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ORIGINAL RESEARCH ARTICLE

Effects of Metformin and Furosemide on Rosuvastatin Pharmacokinetics in Healthy Volunteers: Implications for Their Use as Probe Drugs in a Transporter Cocktail

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Abstract

Background In a recently described probe drug cocktail for clinically relevant drug transporters containing digoxin, furosemide, metformin and rosuvastatin, mutual interactions were essentially absent except for increases in the systemic exposure of rosuvastatin. To optimize the cocktail, we further examined the dose dependence of the effects of metformin and furosemide on rosuvastatin pharmacokinetics.

Methods This was a randomized, open label, single center, six-treatment, six-period, six-sequence crossover trial. Eighteen healthy male subjects received 10 mg rosuvastatin as reference treatment and, as test treatments, 10 mg rosuvastatin combined with 10, 50 or 500 mg metformin (T1, T2 and T3) or with 1 or 5 mg furosemide (T4 and T5). Primary pharmacokinetic endpoints were rosuvastatin C_{max} (maximum plasma concentration) and AUC_{0-tz} (area under

the plasma concentration–time curve from time zero to the last quantifiable concentration).

Results The relative bioavailability of rosuvastatin was essentially unchanged when administered with metformin in T1 and T2, but in T3 it increased to 152% for AUC_{0-tz} (90% CI 135–171%) and 154% for C_{max} (90% CI 132–180%). Coadministration with furosemide did not change rosuvastatin relative bioavailability in T4, but in T5 it increased slightly to 116% for AUC_{0-tz} (90% CI 102–132%) and 118% for C_{max} (90% CI 98–142%).

Conclusion The increased systemic exposure of rosuvastatin when administered as part of the proposed transporter cocktail is most likely attributable to metformin and only to a minor degree to furosemide. Reduction of the doses of metformin and furosemide is expected to eliminate the previously described interaction.

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Key Points

The use of a probe drug cocktail is a promising approach to reduce the number of clinical studies required to investigate drug–drug interactions (DDI), and was recently extended for the first time to a cocktail with four probe drugs exclusively selected to investigate potential transporter-based DDIs.

In this trial with healthy volunteers, doses of metformin and furosemide were optimized to eliminate their interaction with rosuvastatin in the cocktail. Plasma concentrations after dosing of 10 mg oral rosuvastatin were elevated in the presence of 500 mg metformin or 5 mg furosemide (order: metformin > furosemide), but these interactions could be eliminated by reducing the dose of metformin to 50 or 10 mg and furosemide to 1 mg.

Another drug cocktail study, using optimized doses of metformin and furosemide, is currently being evaluated. This cocktail is expected to result in minimal mutual interactions, thus providing a valuable new tool in drug development.

1 Introduction

Membrane-bound drug transporters can play a relevant role in drug absorption, distribution and elimination [1, 2]. Modulation of transporter activity by concomitant administration of drugs acting as inhibitors or inducers can lead to drug–drug interactions (DDI) affecting the safety or efficacy of the victim drug [3]. Accordingly, in drug development, thorough investigation of the potential of a new molecular entity to be a victim or a perpetrator of transporter-mediated DDI is recommended by EMA, FDA and PMDA guidelines [4–6]. Regulatory authorities expect an appropriate assessment of the inhibition potential of a new drug towards P-glycoprotein (P-gp), organic anion transporting polypeptides OATP1B1 and OATP1B3, organic anion transporters OAT1 and OAT3, organic cation transporter 2 (OCT2), multidrug and toxin extrusion proteins MATE1 and MATE2-K and breast cancer resistance protein (BCRP) [4–6].

An elegant and efficient approach to reduce the number of clinical studies in a development program is the probe drug cocktail, which can be used to explore the effect of a new molecular entity on the pharmacokinetics of several probe drugs, given together simultaneously in a single trial. Whereas cocktails of probe drugs for cytochrome P450 (CYP)-mediated DDI are well established [7, 8], until very recently no such cocktail has been reported for drug transporters involved in clinically relevant DDI. To fill this gap, we published a Phase I interaction trial [9] that for the first time investigated mutual pharmacokinetic interactions in a probe drug cocktail that is exclusively intended to evaluate the potential for transporter-based DDI. Recently, another group reported a microdose victim probe drug cocktail focused on detection of interactions mediated by inhibition of OATP1B, BCRP, P-gp, and CYP3A, but not aimed at addressing OATs, OCT2 and MATEs, illustrating the high interest in cocktail approaches for evaluation of transporter-mediated DDI [10].

The cocktail investigated for mutual interactions in our previous study contained the following probe drugs and dose levels (principal transporters indicated in parentheses): 0.25 mg digoxin (P-gp), 5 mg furosemide (OAT1 and OAT3), 500 mg metformin (OCT2, MATE1, and MATE2-K) and 10 mg rosuvastatin (OATP1B1, OATP1B3, BCRP) [9]. The design of the in vivo trial took comprehensive preceding in vitro investigations into account [11].

It was found that potentially relevant interactions between the four probe cocktail drugs in vivo were essentially absent, with the exception that rosuvastatin C_{max} (maximum measured concentration in plasma) and AUC_{0-tz} (area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration) were 39 and 43% higher, respectively, when all four probe drugs were given together as a cocktail compared to when rosuvastatin was given alone [9]. This result was unexpected and not predictable based on available in vitro data. Based on an examination of published literature, it was considered that metformin was likely the perpetrator responsible for the increase in rosuvastatin systemic exposure [9, 12]. Also, inhibition of rosuvastatin transport by furosemide, for example via gastrointestinal efflux pumps, could not be ruled out as a potential underlying mechanism. Digoxin, on the other hand, was judged to be an unlikely perpetrator due to its generally low propensity to cause DDI and the low dose of 0.25 mg.

Because a cocktail without mutual interaction between the probe substrates would be preferable [4–6], the present trial was performed to investigate whether metformin or furosemide caused the increase in systemic exposure of rosuvastatin, and whether the interaction could be mitigated by using lower doses of the cocktail components, with the aim of optimizing the probe drug cocktail for investigation of transporter-mediated DDI.

2 Methods

2.1 Subjects

Eighteen healthy male subjects aged 18–55 years and with a body mass index of 18.5–29.9 kg/m^2 were eligible to

participate in this trial (EudraCT no. 2015-003052-46, ClinicalTrials.gov identifier NCT02574845). Women were not included, to avoid any potential interference of hormonal cycle or hormone-based contraceptives with the trial results. The clinical trial protocol was approved by the Ethics Commission of the State Chamber of Physicians of Baden-Württemberg, Stuttgart, Germany, and the Federal Institute for Drugs and Medicinal Products (BfArM), Bonn, Germany. The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonization guidelines for Good Clinical Practice. All subjects provided written informed consent.

2.2 Trial Objectives, Design and Treatments

The primary trial objective was to determine the relative bioavailability of rosuvastatin when administered orally together with three different oral doses of metformin or two different oral doses of furosemide (test treatments T), compared to rosuvastatin administered alone (reference treatment REF). This was a randomized, open label, single center, six-treatment, six-period, six-sequence crossover trial. Investigational medicinal products were rosuvastatin (Crestor® 10 mg film-coated tablet, AstraZeneca GmbH, Germany), metformin hydrochloride (MetfoLiquid GeriaSan® 500 mg/5 mL oral solution, Infectopharm Arzneimittel und Consilium GmbH, Germany), and furosemide (Lasix® liquidum 10 mg/mL oral solution, Sanofi-Aventis Deutschland GmbH, Germany). The following treatments were to be administered to each of the 18 subjects according to one of the six different randomly assigned treatment sequences, with three subjects per sequence and a washout period of at least 6 days between each individual treatment (based on 5 times the half-life of rosuvastatin, which is approximately 19 h [13]):

Reference (REF): 10 mg rosuvastatin
Test 1 (T1): 10 mg rosuvastatin and 10 mg (0.1 mL) metformin hydrochloride
Test 2 (T2): 10 mg rosuvastatin and 50 mg (0.5 mL) metformin hydrochloride
Test 3 (T3): 10 mg rosuvastatin and 500 mg (5 mL) metformin hydrochloride
Test 4 (T4): 10 mg rosuvastatin and 1 mg (0.1 mL) furosemide
Test 5 (T5): 10 mg rosuvastatin and 5 mg (0.5 mL) furosemide

After an overnight fast of at least 10 h, the trial medication was administered to the subjects in a standing position as a single oral dose together with 240 mL of water. During the first 4 h after medication administration, no food was allowed and subjects were not allowed to lie down.

2.3 Pharmacokinetics

Blood samples (4.9 mL) for the measurement of plasma concentrations of rosuvastatin, metformin and furosemide were taken using K_3-EDTA as anticoagulant from a forearm vein of each subject before dosing and at 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 24 h, 34 h and 48 h after dosing. Pharmacokinetic parameters were calculated using standard noncompartmental methods with the software Phoenix WinNonlin® Professional, version 6.3 (Certara, Princeton, USA).

The primary endpoints were AUC_{0-tz} and C_{max} for rosuvastatin, and the secondary endpoint was $AUC_{0-\infty}$ for rosuvastatin (area under the plasma concentration–time curve extrapolated to infinite time). Further endpoints were: (1) for rosuvastatin, $\%AUC_{tz-\infty}$ (percentage of $AUC_{0-\infty}$ obtained by extrapolation), t_{max} (time to attainment of maximum plasma concentration), $t_{1/2}$ (terminal elimination half-life), CL/F (apparent plasma clearance), V_z/F (apparent volume of distribution during the terminal elimination phase), and MRT_{po} (mean residence time); (2) for metformin and furosemide, AUC_{0-tz} and C_{max}; in addition $AUC_{0-\infty}$ for metformin only. The $t_{1/2}$ calculation of rosuvastatin was based on at least the last 3 plasma concentration time points (24, 34 and 48 h).

2.4 Bioanalytical Methods

Plasma concentrations of rosuvastatin, metformin, and furosemide were determined by fully validated [14, 15] HPLC–MS/MS (high-performance liquid chromatography–tandem mass spectrometry) methods using the isotope-labeled internal standards [$^{13}C_6$]furosemide, [$^{13}C_1$,$^2H_4$]rosuvastatin and [$^2H_6$]metformin. The assay for simultaneous determination of rosuvastatin (0.2–20 nmol/L) and furosemide (0.6–1500 nmol/L) was performed at Boehringer Ingelheim, Drug Metabolism & Pharmacokinetics, Biberach, Germany. It comprised sample clean-up by supported liquid extraction on Biotage Isolute 200 μL SLE+ 96 well plates with isopropyl acetate as extraction medium followed by chromatography on an analytical Waters xBridge BEH C8 2.5 μm analytical column. Monitored MS/MS transitions on a Sciex 6500QTrap mass spectrometer were from m/z 482.2 → 258.1 and 487.2 → 263.0 for rosuvastatin and its internal standard (positive ion mode) as well as from m/z 328.8 → 204.8 and 335.0 → 211.1 for furosemide and its internal standard (negative ion mode).

The assays for determination of metformin in the low (0.6–300 nmol/L) and high (38.7–19,400 nmol/L) concentration ranges were performed at SGS Cephac Europe, Saint Benoît Cedex, France. They comprised sample clean-up by solid phase extraction on Waters Seppack C18 extraction cartridges with MeOH/MeCN (50/50, v/v)

containing 1% of formic acid as elution medium followed by chromatography on analytical Phenomenex Kinetex C18 2.6 μm (low) or Synergi 4 μm polar RP 80A (high) analytical columns. Monitored MS/MS transitions on a Sciex API 4000 (3000) mass spectrometer were from m/z 130.0 (130.2) → 71.1 and 136.1 (136.2) → 77.1 for metformin and its internal standard (positive ion mode).

2.5 Safety Assessment

Safety and tolerability were assessed based on adverse events (AE), safety laboratory, vital signs, and 12-lead ECGs. The treated set (consisting of all subjects treated with at least one study drug) was used for safety analyses. AEs were analyzed according to the concept of treatment-emergent AEs and the number of subjects with AEs. AEs occurring within 4 days after study drug intake were defined as treatment-emergent and assigned to the corresponding treatment. The Medical Dictionary for Regulatory Activities (MedDRA) version 18.1 was used for coding of AEs.

2.6 Statistical Methods

The relative bioavailability of rosuvastatin administered together with the different doses of metformin and furosemide ("test" treatments T1, T2, T3, T4, T5) compared to rosuvastatin given alone ("reference" treatment REF) was investigated based on the geometric mean ratios (GMR) of test/reference and their two-sided 90% confidence intervals (CI) for the primary ($AUC_{0–tz}$, C_{max}) and secondary ($AUC_{0–\infty}$) endpoints. The statistical model used was analysis of variance (ANOVA) on the logarithmic scale and included the effects sequence, subjects within sequence, period and treatment. The effect 'subjects within sequence' was defined as random, whereas the other effects were fixed. The analysis set included all treated subjects who had at least one evaluable primary or secondary endpoint. The statistical methods of the primary analysis were further used to assess the proportionality of $AUC_{0–tz}$, C_{max} and $AUC_{0–\infty}$ among the different doses of metformin and furosemide used in the trial. No corrections were performed for multiple comparisons, due to the exploratory nature of the trial. All statistical calculations were performed with SAS® (version 9.4.by SAS Institute Inc., Cary, NC, USA).

The sample size was based on the expected precision (defined as ratio of upper 90% CI limit over point estimate) of the GMR estimate and the within-subject gCVs (geometric coefficients of variation) of the primary endpoints observed in [9]. The calculation was performed as described by Kupper and Hafner [16] using R Version 3.0.3. Assuming an intra-individual gCV of 20% and 18 evaluable subjects, the precision was expected to be 1.16 and

deemed sufficient for this trial. Based on these assumptions and a GMR of 100%, the 90% CI was expected to range from 86 to 116%. The dropout rate was expected to be considerably less than 6, the number of investigated treatment sequences. To avoid an unbalanced design in case of no dropouts, it was not planned to recruit additional subjects to account for possible dropouts.

3 Results

3.1 Subjects

Eighteen healthy white male subjects were randomized in the trial and treated. Their mean (SD; standard deviation) age was 31.9 (10.0) years and the mean (SD) body mass index was 26.03 (2.41) kg/m². Of the 18 treated subjects, 15 (83.3%) completed the planned observation time according to the protocol. One subject withdrew consent after the first treatment for personal reasons. Two subjects were withdrawn due to AEs that were not treatment-emergent or drug-related. Additionally, two subjects missed the second of the six treatments (not due to AE). For one subject, metformin administration in treatment T2 (rosuvastatin + 50 mg metformin) was incomplete because several drops of the oral solution were lost during dosing. The pharmacokinetic data of the subject for that treatment were not used for assessment of the primary endpoints.

3.2 Pharmacokinetics

3.2.1 Bioanalytical Methods

Assay performance during measurement of the trial samples was assessed by back-calculation of calibration standards, tabulation of the standard curve fit function parameters and measurement of quality control samples. Accuracy, measured as the deviation from target concentrations, ranged from −3.2 to −5.6% for rosuvastatin, −2.5 to −4.2% for furosemide, and −2.0 to 2.2% (low range), −1.7 to 1.2% (high range) for metformin. The assay precision (coefficient of variation) was 5.9–9.0% for rosuvastatin, 5.7–7.7% for furosemide, and 2.1–5.3% (low range), 1.5–2.9% (high range) for metformin. The results documented adequate accuracy, precision and specificity of the assays.

3.2.2 Effect of Metformin on Rosuvastatin Pharmacokinetics

Geometric mean plasma concentration–time profiles of rosuvastatin 10 mg when given alone (reference treatment) or together with increasing doses of metformin (test
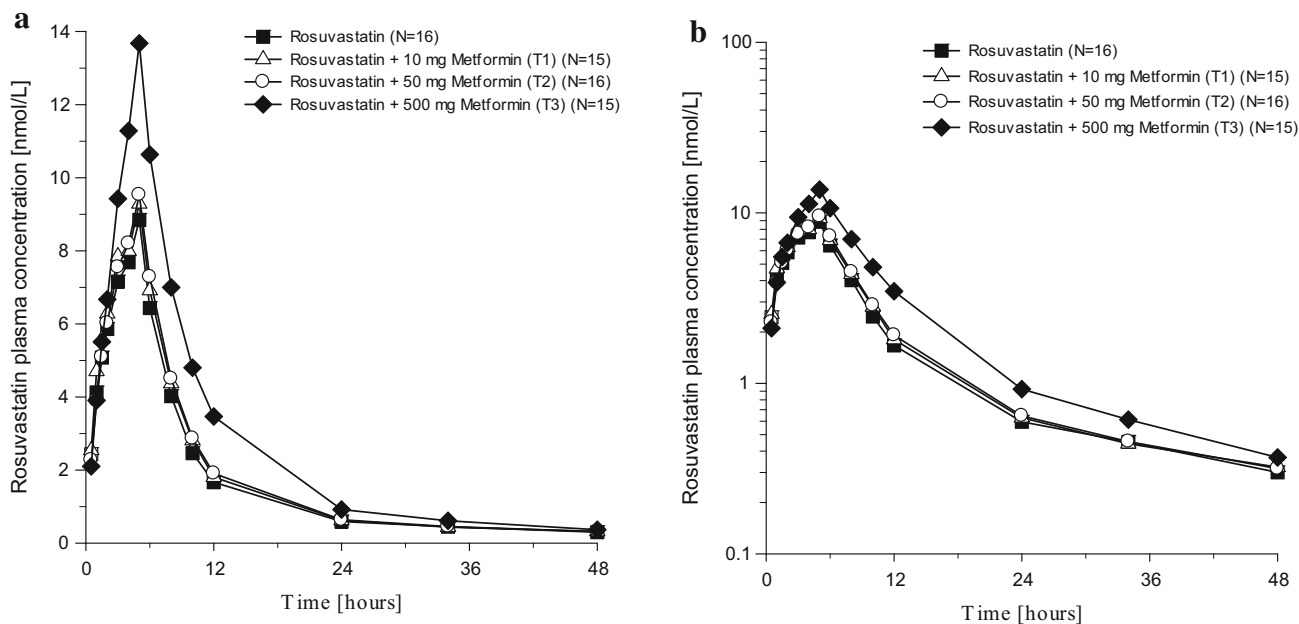
Fig. 1 Geometric mean plasma concentration–time profiles of rosuvastatin (10 mg) after oral dosing alone (reference), and in combination with different doses of metformin **a** linear and **b** semi-log. The doses of metformin were 10 mg (T1), 50 mg (T2) and 500 mg (T3)

treatments T1–T3) are shown in Fig. 1. The pharmacokinetic parameters of rosuvastatin are described in Tables 1 and 2. Maximum plasma concentrations of rosuvastatin were observed after a median t_{max} of 5 h in all treatment periods (Table 2). The profiles of rosuvastatin alone and together with metformin 10 mg (T1) and 50 mg (T2) were almost superimposable, whereas rosuvastatin plasma concentrations were clearly higher when given in combination with metformin 500 mg (T3) (Fig. 1). The relative bioavailabilities of rosuvastatin with metformin 10 mg (T1) and 50 mg (T2) compared to rosuvastatin alone were close to 100% for AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$, and all 90% CI included 100% and were in the range of 87–124%. However, the relative bioavailability of rosuvastatin with respect to these parameters increased to 146–154% when given with metformin 500 mg (T3), and all 90% CIs were in the range of 132–180% (Table 1). The precision of the $AUC_{0-\infty}$ values was considered high, with $\%AUC_{tz-\infty}$ values of around 10% or below (Table 2)

3.2.3 Effect of Furosemide on Rosuvastatin Pharmacokinetics

Geometric mean plasma concentration–time profiles of rosuvastatin 10 mg when given alone (reference treatment) or together with increasing doses of furosemide (test treatments T4 and T5) are shown in Fig. 2. The pharmacokinetic parameters of rosuvastatin are described in Tables 3 and 4. Maximum plasma concentrations of

rosuvastatin were again observed after a median t_{max} of 5 h in all treatment periods (Table 4). There was no change in plasma concentrations of rosuvastatin with furosemide 1 mg (T4) and only a slight increase with furosemide 5 mg (T5) (Fig. 2). The relative bioavailability of rosuvastatin with respect to AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$ was 106–107% with furosemide 1 mg (T4), and increased only slightly to 116–118% with furosemide 5 mg (T5). With furosemide 1 mg, the 90% CIs of the GMRs included 100% and ranged from 92 to 124%, but with furosemide 5 mg the 90% CI included 100% only for C_{max}. For the other endpoints, the lower limit of the 90% CI was already greater than 100% (Table 3). The precision of the $AUC_{0-\infty}$ values was considered high, with $\%AUC_{tz-\infty}$ values of around 10% (Table 4).

3.2.4 Metformin Pharmacokinetics

The pharmacokinetic parameters AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$ of metformin at the doses of 10, 50 and 500 mg administered together with 10 mg rosuvastatin (test treatments T1–T3) and their pairwise relative bioavailabilities are shown in Table 5. On increasing the metformin dose fivefold (from 10 to 50 mg), tenfold (from 50 to 500 mg) and 50-fold (from 10 to 500 mg), the pharmacokinetic parameters showed less than proportional increases; these were 4.1-, 7.9-, and 33-fold respectively for AUC_{0-tz}, and slightly lower factors for C_{max}.

Table 1 Geometric means and relative bioavailabilities for the primary and secondary pharmacokinetic endpoints of oral rosuvastatin 10 mg given alone (reference treatment REF) or together with different doses of metformin (test treatments T1–T3)

Endpoint	Test (T)		Reference (REF)		Ratio T/REF (%)	90% CI (%)	gCV (%)[a]
	Rosuvastatin + 10 mg metformin (T1)		Rosuvastatin alone				
	N	Adj. gMean	N	Adj. gMean			
AUC_{0-tz} (nmol·h/L)	15	85.96	16	84.32	101.95	(89.49; 116.15)	19.3
C_{max} (nmol/L)	15	9.54	16	9.31	102.47	(87.19; 120.42)	24.1
$AUC_{0-\infty}$ (nmol·h/L)	14	101.72	15	100.50	102.22	(89.23; 114.82)	17.8
Endpoint	Test (T)		Reference (REF)		Ratio T/REF (%)	90% CI (%)	gCV (%)[a]
	Rosuvastatin + 50 mg metformin (T2)		Rosuvastatin alone				
	N	Adj. gMean	N	Adj. gMean			
AUC_{0-tz} (nmol·h/L)	16	88.54	16	83.45	106.09	(96.12; 117.11)	14.6
C_{max} (nmol/L)	16	9.90	16	9.25	106.98	(92.54; 123.69)	21.7
$AUC_{0-\infty}$ (nmol·h/L)	16	103.96	15	96.93	107.26	(97.65; 117.81)	12.6
Endpoint	Test (T)		Reference (REF)		Ratio T/REF (%)	90% CI (%)	gCV (%)[a]
	Rosuvastatin + 500 mg metformin (T3)		Rosuvastatin alone				
	N	Adj. gMean	N	Adj. gMean			
AUC_{0-tz} (nmol·h/L)	15	128.94	16	84.73	152.18	(135.12; 171.41)	18.1
C_{max} (nmol/L)	15	14.48	16	9.40	154.07	(131.70; 180.24)	24.0
$AUC_{0-\infty}$ (nmol·h/L)	14	147.48	15	100.71	146.45	(135.21; 158.62)	11.4

AUC_{0-tz} area under the plasma concentration–time curve from time zero to last quantifiable concentration, C_{max} peak plasma concentration, $AUC_{0-\infty}$ area under the plasma concentration–time curve extrapolated to infinity, *CI* confidence interval, *gCV* geometric coefficient of variation, *Adj. gMean* adjusted geometric mean

[a] Within-subject

3.2.5 Furosemide Pharmacokinetics

AUC_{0-tz} and C_{max} of furosemide at the doses of 1 and 5 mg administered together with 10 mg rosuvastatin (test treatments T4 and T5) and the relative bioavailability of the 5 mg compared to the 1 mg dose are shown in Table 6. The GMRs of furosemide between the 5 and 1 mg doses were 5.3 (AUC_{0-tz}) and 5.2 (C_{max}) indicating dose proportionality.

3.3 Safety

Treatment-emergent AEs were reported by 9/18 subjects (50.0%). One subject reported mild or moderate headache that was assessed as drug-related by the investigator after REF, T1, and T2. No other AEs were assessed as drug-related. Two subjects discontinued the study due to AEs not defined as treatment-emergent and assessed as not drug-related. One subject was diagnosed with asymptomatic Wolff–Parkinson–White syndrome, a congenital ECG disorder, before treatment in period 2. In ECGs

before this time point, the abnormality had been hidden. Another subject reported severe back pain that caused him to drop out after period 4. No deaths, other treatment-emergent SAEs, or 'other significant AEs' occurred during the trial. There were no treatment-emergent clinically relevant findings in clinical laboratory, vital signs, or ECG. Rosuvastatin, metformin and furosemide were safe and well tolerated in this trial.

4 Discussion

The pharmacokinetic data obtained in this trial confirm and extend the results presented in our previous report, which was the first clinical investigation of mutual pharmacokinetic interactions within a dedicated drug transporter cocktail containing 0.25 mg digoxin, 5 mg furosemide, 500 mg metformin and 10 mg rosuvastatin [9]. In that initial trial, two additional dosing regimens were also studied that contained higher metformin and furosemide doses (1000 and 20 mg, respectively) to simulate

Table 2 Additional pharmacokinetic parameters of oral rosuvastatin 10 mg given alone (reference treatment) or together with different doses of metformin (test treatments T1–T3)

	Rosuvastatin alone			Rosuvastatin + 10 mg metformin (T1)		
	N	gMean	gCV (%)	N	gMean	gCV (%)
t_{max} (h)[a]	16	5.00	3.00–5.03	15	5.00	3.00–5.03
$t_{1/2}$ (h)	15	24.6	54.2	14	23.4	57.3
V_z/F (L)	15	7390	87.1	14	6650	82.5
CL/F (mL/min)	15	3470	39.0	14	3290	48.0
%$AUC_{tz-\infty}$ (%)	15	10.6	77.2	14	9.85	74.0
MTR_{po} (h)	15	20.3	56.6	14	19.1	61.5
	Rosuvastatin + 50 mg metformin (T2)			Rosuvastatin + 500 mg metformin (T3)		
	N	gMean	gCV (%)	N	gMean	gCV (%)
t_{max} (h)[a]	16	5.00	1.50–6.00	15	5.00	4.00–5.02
$t_{1/2}$ (h)	16	23.0	38.4	14	17.5	32.1
V_z/F (L)	16	6790	84.8	14	3520	80.5
CL/F (mL/min)	16	3410	55.0	14	2330	49.8
%$AUC_{tz-\infty}$ [%]	16	10.1	76.6	14	6.09	60.0
MRT_{po} (h)	16	18.6	40.7	14	15.1	21.2

gMean geometric mean, *CL/F* apparent plasma clearance, *gCV* geometric coefficient of variation, $t_{1/2}$ terminal elimination half-life, t_{max} time to maximum plasma concentration, V_z/F apparent volume of distribution during the terminal elimination phase; %$AUC_{tz-\infty}$ percentage of $AUC_{0-\infty}$ obtained by extrapolation, MRT_{po} mean residence time of the analyte in the body after oral administration

[a] Median and range
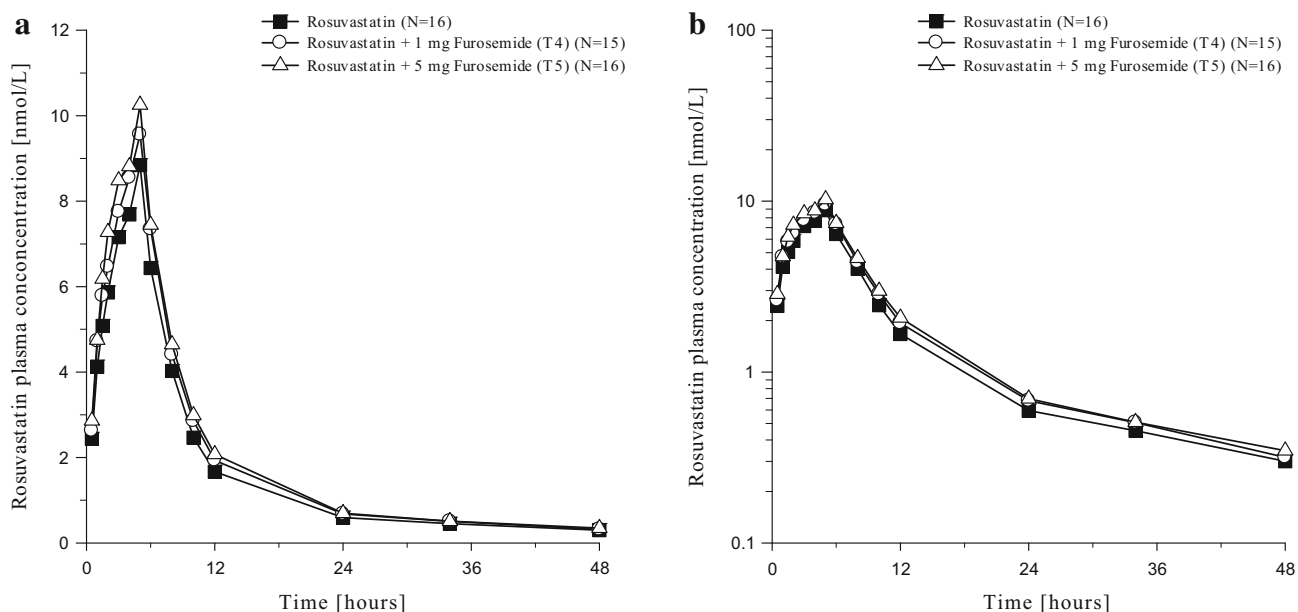
Fig. 2 Geometric mean plasma concentration–time profiles of rosuvastatin (10 mg) after oral dosing alone (reference), and in combination with different doses of furosemide **a** linear and **b** semi-log. The doses of furosemide were 1 mg (T4) and 5 mg (T5)

conditions in which these drugs could act as perpetrators of drug-transporter interactions [9]. It was found that the most pronounced pharmacokinetic change identified in the trial—an approximate 40% increase in rosuvastatin C_{max} and AUC compared to dosing alone—did not increase further at the higher doses of metformin and furosemide. In the present trial, the metformin and furosemide dose ranges were adjusted to substantially lower levels—from 500 to 50 mg and 10 mg for metformin and from 5 to 1 mg for furosemide—in order to establish "no-effect" doses for

Table 3 Geometric means and relative bioavailabilities for the primary and secondary pharmacokinetic endpoints of rosuvastatin 10 mg given alone (reference treatment REF) or together with different doses of furosemide (test treatments T4 and T5)

Endpoint	Test (T)		Reference (REF)		Ratio T/REF (%)	90% CI (%)	gCV (%)[a]
	Rosuvastatin + 1 mg furosemide (T4)		Rosuvastatin alone				
	N	Adj. gMean	N	Adj. gMean			
AUC_{0-tz} (nmol·h/L)	15	90.28	16	84.40	106.97	(94.34; 121.30)	18.9
C_{max} (nmol/L)	15	9.94	16	9.31	106.81	(91.78; 124.30)	22.9
$AUC_{0-\infty}$ (nmol·h/L)	15	106.40	15	100.73	105.63	(92.20; 121.00)	20.4
Endpoint	Test (T)		Reference (REF)		Ratio T/REF	90% CI	gCV (%)[a]
	Rosuvastatin + 5 mg furosemide (T5)		Rosuvastatin alone				
	N	Adj. gMean	N	Adj. gMean			
AUC_{0-tz} (nmol·h/L)	16	94.29	16	81.34	115.92	(101.93; 131.82)	19.7
C_{max} (nmol/L)	16	10.65	16	9.02	117.98	(98.27; 141.65)	28.4
$AUC_{0-\infty}$ (nmol·h/L)	16	113.43	15	96.04	118.10	(106.75; 130.67)	15.4

AUC_{0-tz} area under the plasma concentration–time curve from time zero to last quantifiable concentration, C_{max} peak plasma concentration, $AUC_{0-\infty}$ area under the plasma concentration–time curve extrapolated to infinity, *CI* confidence interval, *gCV* geometric coefficient of variation, *Adj. gMean* adjusted geometric mean

[a] Within-subject

Table 4 Additional pharmacokinetic parameters of oral rosuvastatin 10 mg given alone (reference treatment) or together with different doses of furosemide (test treatments T4 and T5)

	Rosuvastatin alone			Rosuvastatin + 1 mg furosemide (T4)			Rosuvastatin + 5 mg furosemide (T5)		
	N	gMean	gCV (%)	N	gMean	gCV (%)	N	gMean	gCV (%)
t_{max} (h)[a]	16	5.00	3.00–5.03	15	5.00	3.00–5.05	16	5.00	2.03–5.02
$t_{1/2}$ (h)	15	24.6	54.2	15	23.0	61.8	16	25.1	61.8
V_z/F (L)	15	7390	87.1	15	6390	90.6	16	6530	93.8
CL/F (mL/min)	15	3470	39.0	15	3210	47.9	16	3000	42.0
%$AUC_{tz-\infty}$ (%)	15	10.6	77.2	15	9.81	92.5	16	10.8	83.5
MRT_{po} (h)	15	20.3	56.6	15	19.6	69.3	16	20.6	69.7

gMean geometric mean, *CL/F* apparent plasma clearance, *gCV* geometric coefficient of variation, $t_{1/2}$ terminal elimination half-life, t_{max} time to maximum plasma concentration, V_z/F apparent volume of distribution during the terminal elimination phase, %$AUC_{tz-\infty}$ percentage of $AUC_{0-\infty}$ obtained by extrapolation, MRT_{po} mean residence time of the analyte in the body after oral administration

[a] Median and range

these compounds with respect to their influence on the AUC and C_{max} of rosuvastatin.

The increase in AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$ of 10 mg rosuvastatin by 46–54% after coadministration with 500 mg metformin (T3), Table 1, was comparable to that observed after coadministration with 500 mg metformin, 5 mg furosemide and 0.25 mg digoxin in the initial trial, where AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$ values increased by 43, 39 and 28%, respectively [9].

Reduction of the dose of metformin to 50 mg (T2) and 10 mg (T1) in the current trial was sufficient to show

similarity of rosuvastatin AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$ values (Table 1). Additional pharmacokinetic parameters were also not relevantly changed (Table 2). It is therefore feasible to reduce the metformin dose in the original cocktail from 500 to 50 mg or 10 mg to eliminate this interaction. There was a slight increase in AUC_{0-tz}, C_{max} and $AUC_{0-\infty}$ of 10 mg rosuvastatin by 16–18% in this trial with coadministration of 5 mg furosemide (T5) as shown in Table 3. Here also, further reduction of the furosemide dose to 1 mg (T4) resulted in similarity of the rosuvastatin pharmacokinetic parameters, with relative bioavailabilities

Table 5 Dose proportionality assessment for pharmacokinetic parameters of metformin at three doses (test treatments T1–T3) in the presence of 10 mg rosuvastatin

Endpoint	Test (T)		Reference (REF)		Ratio T/REF	90% CI	Dose ratio	(T/REF) as a fraction of dose ratio
	T2 (50 mg metformin)		T1 (10 mg metformin)					
	N	Adj. gMean	N	Adj. gMean				
AUC_{0-tz} (nmol·h/L)	16	6382.07	15	1554.00	4.11	(3.87; 4.36)	5	0.822
C_{max} (nmol/L)	16	990.39	15	273.27	3.62	(3.16; 4.15)	5	0.724
$AUC_{0-\infty}$ (nmol·h/L)	16	6418.12	12	1559.11	4.12	(3.83; 4.43)	5	0.824
Endpoint	Test (T)		Reference (REF)		Ratio T/REF	90% CI	Dose ratio	(T/REF) as a fraction of dose ratio
	T3 (500 mg metformin)		T2 (50 mg metformin)					
	N	Adj. gMean	N	Adj. gMean				
AUC_{0-tz} (nmol·h/L)	15	50027.10	16	6333.14	7.90	(7.55; 8.27)	10	0.790
C_{max} (nmol/L)	15	7129.32	16	999.06	7.14	(6.54; 7.78)	10	0.714
$AUC_{0-\infty}$ (nmol·h/L)	15	50491.90	16	6374.86	7.92	(7.58; 8.28)	10	0.792
Endpoint	Test (T)		Reference (REF)		Ratio T/REF	90% CI	Dose ratio	(T/REF) as a fraction of dose ratio
	T3 (500 mg metformin)		T1 (10 mg metformin)					
	N	Adj. gMean	N	Adj. gMean				
AUC_{0-tz} (nmol·h/L)	15	50547.30	15	1553.37	32.54	(31.12; 34.03)	50	0.651
C_{max} (nmol/L)	15	7193.55	15	266.59	26.98	(23.95; 30.40)	50	0.540
$AUC_{0-\infty}$ (nmol·h/L)	15	51017.10	12	1537.88	33.17	(31.24; 35.22)	50	0.663

AUC_{0-tz} area under the plasma concentration–time curve from time zero to last quantifiable concentration, C_{max} peak plasma concentration, $AUC_{0-\infty}$ area under the plasma concentration–time curve extrapolated to infinity, *CI* confidence interval, *Adj. gMean* adjusted geometric mean

Table 6 Dose proportionality assessment for pharmacokinetic parameters of furosemide at two doses (test treatments T4 and T5) in the presence of rosuvastatin

Endpoint	Test (T)		Reference (REF)		Ratio T/REF	90% CI	Dose ratio	(T/REF) as a fraction of dose ratio
	T5 (5 mg furosemide)		T4 (1 mg furosemide)					
	N	Adj. gMean	N	Adj. gMean				
AUC_{0-tz} (nmol·h/L)	16	980.05	15	183.59	5.34	(4.91; 5.80)	5	1.068
C_{max} (nmol/L)	16	493.03	15	94.72	5.21	(4.53; 5.98)	5	1.042

AUC_{0-tz} area under the plasma concentration–time curve from time zero to last quantifiable concentration, C_{max} peak plasma concentration, *CI* confidence interval, *Adj. gMean* adjusted geometric mean

of AUC and C_{max} (GMRs) around 107% and all 90% CIs including 100% (Table 3), and additional pharmacokinetic parameters were not relevantly changed (Table 4). The furosemide dose in the original cocktail could therefore be reduced from 5 to 1 mg to effectively eliminate this slight interaction. Taken together, the present results indicate that the increase in rosuvastatin bioavailability in the cocktail in the initial trial [9] was most likely principally caused by metformin, but with a small contribution by furosemide.

A relatively minor interaction of metformin with rosuvastatin was also reported by Lee et al. [12] in a steady-state trial in Asian subjects with 750 mg metformin and 10 mg rosuvastatin q.d. In that study, the $AUC_{\tau,ss}$ of rosuvastatin was hardly affected by coadministration of metformin (11% increase, CIs within the acceptance limits), and $C_{max,ss}$ increased slightly by 23% (CIs outside the acceptance limits). These changes—in common with those we observed in this trial and the previous report [9]—are

very small in comparison to those caused by rifampin, which is a potent inhibitor of OATP1B1, OATP1B3 and an inhibitor of BCRP [10, 17]. To our knowledge, no studies have been published to date on any clinical interaction of furosemide with rosuvastatin.

In our previous trial [9], the increase of the systemic rosuvastatin exposure in the cocktail by approximately 40% was accompanied by an increase in the fraction of rosuvastatin excreted in urine without a relevant change in its renal clearance. This indicates that metformin and furosemide interact with rosuvastatin on an extrarenal mechanism. The molecular mechanisms underlying the increased AUC and C_{max} of rosuvastatin caused by the higher doses of metformin and furosemide in this trial require consideration of the known transporters for which rosuvastatin is a substrate. Based on in vitro data, Ebner et al. assessed the risk for interaction within the cocktail at the level of OATP1B- and BCRP-mediated rosuvastatin transport as remote [11]. In addition, rosuvastatin transport was reported for multidrug resistance protein 2 (MRP2) [18, 19], OATP2B1 (transport data are controversial) [18, 20, 21] and sodium taurocholate cotransporting polypeptide (NTCP) [22, 23]. However, the risk for mutual interaction within the cocktail at these transporters is also regarded as remote (Boehringer Ingelheim, data on file). Based on the available data, the precise molecular mechanisms underlying the interactions of rosuvastatin with metformin and furosemide are currently unknown, although mechanisms affecting intestinal absorption or intestinal metabolism may be involved that would require further in vitro and in vivo work for clarification.

This trial also allowed the assessment of the dose proportionality of the pharmacokinetics of metformin and furosemide in the presence of 10 mg rosuvastatin. Metformin exhibited less-than-proportional increases of AUC and C_{max} values with dose (Table 5), similar to the observations in the initial trial using doses of 500 and 1000 mg [9]. Kusuhara et al. reported that AUC_{0-12h} of metformin showed dose proportionality when comparing a microdose of 100 μg and a therapeutic dose of 250 mg in healthy subjects [24], but the dose range used was different and blood sampling was not as extended as in the present trial. Metformin dose non-proportionality could be explained by a decreasing extent of absorption with increasing dose [25]. Non-dose proportional absorption would not be expected to affect the sensitivity of the cocktail for detection of clinically relevant inhibition of OCT2 or MATEs localized in the kidney. The dose-proportionality of furosemide pharmacokinetics observed between 1 and 5 mg (Table 6) is in agreement with and extends literature data describing dose proportionality in the range of 20–80 mg [26].

Another observation in the original cocktail was a decrease of furosemide C_{max} by 19% as compared to furosemide 5 mg alone without a change in furosemide renal clearance, suggesting an extrarenal mechanism [9]. This minor and presumably extrarenal interaction is not expected to affect the sensitivity of the cocktail for inhibition of renal transporters OAT1 and OAT3; however, a fully interaction-free cocktail would be preferable. Our finding is consistent with a previous report of a 31% decrease of furosemide C_{max} when given together with metformin [27]. If indeed metformin is the perpetrator of this relatively minor interaction, a 10- or 50-fold decrease of metformin dose from 500 mg in the original cocktail to 50 or 10 mg could, as a positive secondary effect, free the cocktail from significant changes in furosemide plasma concentrations.

On the basis of the new results gained in this trial, it is proposed that the four-component drug transporter cocktail [9] be re-evaluated and further clinically tested using the doses 0.25 mg digoxin, 1 mg furosemide (previously 5 mg), 10 or 50 mg metformin (previously 500 mg) and 10 mg rosuvastatin. Such a trial is ongoing and will determine the pharmacokinetics in plasma and urine of each of the components alone and in presence of the other three and is expected to show minimal mutual interactions.

5 Conclusion

The increased exposure of rosuvastatin that was observed when administered as part of the proposed transporter cocktail containing digoxin, metformin, furosemide, and rosuvastatin compared to rosuvastatin alone is most likely attributable to metformin, and only to a minor degree to furosemide. Reduced doses of metformin and furosemide are expected to eliminate mutual pharmacokinetic interactions in a drug transporter cocktail, which will be a valuable tool in drug development.

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