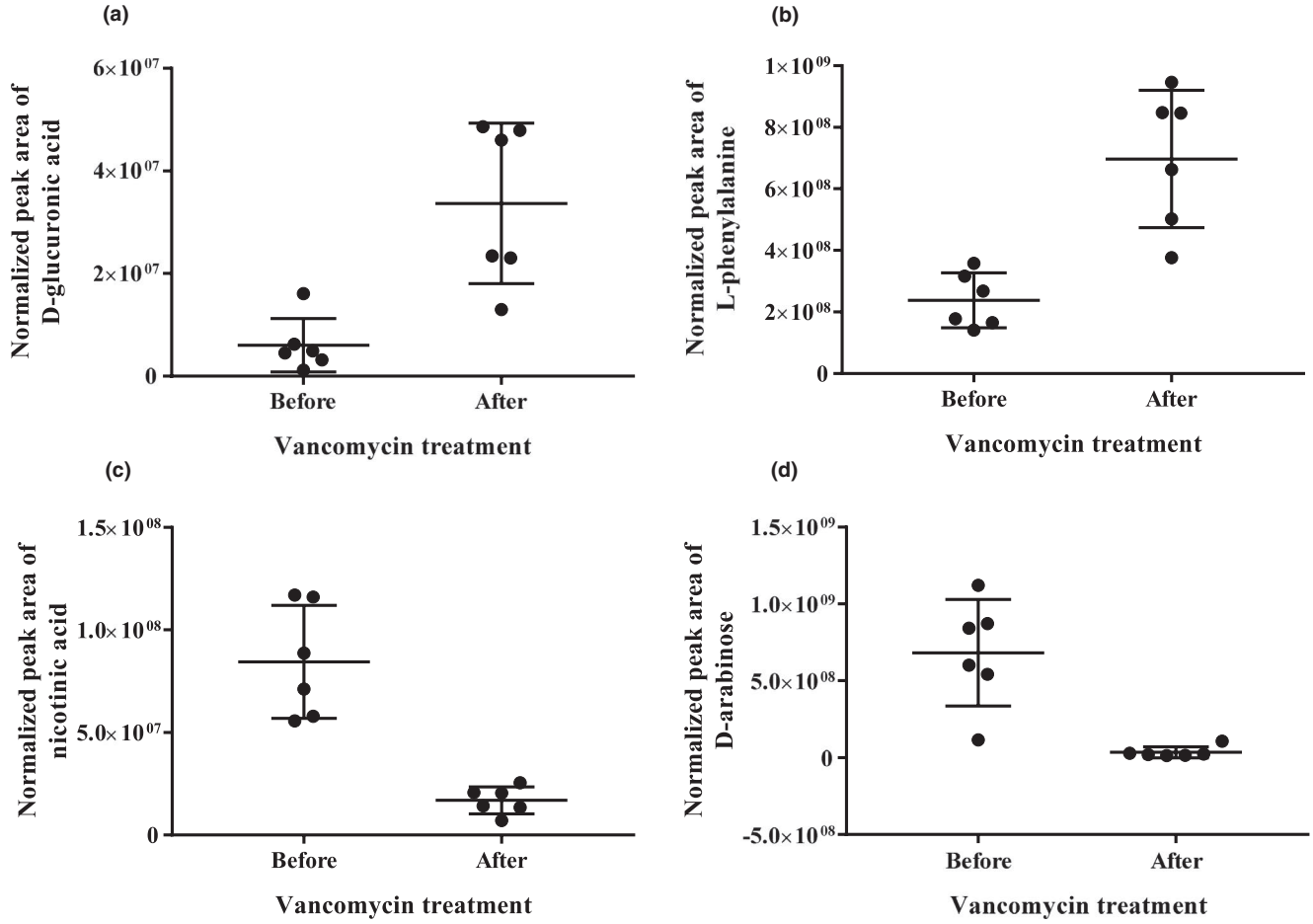

Figure 4 Plots before and after vancomycin treatment showing the change in four markers selected from metabolomics analysis of fecal samples. (**a**) D-glucuronic acid, (**b**) L-phenylalanine, (**c**) nicotinic acid, and (**d**) D-arabinose.

after vancomycin treatment. Other tolerability evaluations were not significantly different before and after vancomycin treatment.

DISCUSSION

The primary hypothesis of this study was that vancomycin treatment might alter the gut microbiota and consequently induce changes in the PK of simvastatin and its metabolite. In this study, obvious taxonomic changes in the gut microbiota were observed after vancomycin treatment; however, no significant changes were observed in the PK of simvastatin and hydroxysimvastatin. Nevertheless, the mean systemic exposure of hydroxysimvastatin increased after vancomycin treatment compared with that before vancomycin treatment in all subjects.

After vancomycin treatment, the relative abundance of the phyla Proteobacteria, Firmicutes, and Fusobacteria increased in the feces. These phyla have been known to produce β–glucuronidase, which plays an important role in the deconjugation of xenobiotics. In addition, D-glucuronic acid, a product of glucuronide deconjugation catalyzed by β-glucuronidase from certain microbial species, was identified as a fecal metabolite related to vancomycin treatment. The change in D-glucuronic acid showed a strong correlation with Proteobacteria, Firmicutes, and Fusobacteria. These results suggest that the relative increase in abundance of Proteobacteria, Firmicutes, and Fusobacteria by vancomycin increased intestinal β-glucuronidase production. However, the relationship between the increased production of intestinal β-glucuronidase and systemic exposure of which can be affected by enterohepatic recirculation was not observed. This phenomenon is presumed that the intestine β-glucuronidase has not changed enough to alter the amount of deconjugation of simvastatin-glucuronide.

The microbiota composition of each subject differed according to the environment, including diet. However, when evaluating the effect of intervention on intestinal microbiota, it is important to homogenize the basal microbiota among the subjects to assess the actual effect of interventions on microbiota while minimizing the bias caused by the basal microbiota. To minimize the heterogeneity, diet was strictly controlled from 1 week prior to the first intervention. In particular, fermented foods or products containing probiotics, which may directly affect the intestinal microbiota, were not provided. Thus, the composition of microbiota became

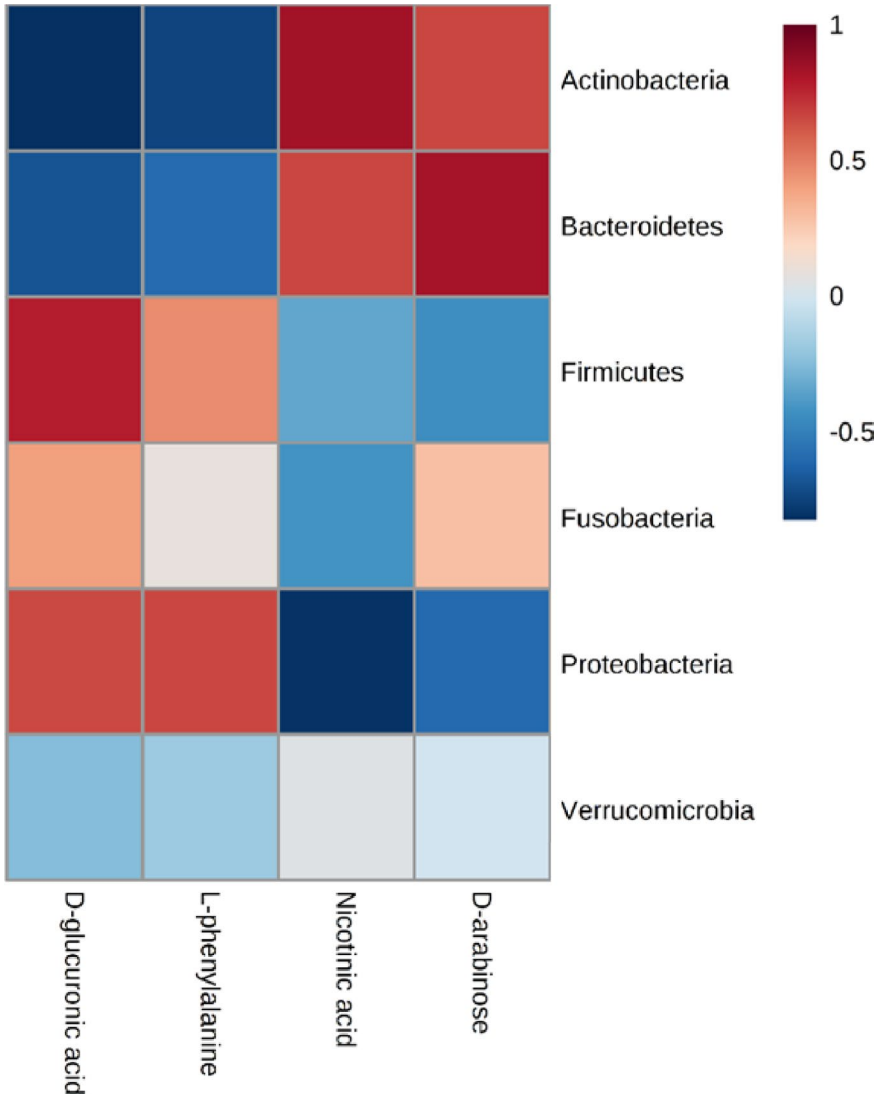

Figure 5 Spearman's rank correlation between four selected markers and gut microbiome before and after administration of vancomycin.

similar among the subjects. Based on these results, it can be suggested that diet control should be considered for future intestinal microbiota studies, even if it requires a relatively long period and extensive efforts.

This study had some limitations. First, although meaningful results have been achieved in this study, this study was a pilot study so that the number of subjects (6) was small to generalize the study results. Further study in a large cohort should be considered. Second, extrapolation to other antibiotics is limited because it is not clear whether the alterations in intestinal microbiota by other antibiotics may have the same effect. Additional evaluation with other antibiotics should be considered. Third, the mechanism underlying the effect of intestinal microbiota on the PK of simvastatin was only speculated. Future *in vitro* or *in vivo* quantitative mechanistic evaluation should be considered.

In conclusion, we found that the composition of intestinal microbiota was changed by oral vancomycin, which also induced changes in fecal metabolites. The effect of these changes on simvastatin were not clearly observed.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Acknowledgments. The authors thank the study participants and the staff of Seoul National University Hospital Clinical Trials Center.

Funding. This study was conducted as an investigator-initiated trial.

Conflict of Interest. All authors declared no competing interests for this work.

Author Contributions. J.S., S.C.J., A.H.K., and S.L. wrote the manuscript. J.S. and S.L. designed the research. J.S. and S.L. performed

the research. J.S., S.C.J., A.H.K., and S.L. analyzed the data. All the authors contributed new reagents/analytical tools.

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