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ARTICLE

# No Effect of Digoxin on Rosuvastatin Pharmacokinetics in Healthy Subjects: Utility of Oita Combination for Clinical Drug–Drug Interaction Study

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**This study evaluated the utility of combination of digoxin (0.25 mg) and rosuvastatin (5 mg) as a new transporter (P-glycoprotein/breast cancer resistance protein/organic anion-transporting polypeptide (OATP)1B1/OATP1B3) probe cocktail (Oita combination) for drug–drug interaction (DDI) studies by demonstrating lack of DDI of digoxin on the pharmacokinetics (PKs) of rosuvastatin, as it was already known that rosuvastatin did not affect digoxin PK. This was an open-label, two-period study in which the primary end points were the geometric mean ratio (GMR) of the area under the plasma rosuvastatin concentration-time curve from time zero to last ($AUC_{last}$) after rosuvastatin administration combined with digoxin to that after rosuvastatin administration alone and its 90% confidence interval (CI). As the GMR of $AUC_{last}$ was 0.974 and its 90% CI was 0.911–1.042, it was judged that digoxin does not affect rosuvastatin PK. Results of this study have rationalized utility of the Oita combination as a transporter probe cocktail for clinical DDI studies.**

### Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**
☑ Regulatory authorities recognize the "cocktail study" usability in evaluation of drugs' inhibition and/or induction potential and various sorts of validated cytochrome P450 enzyme probe cocktails have been used in clinical drug–drug interaction (DDI) studies. As for transporters, there were only a few validated probe cocktails.

**WHAT QUESTION DID THIS STUDY ADDRESS?**
☑ Combination of digoxin and rosuvastatin will be a valuable transporter probe cocktail for clinical DDI studies, if there is no DDI between digoxin and rosuvastatin. It has not been known if digoxin affects the pharamcokinetic (PK) of rosuvastatin, although it is known that rosuvastatin does not affect the PK of digoxin.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**
☑ As digoxin does not affect the PK of rosuvastatin, the rationale to use the combination of digoxin (0.25 mg) and rosuvastatin (5 mg) as a transporter probe cocktail in clinical DDI studies is supported.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**
☑ It becomes possible to evaluate drug's inhibition potential for a wide range of transporters (P-glycoprotein/breast cancer resistance protein/organic anion-transporting polypeptide (OATP)1B1/OATP1B3) in clinical DDI studies with the simple combination of only two drugs, digoxin and rosuvastatin. This new approach will accelerate drug development in a cost and time-efficient manner.

It is important to evaluate drug–drug interaction (DDI) of drugs adequately because efficacy and safety of drugs are often affected by DDI. The regulatory agencies of Japan, the United States, and the European Union have issued guidelines on DDI studies (DDI guidelines)[1–3] and proposed evaluation methods for DDI studies. The guidelines introduce a cocktail study that includes the simultaneous administration of substrates of multiple cytochrome P450 (CYP) enzymes and/or transporters to study subjects. Although the cocktail study is an efficient approach, it must assume there are no interactions among the substrates. However, information on the DDI among probe substrates is limited. Various sorts of combination of CYP enzyme substrates have been proposed and some of them have been validated as CYP enzyme probe cocktails.[4–9] As for transporters, only a few validated probe cocktails for clinical DDI studies have been established.[10,11]

As digoxin is a substrate of P-glycoprotein (P-gp) and rosuvastatin is a substrate of breast cancer resistance

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protein (BCRP), organic anion-transporting polypeptide (OATP1B1) and OATP1B3, the combination of digoxin and rosuvastatin is expected to be a valuable transporter probe cocktail that can be used to simultaneously screen for potential effect of investigational drugs on a wide range of transporters (P-gp/BCRP/OATP1B1/OATP1B3) in clinical DDI studies. It was reported the pharmacokinetics (PKs) of digoxin was not altered with rosuvastatin.[12] Although there is no clinical study report that shows digoxin does not affect the PK of rosuvastatin, there are some pieces of supportive information to suggest lack of effect of digoxin on the PK of rosuvastatin. A report has suggested the DDI between digoxin and rosuvastatin is unlikely, considering *in vitro* inhibitory effects and *in vivo* concentrations of digoxin and rosuvastatin at DDI site.[13] In addition, two *in vitro* studies have reported digoxin is not a substrate of BCRP,[14] OATP1B1, nor OATP1B3.[15] Therefore, the combination of digoxin and rosuvastatin has a potential to be used as a transporter probe cocktail in clinical DDI studies. Recently, Ishii *et al*.[16] reported a clinical DDI study of ravuconazole, an active form of a new antifungal drug fosravuconazole, with two types of cocktails in healthy volunteers. One of the cocktails was a combination of digoxin and rosuvastatin to simultaneously evaluate inhibition potential of ravuconazole for P-gp, BCRP, OATP1B1, and OATP1B3.

We decided to conduct a clinical study to evaluate the effect of digoxin on the PK of rosuvastatin, so as to validate the combination of digoxin and rosuvastatin as a transporter probe cocktail for clinical DDI studies.

## METHODS

The protocol of this study was discussed at the Rosuvastatin and Digoxin Interaction. Study Protocol Committee, which included a biostatistician and an external clinical pharmacologist, then it was reviewed and approved by the Oita University Hospital Institutional Review Board, and conducted in compliance with the current Declaration of Helsinki (2013) and local regulations. Subjects were enrolled at Oita University Hospital. The study was registered to the University Hospital Medical Information Network (UMIN, http://www.umin.ac.jp/english/) prior to start of the study and the UMIN Study ID is UMIN000029232.

### Study design and plan

This was an open-label, two-period study to evaluate the effect of digoxin on the PK of rosuvastatin in Japanese healthy male subjects. The subjects, 20–45 years of age with a body mass index of 18.5–28.0 kg/m$^2$ were enrolled after giving informed consent. This study was conducted in two periods separated by at least 5-day washout. On day 1 of period 1, after a minimum 10-hour fast, the subjects were orally administered a single dose of rosuvastatin (5 mg, Crestor; AstraZeneca K.K., Osaka, Japan) with 200 mL water. On day 1 of period 2, after a minimum 10-hour fast, the subjects were orally administered a single dose of rosuvastatin (5 mg) and digoxin (0.25 mg, Digosin; Chugai Pharmaceutical, Tokyo, Japan) with 200 mL water.

### End points

The primary end points were the geometric mean ratio (GMR) of the area under the plasma rosuvastatin concentration-time curve from zero time to time of last quantifiable concentration ($AUC_{last}$) of rosuvastatin after administration of rosuvastatin combined with digoxin (combined administration) to that after administration of rosuvastatin alone (single administration) and its 90% confidence interval (CI). In this study, we set a criterion that if the 90% CI for the GMR of $AUC_{last}$ is within the range of 0.7–1.43, digoxin does not affect the PK of rosuvastatin.

The secondary end points were as follows:

1. The GMRs of the maximum plasma rosuvastatin concentration ($C_{max}$) and the area under the plasma rosuvastatin concentration-time curve from zero time to infinite time ($AUC_{inf}$) of rosuvastatin after the combined administration to those after the single administration and their 90% CIs
2. Estimation of PK parameters, including $C_{max}$, the time of maximum observed plasma concentration ($t_{max}$), the elimination half-life ($t_{1/2}$), $AUC_{inf}$, $AUC_{last}$, the apparent clearance (CL/F), and the apparent volume of distribution ($V_d$/F) of rosuvastatin after the single administration and the combined administration

Adverse events (AEs), adverse drug reactions (ADRs), and laboratory test values were collected for safety assessments.

### Outcome measures

**PK assessment.** The plasma samples for PK analysis were collected at before dosing, and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 hours after dosing in both periods.

The plasma samples of rosuvastatin were extracted and then the concentrations were measured by validated assay using liquid chromatography-tandem mass spectrometry; Shimadzu LC-10A, Kyoto, Japan; AB Sciex API-4000, Framingham, MA). The extraction method was solid-phase extraction (OASIS HLB plate; Waters, Milford, MA), the lower and upper limit of quantification were 0.05 and 100 ng/mL, respectively. The between-run % coefficient of variation was 0.9–7.0, the maximum % deviation from nominal concentration was 4.0, and the tandem mass spectrometry conditions were 482.0 → 258.0 m/z.[16]

**Safety assessment.** AEs were monitored continuously and graded by intensity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0-Japan Clinical Oncology Group (JCOG) and judged the causal relationship with the study drug(s). Physical examinations were undertaken at screening, the day before the study, day 1 (before dosing and 4 hours after dosing), day 2, and day 3 in the periods 1 and 2, and post–study test. Twelve-lead electrocardiograms were performed at screening, the day before the study, and day 3 in the periods 1 and 2, and post–study test. Hematology, clinical chemistry, and urinalysis were performed at screening, the day before the study, day 2 and day 3 in periods 1 and 2, and post–study test.

### Statistical analysis and sample size

**Statistical analysis.** PK data. A noncompartmental PK method was used to calculate $AUC_{last}$, $AUC_{inf}$, $C_{max}$, $t_{max}$, $t_{1/2}$, CL/F, and $V_d$/F of rosuvastatin after single administration and combined administration by using WinNonlin (Phoenix WinNonlin, Certara LP, Princeton, NJ, USA.) Professional version 7.0. Plasma concentrations and the PK parameters were summarized using descriptive statistics by period. Additionally, the GMRs of $AUC_{last}$, $AUC_{inf}$, and $C_{max}$ and their 90% CIs were calculated to evaluate the effect of digoxin on the PK of rosuvastatin. Statistical analyses were performed using SAS 9.4 (SAS 9.4, SAS Institute Inc., Cary, NC, USA).

*Safety data.* The incidence of AEs, vital signs, physical examination results, 12-lead electrocardiogram results, and changes from baseline laboratory values were summarized for all subjects enrolled in the study, and the appropriate descriptive statistics were provided.

**Sample size.** The target sample size was 10 and was calculated based on the criteria that if the 90% CI for the GMR of the $AUC_{last}$ is within the range of 0.7–1.43, digoxin does not affect the PK of rosuvastatin. Necessary sample sizes that 90% CIs for assumed GMRs of 0.95, 1.00, and 1.05 were within the range of 0.7–1.43, were calculated with the coefficient of variance (0.21) reported by Stopfer *et al*.[17] The calculated necessary sample sizes were 8 for all assumed GMRs of 0.95, 1.00, and 1.05. The target sample size was set at 10 in consideration of the number of cases of dropouts.

## RESULTS

### Subjects

Ten subjects were enrolled, and all subjects completed the study. All subjects were included in the PK set and the safety set. The demographics of subjects are summarized in **Table 1**. All subjects were Japanese male adults. The ages, heights, weights, and body mass indexes were 21–44 years, 166.9–178.4 cm, 56.1–81.7 kg, and 19.8–26.9 kg/$m^2$, respectively.

**Table 1 Demographic characteristics**

| Characteristic | *N* = 10 |
|---|---|
| Gender *n* (%) | |
| Male | 10 (100%) |
| Female | |
| Race *n* (%) | |
| Asian (Japanese) | 10 (100%) |
| Age (year) | |
| *N* | 10 |
| Mean | 31.8 |
| SD | 8.2 |
| Minimum | 21 |
| Median | 31.0 |
| Maximum | 44 |
| Height (cm) | |
| *N* | 10 |
| Mean | 172.12 |
| SD | 4.36 |
| Minimum | 166.9 |
| Median | 172.00 |
| Maximum | 178.4 |
| Weight (kg) | |
| *N* | 10 |
| Mean | 68.70 |
| SD | 9.43 |
| Minimum | 56.1 |
| Median | 68.55 |
| Maximum | 81.7 |
| BMI (kg/$m^2$) | |
| *N* | 10 |
| Mean | 23.14 |
| SD | 2.47 |
| Minimum | 19.8 |
| Median | 23.05 |
| Maximum | 26.9 |

BMI, body mass index.

### PK

**Primary end points.** The geometric means of $AUC_{last}$ of rosuvastatin after the single administration and the combined administration, the GMR of the $AUC_{last}$ and its 90% CI are shown in **Table 2**. The results in which the GMR was 0.974 and its 90% CI was 0.911–1.042 fulfilled the criterion that if the 90% CI for the GMR of the $AUC_{last}$ is within the range of 0.7–1.43, digoxin does not affect the PK of rosuvastatin.

**Secondary end points.**

1. The geometric means of $C_{max}$ and $AUC_{inf}$ of rosuvastatin after the single administration and the combined administration, the GMRs of $C_{max}$ and $AUC_{inf}$, and their 90% CIs are shown in **Table 2**. The GMR of $C_{max}$ was 0.912 and its 90% CI was 0.812–1.023, and the GMR of $AUC_{inf}$ was 0.969 and its 90% CI was 0.900–1.043. The 90% CIs were also within the range of 0.7–1.43.
2. The descriptive statistics of the PK parameters of rosuvastatin after the single administration and the combined administration are shown in **Table 3**. Arithmetic mean ± SD of the PK parameters were 63.5 ± 18.8 ng hour/mL (single administration) and 62.6 ± 20.5 ng hour/mL (combined administration) for $AUC_{last}$, 65.5 ± 18.8 ng hour/mL (single administration) and 64.4 ± 21.0 ng hour/mL (combined administration) for $AUC_{inf}$, 6.79 ± 2.04 ng/mL (single administration) and 6.23 ± 1.97 ng/mL (combined administration) for $C_{max}$, and 11.3 ± 2.9 hour (single administration) and 10.3 ± 1.5 hour (combined administration) for $t_{1/2}$. As for $t_{max}$, median (minimum, maximum) were 3.50 (1.00, 5.00) for the single administration and 4.00 (0.50, 5.00) for the combined administration.
3. The plasma rosuvastatin concentration-time profiles after single administration and combined administration are shown in **Figure 1**. The concentration-time profile after the combined administration was similar to that after the single administration.

### Safety

No deaths, no other serious AEs, and no AEs leading to discontinuation of the study by the study drug administration

**Table 2 GMs, GMRs, and 90% CIs of $AUC_{last}$, $C_{max}$, and $AUC_{inf}$ of rosuvastatin after administration of rosuvastatin alone or administration of rosuvastatin combined with digoxin**

| PK parameter (unit) | Administration | *N* | GM | GMR | 90% CI |
|---|---|---|---|---|---|
| $AUC_{last}$ (ng hour/mL) | Single administration | 10 | 60.3 | 0.974 | 0.911–1.042 |
| | Combined administration | 10 | 58.8 | | |
| $C_{max}$ (ng/mL) | Single administration | 10 | 6.46 | 0.912 | 0.812–1.023 |
| | Combined administration | 10 | 5.89 | | |
| $AUC_{inf}$ (ng hour/mL) | Single administration | 10 | 62.4 | 0.969 | 0.900–1.043 |
| | Combined administration | 10 | 60.5 | | |

$AUC_{inf}$, area under the plasma rosuvastatin concentration-time curve from zero time to infinite time; $AUC_{last}$, area under the plasma rosuvastatin concentration-time curve from zero time to time of last quantifiable concentration; CIs, confidence intervals; $C_{max}$, maximum plasma rosuvastatin concentration; Combined administration, administration of rosuvastatin 5 mg combined with digoxin 0.25 mg; GMs, geometric means; GMRs, geometric mean ratios of PK parameter of rosuvastatin after combined administration with digoxin to those after single administration; PK, pharmacokinetic; Single administration, single administration of rosuvastatin 5 mg.

**Table 3 The descriptive statistics of PK parameters for rosuvastatin after administration of rosuvastatin alone or administration of rosuvastatin combined with digoxin**

| PK parameter (unit) | $C_{max}$ (ng/mL) | | $t_{max}$ (hour) | | $t_{1/2}$ (hour) | | $AUC_{inf}$ (ng hour /mL) | | $AUC_{last}$ (ng hour /mL) | | CL/F (L/hour) | | $V_d$/F (L) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Administration** | **S** | **C** | **S** | **C** | **S** | **C** | **S** | **C** | **S** | **C** | **S** | **C** | **S** | **C** |
| No. of subjects | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Arithmetic mean | 6.79 | 6.23 | 3.40 | 3.45 | 11.3 | 10.3 | 65.5 | 64.4 | 63.5 | 62.6 | 85.2 | 89.4 | 1436 | 1347 |
| SD | 2.04 | 1.97 | 1.43 | 1.38 | 2.9 | 1.5 | 18.8 | 21.0 | 18.8 | 20.5 | 36.3 | 42.0 | 859 | 702 |
| Minimum | 2.93 | 2.53 | 1.00 | 0.50 | 8.7 | 7.6 | 28.9 | 28.8 | 27.9 | 27.5 | 58.4 | 55.6 | 738 | 691 |
| Median | 6.80 | 6.55 | 3.50 | 4.00 | 10.2 | 10.2 | 69.9 | 68.4 | 67.2 | 66.4 | 71.7 | 73.0 | 1130 | 1122 |
| Maximum | 9.59 | 9.55 | 5.00 | 5.00 | 19.0 | 12.4 | 85.5 | 89.9 | 83.9 | 86.3 | 172.8 | 173.6 | 3388 | 2702 |
| Lower limit of 95% CI | 5.33 | 4.82 | 2.38 | 2.46 | 9.1 | 9.2 | 52.1 | 49.3 | 50.0 | 47.9 | 59.2 | 59.4 | 822 | 844 |
| Upper limit of 95% CI | 8.25 | 7.64 | 4.42 | 4.44 | 13.4 | 11.4 | 79.0 | 79.5 | 77.0 | 77.3 | 111.2 | 119.5 | 2051 | 1849 |
| Geometric mean | 6.46 | 5.89 | 3.06 | 2.99 | 11.0 | 10.2 | 62.4 | 60.5 | 60.3 | 58.8 | 80.0 | 82.6 | 1273 | 1216 |
| Lower limit of 95% CI | 5.03 | 4.51 | 2.10 | 1.83 | 9.4 | 9.1 | 48.6 | 45.5 | 46.5 | 44.1 | 62.2 | 62.1 | 901 | 878 |
| Upper limit of 95% CI | 8.30 | 7.70 | 4.45 | 4.89 | 12.8 | 11.4 | 80.3 | 80.5 | 78.3 | 78.4 | 102.9 | 109.8 | 1798 | 1686 |

$AUC_{inf}$, area under the plasma rosuvastatin concentration-time curve from zero time to infinite time; $AUC_{last}$, area under the plasma rosuvastatin concentration-time curve from zero time to time of last quantifiable concentration; C, administration of rosuvastatin 5 mg combined with digoxin 0.25 mg; CI, confidence interval; CL/F, apparent clearance; $C_{max}$, maximum plasma rosuvastatin concentration; PK, pharmacokinetic; S, administration of rosuvastatin 5 mg alone; $t_{1/2}$, elimination half-life; $t_{max}$, time of maximum observed plasma concentration; $V_d$/F, apparent volume of distribution.

were found in this study. Four AEs were found in two subjects and two ADRs were found in one subject in period 2. No AE was found in period 1. In four AEs (feeling hot, hot flush, aspartate aminotransferase increased, and blood creatine phosphokinase increased), feeling hot and hot flush were found in the same subject in the same time were judged as ADRs. All AEs were grade 1 in severity and were recovered promptly without any treatment.

## DISCUSSION

The DDI guidelines[1,2] introduce digoxin as a substrate of P-gp and rosuvastatin as a substrate of BCRP, OATP1B1, and OAPT1B3. The combination of digoxin and rosuvastatin is expected to be a valuable transporter probe cocktail that can be used to simultaneously screen for potential effect of investigational drugs on a wide range of transporters (P-gp, BCRP, OATP1B1, and OATP1B3) in clinical DDI studies. Although it was reported that the PK of digoxin was not affected by rosuvastatin,[12] there is no definitive clinical study report that shows digoxin does not affect the PK of rosuvastatin. We intended to provide an initial estimate for the effect of digoxin on the PK of rosuvastatin in a relatively small study but do so without compromising statistical multiplicity. The present study was initially designed to minimize the sample size to guide a step-wise clinical decision, whereas we set the initial criterion that if the 90% CI for the GMR of $AUC_{last}$ is within the range of 0.7–1.43 (preset decision making boundary), digoxin does not affect the PK of rosuvastatin, in accordance with our strategy. We decided to enroll only male subjects to reduce potential variability and the statistical power to observe the 90% CI fall into 0.7–1.43 was reasonably maintained (>0.8) with a relatively small number of subjects (*N* = 10). If the observed

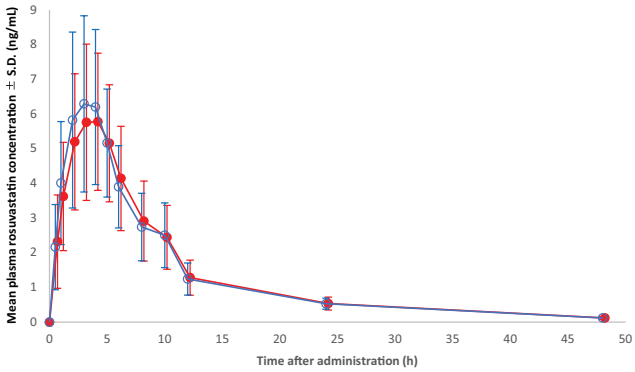


**Figure 1** Plasma rosuvastatin concentration-time profiles after single administration and combined administration. Arithmetic mean value ± SD $n$ = 10. (↔) Combined administration of rosuvastatin 5 mg with digoxin 0.25 mg. (↔) Single administration of rosuvastatin 5 mg.

90% CI fell outside the 0.7–1.43 boundary, then we would have chosen to stop the study or trigger a second study to definitely answer our study question (by increasing the *N*), depending on the probability of success estimated from the actual data.

As the result of this study showed the GMR of $AUC_{last}$ was 0.974 and its 90% CI was 0.911–1.042, we judged that digoxin does not affect the PK of rosuvastatin on the basis of the criterion prespecified in the protocol. The result of this study fulfilled not only this study's criterion but also fulfilled the criterion of the final Japanese DDI guideline[1] that when the 90% CI for GMR of the PK parameter is within the range of 0.8–1.25, it is generally concluded that there is no clinically significant DDI between the drugs. This is inconsistent with the Draft FDA DDI guidance[2] that states when the 90% CIs for systemic exposure ratios fall entirely within the equivalence range of 80–125%, the agency concludes that there is no clinically significant DDI. As for secondary end points, the 90% CIs for GMRs of the $C_{max}$ and the $AUC_{inf}$ were 0.812–1.023 and 0.900–1.043, respectively, and those were also within the range of 0.8–1.25. Other PK parameters ($t_{max}$, $t_{1/2}$, CL/F, and $V_d$/F) after the combined administration with digoxin were similar to those after the single rosuvastatin administration. The plasma rosuvastatin concentration-time profile after combined administration with digoxin was similar to that after single rosuvastatin administration. The results of primary and secondary end points have suggested digoxin has no effect on the PK of rosuvastatin. The rosuvastatin administration combined with digoxin was safe and well tolerated in this study. Results of this study have rationalized utility of the combination of digoxin (0.25 mg) and rosuvastatin (5 mg) as a transporter probe cocktail for clinical DDI studies, considering the report that rosuvastatin did not affect the PK of digoxin.[12]

Ishii *et al.*[16] used this combination to evaluate the inhibition potential of ravuconazole, the active form of the new antifungal drug fosravuconazole, for P-gp, BCRP, OATP1B1, and OATP1B3, although they recognized the combination had not been validated clinically. Their basis for using of this combination was the clinical evidence that rosuvastatin does not affect the PK of digoxin[12] and the *in vitro* evidences that suggested digoxin does not affect the PK of rosuvastatin.[13–15] Our study results provide the clinical evidence to support the methods used in the Ishii *et al.*[16] study and strengthen their conclusion that ravuconazole is unlikely to affect the transporter activity of P-gp, BCRP, OATP1B1, and OATP1B3.

Recently, Stopfer *et al*.[10] conducted a clinical study to optimize the doses of the combination of digoxin (0.25 mg), rosuvastatin (10 mg), furosemide (1 mg), and metformin (10 mg), so as to make the combination be a transporter probe cocktail, responding to their own previous study result that the $C_{max}$ and the $AUC_{last}$ of rosuvastatin after administration of rosuvastatin (10 mg) combined with digoxin (0.25 mg), furosemide (5 mg), and metformin (500 mg) were increased by 38.6% and 43.4%, respectively, compared with those after administration of rosuvastatin alone.[17] However, the 90% CIs for GMRs of the $C_{max}$ and the $AUC_{last}$ of rosuvastatin were within the range of 0.8–1.25 in another study where doses of furosemide and metformin were reduced.[10] The data by Stopfer *et al*.[10] are in accordance with our result that digoxin (0.25 mg) does not affect the PK of rosuvastatin (5 mg).

In conclusion, the rational to use the Oita combination (digoxin (0.25 mg) and rosuvastatin (5 mg)) as a transporter probe cocktail in clinical DDI studies is supported.

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**Conflict of Interest.** N.U. is an employee of Oita University and a partial employee of Osaka University and RIKEN, and is a board member of Clinical Research Support Center Kyushu (CREST) and a member of Technological Review Board for Advance Medicine, Ministry of Health, Labor, and Welfare of Japan. As an Associate Editor for *Clinical and Translational Science*, N.U. was not involved in the review or decision process for this article. N.O., H.W., H.I., and M.K. are employees of Oita University. Ya.I., Yu.I., and A.O. are employees of Sato Pharmaceutical Co. Ltd. O.O. and K.T. are employees of Seren Pharmaceuticals Inc. T.O. is an employee of Kurume University. T.H. is an employee of Kitasato University.

**Author Contributions.** N.U., N.O., H.W., Y. Ishii, Y. Ito, and K.T. wrote the manuscript. N.U., N.O., H.W., Y. Ishii, A.O., O.O., K.T., T.O., and T.H. designed the research. N.U., N.O., H.W., H.I., and M.K. performed the research. N.U., N.O., H.W., H.I., M.K., Y. Ishii, Y. Ito, A.O., O.O., K.T., T.O., and T.H. analyzed the data.

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