Received: 23 November 2020 | Revised: 12 February 2021 | Accepted: 16 February 2021

DOI: 10.1111/cts.13017

ARTICLE

Regulatory guidelines do not accurately predict tolvaptan and metabolite interactions at BCRP, OATP1B1, and OAT3 transporters

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Funding information
These studies were funded by Otsuka Pharmaceutical Development & Commercialization, Inc. (OPDC).

Abstract

Tolvaptan (TLV) was US Food and Drug Administration (FDA)-approved for the indication to slow kidney function decline in adults at risk of rapidly progressing autosomal dominant polycystic kidney disease in 2018. In vitro, TLV was a breast cancer resistance protein (BCRP) inhibitor, whereas the oxobutyric acid metabolite of TLV (DM-4013) was an inhibitor of organic anion transport polypeptide (OATP)1B1 and organic anion transporter (OAT)3. Based on the 2017 FDA guidance, potential for clinically relevant inhibition at these transporters was indicated for the highest TLV regimen. Consequently, two postmarketing clinical trials in healthy subjects were required. In trial 1, 5 mg rosuvastatin calcium (BCRP and OATP1B1 substrate) was administered alone, with 90 mg TLV or 48 h following 7 days of once daily 300 mg TLV (i.e., in the presence of DM-4103). In trial 2, 40 mg furosemide (OAT3 substrate) was administered alone and in presence of DM-4103. For BCRP, rosuvastatin geometric mean ratios (90% confidence intervals [CIs]) for maximum plasma concentration (C_{max}) were 1.54 (90% CI 1.26–1.88) and for area under the concentration-time curve from time 0 to the time of the last measurable concentration (AUC_t) were 1.69 (90% CI 1.34–2.14), indicating no clinically significant interaction. DM-4103 produced no clinically meaningful changes in rosuvastatin or furosemide concentrations, indicating no inhibition at OATP1B1 or OAT3. The BCRP prediction assumed the drug dose is completely soluble in 250 ml; TLV has solubility of ~0.01 g/250 ml. For OATP1B1/OAT3, if fraction unbound for plasma protein binding (PPB) is less than 1%, then 1% is assumed. DM-4103 has PPB greater than 99.8%. Use of actual drug substance solubility and unbound fraction in plasma would have produced predictions consistent with the clinical results.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The US Food and Drug Administration (FDA) created a guidance for estimating the potential for clinically relevant drug-drug interactions (DDIs) at BCRP, OATP1B1,

OAT3, and other transporters. The predictions use various assumptions. For example, for BCRP, it is assumed that inhibitory drug is completely soluble in 250 ml. For OATP1B1 and OAT3, if plasma protein binding for inhibitor is greater than 99%, then fraction unbound is set to 1%.

WHAT QUESTION DID THIS STUDY ADDRESS?

These studies addressed if the predictions for clinically relevant DDIs at BCRP for tolvaptan and at OATP1B1 and OAT3 for the oxobutyric acid metabolite were correct and interactions were observed.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

As there were no clinically relevant interactions, the results support the consideration of drug solubility for BCRP and actual plasma protein binding for OATP1B1 and OAT3 in the predictions of clinically relevant DDIs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Assumptions used in the prediction of clinically relevant DDIs would be revised.

INTRODUCTION

Tolvaptan (TLV) is a selective vasopressin receptor (V_2) antagonist that has been shown to be effective for the treatment of rapidly progressing autosomal dominant polycystic kidney disease (ADPKD).[1,2] The FDA required postmarketing clinical trials following the approval of TLV for the treatment of ADPKD to assess the potential for interaction at breast cancer resistance protein (BCRP), organic anion transport polypeptide (OATP)1B1, and organic anion transporter (OAT)3 transporters.

The highest approved dose regimen is 90 mg in the morning followed by a 30 mg dose given 8–9 h later. The half-life of TLV is about 3.5 h[3] so there is no accumulation and steady-state concentrations are achieved on day 1. TLV has pH independent solubility of about 0.01 g/250 ml.[4] However, TLV tablets contain TLV formulated using a spray-dried technique, which may increase TLV in vitro solubility up to sevenfold.[5,6] The oxobutyric acid metabolite of TLV (DM-4103) is formed by CYP3A4-mediated metabolism of another TLV metabolite; consequently, plasma concentrations are less than 100 ng/ml for the first 2 h following a 90 mg dose (data on file). The elimination half-life is ~180 h[7] so steady-state concentrations are achieved ~8 weeks after the start of TLV dosing. In vitro studies indicated that TLV is a BCRP inhibitor, whereas the oxobutyric acid metabolite (DM-4013) is an inhibitor of OATP1B1 and OAT3.

In 2020, the FDA finalized a guidance document[8] intended to help drug developers determine the in vivo drug-drug interaction (DDI) potential of an investigational drug product. The earlier draft version released in 2017 contained almost identical language with regard to the prediction of the potential for in vivo inhibition at BCRP, OATP1B1, and OAT3 transporters. The FDA guidance is also consistent with guidelines published by the European Medicines Agency in 2013[9] and the Ministry of Labor and Welfare in Japan in 2018.[10]

Based on the FDA website "Drug Development and Drug Interactions; Table of Substrates, Inhibitors and Inducers,"[11] rosuvastatin is considered to be a suitable substrate for use in clinical DDI studies for determining interactions at BCRP and OATP1B1. Other statins are also known to be substrates of OATP1B1. Furosemide is considered to be a suitable substrate for use in clinical DDI studies for determining interactions at OAT3.

In the phase III trials for ADPKD, statin use was unrestricted and no differences in statin use (duration, dose change, statin change, and permanent discontinuation) or incidences of statin-related adverse events were observed for subjects taking TLV alone or taking TLV plus statins (atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, and simvastatin/ezetimibe).[12] In phase II and III trials for congestive heart failure,[13–15] although tested daily TLV doses may have been lower and treatment durations shorter than those used in ADPKD trials, DM-4103 concentrations at the minimum level predicted to be inhibitory at OAT3 were achieved (data on file). Furosemide was used by greater than 80% of congestive heart failure subjects enrolled in TLV clinical trials (data on file) and the incidence of adverse events was similar for subjects taking TLV + furosemide compared with placebo + furosemide. Despite the lack of an interaction based on clinical data, the FDA required postmarketing clinical trials.

The primary objective of trial 1 was to determine the potential inhibitory effect of TLV on the BCRP transporter substrate (i.e., rosuvastatin), and the potential inhibitory effect of the TLV DM-4103 metabolite on an OATP1B1 transporter substrate (i.e., rosuvastatin). The primary objective of trial 2

was to determine the potential inhibitory effect of DM-4103 on an OAT3 substrate (i.e., furosemide).

METHODS

Transporter assays

BCRP: Prazosin transport was evaluated in MDCKII BCRP expressing cells (SOLVO Biotechnology) precultured for 6–8 days. Mannitol transport was unaffected by TLV at 10 μM. Ko143 hydrate, a BCRP inhibitor, decreased prazosin transport by 96.8%. TLV concentrations of 0.10, 0.30, 1.0, 3.0, and 10 μM were tested.

OAT3: Estrone 3-sulfate transport was evaluated in HEK293 cells stably expressing human OAT3 (kit with ready to use 24-well plates were obtained from GenoMembrane). Probenecid, 30 μM, inhibited estrone 3-sulfate transport by 91.1%. DM-4103 concentrations of 0.064, 0.32, 1.6, 8.0, 40, and 200 μM were tested.

OATP1B1: Estradiol 17β-D-glucuronide transport was evaluated in HEK293 cells stably expressing human OATP1B1 (kit with ready to use 24-well plates were obtained from GenoMembrane). Rifampicin, 10 μM, inhibited estradiol 17β-D-glucuronide transport by 91.1%. DM-4103 concentrations of 0.064, 0.32, 1.6, 8.0, 40, and 200 μM were tested.

Informed consent and ethics

These trials were conducted in compliance with FDA regulations, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use–Good Clinical Practice (ICH GCP) Guideline (E6), and international ethical principles derived from the Declaration of Helsinki and Council for International Organizations of Medical Science (CIOMS) guidelines. The protocols for both trials were approved by the Advarra Institutional Review Board (Columbia, MD); subjects were treated at Pharmaceutical Research Associates, Inc. (Salt Lake City, UT).

Trial designs

Figure 1 highlights the treatments administered in each trial and the comparisons that were made.

Trial 1

Trial 1 was designed as an open-label, 3-period, sequential crossover. Screening was conducted in the 28 days prior to dosing. Subjects checked into the clinic on day −1 and remained in-clinic for the duration of the study. On day 1, 5 mg rosuvastatin calcium was administered alone. On day 4, 5 mg rosuvastatin calcium was co-administered with 90 mg TLV. On days 7 to 13, 300 mg TLV was administered q.d. On day 15, 5 mg rosuvastatin calcium was administered alone. Subjects were discharged from the clinic on day 18 and a follow-up telephone call was conducted on day 22.

Doses on pharmacokinetic (PK) sampling days were administered in the fasted state with 240 ml of room temperature still water. No food was allowed from 10 h prior to dosing until lunch was given following the 4-h postdose assessments; water was available ad libitum except for +/− 2 h around dosing. On non-PK sampling days, TLV was administered at least 30 min prior to breakfast. Safety was evaluated by laboratory values, vital signs, and 12-lead electrocardiogram (ECG) at screening, day −1, and discharge (no ECG), and adverse event reports.

The 5 mg dose of rosuvastatin calcium was chosen based on the high predicted value for a BCRP interaction and that increases in rosuvastatin maximal peak plasma concentration

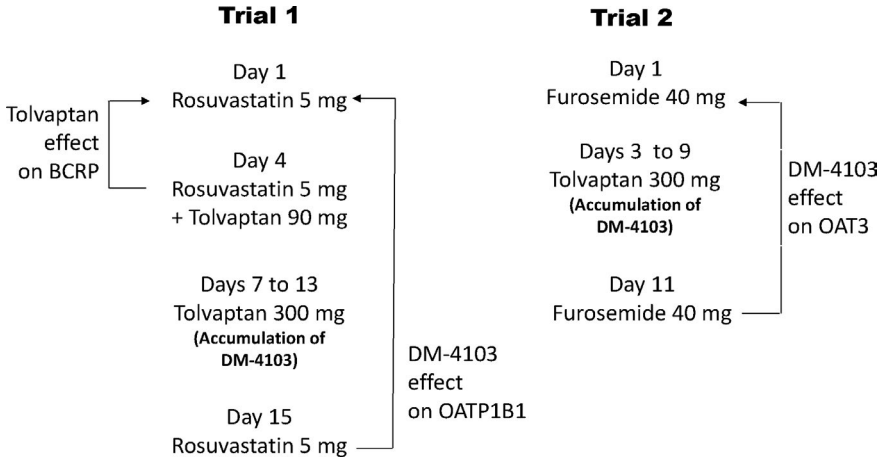

FIGURE 1 Diagram of treatments administered and comparisons

(C_{max}) up to 11-fold have been observed.[16] A 90-mg dose of TLV was chosen as it is the highest dose administered at any given time in the treatment of ADPKD. The FDA required that DM-4103 concentrations tested in this trial be similar to those observed at steady-state in ADPKD trials (~7500 ng/ml), therefore, TLV doses of 300 mg were administered for 7 days. Previously, 300 mg TLV administered for 5 days to healthy subjects was shown to be safe and well-tolerated.[17] After 48 h, TLV would be completely eliminated due to its short half-life, leaving only DM-4103 concentrations present in the plasma.

Trial 2

Trial 2 was designed as an open-label, 2-period, sequential crossover. Screening was conducted in the 28 days prior to dosing. Subjects checked into the clinic on day −1 and remained in-clinic for the duration of the study. On day 1, 40 mg furosemide was administered alone. On days 2 to 8, 300 mg TLV was administered q.d. On day 10, 40 mg furosemide was administered alone. Subjects were discharged from the clinic on day 11 and a follow-up telephone call was conducted on day 17.

Treatments were administered and safety assessed as described for Trial 1.

A 40 mg dose of furosemide was chosen as it was expected that furosemide concentrations would be measurable up to 24 h and that this dose would have at least a 2-fold safety margin, as up to 80-mg doses have been administered safely to healthy subjects.[18]

Sample collection

For trial 1, blood samples for rosuvastatin (4 ml using dipotassium ethylenediaminetetraacetic acid [K_2EDTA] as anticoagulant) were taken at predose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, 56, and 72 h postdose on days 1, 4, and 15. Samples for TLV (4 ml with sodium heparin as anticoagulant) were taken at 2 and 3 h postdose on day 4. Samples for DM-4103 (4 ml with sodium heparin as anticoagulant) were taken at predose and 24, 48, 72, 96, and 120 h postdose on day 13 (7th day of dosing).

For trial 2, blood samples for furosemide (4 ml using K_2EDTA as anticoagulant) were taken at predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h on days 1 and 10. Samples for DM-4103 (4 ml with sodium heparin as anticoagulant) were taken at predose, 24, 48, and 72 h on day 8 (7th day of dosing).

Plasma was evenly pipetted into 2 aliquots and stored at –70°C or colder (rosuvastatin, TLV, and DM-4103) or −20°C or colder (furosemide) until analyzed.

Bioanalytical

Plasma rosuvastatin concentrations were determined using a specific and validated reversed-phase high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS). Rosuvastatin and the internal standard rosuvastatin-d6 were extracted from human plasma containing K_2EDTA as an anticoagulant using solid phase extraction. The method was validated for concentrations ranging from 0.500 to 300 ng/ml for rosuvastatin. All validation work and sample analysis were performed at ICON Laboratory Services, Inc. (Whitesboro, NY).

The assay used to determine plasma TLV concentrations[19] also included DM-4103 as a standard. The precursor→product ion was m/z 479 → 252. DM-4103 concentrations were linear over the range of 12.5 to 12,500 ng/ml. All validation work and sample analysis were performed at ICON Laboratory Services, Inc. (Whitesboro, NY).

Plasma furosemide concentrations were determined using a specific and validated reversed-phase HPLC-MS/MS method. Furosemide and the internal standard, furosemide-d5, were extracted from human plasma containing K_2EDTA by protein precipitation. The method was validated for concentrations ranging from 5.00 to 5000 ng/ml. All validation work and sample analysis were performed at Altasciences (Quebec, Canada).

Noncompartmental analysis

Rosuvastatin and furosemide concentrations were analyzed using noncompartmental methods (SAS version 9.4). For calculation of descriptive statistics and PK parameters, plasma concentration values below the lower limit of quantitation (LLOQ) prior to first measurable concentrations were imputed to 0. Concentration values below the LLOQ following the last sample with a measurable concentration were excluded from the analyses. Actual blood sample times postdose were determined and reported as 2 decimals. Values for C_{max} and the time of maximal plasma concentration (T_{max}) were determined directly from the observed data. Values of area under the concentration-time curve from time 0 to the time of the last measurable concentration (AUC_t) were estimated using the linear trapezoidal rule. The terminal-phase elimination rate constant (λ_z) was estimated by a log-linear regression of at least three non-zero concentrations; regressions with r^2 values less than 0.8 were not reported. The terminal-phase elimination half-life ($t_{1/2,z}$) was determined as $(\ln 2)/\lambda_z$. Values of AUC from time 0 to infinity (AUC_∞) were determined as $AUC_t + C_{last}/\lambda_z$. The value of apparent clearance from plasma following extravascular drug administration (CL/F) was determined as dose/AUC_∞.

Descriptive statistics were presented by treatment and analyte. A maximum of 3 significant figures were used for table presentations except for T_{max}, which was reported to 2 decimals and $t_{1/2,z}$, which was reported to 1 decimal.

Statistical

The geometric mean ratios with 90% confidence intervals (CIs) of C_{max}, AUC_t, and AUC_∞ were determined for rosuvastatin with TLV and rosuvastatin in the presence of DM-4103 compared with rosuvastatin alone (periods 2 or 3 compared with period 1) and for furosemide in the presence of DM-4103 compared with furosemide alone (period 3 compared with period 1). An analysis of variance with a factor of period and random effect of subjects was performed on the natural-log transformed PK parameters using the MIXED procedure in SAS. From each analysis, the least square means, their difference, and the 90% CI for their difference were derived. Then, the anti-logs of the difference and the confidence limits provided the estimate and 90% CI for the ratio of the geometric means of the test treatment to the reference treatment. Only subjects with values in both treatments were used in the analysis.

RESULTS

TLV inhibition of prazosin transport in BCRP overexpressing MDCKII cells provided a half-maximal inhibitory concentration (IC_{50}) estimate of 8.32 μM. The FDA guidance predicts an interaction when "...drug is administered orally, and the I_{gut}/IC_{50} or $K_i \geq 10$ where I_{gut} = dose of inhibitor/250 ml." A 90 mg dose of TLV is 200 μmol, which results in a predicted $K_i = 800/8.32 = 96.4$, indicating potential for an interaction.

DM-4103 inhibition of estradiol 17β-D-glucuronide transport in OATP1B1 overexpressing HEK293 cells provided an IC_{50} estimate of 0.255 μM. The FDA guidance states that potential for a clinically relevant interaction exists if $R = 1 + ([f_{u,p} \times I_{in,max}]/IC_{50})$ greater than or equal to 1.1 where **R** is the predicted ratio of the victim drug's AUC in the presence and absence of the investigational drug as the inhibitor, $\mathbf{f_{u,p}}$ is the unbound fraction in plasma, $\mathbf{IC_{50}}$ is the half-maximal inhibitory concentration, and $\mathbf{I_{in,max}}$ is the estimated maximum plasma inhibitor concentration at the inlet to the liver. Considering uncertainties in the protein binding measurements, the unbound fraction ($f_{u,p}$) should be set to 1% if experimentally determined to be less than 1%. As a metabolite formed slowly by metabolism after absorption of TLV, DM-4013 has no intestinal availability and portal vein concentrations are negligible compared with circulating concentrations at steady-state, so $I_{in,max}$ was set equal to the circulating concentrations of DM-4103. Following the 90 + 30 mg dose regimen of TLV in subjects with ADPKD, mean DM-4103 concentrations were ~7500 ng/ml or 15.7 μM (data on file). DM-4103 is greater than 99.8% plasma protein bound so $f_{u,p}$ was set to 0.01. Consequently, for DM-4103, $R = 1 + ([0.01 \times 15.7]/0.255) = 1.62$, indicating potential for an interaction.

DM-4103 inhibition of estrone 3-sulfate transport in OAT3 overexpressing HEK293 cells provided an IC_{50} estimate of 0.425 μM. The FDA guidance predicts an interaction if "...the $I_{max,u}/IC_{50}$ value is ≥0.1." For DM-4103, 15.7*0.01/0.425 = 0.37, indicating potential for an interaction. The ratio is equal to 0.1 when DM-4103 concentration is ~2034 ng/ml.

For trial 1, 16 subjects were enrolled and 14 completed. One subject was discontinued due to a treatment-emergent adverse event (TEAE) of palpitations at 70 h following rosuvastatin dosing on day 1. One subject withdrew following rosuvastatin dosing on day 1. For trial 2, 14 subjects were enrolled and completed. The demographic characteristics of enrolled subjects are summarized in Table 1.

Plots of mean (SD) rosuvastatin and furosemide plasma concentrations are presented in Figures 2 and 3, respectively. PK parameters for rosuvastatin and furosemide are presented in Tables 2 and 3, respectively. Geometric mean ratios and 90% CIs are presented in Table 4.

TLV administration concomitant with rosuvastatin administration was confirmed as mean (SD) concentrations at 2 and 3 h postdose were 422 (241) and 409 (184) ng/ml, respectively.

Mean (SD) DM-4103 concentrations at 48 h postdose (i.e., predose of the last dosing period) were 7240 (1880) ng/ml and 6430 (2030) ng/ml in trial 1 and trial 2, respectively, well above the predicted inhibitory levels.

TABLE 1 Demographic characteristics for enrolled subjects

Demographics	Trial 1 (*N* = 16)	Trial 2 (*N* = 14)
Age, Mean (range)	27.3 (20–42)	28.1 (19–43)
Weight, Mean (range)	70.8 (48.5–92.4)	70.3 (53.7–87.2)
Sex, *n* (%)		
Male	10 (62.5)	7 (50.0)
Female	6 (37.5	7 (50.0)
Race, *n* (%)		
White	13 (81.3)	12 (85.7)
Black	2 (12.5)	1 (7.1)
American Indian or Alaskan Native	1 (6.3)	1 (7.1)
Ethnicity, *n* (%)		
Hispanic or Latino	1 (6.3)	4 (28.6)
Not Hispanic or Latino	15 (93.8)	10 (71.4)

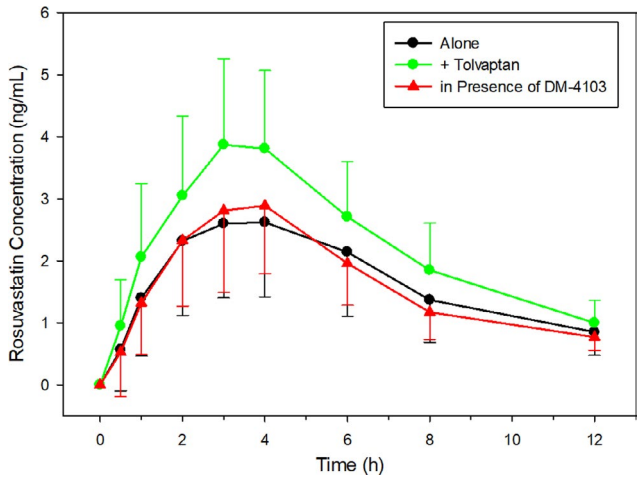

FIGURE 2 Mean (SD) rosuvastatin plasma concentrations after administration of 5 mg rosuvastatin calcium alone, with 90 mg of tolvaptan or 48 h following 7 days of 300 mg tolvaptan (TLV) q.d. (i.e., in the presence of DM-4103) to 14 healthy adult subjects. No clinically relevant inhibition of BCRP by TLV and no inhibition of OAPT1B1 by DM-4103 is observed

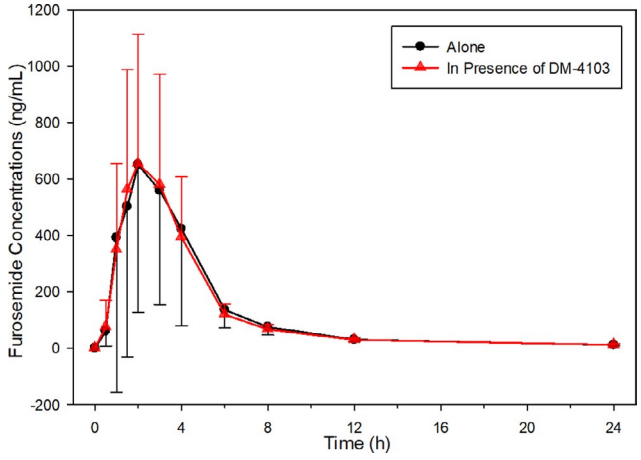

FIGURE 3 Mean (SD) furosemide plasma concentrations after administration of 40 mg furosemide alone or 48 H following 7 days of 300 mg tolvaptan q.d. (i.e., in the presence of DM-4103) to 14 healthy subjects. No inhibition of OAT3 by DM-4103 is observed

For trial 1, TEAEs reported in at least 2 subjects/period were, in period 1 (rosuvastatin), nausea (12.5%), in period 2 (rosuvastatin + TLV), pollakiuria (35.7%), thirst (50%), dysgeusia (14.3%), and headache (14.3%), in period 3 (TLV alone), pollakiuria (71.4%), thirst (41.9%), nausea (21.4), and constipation and dry mouth (both 14.3%), and in period 3 (rosuvastatin in presence of DM-4103), headache (14.3%).

For trial 2, TEAEs reported in at least 2 subjects/period were, in period 1 (furosemide alone), polyuria (64.3%), in period 2 (TLV alone), polyuria (100%), thirst (92.9), dry mouth (21.4%), constipation, dizziness, headache, and insomnia (all 14.3%) and, in period 3 (furosemide in presence of DM-4103), and headache (21.4%).

All incidences of pollakiuria, polyuria, dry mouth, and thirst were considered to be related to the tolvaptan. No subjects had AEs related to changes in laboratory values or vital signs.

TABLE 3 Mean (SD) plasma furosemide pharmacokinetic parameters in 14 subjects

Parameters	40 mg Furosemide alone	40 mg Furosemide in presence of DM−4103
C_{max}, ng/ml	992 (580)	873 (475)
T_{max}, h[a]	2.00 (1.00–4.00)	2.00 (1.00–4.00)
AUC_t, ng·h/ml	2920 (1400)	2900 (1140)
AUC_∞, ng·h/ml	2950 (1390)[b]	3070 (1150)[b]
$t_{1/2,z}$, h	6.1 (2.4)[b]	6.7 (1.3)[b]
CL/F, ml/min/kg	3.88 (1.55)[b]	3.53 (1.12)[b]

Abbreviations: AUC_t, area under the concentration-time curve from zero to time of last measurable concentration; AUC_∞, area under the concentration-time curve from zero to infinity; C_{max}, maximal peak plasma concentration; CL/F, apparent clearance from plasma following extravascular administration; $t_{1/2,z}$, terminal phase elimination half-life; T_{max}, time of maximal peak concentration.

[a]Values are median (minimum-maximum); [b]$n = 13$.

TABLE 2 Mean (SD) ROS plasma pharmacokinetic parameters in 14 healthy adults subjects

Parameters	5 mg ROS alone	5 mg ROS + 90 mg TLV	5 mg ROS in presence of DM−4103
C_{max}, ng/ml	2.81 (1.27)	4.01 (1.30)	3.03 (1.20)
T_{max}, h[a]	3.50 (2.00–6.00)	3.03 (2.00–4.12)	4.00 (3.00–6.00)
AUC_t, ng·h/ml	18.9 (10.1)	29.7 (14.6)	18.4 (8.26)
$t_{1/2,z}$, h	2.4–4.3[b]	4.5 (1.5)[c]	3.6 (0.6)[d]
AUC_∞, ng·h/ml	20.3–36.0[b]	38.0 (14.6)[c]	27.2 (8.21)[d]
CL/F, ml/min/kg	28.5–68.2[b]	36.6 (16.4)[c]	44.3 (16.6)[d]

Abbreviations: AUC_t, area under the concentration-time curve from zero to time of last measurable concentration; AUC_∞, area under the concentration-time curve from zero to infinity; C_{max}, maximal peak plasma concentration; CL/F, apparent clearance from plasma following extravascular administration; ROS, rosuvastatin; $t_{1/2,z}$, terminal phase elimination half-life; TLV, tolvaptan; T_{max}, time of maximal peak concentration.

[a]Values are median (minimum-maximum); [b]Values are minimum-maximum, $n = 7$; [c]$n = 11$; [d]$n = 8$.

TABLE 4 Geometric mean ratios and 90% confidence intervals for rosuvastatin (BCRP, OATP1B1) and furosemide (OAT3)

Comparison[a]	C_{max}	AUC_t	AUC_∞
BCRP inhibition			
Rosuvastatin + tolvaptan (T) vs. rosuvastatin alone (R), $n = 14$	1.538 1.255–1.883	1.691 1.335–2.141	1.281[a] 1.148–1.429
OATP1B1 inhibition			
Rosuvastatin in presence of DM−4103 (T) vs. rosuvastatin alone (R), $n = 14$	1.129 0.995–1.335	1.045 0.881–1.239	0.998[b] 0.876–1.138
OAT3 inhibition			
Furosemide in presence of DM−4103 (T) vs. furosemide alone (R), $n = 14$	0.907 0.794–1.035	1.04 0.938–1.152	1.018[c] 0.916–1.131

Abbreviations: AUC_t, area under the concentration-time curve from zero to time of last measurable concentration; AUC_∞, area under the concentration-time curve from zero to infinity; C_{max}, maximal peak plasma concentration; R, reference; T, test.

Number of subjects with parameter in both treatment periods: [a]$n = 6$, [b]$n = 5$, and [c]$n = 12$.

DISCUSSION

Rosuvastatin C_{max} and AUC_t were increased 1.54- and 1.69-fold, respectively, when administered with 90 mg TLV. Table 4 in the prescribing information for CRESTOR[19] indicates that interactions are only considered clinically significant if the increases in AUC are at least 1.9-fold and the increases in C_{max} are at least 2.2-fold. Therefore, the increases observed in this trial would not be considered clinically significant.

Based on the guidances for prediction of potential for interaction at BCRP, it is assumed the dose is completely soluble in 250 ml; a 90-mg TLV dose (200 μmol) in 250 ml has a predicted concentration of 800 μM. However, TLV has pH independent solubility of about 0.01 mg/250 ml (~0.09 μM). Even if TLV solubility was increased sevenfold, due to spray-drying, TLV would not be predicted to have clinically relevant interactions at BCRP transporters.

A potential mechanism behind the observed interaction could be TLV inhibition of the sodium-dependent taurocholate co-transporting polypeptide (NTCP). It has been reported that rosuvastatin is transported by NTCP with human hepatocyte studies suggesting that NTCP alone could account for ~35% of rosuvastatin uptake.[20] TLV was shown to be an inhibitor of NTCP, although the IC_{50} value was higher than C_{max}.[21] However, liver concentrations of TLV were predicted to be much higher than blood concentrations (liver:blood ratio 38.76)[22] and TLV was shown to accumulate at least 10-fold in sandwich-cultured human hepatocytes[23]; "The total cellular concentration of tolvaptan ranged from ~2 μM to 500 μM and was greater than the respective incubation concentration (0.15–50 μM)." Thus, liver concentrations of TLV may be reaching inhibitory levels.

As shown in Table 4, the lower bounds of the 90% CI for C_{max} and AUC_t are less than 1 for both rosuvastatin in the presence of DM-4103 compared with rosuvastatin alone and furosemide in the presence of DM-4103 versus furosemide alone, therefore we can conclude that DM-4103 is not an inhibitor of OATP1B1 or OAT3 at clinically relevant concentrations.

For OATP1B1 and OAT3, the FDA guidance instructs that fraction unbound for plasma protein binding less than 1% be set to 1% "considering uncertainties in protein binding measurements." DM-4103 protein binding was determined to be greater than 99.8% using a micropartition device (Centrifree YM-30) followed by HPLC-MS/MS detection of concentrations in the ultrafiltrate. The effect of 6 other highly plasma protein bound compounds on DM-4103 binding was also evaluated; no interactions were detected with DM-4013 binding greater than 99.8% in all samples (data on file). No potential for interaction at either transporter would have been predicted if a free fraction of 0.1% had been used, consistent with the clinical findings.

These results support the use of drug substance solubility and actual unbound fraction in plasma in the predictions for clinically significant drug interactions.

DATA SHARING

To submit inquiries related to Otsuka Clinical Research, or to request access to individual participant data (IPD) associated with any Otsuka clinical trial, please visit https://clinical-trials.otsuka.com/. For all approved IPD access requests, Otsuka will share anonymized IPD on a remotely accessible data sharing platform.

ACKNOWLEDGMENTS

Editorial support and submission assistance was provided by BioScience Communications, Inc (New York, NY, USA) and funded by Otsuka Pharmaceutical Development & Commercialization, Inc.

CONFLICT OF INTEREST

All authors are employees of Otsuka Pharmaceutical Development & Commercialization, Inc. (OPDC).

AUTHOR CONTRIBUTIONS

S.E.S. wrote the manuscript. S.E.S., P.B.. and J.R.G. designed the research. P.B. and J.R.G. performed the research. S.E.S. analyzed the data.

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How to cite this article: Shoaf SE, Bricmont P, Repella Gordon J. Regulatory guidelines do not accurately predict tolvaptan and metabolite interactions at BCRP, OATP1B1, and OAT3 transporters. *Clin Transl Sci*. 2021;14:1535–1542. https://doi.org/10.1111/cts.13017