

Dose, mg	Mean AUC0-23 Na ⁺ ^a mEq • h	Mean AUC0-23 Na ⁺ ^b mEq • h	Mean AUC0-23 OPC-41061 ng • h/mL
10	na	na	na
15	-5.5	30.7	1159
30	42.4	78.6	4357
60	7.6	43.8	7264
90	-5.5	27.9	5814
120	58.7	94.9	8524

^a Uncorrected for pooled placebo ^b Pooled placebo corrected na= not available

There is no apparent relationship between mean change from baseline in AUC0-23 of plasma Na⁺ and mean AUC0-23 of OPC-41061 in CHF patients..

Conclusions

PK

The pharmacokinetics of OPC-41061 in patients with hypervolemic CHF can be considered dose proportional. There is substantial inter-subject variation in peak and average exposure to OPC-41061 in patients with mild to moderate CHF. Oral clearance ranges between 0.93 and 3.69 mL/min/kg. Regimens with qd administration of OPC-41061 result in a mean accumulation of 1.1. The individual C_{max} and C₂₃ values and their ratio on Days 1 and 13 among groups and subjects vary significantly. However, the respective values of C_{max}, C₂₃ and their ratio on Days 1 and 13 in the individual patients are remarkably similar indicating small intra-subject variability. Variable absorption determines the C_{max} to C₂₃ ratio and the decline of the post C_{max} concentrations of OPC-41061.

PD

The net effect of OPC-41061 of the OPC-410-61 regimens with 30, 90 and 120 mg qd appears to be an increase in the plasma Na⁺ concentrations. [REDACTED]

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[REDACTED]. There is no overt evidence that OPC-41061 in the tested doses impacts plasma K⁺.

OPC-41061 consistently and dose dependently increases the 24 h urine volume during the first 3 treatment days more than placebo. The increase in urine volume at the 60, 90, and 120 mg dose levels on Days 2 and 3 appears to be smaller than on Day 1 suggesting increased retention of fluid and/or decreased fluid intake. A continuous aquaretic effect (net rate ≥ 1 mL/min) is attained on Day 1 with dose levels of ≥ 30 mg. At these dose levels the onset of aquaretic activity is observed in the first collection interval of 0-4 h post-dose. Peak rates are seen in the 0-4 or 4-8 h interval and the time duration of the aquaretic effect ranges between 6 and 18 h at dose levels ≥ 30 mL/min.

The mean reduction in urine osmolality in the patients receiving OPC-41061 at all dose levels appears to be consistently greater than in the patients receiving placebo. The reduction in urine osmolality appears to be similar on treatment Days 1, 2 and 3. The patients receiving 30 mg to 120 mg OPC-41061 qd tend to show a greater reduction in mean 24 h sodium excretion in the first 3 days of treatment compared to baseline than the patients receiving placebo.

PD-PK Correlation

The mean net 24 h urine excretion rate appears to increase with increasing exposure to tolvaptan in the patients with CHF on Day 1 of the treatment.

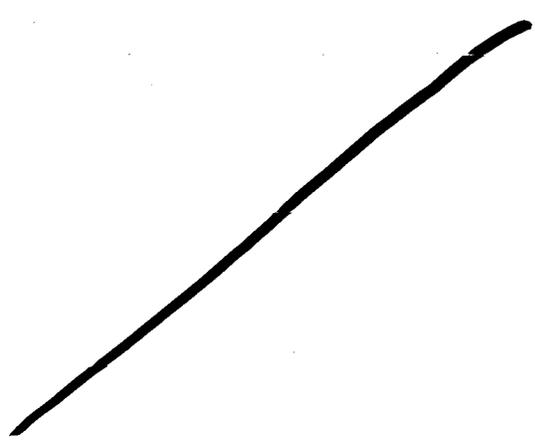
Comments

1. Patients had to exhibit extra-cellular volume expansion in the opinion of the investigator to be enrolled in the study. The inclusion criteria do not state the criteria to be met for diagnosing extra-cellular volume expansion.
2. The inter-subject and intra-subject variation in peak and trough concentrations of OPC-41061 should be assessed
3. The estimates for λ_z were obtained from 3 non-zero concentrations measured during intervals 12-16 h. The mean $t_{1/2z}$ estimates by the sponsor ranged between 8 h and 13 h. λ_z (or $0.693/t_{1/2z}$) should not be estimated from an interval that is clearly smaller than $3 \cdot t_{1/2z}$. Thus, λ_z and the derived parameters including $t_{1/2z}$, $AUC_{0-\infty}$ and CL/F are not reliable.
4. The accumulation ratio should be determined from AUC not from C_{max} . The accumulation ratio RAUC should be determined from $AUC_{ss,\tau}(\text{Day } 13)/AUC_{0-24}(\text{Day } 1)$, not from $AUC_{ss,\tau}/AUC_{\infty}$.
5. Blood samples should have been collected at 24 h post dose on Days 1 and 13.

Study Report No. 156-01-231: "A Multicenter, Randomized, Double-Blind, Parallel-Group Study to Compare the Effects 30 mg QD versus 15 mg BID Oral Administration of Tolvaptan (OPC-41061) in Patients with Congestive Heart Failure"

Investigators and Study Sites

The 10 participating investigators and Centers are listed below:



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Objectives

Primary: to assess the pharmacodynamic and clinical effects of tolvaptan 30 mg once daily (qd) versus tolvaptan 15 mg twice daily (BID) over a period of 7 days.

Secondary: to evaluate the pharmacokinetics of tolvaptan 30 mg qd and tolvaptan bid over a period of 7 days.

Investigational Drugs and Formulations

Tolvaptan 15 mg tablets (Lot No. 99E87A015) and 30 mg tablets (Lot No. 99E87A030A) and matching placebo tablets (Lot No. 99D96P000) were provided by the sponsor.

Design

This was a multicenter, randomized, double-blind study in 2 parallel groups. The study consisted of a screening day (Day -5) and a 48 h washout from current diuretics (Days -2 and -1) followed by 7 days of dosing (Days 1-7). Subjects were hospitalized from Day -1 through Day 2. Subjects were readmitted to the hospital on Day 7 and discharge on Day 8. Two groups (1:1 ratio) were randomized to receive either tolvaptan 30 mg qd or tolvaptan 15 mg bid. One group of patients received a single dose 30 mg tolvaptan in the morning and matching placebo tablet in the afternoon. An asymmetrical dosing interval was selected: the morning dose was administered at 0800 and the afternoon dose was administered at 1600 in order to minimize disruption of sleep. A maximum diuretic effect of tolvaptan is reportedly seen 8 h after administration. The second group received one 15 mg tablet in the morning and one tablet in the afternoon. The first dose each day was administered with the subjects in the fasted state, while this was not required for the second dose. A previous study had shown that the extent of drug absorption from tablets is not impacted by food, only the rate of absorption is slowed by food. All subjects were prescribed a 2 g/day Na⁺ starting at screening (Day -5) and maintained throughout the study. The duration of treatment was 7 days. A follow-up telephone contact occurred 7 days after the last dose of study drug.

Secondary pharmacodynamic variables included urine volume, urine excretion rate, serum electrolytes (Na⁺, K⁺ and Mg²⁺), serum osmolality, creatinine clearance, dyspnea, orthopnea, edema (pedal), jugular venous pressure, rales and hepatomegaly.

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Exclusion and inclusion criteria are listed below:

1.	Age greater than or equal to 18 years.
2.	Gender: male and female subjects that were surgically sterile or were prepared to agree to practice a double-barrier form of birth control from the screening visit through 30 days after the last dose of study medication (refer to Protocol Section 5.4). A negative serum pregnancy test was confirmed prior to first dose for women of childbearing potential. Females who were more than 12 months postmenopausal and not on hormone replacement therapy were also eligible to participate in the study.
3.	Informed Consent: eligible subjects were required to sign an IRB-approved informed consent form prior to the initiation of any study procedures.
4.	History of congestive heart failure.
5.	Heart failure symptoms classified as New York Heart Association (NYHA) class II or III for at least 30 days prior to the screening day.
6.	In the judgment of the investigator, the subject had persistent water overload with evidence of current hepatjugular reflux, jugular venous distention, pitting edema, or rales.
7.	Ejection fraction \geq 40% within one year assessed by either 2D-echo, radionuclide ventriculogram, angiography, or gated single photon emission computed tomography (SPECT).

Source: Appendix F-1.

1.	Women who were breast-feeding and females of childbearing potential who were not using acceptable double-barrier contraceptive methods (such as barrier contraceptives or methods that result in a low failure rate of less than 1%). Hormonal contraceptives were not permitted. All females of childbearing potential were required to have a negative serum pregnancy test with results available prior to receiving study drug. Non-childbearing potential was defined as either postmenopausal (12 consecutive months without menses) or surgically sterile. Tubal ligation or vasectomy was not considered an acceptable surgical sterilization. Another form of contraception was required.
2.	Cardiac surgery within 90 days.
3.	Percutaneous coronary interventions and implantable cardioverter defibrillator (ICD) implant within 30 days of potential study enrollment.
4.	History of sustained ventricular tachycardia or ventricular fibrillation, or discharge of an automatic ICD within 30 days of enrollment.
5.	CHF related to tachy- or bradyarrhythmias.
6.	Unstable angina or myocardial infarction (documented by ECG or enzymes) within 3 months of study enrollment.
7.	Systolic arterial blood pressure \leq 90 mmHg (sitting and standing).
8.	Symptomatic ventricular arrhythmia, or atrial fibrillation with ventricular rate $>$ 118 beats per minute (bpm).
9.	Hemodynamically significant primary cardiac valvular disease.
10.	Hypertrophic cardiomyopathy (obstructive or non-obstructive).
11.	History of a cerebrovascular accident within the last 6 months.
12.	Significant hepatic, renal or hematological disorder or dysfunction beyond that expected from CHF alone.
13.	Use of drugs or substances known to inhibit telapristin metabolism within 30 days of dosing or during the course of the study. (See Table 5.4.6-2)
14.	Use of xanthine-containing food or drinks, grapefruit, grapefruit juice products, Seville oranges and alcohol containing drinks within 72 hours of admission to the study unit and for the duration of the study.
15.	History of hypersensitivity and/or idiosyncratic reaction to benzazepine derivatives (such as benzazepin).
16.	A requirement for treatment with nonsteroidal anti-inflammatory agents, or aspirin at a dose \geq 750 mg/day.
17.	History of drug or medication abuse within the past year, or current alcohol abuse.

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18.	Positive drug/alcohol test at screening.
19.	History of hypernatremia.
20.	Uncontrolled diabetes mellitus.
21.	History of positive human immunodeficiency virus (HIV) test or acquired immunodeficiency syndrome (AIDS).
22.	Urinary tract obstruction.
23.	Morbid obesity, as defined by the investigator.
24.	Previous participation in this or any other OPC-1061 clinical trial.
25.	Volunteers having taken an investigational drug within the four weeks which preceded study entry.
26.	The donation of blood or plasma within 30 days prior to study enrollment.
27.	Presence of malignancy at screening, excluding basal cell carcinoma.
28.	Acute pulmonary edema.
29.	Serum creatinine > 3.0 mg/dL or blood urea nitrogen > 60 mg/dL.
30.	Serum potassium < 4.0 mEq/L.
31.	Prothrombin time (PT) value > 18.5 seconds.
32.	Unable to tolerate the 48-hour washout period.
33.	Terminally ill or moribund condition with little chance of short-term survival.
34.	Any subject who, in the opinion of the sponsor or investigator, should not participate in the study.

Source: Appendix I-1.

The medications permitted and prohibited prior to and during the study are shown in the below 2 tables:

1.	ACE-inhibitors
2.	Beta-blockers
3.	Digoxin
4.	Vasodilators (including nitrates and hydralazine)
5.	Angiotensin II receptor blockers
6.	Calcium channel blockers

Source: Appendix I-1.

1.	Ketoconazole
2.	Troleandomycin
3.	Miconazole
4.	Amiodarone
5.	Cimetidine
6.	Clotrimazole
7.	Danazol
8.	Dexamethasone
9.	Diltiazem
10.	Erythromycin
11.	Fluconazole
12.	Griseofulvin
13.	Indinavir
14.	Itraconazole
15.	Trisacryloylchondromycin
16.	Quinidine
17.	Quinine
18.	Ritonavir
19.	Saquinavir
20.	Verapamil
21.	Zalcitabine
22.	Zidovudine
23.	Diuretics
24.	Hormonal contraceptives

Source: Appendix I-1 and Section 13.

The scheduled study activities are listed in the below scheme:

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Table 5.5-1 Schedule of Assessments I

Period	Screening ^a		48-Hour Washout		Double-blind Treatment							
	-5	-1	-2	-1	Inpatient		Outpatient				Inpatient	
Day	-5	-1	-2	-1	1	2	3	4	5	6	7	8
Procedure												
Informed Consent	X											
Inclusion/Exclusion Criteria	X			X								
Medical History & Prior Medications ^b	X											
Physical Examination (including weight) ^c	X			X	X	X					X	X
Vital Signs ^d	X			X	X	X					X	X
12-Lead ECG ^e	X			X	X	X					X	X
Serum Pregnancy (if applicable)	X											
Urine Pregnancy (if applicable)				X ^f							X	
Safety Labs ^g	X			X	X	X					X	X
Drug & Alcohol Screen				X ^h							X ⁱ	
2 g/day sodium diet	X	X	X	X	X	X	X	X	X	X	X	X
Telephone Notification to Start Washout		X										
Withdrawn From Diuretic Therapies			X	X	X	X	X	X	X	X	X	X ^h
Randomization				X								

Table 5.5-1 Schedule of Assessments I

Period	Screening ^a		48-Hour Washout		Double-blind Treatment							
	-5	-1	-2	-1	Inpatient		Outpatient				Inpatient	
Day	-5	-1	-2	-1	1	2	3	4	5	6	7	8
Procedure												
Admit to Hospital				X							X ⁱ	
Study Drug Dosing at 0800 and 1600				X	X	X	X	X	X	X	X	X
24-Hour Urine Collection ^j				X	X						X	X
PK Sampling ^k				X	X	X					X	X
Serum Creatinine ^l				X	X						X	
Pharmacodynamic and Cardiovascular Assessments ^m	X			X	X	X					X	X
Discharge From Hospital						X ⁿ						X
Adverse Event Assessment	X	X		X	X	X					X	X
Concomitant Medications	X	X		X	X	X					X	X

^a Results of all screening assessments were required to be available prior to the start of the 48-hour washout period.
^b History included a medical and cardiovascular history. All medications taken by the subject in the past 30 days were recorded.
^c Physical examination, 12-lead ECG and safety labs were conducted prior to the 0800 dose. Weight was measured prior to the 0800 dose after the subject had voided.
^d Vital signs included blood pressure, heart rate, and respiratory rate. Vital signs were obtained again after ≥ 5 minutes and again after ≥ 2 hours. Vital signs were collected prior to each inpatient dose of study medication.
^e Results were required prior to randomization.
^f Hematology, chemistry, urinalysis. Collected prior to the 0800 dose (if applicable).
^g Performed by the site's local lab.
^h Standard diuretic therapy was resinitiated after completion of assessments.
ⁱ Study medication was collected from subject.
^j Collection was started at 0800 hours. 24-hour collection on Days -1, 1, and 7. (Urine from each 24-hour period was pooled and urine volume, osmolality, pH, sodium and creatinine concentrations were recorded.)
^k See Section 3.5.1.
^l Serum creatinine was collected 12 hours after the 0800 dose. On Day -1, serum creatinine was collected at 2600 hours.
^m Pharmacodynamic and cardiovascular assessments included: edema (pedal and sacral), JVP, rales, hepatomegaly, dyspnea, orthopnea, fatigue, heart sounds, palpitation, murmur, NYHA classification, Heart Failure Score, serum electrolytes (sodium, potassium, magnesium), and serum osmolality. All assessments were to be completed at the same time each day, prior to the 0800 dose.
ⁿ Study medication was dispensed to subject.

Table 5.5-2 Schedule of Assessments II

Period	Follow-up		
	9	10	14/ET
Procedure			
PK Sampling ^a	X ^b	X ^c	X ^d
Physical Exam (including weight)			X
Vital Signs			X
12-Lead ECG			X
Safety Labs			X
Pharmacodynamic and Cardiovascular Assessments			X
Discharge From Study			X
Adverse Event Assessment	X	X	X
Concomitant Medications	X	X	X

^a Follow-up phone-call was completed 7 days after the last dose of study medication.
^b See Section 3.5.1.
^c PK samples at 40 hours post Day 7 Dose 2.
^d PK samples at 64 hours post Day 7 Dose 2.
^e PK samples at 88 hours post Day 7 Dose 2.

Safety

Safety data included adverse events, clinical laboratory tests, physical examination, vital signs, 12-Lead ECGs and medical history. The activities were performed according to the schedule shown above.

Pharmacokinetic Profiling

Blood samples for the determination of OPC-41061 were collected on:

Days 1 and 7: pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 24 h after administration
Additional blood samples were collected on Day 8 (24 h after administration of the last dose), Day 9 (48 h after administration of last dose) and Day 10 (72 h after administration of the last dose).

Bioassay

The plasma concentrations of OPC-41061 and its metabolite were determined by a validated LC/MS/MS method with an internal standard. The method for OPC-41061 and the metabolite is linear in the concentration range 5.00 ng/mL to 1000 ng/mL and 12.5 to 2500 ng/mL, respectively. The metabolite concentrations were not reported for this protocol. The mean correlation coefficient for OPC-41061 was 0.9976. The mean accuracy ranges between -8.14 % and 1.74 % and the mean precision is $\leq 7.04\%$ with QC samples that were analyzed along the samples with unknown OPC-41061 concentrations. Stability of OPC-41061 was demonstrated by exposure of plasma for 24 h to room temperature, long term freezer conditions at -70° for 22 days and 4 freeze/thaw cycles, and exposure of the extract at 4° C for 67 h. b(4)

Pharmacokinetic Data Analysis

The following PK parameters were determined: C_{max} , t_{max} and AUC_{24} were determined with the Day 1 data. $C_{min,ss}$, $C_{max,ss}$, λ_z , AUC_{τ} , $C_{avg,ss}$, CL_{ss}/F and V_z/F were determined with the Day 7 data. In addition accumulation of OPC-41061 was estimated from $R_{C_{max}} (=C_{max,ss}/C_{max1})$ and $R_{AUC_{\tau}} (=AUC_{\tau}/AUC_{24})$. Non-compartmental methods were applied using WinNonlin®, Version 3.3 (Pharsight Corporation, Mountain View, CA). If $t_{last} < 24$ h, AUC_{τ} was obtained by extrapolation using λ_z . CL_{ss}/F and V_z/F were only estimated in the subjects receiving the 30 mg dose, as these parameters could not be estimated in the 15 mg OPC-41061 bid group.

Pharmacodynamic Profiling

Serum Na^+ , K^+ and Mg^{++} and Osmolality

Serum electrolytes, and osmolality were measured prior to 0800 at the same time on:
Days -5, -1, 1, 2, 7, 8 and 11

24h Urine Volume, Urine Osmolality, pH, Na^+ and Creatinine

24 h urine collections were started at 0800 on:

Days -1, 1, and 7 and urine volume, osmolality, pH Na^+ and K^+ in the pooled urine samples measured.

Days -5, -1, 1, 2, 7, and 11

Dyspnea, Orthopnea, Edema (Sacral, Pedal), JVP, Rales, Hepatomegaly

These variables were measured prior to the 0800 dose each day on:

Days -5, -1, 1, 2, 7, 8 and 11

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Serum Creatinine

Creatinine in serum was measured on:

Day -1: at 2000
Days 1 and 7: 12 h after the 0800 dose

Baseline for all pharmacodynamic parameters, except for urine output, was defined as the last observation before beginning the double-blind treatment. Baseline for urine output was defined as the 24 h urine collection obtained on the last day of the washout period (Day-1).

Additional Assessments

Additional assessments included fatigue, heart sounds, palpitation, murmur, and NYHA classification. All assessments were to be completed at the same time of the day, prior to the 0800 dose on:

Days -5, -1, 1, 2, 7, 8 and 11

The Heart Failure Score was derived from the data collected. The total score was equal to the sum of the responses for rales, pedal edema, sacral edema, right heart failure (JVP), hepatomegaly, orthopnea, dyspnea, and fatigue. Possible scores ranged from 0 to 26, with higher scores signifying worse heart failure.

Pharmacokinetic-Pharmacodynamic Correlations

None were attempted

Statistical Analysis

Sample Size and Power

A total of 40 subjects were studied to compare [REDACTED] the two dosage regimens. This sample size was not determined with respect to any power requirements.

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The ITT population provided the data set. The ITT population consisted of all randomized subjects. As an exploratory analysis, p-values were determined for treatment comparisons for the pharmacodynamic variables (except urine excretion rate), using an analysis of covariance (ANCOVA) for continuous outcome variables (with terms for treatment, center, and baseline observations, and the Mantel-Haenszel mean score test (with rank option stratified by center) for categorical outcome variables. SAS (version 8) was used for this analysis. Urine excretion rate for the ITT population and the descriptive statistics used to summarize these data by time point and treatment were determined by Microsoft® Excel 2000.

RESULTS

The demographics of the participating patients are described in the below table:

Parameter	Statistic	Dosage Regimen	
		Tolvaptan 30 mg QD (N=21)	Tolvaptan 15 mg BID (N=19)
Age (years)	Mean (SD)	54 (14)	52 (14)
Weight (kg) ^a	Mean (SD)	93.1 (19.3)	97.1 (27.8)
Height (cm)	Mean (SD)	172 (12)	171 (16)

Parameter	Statistic	Dosage Regimen	
		Tolvaptan 30 mg QD (N=21)	Tolvaptan 15 mg BID (N=19)
Race ^b	Caucasian	9 (42.9)	9 (47.4)
	Black	4 (19.0)	5 (26.3)
	Hispanic	8 (38.1)	5 (26.3)
Gender ^b	Male	15 (71.4)	15 (78.9)
	Female	6 (28.6)	4 (21.1)

^aWeights obtained from Day 1.
^bValues are N (% of subjects in group).

Forty subjects, 21 in the 30 mg qd group and 19 in the 15 mg bid, were enrolled in the study. Mean age, body weight, gender and race of the subjects in both groups were comparable.

The previous cardiovascular history of the patients is summarized in the next table:

Parameter	Statistic	Dosage Regimen	
		Tolvaptan 30 mg QD (N=21)	Tolvaptan 15 mg BID (N=19)
Race ^b	Caucasian	9 (42.9)	9 (47.4)
	Black	4 (19.0)	5 (26.3)
	Hispanic	8 (38.1)	5 (26.3)
Gender ^b	Male	15 (71.4)	15 (78.9)
	Female	6 (28.6)	4 (21.1)

^aWeights obtained from Day 1.
^bValues are N (% of subjects in group).

In the group receiving the 30 mg qd regimen 10 patients had NYHA Class II CHF and 10 had NYHA Class III CHF, whereas the corresponding numbers in the 15 mg bid receiving group were 8 and 11, respectively. Orthopnea was reported by 14.3 % of the patients receiving the 30 m bid regimen and by 47.4% of the patients receiving the 15mg bid regimen.

The disposition of the subjects is shown in the next table:

Subjects	Tolvaptan 30 mg QD (N = 21) n (%)	Tolvaptan 15 mg BID (N = 19) n (%)	Total (N = 40) n (%)
Screened			68
Randomized	21 (100)	19 (100)	40 (100)
Treated	21 (100)	19 (100)	40 (100)
Completed	20 (95.2)	19 (100)	39 (97.5)
Discontinued	1 (4.8)	0	1 (2.5)
Analyzed for efficacy	21 (100)	19 (100)	40 (100)
Analyzed for Safety	21 (100)	19 (100)	40 (100)

Source: CST-1, Appendix IV-2.1, and Section 13.

Of the forty subjects enrolled, 39 completed the study and 1 subject withdrew consent on Day 6 of treatment.

Prior to study start use of diuretics and antacids and other gastrointestinal treatments was higher in the 30 mg qd group and use of K⁺ supplements, anti-gout preparations, and thyroid therapy was more prevalent in the 15 mg bid group.

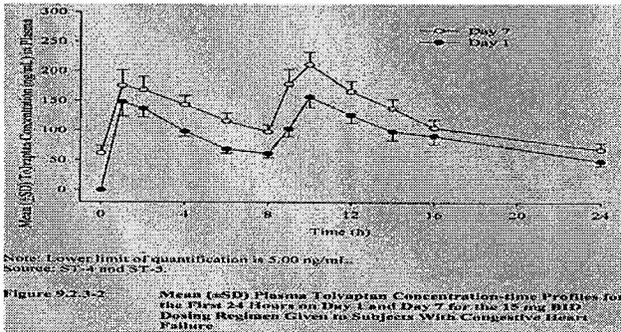
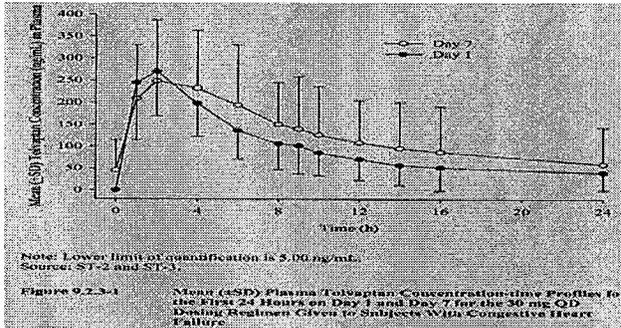
Use of diuretics during washout and treatment periods was allowed. Five subjects on isosorbide continued to use it throughout the study. Administration of spironolactone at a daily dose of 25 mg was allowed as well. Of 17 subjects receiving spironolactone prior to treatment with study drug, 9 subjects (7 in the 30 mg qd group and 2 in the 15 mg

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bid group) continued this treatment during the study. In addition, one subject continued treatment with furosemide and one subject continued treatment with metolazone, although disallowed by the protocol.

Pharmacokinetics

The sponsor considered the data assessable from 20 patients who received the 30 mg qd treatment and from 16 patients who received the 15 mg bid treatment. These 36 patients had usable plasma concentration data on both Days 1 and 7. Linear plots of the mean plasma concentrations of tolvaptan from both groups are shown in the below 2 figures:



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With both regimens the mean plasma concentrations on Day 7 are greater than on Day 1 indicating some accumulation. The mean peak concentrations on Days 1 and 7 in the subjects receiving the 30 mg qd treatment are greater than in the subjects receiving the 15 mg bid regimen. In contrast, the mean trough concentrations on Days 1 and 7 of the subjects receiving the 15 mg bid regimen are greater than in the subjects on the 30 mg qd treatment. These findings are logical consequences of the greater dose and dose interval used in the 30 mg qd regimen than in the 15 mg bid regimen.

The sponsor's estimated pharmacokinetic parameters after single and multiple dose administration of the 30 mg qd and 15 mg bid regimens on Days 1 and 7 are listed in the below table:

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Mean (SD) Tolvaptan Pharmacokinetic Parameters for the Two Dosing Regimens		
Pharmacokinetic Parameters	30 mg QD	15 mg BID
Day 1		
C _{max} (ng/mL)	306 (96)	194 (77)
t _{max} (h) ^a	1.10, 0.98-4.00	9.99, 1.00-16.05
AUC ₀₋₂₄ (ng·h/mL)	2614 (1289)	2205 (875)
Day 7		
C _{max} (ng/mL)	283 (138)	240 (85)
t _{max} (h) ^a	2.00, 1.00-6.03	9.63, 1.00-11.95
t _{1/2} (h)	5.4 (4.6)	9.3 (5.6)
AUC _τ (ng·h/mL)	3087 (2464)	3001 (1375)
C _{min,ss} (ng/mL)	48 (69)	60 (50)
C _{avg,ss} (ng/mL)	129 (103)	125 (57)
V _{d,ss} /F (L/kg)	1.76 (0.76)	ND
CL _{ss} /F (mL/min/kg)	2.73 (1.87)	ND
R _{ac} (AUC _τ)	1.19 (0.34)	1.35 (0.45)
R _{ac} (C _{max})	0.94 (0.36)	1.32 (0.42)

^a For t_{max} the median and range are given.
SD = standard deviation, ND = not determined.

The plasma concentrations of OPC-41061 with the 30 mg qd regimen are measurable for 24 h on Days 1 and 7. As discussed in detail in the review of report 156-98-202 the 0-24 h observation interval is not long enough to determine λ_z and derived parameters including t_{1/2z} and V_z/F. The estimates for C_{max} and t_{max} on Days 1 and 7 and AUC₀₋₂₄ on Day 1 and AUC_τ on Day 7 as well as CL/F and R_{ac} (AUC) can be considered reliable. The median t_{max} is 1.1 h and 2.0 h on Day 1 and Day 7, respectively. The coefficient of variation about mean C_{max} and AUC₀₋₂₄ on Day 1 is 31 % and 48 %, respectively. The corresponding figure for C_{max} and AUC_τ on Day 7 is 49 % and 80 %, respectively, suggesting marked inter-subject variation. The mean CL/F is 2.73 mL/min/kg and the accumulation factor is 1.19.

The plasma concentrations of OPC-41061 on Days 1 and 7 are measurable up to 24 h post-dose with the 15 mg bid treatment. Hence, the estimates for C_{max}, AUC₀₋₂₄ and AUC_τ can be considered reliable. The median t_{max} is 2.0 h and 1.6 h after the afternoon dose on Days 1 and 7, respectively. The percent coefficient of variation about mean C_{max} and AUC₀₋₂₄ on Day 1 is an identical 40 %. The corresponding figure for C_{max} and AUC_τ on Day 7 is 35% and 46%, respectively. The mean accumulation factor is 1.35.

The mean respective AUC_τ on Day 7 of the 15 mg bid regimen is close to that of the 30 mg qd regimen indicating identical exposure and CL/F values with both treatments. The median pre-dose plasma concentration of OPC-41061 on Day 7 with the 15 mg bid regimen is about 2 fold greater than with the 30 mg qd regimen.

The below 2 tables list the peak and trough concentrations and their ratio on Days 1 and 7 for the 30 mg qd and 15 mg bid regimens:

Individual Plasma Concentrations at Peak and Trough and their Ratio on Days 1 and 7 of a Dose Regimen with 30 mg OPC-41061 QD in CHF Patients

Subject	Individual Plasma Concentrations of OPC-41061 at Peak and Trough ^b ng/ml					
	Day 1			Day 7		
	C _{max}	C _{trough} ^b	Ratio	C _{max}	C _{trough} ^b	Ratio
2		14	23		24	10
3		54	5.0		59	5.1
7		12	20		12	27
15		142	3.8		268	2.1

b(4)

25		11	26		12	16
31		17	24		19	11
34		34	9.8		69	3.4
39		61	6.6		121	3.1
41		43	6.6		69	3.5
42		31	4.3		40	8.9
49		31	9.4		54	7.2
53		20	11		27	8.2
57		127	2.8		310	1.8

b(4)

^a Only subjects considered with complete information ^b Ctrough Day 1 measured 24 h after the first dose and Ctrough Day 7 measured 24 h after the last dose on Day 7

Individual Plasma Concentrations at Peak and Trough and their Ratio on Days 1 and 7 of a Dose Regimen with 15 mg OPC-41061 Q8 alternating with Q16 in CHF Patients

Subject	Individual Plasma Concentrations of OPC-41061 at Peak and Trough ^a , ng/ml					
	Day 1			Day 7		
	Cmax	Ctrough ^b	Ratio	Cmax	Ctrough ^b	Ratio
1		74	3.3		96	1.7
4		70	2.7		107	1.7
5		41	4.1		111	1.5
6		44	2.5		72	1.3
11		11	5.1		25	2.4
13		49	3.3		158	1.7
16		78	4.9		69	2.6
26		33	4.2		38	5.5
40		69	1.7		87	1.1
43		40	3.2		114	1.8
44		51	5.2		105	2.5
45		84	1.0		200	1.3
47		97	1.5		144	1.7
61		87	2.1		142	2.3

b(4)

^a Only subjects considered with complete information ^b Ctrough Day 1 measured 24 h after the first dose and Ctrough Day 7 measured 24 h after the last dose on Day 7

With the 30 mg qd regimen the Cmax/Ctrough ratio on Day 1 varies between 2.8 and 26 and on Day 7 between 1.8 and 27. In all subjects the trough concentrations on Day 7 are slightly greater than on Day 1 in accordance with a small accumulation with the 30 mg qd regimen of OPC-41061. With the 15 mg bid regimen the Cmax/Ctrough concentration ratio on Day 1 varies between 1.0 and 5.1 and on Day 7 between 1.1 and 5.5. The significantly reduced peak to trough fluctuation with the bid regimen is a consequence of the smaller dose interval.

Pharmacodynamics

The mean change from baseline for  urine- and serum parameters is shown in the below table:

b(4)

Mean (SD) ^c from Baseline at Day 1 (Urine Parameters) or Day 2 (Serum Parameters) and Day 7

Pharmacodynamic Parameters	30 mg QD		15 mg BID	
	Day 1 or 2	Day 7	Day 1 or 2	Day 7
Urine volume (mL) ^a	4278 (2360)	3324 (1634)	4197 (3392)	3067 (1928)
Urine Excretion Rate (mL/min) ^b	2.97 (1.64)	2.45 (1.13)	2.92 (2.36)	2.13 (1.34)
Serum Sodium (mEq/L)	2.52 (2.52)	0.95 (2.14) ^c	3.84 (3.00)	3.53 (2.78) ^c
Serum Potassium (mEq/L)	0.00 (0.35)	-0.06 (0.57)	0.17 (0.39)	0.01 (0.45)
Serum Magnesium (mEq/L)	0.09 (0.13)	-0.02 (0.14)	0.14 (0.14)	0.06 (0.21)
Serum Osmolality (mOsm/kg)	2.43 (7.41)	-1.40 (8.44)	5.58 (10.35)	1.58 (11.77)
Urine Osmolality (mOsm/kg) ^a	-325 (168)	-326 (167)	-247 (222)	-323 (276)
Creatinine Clearance (mL/min) ^a	-16 (39)	-11 (31)	-6 (24)	-12 (27)

^a Day 1 and 7 values are truncated to integers from source tables.
^b Values not statistically compared.
^c Value from 15 mg BID compared to 30 mg QD was found to have a p = 0.0028.

b(4)

With both treatments the serum sodium is increased on Day 1, but only with the bid regimen this effect is sustained on Day 7. This may be explained by the more sustained concentrations of OPC-41061 towards the end of the dose interval with the 15 mg bid regimen when the serum Na⁺ concentrations were measured. Both treatments increase the net 24 h urine volume/excretion rate. The net urine volumes/excretion rates on Day 7 are 82 % and 73 % of those on Days 1 or 2 with the 30 mg qd and 15 mg bid regimens, respectively, confirming a rebound effect with more retention of fluid. With both treatments the mean net plasma osmolality tends to increase, whereas serum K⁺ and Mg⁺⁺ show no change from baseline. Urine osmolality is decreased with both regimens and this effect is sustained on Day 7. Creatinine clearance is not affected differently by the two regimens.

b(4)

The proportion of improved, unchanged and worsened cardiovascular status in the two groups is similar.

Safety

A summary of all adverse events is given in the below table:

Parameter	Tolvaptan 30 mg QD (N = 21) n (%)	Tolvaptan 15 mg BID (N = 19) n (%)	Total (N = 40) n (%)
Subjects treated	21	19	40
Subject days of drug exposure	146 ^a	133	279 ^a
Subjects with adverse events	10 (47.6)	8 (42.1)	18 (45.0)
Adverse events	30	32	62
Subjects with treatment-emergent adverse events	10 (47.6)	8 (42.1)	18 (45.0)
Treatment-emergent adverse events	23	27	50
Subjects with serious treatment-emergent adverse events	0 (0.0)	0 (0.0)	0 (0.0)

Parameter	Tolvaptan 30 mg QD (N = 21) n (%)	Tolvaptan 15 mg BID (N = 19) n (%)	Total (N = 40) n (%)
Subjects with severe treatment-emergent adverse events	1 (4.8)	1 (5.3)	2 (5.0)
Subjects discontinued due to adverse events	0 (0.0)	0 (0.0)	0 (0.0)

^a147 (280 total) including an extra day of dosing for subject 0053 in the 30 mg QD group. Source: CST-8.1, Appendix IV-8.1, and Section 13.

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There was no important difference in any of the adverse events between the 2 groups tested. The most commonly reported treatment emergent events were headache and thirst.

Conclusions

PK

With the 30 mg qd regimen the percent coefficients of variation about mean peak and average exposure range between 31 % and 49 % and 48 % and 80 %, respectively. With the 15 mg regimen the coefficients of variation about mean C_{max} and AUC_τ range between 35 % and 40 % and 40 %-48 %, respectively. The mean CL/F for OPC-41061 is 2.73 mL/min/kg and the accumulation factor is 1.19. With the 15 mg bid regimen the mean CL/F is similar, and the accumulation factor of 1.35 is greater because of the shorter dose interval. The mean AUC_τ on Day 7 of the 15 mg bid regimen is similar to that of the 30 mg qd regimen .

PD


 With both treatments the serum sodium is increased on Day 1, but only with the bid regimen this effect is sustained on Day 7. This may be explained by the more sustained concentrations of OPC-41061 towards the end of the dose interval with the 15 mg bid regimen when the serum Na⁺ concentrations were measured. Both treatments increase the net 24 h urine volume/excretion rate. The net urine volumes/excretion rates on Day 7 are 82 % and 73 % of those on Days 1 or 2 with the 30 mg qd and 15 mg bid regimens, respectively, confirming a rebound effect with more retention of fluid. With both treatments the mean net plasma osmolality tends to increase, whereas serum K⁺ and Mg⁺⁺ show no change from baseline. Urine osmolality is decreased with both regimens and this effect is sustained on Day 7. Creatinine clearance is not affected differently by the two regimens.

b(4)

Comments

1. A possible correlation between exposure and response should be evaluated
2. The report states (p.61):" The administration of concomitant medications did not appear to affect the pharmacokinetic profile of tolvaptan." Evidence supporting this conclusion is not provided.
3. The sensitivity of the assay does not allow following the plasma concentration time curve of OPC-41061 for an interval long enough to determine λ_z.
4. The protocol indicates that the metabolite concentrations in plasma were to be measured. However, this was not done. The report does not state what metabolite is referred to. A rationale for this decision is not provided.

Study Report No. 156-04-247:" A Multi-Center, Randomized, Double-Blind, Placebo Controlled Study to Evaluate the Effect of Single Oral Tolvaptan Tablets on Hemodynamic Parameters in Subjects with Heart Failure"

Investigators and Study Sites

Forty-Nine (49) investigators and centers in the US, Bulgaria and Romania participated in the study.

Objectives

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and AUC0-8h

free water clearance, and urine osmolality, and to study Cmax, tmax

Investigational Drugs and Formulations

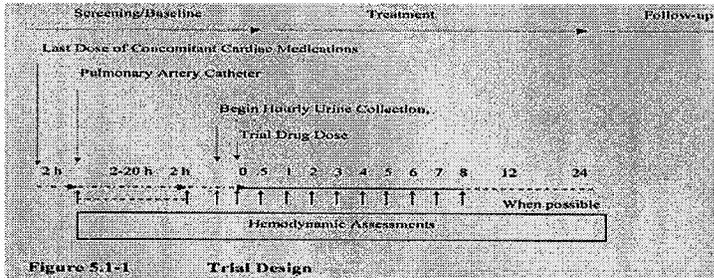
The trial medication consisting of 15 mg, 30 mg and 60 mg tolvaptan tablets and matching placebo tablets was provided by the sponsor. The Lot Nos. are listed below:

Table 5.4.3-1 Batch Numbers of Trial Medication	
Trial Medication	Batch Number(s)
Tolvaptan 15 mg	02C80A015A, 04C77A015, and 05D73A015A
Tolvaptan 30 mg	02C80A030A, 03L73A030E, and 05D73A030A
Tolvaptan 60 mg	02C80A060, 04L93A060, and 05D73A060
Placebo	02C80P000B, 04A95P000E, and 05D73P000A

Source: certificates of analysis.

Design

This is a multi-center, randomized, double-blind, placebo controlled, single dose trial to determine the hemodynamic effects of single doses of 15 mg, 30 mg, and 60 mg tolvaptan and placebo in patients with heart failure. Suitable patients underwent insertion of a balloon-flotation pulmonary artery catheter to assess final eligibility criteria. Final doses of concomitant medications were administered at least 2 h prior to catheter insertion. Following catheter insertion, patients entered into a 2-20 h stabilization period. Hemodynamic assessments were completed periodically during this time to determine final subject eligibility. Potential patients then entered a 2 h baseline period. If all criteria were met during the baseline period, subjects were randomized to one of 4 groups and received either a single oral tablet of placebo or tolvaptan 15 mg, 30 mg or 60 mg. Subjects were followed for up to 24 h for PD measurements. Blood samples were collected for assessing the PK of tolvaptan and plasma osmolality and urine was collected hourly beginning 1 h before initiation of the treatments to determine volume and osmolality. Safety was assessed by evaluating AEs, vital signs and clinical laboratory parameters. A follow-up telephone call was made to the subjects between 7 and 9 days after the dosing. A schematic of the study design is shown below:



Exclusion and inclusion criteria are listed below:

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1.	Male or female.
2.	Age greater than or equal to 18 years.
3.	New York Heart Association Class III or IV heart failure for at least 3 months duration due to left ventricular systolic dysfunction at the time of screening.
4.	Left ventricular ejection fraction \leq 40% at the last measurement, which must be within one year of screening.
5.	Two consecutive PCWP measurements \geq 18 mmHg, as measured during baseline period and within 2 hours of randomization. The lower of the two measurements was to be within \pm 10% of the higher measurement.
6.	Standard background therapy for at least one month including ACE inhibitors, ARBs, and beta-blockers (unless intolerance has been demonstrated) and may have included aldosterone receptor antagonists.

Source: protocol.

5.2.2 Exclusion Criteria

Subjects were excluded if they met any of the exclusion criteria listed in Table 5.2.2-1:

1.	Women who were breast-feeding and/or would not adhere to the reproductive precautions as outlined in the ICF.
2.	Positive serum pregnancy test.
3.	Inability to provide written informed consent.
4.	Supine systolic arterial BP $<$ 90 mmHg or uncontrolled hypertension.
5.	Item removed by Amendment 1.
6.	Uncontrolled bradyarrhythmias or tachyarrhythmias.
7.	Hypertrophic cardiomyopathy (obstructive).
8.	Significant uncorrected valvular or congenital heart disease.
9.	Severe obstructive pulmonary disease.
10.	Significant renal impairment (serum creatinine $>$ 3.0 mg/dL).
11.	Continuous IV inotropic therapy within 48 hours of randomization.
12.	CYP3A4 inhibitors, except amiodarone, used within 30 days of trial drug dosing that were determined by the sponsor to have a potential metabolic interaction with tolvaptan.
13.	Current (within 24 hours of randomization) treatment with nesiritide or intravenous nitroglycerin.
14.	History of hypersensitivity and/or idiosyncratic reaction to benzazepine derivatives (such as benazapril).
15.	History of drug or medication abuse within the past year, or current alcohol abuse.
16.	Inability to take oral medications.
17.	Participation in another clinical drug or device trial where the last dose of drug was within the past 30 days or an investigational medical device was currently implanted.
18.	The donation of blood or plasma within 30 days prior to trial enrollment.
19.	Previous participation in this or any other tolvaptan clinical trial.

CYP = cytochrome P450.

Source: protocol.

Medications prohibited before and during the trial are listed below:

Prohibited Prior to Trial	
1.	Unless approved by the sponsor, CYP3A4 inhibitors with the exception of amiodarone (eg, amprenavir, atorvastatin, aprepitant, chloramphenicol [not eye drop], cimetidine, clarithromycin, clemastine, clemastine fumarate, danazol, delavirdine, diltiazem, erythromycin, fluconazole, fluvoxamine, indinavir, isoniazid, itraconazole, josamycin, ketoconazole, nelfinavir, nefazadone, quinupristin/dalfopristin, ritonavir, saquinavir, troleandomycin, verapamil). ^a
2.	Nesiritide
3.	IV nitroglycerin
4.	IV inotropic therapy
Prohibited During Trial	
1.	ACE inhibitors
2.	ARBs
3.	Beta-blockers
4.	Diuretics
5.	Nesiritide
6.	Nitroglycerin
7.	Inotropes
8.	Other medications for cardiovascular indications

CYP = cytochrome P450.

^a Washout times for CYP3A4 inhibitors were supplied by the sponsor.

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The scheduled study activities are shown in the below schemes:

Table 5.6-1 Schedule of Assessments for Screening and Baseline

Procedures	Screening		Baseline Trial Hour		
	Days -14 to -1	Day -1 (-20 to -2 h)	-2	-1	Pre-dose
Informed consent	X				
Review inclusion/exclusion	X				
Demographic information	X	X			
Medical history	X				
Concomitant medication and adverse events	X	X	X		
Vital signs ^a	X	X	X		
Physical exam	X				
Cardiovascular history/assessment	X	X			
Clinical chemistry, hematology, urinalysis	X	X			
Pregnancy test ^b	X	X ^c			
Pulmonary artery catheterization		X ^c			
Hemodynamic assessments		X	X		
Caloric and fluid restriction ^d			X		
Blood sample for tolvaptan PK					X
Blood sample for plasma osmolality					X
Hourly urine collection, urine osmolality samples ^e				X	X
Randomization via IVRS					X

- D/C = discharge.
- ^a Vital signs from -2 to 24 hours included BP and HR only. Vital signs at screening, discharge, and ET included BP, HR, temperature, and respiration rate after 5 minutes of supine rest. Vital signs were obtained at Hour 12 and 24 only if a hemodynamic assessment was made.
 - ^b The pregnancy test had to be negative prior to randomization.
 - ^c Last doses of cardiac medications were given at least 2 hours prior to catheterization. Subjects were not given additional cardiac medications until all hemodynamic measurements were completed.
 - ^d Subjects were allowed a limited caloric intake of ≤150 calories and ≤250 mL of fluid every 2 hours beginning 2 hours prior to start of baseline hemodynamic measurements and continuing through Hour 8 of hemodynamic measurements. Hemodynamic bolus injectate was not included in the 250-mL limit.
 - ^e Total urine volume was determined for each hour between -1 hours predose to 8 hours postdose. A urine sample for determination of osmolality was taken from each hourly total urine volume. Source: protocol.

Table 5.6-2 Schedule of Assessments for Treatment, Discharge/Early Termination, and Follow-up

Procedures	Treatment Trial Hour												D/C	ET	Follow-up			
	0	0.5	1	2	3	4	5	6	7	8	12, 24 ^a							
Concomitant medication and AEs	X																	
Vital signs ^b	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam																	X	X
Clinical chemistry, hematology, urinalysis																	X	X
Pregnancy test																	X	X
Hemodynamic assessments	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Caloric and fluid restriction ^c	X																	
Blood sample for tolvaptan PK			X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood sample for plasma osmolality			X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Hourly urine collection, urine osmolality sample ^d	X																	
Dispense drug	X																	
Follow-up phone call ^e																		X

- D/C = discharge.
- ^a Assessments were made if the pulmonary artery catheter was still in place for medical necessity.
 - ^b Vital signs from -2 to 24 hours included BP and HR only. Vital signs at screening, discharge and ET included BP, HR, temperature, and respiration rate after 5 minutes of supine rest. Vital signs were obtained at Hour 12 and 24 only if a hemodynamic assessment was made.
 - ^c Subjects were allowed a limited caloric intake of ≤150 calories and ≤250 mL of fluid every 2 hours beginning 2 hours prior to start of baseline hemodynamic measurements and continuing through Hour 8 of hemodynamic measurements. Hemodynamic bolus injectate was not included in the 250-mL limit.
 - ^d Total urine volume was determined for each hour between -1 hours predose to 8 hours postdose. A urine sample for determination of osmolality was taken from each hourly total urine volume.
 - ^e All subjects were contacted 7 (+ 2) days after the last dose of trial medication to collect safety information. Any AEs identified during this contact were entered on the CRF. Source: protocol.

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It should be noted that the subjects were allowed ≤250 mL fluid every 2 h beginning 2 h prior to start of the baseline hemodynamic measurements and continuing through hour 8. Hemodynamic bolus injectate was not included in the 250 mL limit.

Pharmacokinetic Profiling:

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Blood samples for the determination of the plasma concentration of tolvaptan were collected at the following times:

Pre-dose, 1, 2, 3, 4, 5, 6, 7, and 8 h post-dose. Additional sample were collected at 12 h and 24 h after dosing if [redacted] as measured at these time points.

Bioassay

The plasma concentrations of tolvaptan were determined by a LC/MS/MS method. The calibration curve is linear over the range 5 ng/mL-1000 ng/mL with a mean correlation coefficient of 0.9980. Using QC samples the accuracy of the assay ranges between -5.88 % and 2.00 % and the precision is ≤ 8.82%. The freeze/thaw cycle-, in-process and refrigeration stability of the analytes was demonstrated. The assays were performed by [redacted]

b(4)

Hemodynamics

Hemodynamic parameters were measured at the following times: 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h post-dose

Pharmacodynamics

Plasma Osmolality

Blood samples for the determination of plasma osmolality were obtained pre-dose, 2, 4, 6, and 8 h after dosing.

Urine Volume and Osmolality

Urine volumes were collected in hourly intervals for 8 h beginning 1 h before dosing. Urine collection began within 15 min of the pre-dose time point (1 h before dosing) by voiding completely and then collecting all subsequent voids of newly accumulated urine.

Data Analysis

PK

Cmax and tmax were taken directly from the data sets. AUC0-8h was obtained on application of the linear trapezoidal rule. WinNonlin (Pharsight Corporation, Version 4.0) was used.

Sample Size and Power

It was estimate that 45 patients per treatment group (180 patients in total) would provide 80 % power to detect a difference of 3 mm Hg in peak change from baseline in [redacted] from 3 h to 8 h, using a SD of 5 mm Hg and a nominal alpha level of 0.05.

b(4)

Hemodynamic Efficacy Variables

[redacted]

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[redacted] change from baseline in urine volume, free water clearance and urine osmolality. [redacted]

[redacted] An ANCOVA model with terms for treatment, country, and baseline observation as covariates was used for the analysis of [redacted] variables. Contrasts statements under the linear model were constructed

to make treatment comparisons for tolvaptan versus placebo. Each contrast of a variable was tested at a significance level of 0.05.

Pharmacodynamic Variables

Free water clearance was obtained as described below:

$$CL_{H_2O} = V - C_{osm}$$

Where, C_{osm} is the osmolar clearance ($U_{osm} \times V/P_{osm}$), and where U_{osm} is the urine osmolality (mOsm/kg H₂O), V is the urine excretion rate (mL/min), and P_{osm} is the plasma osmolality (mOsm/kg H₂O). Urine excretion rate (mL/min) is equal to $(Vol_1 + Vol_2)/120$ for each 2 hour postbaseline interval, where Vol_1 is the first hour urine volume and Vol_2 is the second hour urine volume. The urine osmolality of a 2-hour postbaseline interval is equal to $(U_1Vol_1 + U_2Vol_2)/(Vol_1 + Vol_2)$, where U_1 is the first hour urine osmolality and U_2 is the second hour urine osmolality. Baseline urine volume interval is 1 hour so that baseline urine excretion rate (mL/min) is equal to $Vol/60$. The plasma osmolality during an interval is $(P_{osm,1} + P_{osm,2})/2$, where $P_{osm,1}$ and $P_{osm,2}$ are the plasma osmolality values at the beginning and end of the collection interval, respectively.

The baseline for hourly urine volume, free water clearance and urine osmolality was the value based on the -1-0 h interval. The baseline for plasma osmolality was the pre-dose sample.

RESULTS

Disposition of Subjects

181 subjects were enrolled in the study and 177 subjects (97.8%) completed the trial. The disposition of the subjects is summarized in the below table:

Subjects	Tolvaptan 15 mg N (%)	Tolvaptan 30 mg N (%)	Tolvaptan 60 mg N (%)	Placebo N (%)
Randomized	44 (100.0)	43 (100.0)	46 (100.0)	48 (100.0)
Withdrawn:				
Adverse experience	0 (0.0)	0 (0.0)	1 (2.2)	3 (6.3)
Investigator discretion	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
Efficacy ^a	44 (100.0)	43 (100.0)	46 (100.0)	48 (100.0)
Completed ^b	44 (100.0)	43 (100.0)	45 (97.8)	45 (93.8)
Safety ^c	44 (100.0)	43 (100.0)	46 (100.0)	48 (100.0)

^aIncludes all randomized and treated subjects with hemodynamic measurements at baseline and at least once between 3 and 8 hours postdose.
^bIncludes all randomized and treated subjects who underwent hemodynamic assessments for at least 8 hours postdose.
^cIncludes all randomized subjects who received trial medication.

Demographics of Subjects

The below table shows the demographic of the subjects:

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Table 8.2-1 Demographic and Baseline Characteristics

Characteristic	Tolvaptan 15 mg (N = 44)	Tolvaptan 30 mg (N = 43)	Tolvaptan 60 mg (N = 46)	Placebo (N = 48)	Total (N = 181)
Sex, n (%)					
Male	32 (72.7)	36 (83.7)	36 (78.3)	40 (83.3)	144 (79.6)
Female	12 (27.3)	7 (16.3)	10 (21.7)	8 (16.7)	37 (20.4)
Race, n (%)					
Caucasian	32 (72.7)	31 (72.1)	31 (67.4)	34 (70.8)	128 (70.7)
Black	10 (22.7)	8 (18.6)	10 (21.7)	14 (29.2)	42 (23.2)
Hispanic	1 (2.3)	3 (7.0)	3 (6.5)	0 (0.0)	7 (3.9)
Asian	1 (2.3)	0 (0.0)	1 (2.2)	0 (0.0)	2 (1.1)
Other	0 (0.0)	1 (2.3)	1 (2.2)	0 (0.0)	2 (1.1)
Age (years)					
Mean (SD)	60.3 (11.7)	59.7 (13.4)	61.0 (11.9)	58.9 (14.0)	60.0 (12.7)
Range	33 - 87	29 - 89	26 - 89	28 - 87	26 - 89
Weight (kg)					
Mean (SD)	87.6 (20.6)	94.3 (23.2)	90.3 (21.3)	95.9 (25.0)	92.1 (23.2)
Range	51.2 - 138.3	48.6 - 175.0	49.4 - 136.8	51.5 - 177.9	48.6 - 177.9
Height (cm)					
N	44	42	45	48	179
Mean (SD)	170.7 (10.7)	173.1 (11.5)	171 (9.2)	174.9 (8.7)	172.5 (10.1)
Range	141.0 - 193.0	135.0 - 191.0	150.0 - 189.0	157.0 - 189.0	135.0 - 193.0

Source: CT-3.1.

Pharmacokinetics

The mean (SD) plasma concentrations of tolvaptan following a single dose of 15 mg, 30 mg and 60 mg and derived parameters are shown in the below figure and table:

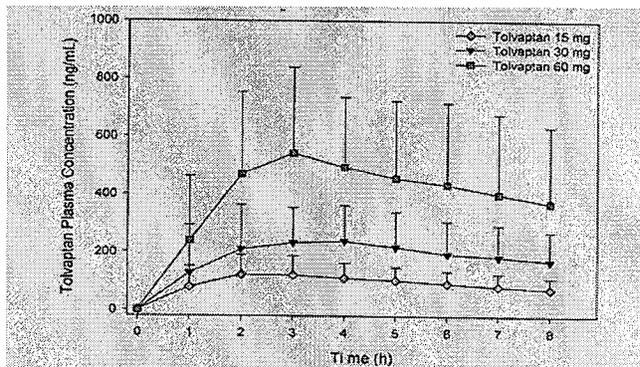


Figure 10.2.4.1-1 Mean (SD) Tolvaptan Plasma Concentrations Following a Single Oral Dose of Tolvaptan to Heart Failure Subjects

The mean peak plasma concentrations of tolvaptan are attained between 2 and 3 h post-dose , but the individual values range from 1- 8 h in the CHF patients suggesting variable absorption. The Cmax appears to increase dose proportionally. The percent coefficient of variation about the mean Cmax in the CHF patients ranges between 35 % and 46 % at the different dose levels.

Hemodynamics

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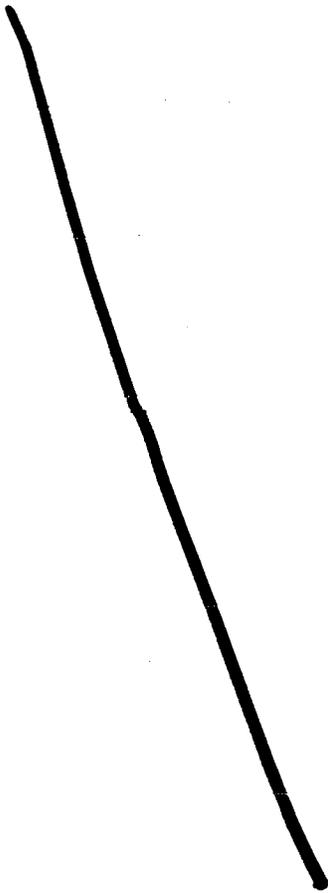
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Pharmacodynamics

Mean change from baseline in urine volume, urine osmolality and free water clearance with the treatments are shown in the below figures:

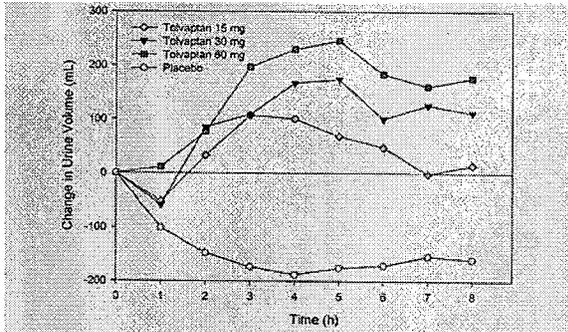


Figure 9.4.3.1-1 Mean Change From Baseline in Urine Volume at the End-time of the Collection Interval

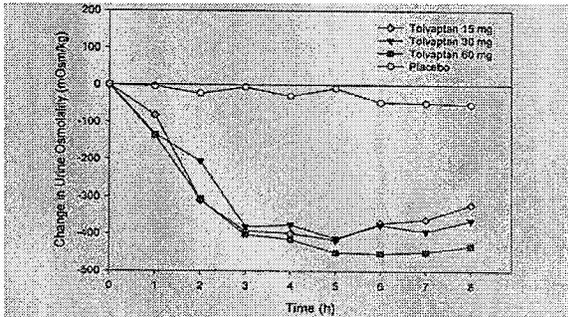


Figure 9.4.3.1-2 Mean Change From Baseline in Urine Osmolality at the End-time of the Collection Interval

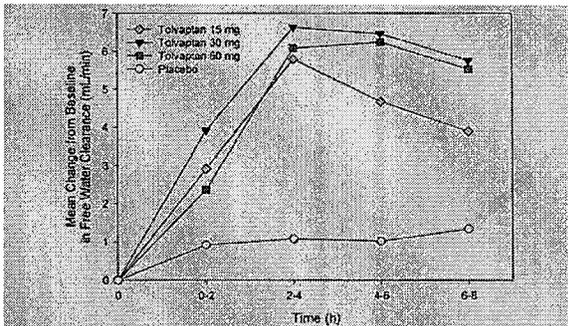


Figure 9.4.3.1-3 Mean Change From Baseline in Free Water Clearance at the End Time of the Collection Interval

The results indicate that single doses of 15 mg, 30 mg and 60 mg tolvaptan increase the baseline corrected urine volumes and the free water clearance and decrease urine osmolality up to 8 h after dosing. Peak effects are observed 1-4 h after administration in agreement with the peak plasma concentrations which are observed 2-3 h after dosing.

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The table below lists the mean treatment effect on urine excretion rate in the intervals 0-2, 2-4, 4-6 and 6-8 h after dosing and the mean plasma concentrations at mid time of tolvaptan:

Dose mg	Time after Dosing h	Mean Concentration ng/mL	Collection Interval ^a h	Mean Urine Excretion ^b Rate mL/min
15	1	79.2	0-2	1.9
	3	120	2-4	4.4
	5	103	4-6	3.3
	7	83.1	6-8	2.3
30	1	128	0-2	1.9
	3	233	2-4	4.4
	5	219	4-6	4.6
	7	185	6-8	3.7
60	1	240	0-2	1.6
	3	543	2-4	5.0
	5	457	4-6	5.4
	7	401	6-8	4.3

^a The mean urine volumes collected in adjacent intervals were combined (e.g. the median urine volume of intervals 0-1 h and 1-2 h etc. were combined and then divided by 120 min) ^b Sponsor's ANOVA model with treatment and country as factors

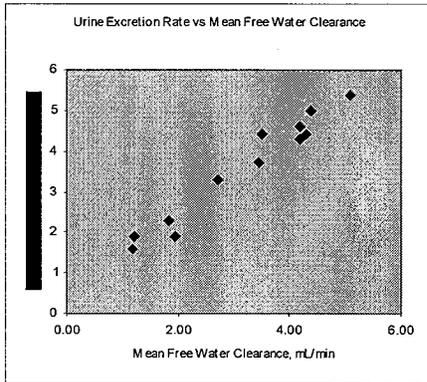
The data from the above table indicate that the onset of the aquaretic action of tolvaptan in the patients with CHF starts in the 0-2 h post-dose interval (net increase in urine excretion rate ≥ 1.0 mL/min. Mean peak excretion rates are observed in the 2-4 h and 4-6 h post-dose intervals. The mean peak rates increase in the order of the administered doses.

The below table summarizes the findings on free water clearance:

Mean Free Water Clearance

Collection Interval, h	Mean Free Water Clearance, mL/min			
	0-2	2-4	4-6	6-8
Dose, mg				
15	1.22	3.51	2.73	1.85
30	1.96	4.29	4.20	3.44
60	1.20	4.40	5.10	4.20

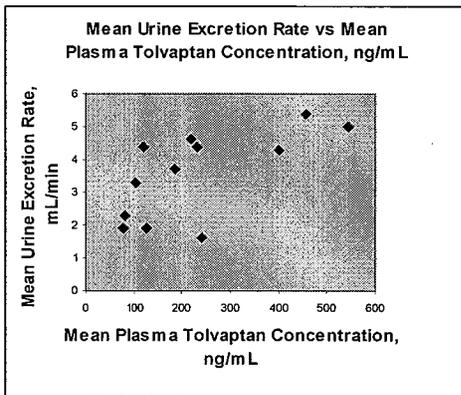
The treatment effects on mean urine excretion rates and free water clearance are correlated as shown in the below plot:



The data indicate good agreement between the treatment effect on urine excretion rate and free water clearance of tolvaptan.

The next plot shows the relationship between mean urine excretion rate and plasma concentration of tolvaptan:

Plot of the Treatment Effect on Urine Excretion Rate vs Mean Mid Point Plasma Concentration of Tolvaptan



The results indicate a saturable aquaretic effect to plasma concentration relationship for tolvaptan. It should be noted that the lagging of the aquaretic effect behind the plasma concentration of tolvaptan (counter-clockwise hysteresis) contributes to the variability of the data.

Conclusions

PK

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Peak plasma concentration and AUC0-8h of tolvaptan appear to increase dose proportionally. Peak concentrations of tolvaptan are reached on average 2.0-3.0 h after administration, but the individual values vary significantly from 1-8 h after administration. Marked inter-subject variation of Cmax is also observed in the CHF patients.

PD

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Tolvaptan increases urine volumes and excretion rates, urine osmolality and free water clearance. There is good agreement between the treatment effects of tolvaptan on urine excretion rate and free water clearance. The time profile of the aquaretic and hemodynamic effects of tolvaptan appear to be similar. The onset of the aquaretic effect occurs in the 0-2 h post-dose interval. Mean peak excretion rates are observed in the 2-4 h or 4-6 h post-dose intervals. The urine excretion rate depends on the plasma concentration of tolvaptan. The relationship is nonlinear: the effect increments associated with higher plasma concentrations become increasingly smaller.

Report 156-98-201: A Randomized Double-Blind Study to Assess the Safety and Pharmacokinetics of Concomitant Administration of Orally Administered OPC-41061 Tablets and Ketoconazole in Healthy Adult Male and Female Volunteers

Investigator and Study Site

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Objectives

To determine the safety and pharmacokinetics of concomitant administration of OPC-41061 and ketoconazole in healthy adult, male and female volunteers

Investigational Drugs and Formulations

15 mg OPC-41061 tablets (Lot No. 97B84A015) and placebo tablets (Lot No. 5L80P) were provided by the sponsor. 200 mg ketoconazole tablets (Lot No. 98P0149E). The protocol does not indicate manufacturer or provider.

Design

This was a single-center, randomized, double-blind, placebo controlled, cross-over study conducted in healthy male and female subjects. Twenty five healthy female or male subjects in the age between 18 and 45 years were enrolled in the study with 5 subjects randomly assigned to the placebo arm and 20 assigned to the OPC-41061 arm. Subjects assigned to the OPC-41061 arm received a 3 µCi intravenous infusion of ¹⁴C-N-methylerythromycin for an erythromycin breath test (ERBT) on Day -1. On the following day (Day 1), the subjects were given a single oral dose of 30 mg OPC-41061 and an ERBT 2 h later. On Day 4 200 mg ketoconazole was administered and an ERBT was performed 2 h later. On Day 5, 200 mg ketoconazole was given together with a single oral dose of 30 mg OPC-41061 and an ERBT was performed 2 h later. Ketoconazole was also given on Day 6. Subjects in the placebo arm followed the same treatment and sampling schedule, but were given placebo instead OPC-41061.

The 30 mg OPC-41061 or placebo tablets were administered together with 240 mL water. The subjects were requested to remain in a sitting position and to abstain from food for the first 4 h post-dose. The protocol does not specify whether the ketoconazole tablets were also given together with 240 mL water. Alcohol consumption and use of xanthine-containing products were not permitted within 48 h of admission to the Unit and for the duration of the study. During the study the subjects were not allowed to ingest grapefruit containing products or tobacco products.

The scheduled study activities are shown in the below scheme:

TABLE 4.6-1 SCHEDULE OF ASSESSMENTS AND PROCEDURES

Day	-21	-1	1	2	3	4	6	8	12	24	36	48	72
Hours			0.5	0	0.5	1	1.5	2	3	4	6	8	12
Medical History	*												
Consent	*												
Inclusion/Exclusion Criteria	*	*											
Physical Examination	*	*											
ECG	*	*	*										
Vital Signs	*	*	*	*	*	*	*	*	*	*	*	*	*
Serum Chemistry & Hematology	*	*											
Serum Free	*	*											
Hepatitis and HIV Screen	*	*											
Urinalysis	*	*											
Alcohol & Drug Screen	*	*											
Ethnic Collection	*	*											
IV Erythromycin and Breath Test		*											
PK Plasma Sample		*	*	*	*	*	*	*	*	*	*	*	*
Adverse Events													
Ketoconazole Dose													*
Diagnose OPC-41061		*											
Placebo Medication			*										

*Included height, weight and heart rate (Razif) and pulse rate on Day -21 only.
 *After eating (23 minutes) - Blood pressure, heart rate, and body temperature.
 *Fasting subject only.
 *¹⁴C-N-methyl erythromycin IV injection and breath samples prior to injection and at 10, 20, 30, 45 and 60 minutes after injection.
 *Breath samples before dosing and urine was collected at intervals of 0-24, 24-48, and 48-72 hours post OPC-41061/placebo dose.
 *Day 4 Study Hour 72, ¹⁴C-N-methyl erythromycin IV injection was given at 2 hours post-dose.
 On Day -1, dinner and a snack were provided. Lunch and an evening meal and a snack were provided at approximately 8, 10 and 13 hours, respectively, after dosing with OPC-41061 or placebo on Days 1 and 5. On study days 2 to 4 and 6 to 7, breakfast was provided after the morning blood sample (after the ketoconazole dose on Days 4 and 6) had been taken and other meals at the same times as on Day 1.

TABLE 4.6-1 SCHEDULE OF ASSESSMENTS AND PROCEDURES (Continued)

Day	5	6	7	8	10
Hours	0.5	1	1.5	2	3
Medical History					
Consent					
Inclusion/Exclusion Criteria					
Physical Examination					*
ECG	*	*	*	*	*
Vital Signs	*	*	*	*	*
Serum Chemistry & Hematology					*
Serum Free					*
Hepatitis and HIV Screen					*
Urinalysis					*
Alcohol & Drug Screen					*
Ethnic Collection	*	*	*	*	*
IV Erythromycin and Breath Test		*			
PK Plasma Sample	*	*	*	*	*
Adverse Events					
Ketoconazole Dose					*
Diagnose OPC-41061	*				
Placebo Medication					*
30 Day FU					*

*After eating (23 minutes) - Blood pressure, heart rate, and body temperature.
 *Fasting subject only.
 *¹⁴C-N-methyl erythromycin IV injection and breath samples prior to injection and at 10, 20, 30, 45 and 60 minutes after injection.
 *Breath samples before dosing and urine was collected at intervals of 0-24, 24-48, and 48-72 hours post OPC-41061/placebo dose.
 On Day -1, dinner and a snack were provided. Lunch and an evening meal and a snack were provided at approximately 8, 10 and 13 hours, respectively, after dosing with OPC-41061 or placebo on Days 1 and 5. On study days 2 to 4 and 6 to 7, breakfast was provided after the morning blood sample (after the ketoconazole dose on Days 4 and 6) had been taken and other meals at the same times as on Day 1.

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Pharmacokinetic Profiling:

OPC-41061

Blood samples for the analysis of OPC-41061 were collected at the following times on Days 1 and 5:

Pre-dose, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 h post-dose.

ERB

Injections of ¹⁴C-N-methyl-erythromycin were administered on Days 1 at -1 h pre-dose of OPC-41061 and 2 h after administration of OPC-41061; on Day 4, 2 h after administration of ketoconazole (72 h after OPC-41061 administration), and on Day 5, 2 h after administration of OPC-41061 & ketoconazole.

Breath samples were obtained prior to intravenous injection of ¹⁴C-N-methyl-erythromycin and at 10, 20, 30, 45 and 60 min after injection.

Bioassay

OPC-41061

Plasma and urine samples were analyzed for OPC-41061 and its metabolites using a HPLC/MS/MS method with an internal standard. The assay is linear in the range between 5.0 and 1000 ng/mL for OPC-41061 and 6 metabolites. For DM-4103 the linear range of the assay is between 12.5 and 2500 ng/mL. Coefficients of correlation for the fits of $1/y^2$ weighted linear functions to the calibration standards are not available. The accuracy of the method for OPC-41061 in plasma ranges between -5.76% and 9.01% and the precision is $\leq 5.83\%$. The accuracy of the method for OPC-41061 in urine ranges between -2.83% and 9.49% and the precision is $\leq 6.12\%$. Stability of the analytes in plasma was demonstrated by exposure to 3 freeze/thaw cycles, 24 h at room temperature, long term freezer conditions at -80°C for 201 days. The assays were performed by [REDACTED]

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Ketoconazole

A validated HPLC/UV method with internal standard (proprietary method of [REDACTED]) was used for the determination of the plasma concentrations of ketoconazole. The assay is linear in the concentration range 0.050 $\mu\text{g/mL}$ and 10.0 $\mu\text{g/mL}$. The mean correlation coefficient is 0.9997. The accuracy ranges between -0.68% and -0.281. The precision is $\leq 7.61\%$. The stability of the analyte during long term freezer storage, freeze/thaw cycles, at room temperature and during processing is not reported.

The assays was performed by [REDACTED]

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PK Analysis

OPC-41061 and Metabolites

C_{max} , t_{max} , AUC_t , AUC_∞ and the percent that is extrapolated from the last observed time point ($\text{AUC}\%$ extrapolated), $t_{1/2z}$ were computed for OPC-41061 and metabolites. CL/F , CL_r , Ae_{0-72} and Vz/F were only computed for OPC-41061. The λ_z was determined by least square regression of the log transformed data using at least 3 measurable concentrations. Renal clearance, CL_r , was calculated from $\text{Ae}_{0-72}/\text{AUC}_\infty$. A non-compartmental approach using the software WinNonlin (Professional Edition, Version 2.1) was used.

ERB

The area under the dpm-time curve from time 0 to 1 h was determined for each subject; Day -1 values were compared with Day 4 and 5 values and the percent decrease determined.

Statistical Analysis

5.3 Statistics
The primary assessment of the influence of co-administration of ketoconazole on OPC41061 pharmacokinetics was based on a paired t-test of pharmacokinetic parameters. Adherence to normality assumptions was tested for both log transformed and raw data. Log transformed data was used in cases where it better satisfied the assumption of normality. If neither raw nor log transformed data adequately satisfied the normality assumption, the Wilcoxon signed rank test was used. For t_{max} , only the Wilcoxon signed rank test was used.

RESULTS

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Disposition and Demographics of Subjects

Seventeen subjects completed the OPC-41061 arm and 5 subjects completed the placebo arm. One screened subject did not return for dosing. Two subjects withdrew on Day 1 after receiving the OPC-41061 dose for personal reasons. Mean age and weight of the subjects were 32 (9.4) years and 75 (9.4) kg. There were 12 males and 7 females.

PK

OPC-41061 and Metabolites

A semi-logarithmic plot of the mean plasma concentrations of OPC-41061 in the absence and presence of ketoconazole and the corresponding parameters of OPC-41061 are shown in the below figure and table, respectively:

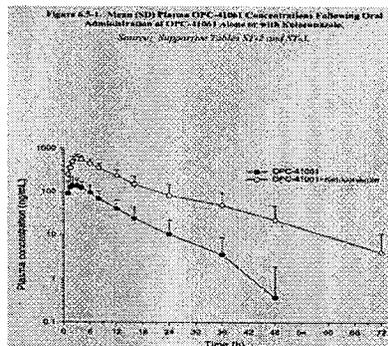


Table 6.5.1. Summary of Pharmacokinetic Parameters for OPC-41061 Following Administration of 30 mg OPC-41061 Alone or with 200 mg Ketoconazole

	OPC-41061 alone		OPC-41061 + ketoconazole		OPC-41061 alone		OPC-41061 + ketoconazole		OPC-41061 alone		OPC-41061 + ketoconazole					
	N	Mean	SD	Median	CV%	QM Mean	LL of 95% CI	UL of 95% CI	N	Mean	SD	Median	CV%	QM Mean	LL of 95% CI	UL of 95% CI
C_{max}	17	173.79	64.71	173.73	37.24	162.58	140.51	207.07	17	605.61	140.89	277.78	23.26	289.42	539.15	678.07
t_{max}	17	2.71	1.35	3.00	0.78	2.38	2.61	3.40	17	3.34	1.04	3.02	1.27	3.18	2.40	3.87
AUC₀₋₂₄	17	1364.11	633.34	1299.07	46.44	1221.96	1034.29	1889.92	17	7673.12	3691.13	6856.01	40.29	7157.40	4683.41	9262.82
AUC_{0-∞}	17	1459.52	651.38	1352.70	44.76	1321.67	1123.50	1795.54	17	7876.75	3145.03	6962.37	39.93	7391.63	6259.31	9494.18
t_{1/2z}	17	5.89	3.29	6.27	5.13	6.22	5.26	8.58	17	10.51	2.67	9.97	25.43	10.24	9.19	11.89
CL/F	17	5.03	2.68	6.27	5.13	6.08	4.36	6.91	17	0.92	0.34	0.94	34.01	0.91	0.81	1.13
V_Z/F	17	3.14	1.90	2.25	60.83	2.74	2.23	4.04	17	0.85	0.26	0.79	30.43	0.81	0.74	0.97
A₀₋₂₄	17	29796.90	28783.98	34020.00	58.60	16114.14	16114.14	43479.66	17	24210.91	155346.63	205838.23	55.83	204211.73	128872.99	306749.34
CL_r	17	0.34	0.38	0.31	111.76	0.16	0.16	0.33	17	0.62	0.48	0.46	75.07	0.46	0.40	0.63

Not determined:
Source: Supportive Tables ST-17 and ST-18

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The plasma concentrations of OPC-41061 in the absence of ketoconazole are measurable up to 24 h post-dose and in the presence of ketoconazole up to 36 h post-dose. The respective observation intervals for determining λ_z and derived parameters are too short for reliable estimates and the so obtained estimates for λ_z and derived parameters including $t_{1/2z}$, AUC, CL/F and V_z/F have to be interpreted with caution. However, it is not anticipated that the bias in AUC_{∞} is large so that a crude assessment of the impact of ketoconazole on the exposure to OPC-41061 based on the sponsor computed measures is possible. There is a significant increase in C_{max} and AUC_{∞} and a trend for an increase in t_{max} during the co-administration treatment with ketoconazole indicating a significant inhibition of the CYP 3A mediated metabolism of OPC-41061. As shown in other studies the excretion of unchanged OPC-41061 in urine is < 1% of the dose and the renal clearance is negligible.

The below table lists the percent change in the PK parameters of OPC that were found to be significantly different following co-administration of ketoconazole with OPC-41061 and alone administration of OPC-41061:

Table 6.5.2. Percent Change in Pharmacokinetic Parameters that were Significantly Different (p<0.05) Following Co-administration of 30 mg OPC-41061 with 200 mg ketoconazole Compared to 30 mg OPC-41061 Alone

Compound	C _{max} (ng/mL)	t _{max} (h)	AUC ₀₋₆ (ng·h/mL)	AUC _{0-∞} (ng·h/mL)	t _{1/2,z} (h)	CL/F (mL/min/kg)	CL _r (mL/min)
OPC-41061	+248	—	+462	+440	+53	-83	+82
DM-4103	-19	+83	—	—	—	NC	NC
DM-4104	-73	+222	-37	—	+99	NC	NC
DM-4105*	—	—	—	—	—	—	—
DM-4107	-58	+255	—	—	—	NC	NC
DM-4110	—	+241	+145	+99	+41	NC	NC
DM-4111	-51	+365	—	NC	NC	NC	NC
DM-4119	-47	+276	—	NC	NC	NC	NC

— value was not significantly different from that calculated following administration of OPC-41061 alone

NC value was not calculated for this compound

* all plasma concentrations were BQL following co-administration of OPC-41061 with ketoconazole.

Source: Supportive Tables ST-17 through ST-31.

The sponsor's estimates for C_{max} and AUC_∞ suggest that ketoconazole co-administration increases the exposure to OPC-41061 significantly about 3.5 and 5.6 fold, respectively. The above mentioned assay sensitivity related limitations for the interpretation of the parameter estimates apply also for the metabolites. Because the metabolites are not active knowledge of the quantitative impact of ketoconazole on their exposure is not considered critical.

ERBT

The below table summarizes the results on the ERBT.

Table 6.5.3 Summary of Mean (SD) ERBT data for Baseline (Day -1), 30 mg OPC-41061 alone (Day 1), 200 mg ketoconazole alone (Day 4), and 30 mg OPC-41061 coadministered with 200 mg ketoconazole (Day 5)

Variable ^a	Baseline (Day -1)	30 mg OPC-41061 Alone (Day 1)	200 mg Ketoconazole Alone (Day 4)	30 mg OPC-41061 + 200 mg Ketoconazole (Day 5)
N	17	17	17	17
AUC ₀₋₆₀	26493.24 (7577.16)	26700.44 (5583.17)	12916.91 ^b (3893.46)	9409.41 ^b (3043.20)

a: Unit of AUC₀₋₆₀ is dpn³·min

b: p < 0.05 when compared to baseline.

The AUC₀₋₆₀ values for ERBT after administration of ketoconazole alone and co-administration of ketoconazole and OPC-41061 compared to the baseline value are statistically significantly decreased to about 51% and 37%, respectively. OPC-41061 alone does not impact ERBT. The findings confirm that CYP 3A plays a central role in the elimination of OPC-41061.

Conclusions

Co-administration of ketoconazole 200 mg and OPC-41061 30 mg results in an approximately 3.5 and 5.6 fold increase in C_{max} and AUC, respectively. The ERBT results confirm that metabolism by CYP 3A is the major route of elimination of OPC-41061. OPC-41061 appears not to have an impact on ERBT. The maximum labeled dose of ketoconazole is 400 mg. The extent of inhibition of OPC-4106 when co-administered with OPC-41061 cannot be estimated from the result of the present study that used 200 mg ketoconazole.

Comments

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1. The sponsor should have evaluated the impact of 400 mg ketoconazole on co-administered OPC-41061.
2. The sensitivity of the assay for OPC-41061 and the metabolites is not sufficient to measure reliably the relevant PK parameters. It would have been important to assess t_{1/2} of OPC-41061 after OPC-41061 alone and co-administration with ketoconazole. Based on reliably assessed t_{1/2} values a determination could have been made as to whether the inhibition of OPC-41061 by ketoconazole is pre-systemic or pre-systemic and systemic.

Study Report 156-03-239: "An Open-Label, Sequential Study of the Effects of Rifampin Administration on Tolvaptan Pharmacokinetics and Pharmacodynamics in Normal, Healthy Volunteers"

Investigator and Study Site



b(4)

Objectives

Primary

- To evaluate the effect of multiple dose 600 mg rifampin pretreatment and concomitant administration on tolvaptan PK

Secondary

- To determine the safety of concomitant administration of tolvaptan and rifampin
- To evaluate the effect of multiple dose 600 mg rifampin pretreatment and concomitant administration of rifampin on tolvaptan and metabolite PK
- To determine rifampin concentrations to verify dosing
- To determine the effect of multiple dose 600 mg rifampin pretreatment and concomitant administration on urine volume, fluid intake, fluid balance and urine osmolality

Investigational Drugs and Formulations

Tolvaptan 60 mg tablets (Lot No. 04L93A060) and rifampin (Rifadin®, Eon Labs, Lake Success, NY) 300 mg capsules were supplied to the Unit by the sponsor.

Design

This was a single-center, open-label, sequential study of the effects of multiple doses of 600 mg rifampin qd on the PK and PD of tolvaptan 240 mg in normal healthy volunteers. Fifteen subjects were enrolled in the study. The study duration was approximately 3 months with expected study participation of 39 days for each subject. The subjects remained in the Unit for the 10 day duration of the study for a total of 11 days. On Day 1 a single dose of 240 mg (4 x 60 mg tablets) tolvaptan was administered to the subjects. On Days 3 to 8 and 10 rifampin 600 mg qd (2x 300mg tablets) was administered to the subjects. On Day 9 tolvaptan 240 mg and rifampin 600 mg were co-administered. All study drugs were administered together with 240 mL of water.

No medication other than the planned study drugs were to be taken by the subjects within 14 days prior to the start of the study and during the study. The intake of antibiotics was prohibited within 30 days of the study or during the study. Grapefruit- and Seville orange containing products were prohibited from 72 h prior to study start and during the study. Use of alcohol- or xanthine-containing products was prohibited within 72 h prior to dosing and during the study.

The scheduled study activities are listed in the below tabulations:

Day	Screening	Check-in	Treatment							7 day FU
	-21 to -2	Day -1	Day 1	Day 2	Day 3-8	Day 9	Day 10	Day 11/E/T		
Informed consent	X									
Inclusion/Exclusion criteria	X	X								
Medical history	X									
Physical exam (with weight)	X ^a	X						X		
Vital signs ^b	X	X	X				X	X		
Resting 12-lead ECG ^c	X	X	X				X	X		
Enrollment			X							
Serum Chemistry, Hematology, urinalysis	X	X						X		
Hepatitis B, C, and HIV	X									
Alcohol and drug screen	X	X								
Serum pregnancy test (WOCBP)	X	X						X		
Tolcaptan PK blood draws			X ^d	X ^e	X ^f	X ^g	X ^h	X ⁱ		
Rifampin PK blood draws			X ^j		X ^k					
Urine volume and fluid intake			X ^l	X ^m	X ⁿ	X ^o	X ^p	X ^q		
Begin fasting at 10 PM (2200 hr) ^r		X			X ^s					
Admit to clinic in PM		X								
Record AEs	X	X	X	X	X	X	X	X	X	
Record concomitant meds	X	X	X	X	X	X	X	X	X	

Day	Screening	Check-in	Treatment							7 day FU
	-21 to -2	Day -1	Day 1	Day 2	Day 3-8	Day 9	Day 10	Day 11/E/T		
Tolcaptan dosing ^a			X			X				
Rifampin dosing ^b					X	X	X			
Meals ^c		X	X	X	X	X	X	X		
*Day 11 telephone call									X	

^aE.T. = early termination; FU = follow-up; Hep. = hepatitis; HIV = human immunodeficiency virus; WOCBP = woman at childbearing potential
^bPhysical examination included elbow breadth and height at Screening.
^cVital signs (blood pressure, pulse, and temperature) were assessed after the subject had been in a supine position for ≥ 3 minutes.
^dStandard 12-lead ECGs were performed after the subject had been recumbent and at rest for at least 10 minutes prior to the ECG.
^ePK blood draws: pre-dose, followed by 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 16 hours post-dose.
^fPK blood draws: 24, 30, and 36 hours post-dose.
^gPK blood draws: 48 hours post-dose.
^hPK blood draws: pre-kick only.
ⁱPK blood draws: 2 hours post-dose.
^jUrine volume and intake measurements during the following intervals: 0-2, 2-4, 4-6, 6-8, 8-12, 12-16, 16-24 hours post-dose. A sample from each was taken for urine osmolality.
^kUrine volume and intake measurements during the following intervals: 24-26, 26-28, 28-30, 30-32, 32-36, and 36-40 hours post-dose. A sample from each was taken for urine osmolality.
^lUrine volume and intake measurements 40-48 hours post-dose on Day 3 and 11.
^mFasting on Day 8 only.
ⁿDosing at approximately 1600 hours. Dosing occurred in the fasted state on Day 1 and 9.
^oDosing of rifampin occurred at least 1 hour prior to breakfast, except on Day 9.
^pMeals were breakfast (~8 AM), lunch (~noon), dinner (~6 PM) and evening snack (~10 PM) except on Day -1, where only dinner was served, and Day 11, where only breakfast was served. No breakfast on Day 1 and Day 9.

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Pharmacokinetic Profiling

Blood samples for the determination of the plasma concentrations of tolvaptan and metabolites were collected on:

Days 1 and 9: Pre-dose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h post-dose

Days 7 and 8: Pre-dose and 2 h post-dose

Bioassay

OPC-41061 and DM-4103 and DM-4107

The plasma concentrations of OPC-41961 and metabolites were measured by a validated HPLC/MS/MS method with an internal standard. The assay is linear (1/y²) over the range 5.0 ng/mL-1000 ng/mL for OPC-41061 and 12.5

ng/mL- 2500 ng/mL for the metabolites DM-4103 and DM 4107. The mean correlation coefficient for OPC-41061, DM-4103 and DM-4107 is 0.9970, 0.9976 and 0.9972, respectively. The stability of the analytes was confirmed by exposure to repeated freeze/thaw cycles, during sample processing, and during refrigeration at -80° C using spiked QC samples that were exposed for the same length as the plasma samples with unknown concentrations. The accuracy of the method for OPC-41061 ranges between -1.13% and 6.88% and the precision is \leq 9.32%. The accuracy for DM 4103 ranges between -1.00% and 3.30% and the precision is $<$ 10.6%. The accuracy for DM-4107 is 0.5% and 4.70% and the precision is \leq 9.05%. The assays were performed by [REDACTED]

b(4)

Rifampin

[REDACTED] proprietary validated HPLC/UV method with an internal standard was used for the determination of rifampin in plasma. The method is linear ($1/Y^2$) in the range between 100-20000 ng/mL with a mean correlation coefficient of 0.9952. The accuracy ranges between -4.13% and 2.33 % and the precision is \leq 11.5%. No information on the stability of the analyte during exposure of the matrix to freeze/thaw cycles during storage at room temperature of sample processing is provided. The assay was performed in [REDACTED]

b(4)

PD Profiling

Urine volumes were measured on:

Days 1 and 9: 0-2, 2-4, 4-6, 6-8, 8-12, 12-16, 16-24, 24-26, 28-30, 30-32, 32-36, 36-40, and 40-48 h post-dose. A single sample was taken from each collection interval for determination of urine osmolality.

Fluid intake [REDACTED] were measured. [REDACTED]

b(4)

For urine osmolality the product of urine osmolality and duration of each collection interval over a period of 24 h was computed (AUC0-24). The time duration during which urine osmolality remains below 300 mOsm was determined as well.

PK Data Analysis

Non-compartmental methods were used. The following parameters were estimated: C_{max} , t_{max} , AUC0-24, AUC_t, $t_{1/2z}$, CL/F for tolvaptan and t_{max} , C_{max} and AUC_t for the metabolites.

Statistical Evaluation

Statistics: The primary endpoints were the PK parameters C_{max} and AUC₀₋₂₄ for tolvaptan following a single oral dose of tolvaptan with rifampin versus tolvaptan alone. As AUC₀₋₂₄ could not be determined for subjects following the tolvaptan + rifampin treatment, AUC_t was used. A paired t-test, with two-sided alpha of 0.05, was used to determine if rifampin treatment produced a significant change in tolvaptan C_{max} or AUC_t.

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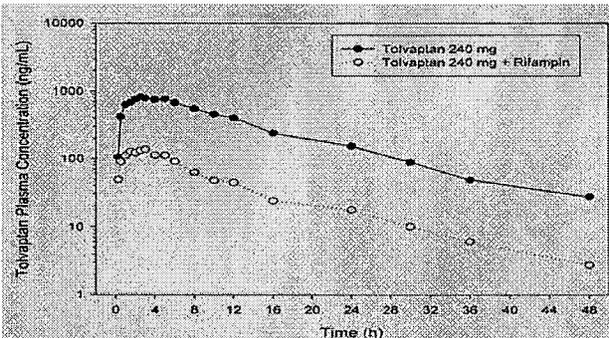
RESULTS

Disposition and Demographics

Fifteen subjects were enrolled in the study and completed the protocol. The mean age and body weight of the subjects was 26 (8) years and 72(11) kg. There were 9 males and 6 females.

PK

A semi-logarithmic plot of the plasma concentrations of tolvaptan against time in the presence and absence of rifampin and the corresponding parameters for tolvaptan is shown in the figure and table, respectively, below:



Note: The lower limit of quantitation (LLOQ) of tolvaptan is 5.00 ng/mL.
Source: S1-2 to S1-3

Figure 9.2.3.1-1 Mean Tolvaptan Plasma Concentrations Following a Single Oral 240-mg Dose of Tolvaptan Alone or with Rifampin at Steady State (600 mg QD) to 15 Normal Healthy Subjects

Parameter	240 mg Tolvaptan (N = 15)	240 mg Tolvaptan + Rifampin (N = 15)
C _{max} (ng/mL)	1000 (936) ^a	168 (73.3)
t _{max} (h) ^b	2.52 (1.00-12.00)	3.00 (1.20-6.02)
AUC _{0-24h} (ng·h/mL)	10000 (3500) ^a	1320 (521)
AUC _t (ng·h/mL)	11600 (4060)	1470 (686)
t _{1/2z} (h)	7.7 (3.9) ^c	ND
AUC _∞ (ng·h/mL)	12100 (3140) ^a	ND
CL/F (mL·min/kg)	5.23 (1.07) ^c	ND
C _{max} ratio ^d	—	0.17 (0.05)
AUC _t ratio ^d	—	0.13 (0.05)

ND = Not determined.
^a Values significantly higher as determined by paired t test, p < 0.0001.
^b Values are median (minimum-maximum).
^c N = 12.
^d Tolvaptan + Rifampin/Tolvaptan alone.

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After a single dose of tolvaptan 240 mg given alone the plasma concentrations are measurable in all subjects up to 36 h only post-dose. In the presence of rifampin the plasma concentrations of OPC-41061 are only measurable only up to 16 h post-dose in all tested subjects. The observation interval for determining λ_z and derived parameters is too short and the sponsor's estimates for λ_z and derived parameters including t_{1/2z}, AUC, CL/F and V_z/F have to be interpreted with caution. However, it is not anticipated that the bias in AUC_∞ is large so that a crude assessment of the impact of rifampin on the exposure to OPC-41061 based on the sponsor computed exposure measures is possible. The data indicate a significant reduction of OPC-41061's C_{max} to about 20 % and AUC_∞ to about 10% with co-administered rifampin indicating a significant induction of the CYP 3A mediated metabolism and/or MDR1 extrusion of OPC-41061.

The follow-up time for the time profiles of the metabolites DM-4103 and DM-4107 is not adequate and thus the interpretation of the exposure parameters presented by the sponsor is limited. Since none of the metabolites is active the assessment of the quantitative impact of rifampin on the exposure to the measured metabolites is not considered critical.

The rifampin concentrations measured during the co-administration phase confirm the subjects' compliance with the protocol.

PD

The 24 h urine volumes after alone administration of tolvaptan are clearly greater than those after co-administration of tolvaptan and rifampin. In the presence of rifampin the reduction in the aquaretic effect of tolvaptan appears to be smaller than the reduction in exposure to tolvaptan which supports the notion of a saturable, nonlinear relationship between exposure and response of tolvaptan.

Conclusion

PK

Rifampin co-administration reduces Cmax and AUC to about 10 % and 20 %, respectively, suggesting that rifampin impacts tolvaptan elimination and/or extrusion by inducing CYP3A and/or MDR1.

PD

The reduction of the aquaretic effect of tolvaptan in the presence of rifampin appears to be smaller than the reduction in the exposure to tolvaptan in accordance with a nonlinear dose-response relationship.

Comments

1. Rifampin is known to inhibit and induce the metabolism and transport of other drugs. In this study a possible interaction between tolvaptan and rifampin was only studied after steady-state of rifampin is attained. Thus, an initial inhibition of tolvaptan's possible hepatic influx and efflux by rifampin may have been missed.
2. No information on the stability of rifampin during exposure of the matrix to freeze/thaw cycles during storage at room temperature and sample processing is provided.

Study Report: 156-01-223:"A Study to Determine the Effects of Single Dose Administration of OPC-41061 on the Single Dose Pharmacokinetic Profile of Lovastatin in Healthy Male Male and Female Subjects"

Investigator and Study Site

b(4)

Objectives

Primary

To determine the effects of OPC-42061 60 mg on the pharmacokinetics of lovastatin

Secondary

To characterize the pharmacokinetics of OPC-41061 60 mg and OPC-41061 90 mg in the presence of lovastatin; and to determine the effects of OPC-41061 90 mg on the pharmacokinetics of lovastatin

Investigational Drugs and Formulations

Lovastatin (Mevacor®) 80 mg (2x 40 mg tablets, Lot No. K1123) and OPC-41061 60 mg (one 60 mg tablet Lot No. 99E87A030A) and 90 mg (one 60 mg and one 30 mg tablet (Lot No. 99E87A060) .

Design

This was a single-center, open-label, 1 arm, 3-period, sequential treatment, drug interaction study conducted in 15 healthy male and female subjects in the age of 18-45 years. Each subject received a single oral dose of 80 mg lovastatin during Period 1, a single 80 mg dose of lovastatin given concomitantly with a single dose of 60 mg OPC-41061 during Period 2, and a single oral dose of lovastatin 80 mg given concomitantly with a single dose of 90 mg OPC-41091 in Period 3. Dosing was separated by a washout of at least 5 days. Blood samples were collected at scheduled times for up to 96 h and were analyzed for lovastatin, lovastatin β-hydroxy acid, and OPC-41061. Prior to dosing subjects were restricted from intake of food and beverages (other than water) for 9 h. On dosing days a standard breakfast was served approximately at 0700 and subjects had to fast until 1300. In addition, except for the 240 mL water administered with each dose subjects could not consume water within 2 h of study medication. Subjects had to meet restrictions on birth control, exercise, and the use of concomitant medications, alcohol and food/beverages.

The scheduled study activities are listed in the below schemes;

TABLE 44-1 SCHEDULE OF ASSESSMENTS: SCREENING AND STUDY DAY -1

Study Procedure	Study Day (D)	Screening: D-21 to D-2	Check-in: D-1
Admission to clinic (approximately 7:00 PM)			X
Informed consent	X		
Medical history	X		
Medication history	X		
Physical performance criteria	X		
Physical examination (including body weight)	X		
Vital signs	X		
Laboratory tests	X		
Serum pregnancy test for female subjects	X		
ECG (drug screen)	X		
Urine alcohol	X		
12-lead ECG	X		
Meal ^a			X

^a Height and weight for this follow breakfast at screening visit only.
^b Sitting blood pressure and pulse; Standing blood pressure and pulse (after sitting/standing 2 minutes). The same arm was used for each assessment.
^c Hematology, chemistry, serology and urinalysis.
^d Dinner at approximately 6:00 PM and snack at approximately 9:30 PM (except fasting at approximately 10:00 PM).

TABLE 44-2 SCHEDULE OF ASSESSMENTS FOR PERIOD 1

STUDY PROCEDURE	DAY	DAY 1															DAY 2	DAY 3	DAY 4	DAY 5
		Hour	-2	-1	0	0.5	1	2	3	4	5	7	11	12.5	20	24	48	72	96	
Vital signs ^a		X					X								X				X	
Laboratory tests ^b		X																	X	
12-lead ECG ^c		X					X							X					X	
Lovastatin Dosing			X																	
Blood collection for Lovastatin & lovastatin β-hydroxy acid			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Meal ^d		X ^e						X ^f		X ^g		X ^h		X ⁱ		X ^j		X ^k		
Blood collection for OPC-41061 (at screening and follow-up)			X																	
Adverse events and laboratory monitoring																				

^a Sitting blood pressure and pulse; Standing blood pressure and pulse (after sitting/standing 2 minutes). The same arm was used for each assessment.
^b Hematology, chemistry, and serology.
^c 12-lead ECG blood sample could be done on 10 or 11 minutes post-dose.
^d Standard breakfast 2 hours post-dose (approximately 10:00 AM).
^e Lunch approximately 6 hours post-dose (approximately 1 PM).
^f Dinner approximately 9 hours post-dose (approximately 6 PM).
^g Evening snack (approximately 12.5 hours post-dose (approximately 9:30 PM)).
^h Breakfast, snack, dinner, and evening snack.
ⁱ Blood collection could be done 15 to 30 hours post-dose.

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TABLE 4.6-3 SCHEDULE OF ASSESSMENTS FOR PERIODS 2 and 3

STUDY PROCEDURES	DAY	Day 1													Day 2		Day 3	Day 4	Day 5	Follow-up (72 Days)	
		Hour	-2	-1	0	0.5	1	2	3	4	8	12	12.5	24	36	48	72	96			
Vital signs ^a		X						X							X					X	
Laboratory tests ^b		X																			X
12-lead ECG ^c		X						X							X						X
Adverse Event Monitoring ^d			X																		
Blood collection for lovastatin & lovastatin β-hydroxy acid			X ^e	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X
Blood collection for OPC-41061			X ^e	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X
Blood collection for serum albumin								X			X				X						
Urinalysis		X						X ^e	X ^e		X ^e	X ^e									
Adverse events and concomitant medications																					X ^f
Physical exam, including weight																					X ^g
Discharge from the clinic																					X ^h

Pharmacokinetic Profiling

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Blood samples for the determination of the plasma concentrations of lovastatin, lovastatin β-hydroxy acid and OPC-41061 were collected at the following times:

Pre-dose, 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48, 72, and 96 h post-dose. One additional blood sample was collected for the determination of the plasma protein binding of OPC-41061.

Bioassay

OPC-41061

Plasma concentrations of OPC-41061 were analyzed by a validated HPLC/UV method using an internal standard. The method is linear (1/y) over the concentration range 5.00 ng/mL and 1000.00 ng/mL. The mean coefficient of correlation is 0.998. The accuracy of the assay ranges between -3.7 % and 12.6 % and the precision is ≤ 8.3 %. Stability of the analyte was demonstrated during processing for 49 h on the autosampler and for 4 h at room temperature. Results of exposure to freeze/thaw cycles and prolonged deep freezer conditions are not reported. The measurements were performed by [REDACTED]

b(4)

Lovastatin and Lovastatin β-Hydroxy Acid

The concentrations of lovastatin and its metabolite were measured by a LC/MS/MS method using an internal standard. The assay is linear over a concentration range of 0.10 and 10.0 ng/mL. The mean coefficient of correlation for lovastatin and lovastatin β-hydroxy acid is 0.9986 and 0.9982, respectively. The accuracy of the assay for lovastatin ranges between -6.00% and -0.31% and the precision is ≤ 28.0%. The accuracy for lovastatin β-hydroxy acid ranges between -3.65% and 1.67% and the precision is ≤ 23.2%. The precision values for both lovastatin and its metabolites exceed the upper limit of 15%. The stability of the analytes in plasma was confirmed by exposing plasma samples to long term storage at -70 °C, room temperature for > 23 h and 5 freeze-thaw cycles. The assay was performed by [REDACTED]

b(4)

Plasma Protein Binding

Plasma protein binding of OPC-41061 was measured using radio-labeled OPC-41061 and a validated equilibrium dialysis method. The measurements were performed by [REDACTED]

b(4)

Pharmacokinetic Data Analysis

The following parameters were determined for OPC-41061 and lovastatin: C_{max}, t_{max}, AUC_t, AUC_∞, Vz/F, CL/F, and λ_z. C_{max} and t_{max} were taken directly from the data. AUC_t was determined by applying the linear trapezoidal rule. λ_z was determined by log linear regression of the post peak concentrations. The software WinNonlin Professional (Version 3.01) was used.

Statistical Analysis

5.3 Statistics
 All plasma concentration data and all pharmacokinetic parameter estimates (except t_{max}) were summarized by treatment within each dose group using descriptive statistics including the following: number of subjects (n), mean, standard deviation (SD), 95% CI, minimum, median, and maximum. Only median, minimum, and maximum were determined for t_{max}. The pharmacokinetic parameters of lovastatin and lovastatin β-hydroxy acid were checked for adherence to normality. Since some of the pharmacokinetic parameters did not pass the normality test (p<0.05), log-transformed data were used. The influence of co-administration of OPC-41061 on lovastatin and its metabolite pharmacokinetics (C_{max}, t_{max}, AUC_t, AUC_∞, CL/F, and Vz/F) for lovastatin and C_{max}, t_{max}, AUC_t, t_{1/2}, and AUC_∞ for lovastatin β-hydroxy acid was assessed using an independent t-test. Since some of the pharmacokinetic parameters such as AUC_∞ and CL/F could not be determined for several subjects, the paired t-test could not be used as mentioned in the protocol. For t_{max}, the Wilcoxon rank sum test was used. A p-value of 0.05 or less was considered significant. The analyses were performed using Microsoft Excel 2000 and SAS version 6.12.

RESULTS

Demographic and Disposition

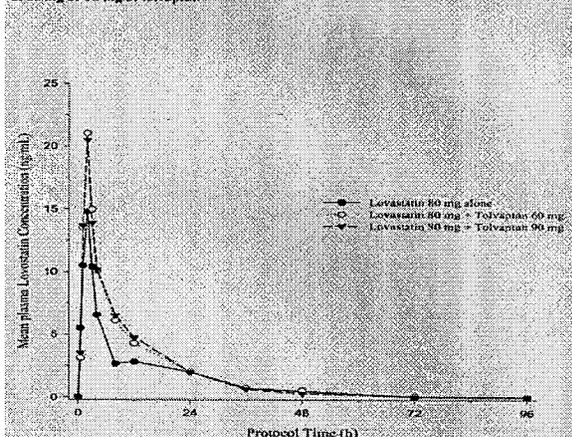
A total of 15 subjects were enrolled and all completed the study. The mean age and body weight of the subjects was 33.8 (7.3) years and 80.3 (8.4) kg, respectively. Nine of the subjects were male and 6 female.

Pharmacokinetics

Impact of Tolvaptan on Lovastatin and Metabolite

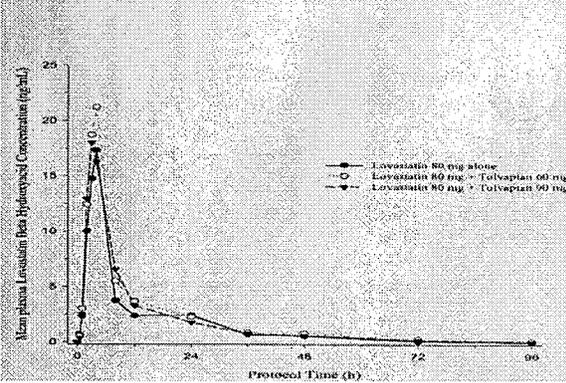
Linear plots of the mean plasma concentration time profiles of lovastatin and lovastatin β-hydroxy acid after 80 mg lovastatin administered alone and after co-administration of OPC-41061 60 mg or 90 mg are shown in the 2 figures below:

Figures 6-4-1 Mean plasma lovastatin concentration - time profiles following a single oral administration of 80 mg of lovastatin alone or in combination with either a single oral dose of 60 mg or 90 mg of tolvaptan



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Figure 6.5-2 Mean plasma lovastatin β-hydroxy acid concentration – time profiles following a single oral administration of 80 mg of lovastatin alone or in combination with either a single oral dose of 60 mg or 90 mg of tolvaptan



The mean plasma concentrations of lovastatin and its metabolite appear to be slightly greater during the co-administration treatment with OPC-41061 than after the alone treatment of lovastatin.

The parameters for lovastatin alone and in the presence of OPC-41061 are listed in the below tables:

Table 6.5-1 Summary of lovastatin pharmacokinetic parameters following a single oral administration of 80 mg of lovastatin

	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_0-t (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	CL/F (mL/min/kg)	V _D /F (L/kg)
N	15	15	11	15	11	11	11
Mean	15.91	—	12.19	122.59	134.69	144.36	154.96
SD	7.35	—	7.58	57.56	64.01	56.93	133.29
Median	14.9	2.00	12.84	91.3	122.99	129.36	113.83
%CV	46.21	—	62.14	46.95	47.52	39.44	86.01

— not determined.
Source: ST-10

Table 6.5-2 Summary of lovastatin pharmacokinetic parameters following a single oral administration of 80 mg of lovastatin in combination with either a single oral dose of 60 mg or 90 mg of tolvaptan

Treatment: Lovastatin 80 mg + Tolvaptan 60 mg

	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_0-t (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	CL/F (mL/min/kg)	V _D /F (L/kg)
N	14	14	9	14	9	9	9
Mean	21.61	—	10.12	174.44	189.40	107.38	85.74
SD	11.11	—	4.9	73.31	84.85	58.10	42.82
Median	19.54	2.00	7.91	172.32	177.48	91.80	80.55
%CV	51.43	—	48.37	42.02	44.80	54.10	49.94

— not determined.
Source: ST-11

Treatment: Lovastatin 80 mg + Tolvaptan 90 mg

	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_0-t (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	CL/F (mL/min/kg)	V _D /F (L/kg)
N	15	15	12	15	12	12	12
Mean	21.38	—	9.75	169.79	187.74	107.19	89.94
SD	13.3	—	4.74	64.53	70.75	53.42	59.57
Median	17.47	2.00	8.63	132.1	160.85	98.77	78.21
%CV	62.18	—	48.56	38.01	37.68	49.83	63.89

— not determined.

The parameters for lovastatin β-hydroxy acid are tabulated in the below tables:

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Table 6.5-3 Summary of lovastatin beta-hydroxy acid pharmacokinetic parameters following a single oral administration of 80 mg of lovastatin

	C _{max} (ng/mL)	t _{max} (h)	t _{1/2z} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)
N	15	15	7	15	7
Mean	18.97	—	13.89	152.01	217.84
SD	14.54	—	5.2	113.86	139.79
Median	14.72	4.00	14.73	97.840	147.02
%CV	76.63	—	37.45	74.9	64.20

— not determined.

Source: ST-13

Table 6.5-4 Summary of lovastatin beta-hydroxy acid pharmacokinetic parameters following a single oral administration of 80 mg of lovastatin in combination with either a single oral dose of 60 mg or 90 mg of tolvaptan

Treatment: Lovastatin 80 mg + Tolvaptan 60 mg

	C _{max} (ng/mL)	t _{max} (h)	t _{1/2z} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)
N	14	14	7	14	7
Mean	22.96	—	16.1	195.60	191.08
SD	17.6	—	12.35	159.75	187.48
Median	17.02	3.00	12.68	135.76	126.65
%CV	76.65	—	76.87	81.67	98.12

— not determined.

Source: ST-14

Treatment: Lovastatin 80 mg + Tolvaptan 90 mg

	C _{max} (ng/mL)	t _{max} (h)	t _{1/2z} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)
N	15	15	7	15	7
Mean	19.34	—	12.41	164.69	208.35
SD	14.76	—	16.07	138.99	169.71
Median	15.29	3.00	9.82	120.83	157.85
%CV	76.33	—	92.34	72.20	81.50

— not determined.

Source: ST-15

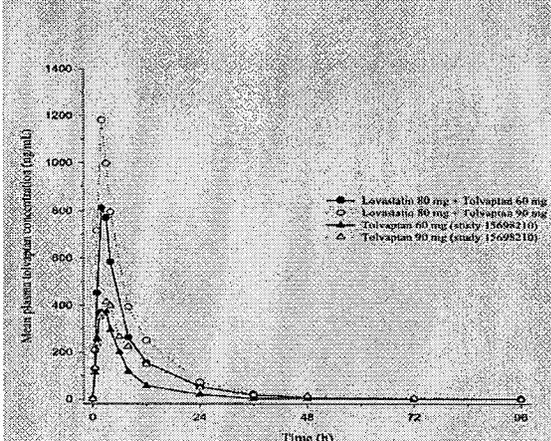
The plasma concentrations of lovastatin and its metabolite after alone treatment are measurable up to 12 h in all tested subjects. After single dose co-administration of tolvaptan 60 mg or 90 mg lovastatin and its metabolite are measurable up to 24 h after administration suggesting that tolvaptan co-administration may increase the exposure to lovastatin and its active metabolite.

The sponsor estimate mean t_{1/2z} of about 12 h for lovastatin and its metabolite from data followed for 12 or 24 h. This is a too short time interval for reliably measuring λ_z. However, the respectively extrapolated AUC values are probably relatively small so that the bias in the estimates may be minor.

Thus, a comparison of the treatments based on the sponsor's average exposure values may allow a crude assessment of the possible impact of OPC-41061 on lovastatin and its metabolite. The arithmetic mean ratio of C_{max} and AUC_t of lovastatin in the presence and absence of tolvaptan 60 mg is each about 1.4. The arithmetic mean ratios of C_{max} and AUC_t for lovastatin β-hydroxy acid in the presence and absence of tolvaptan 60 mg are about 1.2 and 1.3, respectively. After co-administration of 90 mg tolvaptan the arithmetic mean ratios for C_{max} and AUC_t are about 1.3 and 1.4, respectively, and for lovastatin β-hydroxy acid, about 1.0 and 1.1 respectively.

The below figure shows the mean plasma concentration time profiles of tolvaptan after co-administration of tolvaptan 60 mg or 90 mg with 80 mg lovastatin (present study). The below figure also displays the mean plasma concentrations of tolvaptan after 60 and 90 mg alone administration obtained in a previous study (Study Report 156-98-210):

Figure 6.5.3. Mean plasma tolvaptan concentration - time profiles following either a single oral dose of 60 mg or 90 mg of tolvaptan co-administered with 80 mg of lovastatin (this study) or a single oral administration of 60 mg or 90 mg of tolvaptan alone (previously reported data).



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The across study results appear to suggest that the mean profiles of tolvaptan in the presence of lovastatin are greater than in its absence. However, the possible impact of lovastatin co-administration on the PK of tolvaptan should be studied in the same subjects and in the same study.

Conclusion

Co-administration of a single dose of tolvaptan 60 mg increases peak and average exposure to lovastatin after a single dose administration of 80 mg about 1.4 fold. The respective figures for lovastatin β -hydroxy acid are about 1.2 and 1.3, respectively. Co-administration of 90 mg tolvaptan increases peak and average exposure to lovastatin by about a factor of 1.3 and 1.4, respectively. The peak and average exposure to lovastatin β -hydroxy acid in the presence of tolvaptan 90 is increased about 1.0 and 1.1 fold. The mean percentage of tolvaptan unbound in plasma ranges between 0.90 % and 0.94 %.

Comments

1. The sensitivity of the assays for tolvaptan and lovastatin and its metabolite is not appropriate. The precision values for both lovastatin and its metabolites exceed the upper limit of 20%.
2. Geometric means should be calculated and the one-sided test applied to the lovastatin data in the presence and absence of tolvaptan.
3. The description of the equilibrium dialysis method used and information on the radiochemical purity of the labeled tolvaptan could not be found.
4. The study should include arms with tolvaptan 60 mg and 90 mg alone treatments.

Study Report 156-01-226: "An Open-Label, Single Arm, Sequential Design Study to Evaluate the Effects of Tolvaptan (OPC-41061) on the Pharmacokinetics of Oral Maintenance Amiodarone Therapy in Patients with Cardiac Arrhythmias"

Investigators and Study Site

b(4)

Objectives

Primary

To assess the potential pharmacokinetic effect of tolvaptan mediated CYP 3A4 inhibition on the metabolism of amiodarone and its metabolite desethylamiodarone (DEA)

Secondary

To characterize the pharmacokinetics of tolvaptan following single 30 mg and 60 mg doses in the presence of amiodarone maintenance therapy.

Investigational Drugs and Formulations

Tolvaptan 30 mg tablets (Lot No. 99E87A030A) were supplied by the sponsor. Amiodarone (Cordarone) 200 mg tablets (Lot Nos. A03875 and A05687).

Design

This was a multi-center, open-label, 1 arm, 3-period, sequential treatment, drug interaction study conducted in 20 adult (>18 years of age) male and female subjects with cardiac arrhythmias who were otherwise healthy. Each subject received single doses of amiodarone 200 mg each morning for 5 days. Subjects also received a single 30 mg tolvaptan dose on Day 3 and a 90 mg dose on Day 4. The study medications were ingested approximately 15-20 min prior to eating a standardized breakfast.

The inclusion and exclusion criteria are tabulated below:

1.	Male and female subjects \geq 18 years of age that were surgically sterile or prepared to agree to practice a double-barrier form of birth control from the screening visit through 6 months after the last dose of study medication. A negative serum pregnancy test must have been confirmed prior to the first dose for women of childbearing potential. Females who were more than 12 months post-menopausal and not on hormone replacement therapy were also eligible to participate in the study.
2.	Subjects with a history of arrhythmia and who were on oral amiodarone maintenance therapy of 200 mg/day for at least 10 months.
3.	With the exception of arrhythmia, subjects must have been otherwise healthy as determined by a medical history, physical examination, serum/urine biochemistry, hematology and serology tests.
4.	Must have had a body weight within \pm 20 percent of ideal body weight as defined in the 1983 Metropolitan Height and Weight Tables.

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Table 4.2-2 Exclusion Criteria

1.	History of or presence of clinically significant acute or unstable cerebrovascular, renal, hepatic, gastrointestinal, pulmonary, immunological, endocrine, or central nervous disorder.
2.	Any surgical or medical condition (active or chronic) that may have interfered with drug absorption, distribution, metabolism, or excretion, or any other condition that may have placed the subject at risk.
3.	History of drug and/or alcohol abuse within two years prior to screening.
4.	A positive urine alcohol test and/or urine drug screen for substances of abuse at screening or upon admission to the study center.
5.	History of or current clinically significant mental disorder or an antagonistic personality that would have compromised the validity of the informed consent.
6.	Donation of blood or plasma within 30 days prior to dosing, or loss of more than 1200 mL of blood within four months prior to screening.
7.	History or presence of hepatitis. Acceptance must have been discussed with Sponsor on a case by case basis and reasons documented.
8.	History of AIDS.
9.	Use of an investigational drug or product, or participation in a drug or product study within 30 days prior to dosing.
10.	Consumption of grapefruit or grapefruit juice within the 3 days prior to dosing.
11.	Use of alcohol as well as food and beverages containing methylxanthine (i.e. caffeinated coffee, caffeinated tea, caffeinated soda, and chocolate) within the 1 day prior to dosing of tolvaptan.
12.	Subjects who were pregnant, breast-feeding, or planning to conceive or father a child in the period surrounding the study as described in the informed consent.
13.	Subjects who had previously received OPC-41061.
14.	Subjects who were unable to abstain from vigorous exercise (examples: running more than 2 miles per day, lifting weights for more than 1 hour a day) from 7 days prior to study entry until discharge from the study.

The scheduled study activities are provided in the below scheme:

Table 4.6-1 Schedule of Assessments

Study Procedures	Screen (Day -5-0)	Hour	-1	-0.5	0	2	4	6	8	10	12	Day 15
Admission to Clinic	X (Day 0)											
Informed Consent	X											
Medical History	X											
Urine Drug and Alcohol Screen	X											
Adverse Events and Previous Medications	X											
Physical Exam (including weight)	X		X									
Vital Signs ^a	X		X									
Laboratory Tests ^b	X		X		X	X						
ECG ^c	X		X		X	X						
Amiodarone Dosing				X								
Tolvaptan Dosing				X								
PK Sampling for Amiodarone and DEA				X ^d		X ^e	X ^f	X ^g	X ^g	X ^g		X ^h
PK Sampling for Tolvaptan				X ^d		X ^e	X ^f	X ^g	X ^g	X ^g		X ^h
Adverse Events and Concomitant Medications ⁱ												
Micta	X			X			X				X	
Urine Pregnancy Test	X						X					
Discharge ^j							X					
Following telephone call												X

^a At sitting and standing blood pressures and pulse; subjects should have been sitting/standing for 2-3 minutes (no same arm) for each assessment.
^b Hematology, chemistry and urinalysis; At screening and Days 3, 4, and 5 only.
^c At screening and Days 2, 3, and 4 only.
^d Days 3 and 4 only; Day 3 = 30 mg tolvaptan; Day 4 = 90 mg tolvaptan dosing.
^e Day 5 only.
^f Days 3, 4, and 5 only.
^g Discharge only after laboratory test results were reviewed by the PI.
^h Assuming 121 inclusion weight and frame size (150 lb/68 cm height).
ⁱ At screening only; adverse event and previous medication information is collected.
 Source: Appendix 1.1 (Protocol)

Pharmacokinetic Profiling

Tolvaptan

Blood samples for the determination of tolvaptan plasma concentrations were collected at the following times: Pre-dose, and 2, 4, 6, and 12 h post-dose on Day 3 following administration of 30 mg tolvaptan and on Day 4 following administration of 90 mg tolvaptan.

Amiodarone and DEA

Blood samples for the determination of the plasma concentrations of amiodarone and DEA were collected at the following times:

At screening and on Days 2, 3, and 4: pre-dose, and 2, 4, 6, 8, and 12 h post-dose
 On Day 5: pre-dose

Bioassay

Tolvaptan

Tolvaptan plasma concentrations were measured by a HPLC/UV method using an internal standard. The assay is linear over the range 5.00 ng/mL to 1000.00 ng/mL with a mean correlation coefficient of 0.9980. The accuracy ranges between -5.2% and -2.4% and the precision is $\leq 8.2\%$ for tolvaptan. Stability of the analyte at room temperature for 4 h and after extraction on the autosampler for 49 h was demonstrated. However, results on the stability of the analyte when exposed to freeze/thaw cycles and long term freezer conditions are not provided. The assay was performed by [REDACTED] b(4)

Amiodarone and DEA plasma concentrations were measured by a LC/MS/MS method using an internal standard. The assay is linear over the range 100.18 ng/ml to 4007.00 ng/mL for amiodarone and 99.88 ng/mL and 3995.00 ng/mL for DEA. The accuracy ranges between -4.76 % and 0.60 % and the precision is $\leq 6.09\%$ for amiodarone. The accuracy ranges between -8.89 % and 3.25 % and the precision is $\leq 6.23\%$ for DEA. The stability of amiodarone and DEA at room temperature for 120 h following sample processing, for 12 h in the biological matrix at room temperature, after 5 freeze/thaw cycles storage at -20°C and -80°C and in solution for 1340 days at -20°C was confirmed. The analyses for amiodarone and DEA were done at [REDACTED] b(4)

PK Data Analysis

The following parameters were determined for amiodarone and DEA: $C_{ss,max}$, t_{max} and $C_{ss,min}$ were directly taken from the data. AUC_{τ} was calculated using the linear trapezoidal rule. AUC_{τ} was obtained by WinNonlin Pro (Version 3.1, Pharsight Corporation Mountain View, CA). $C_{ss,min}$ and the ratio of $C_{max,ss}$ and $C_{ss,min}$ in the presence and absence of tolvaptan were obtained "manually".

Statistical evaluation

The confidence interval approach using the two-one sided test was used for the comparison of $C_{ss,min}$, $C_{ss,max}$ and AUC_{τ} of amiodarone in the presence and absence of tolvaptan.

RESULTS

Demographics and Disposition

Twenty-two subjects were enrolled and 21 completed the study per protocol. One subject was found to be taking zolpidem (Ambien®), an excluded medication, and was not allowed to complete the study. The mean age and weight of the subjects was 65.9 (9.5) kg and 74.1 (11.8) kg. There were 11 females and males.

Pharmacokinetics

Among the completing subjects the sponsor excluded 4 subjects with measurable pre-dose plasma concentrations of tolvaptan on Day 3 ("the reason for this is still under investigation"). A fifth subject was excluded from the analysis of DEA because all DEA concentrations were BQL.

The median plasma concentrations of amiodarone and DEA following a 200 mg dose of amiodarone alone and co-administration of 60 mg or 90 mg tolvaptan.

Figure 6.5-1 Median plasma concentrations of amlodaronc following a 200 mg dose of amlodaronc at steady state alone (Days 1 and 2) or with 30 mg tolvaptan (Day 3) or 90 mg tolvaptan (Day 4) in subjects with cardiac arrhythmias

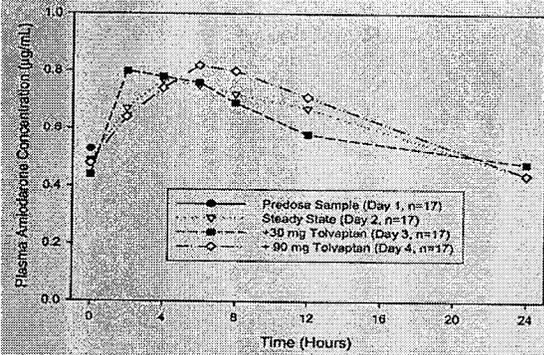
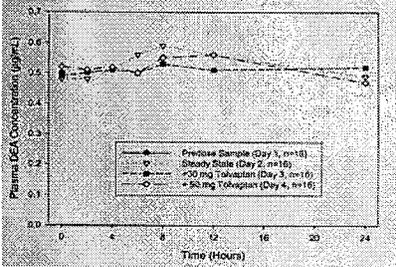


Figure 6.5-2 Median plasma concentrations of desethylamlodaronc (DEA) following a 200 mg dose of amlodaronc at steady state alone (Days 1 and 2) or with 30 mg tolvaptan (Day 3) or 90 mg tolvaptan (Day 4) in subjects with cardiac arrhythmias



The geometric mean ratios of the target parameters C_{ssmin} , C_{ssmax} , t_{max} and AUC_{τ} of amlodaronc and DEA in the presence and absence of tolvaptan are shown in the below tables:

Table 6.5-1 Summary of amlodaronc pharmacokinetic parameters following 200 mg doses of amlodaronc at steady state alone (Day 2) or with 30 mg tolvaptan (Day 3) or 90 mg tolvaptan (Day 4) in 17 subjects with cardiac arrhythmias

Parameter	Statistic	Day 2 alone	Day 3 +30 mg tolvaptan	Day 4 +90 mg tolvaptan
C_{ssmin} (µg/mL)	Median	0.43	0.43	0.41
	Mean	0.61	0.66	0.53
	SD	0.45	0.53	0.47
	%CV	73.4	79.3	74.6
	Minimum	0	0.10	0.16
	Maximum	1.78	2.17	1.85
C_{ssmax} (µg/mL)	Median	0.79	0.83	0.86
	Mean	1.16	1.14	1.10
	SD	0.76	0.71	0.60
	%CV	65.4	62.2	54.7
	Minimum	0.52	0.53	0.56
	Maximum	3.43	3.29	2.91
t_{max} (h)	Median	6.07	6.00	6.00
	Minimum	2.00	1.92	0
	Maximum	12.50	24.00	13.93
AUC_{τ} (µg·h/mL)	Median	13.3	14.7	14.6
	Mean	21.0	21.0	20.2
	SD	14.3	14.4	13.1
	%CV	68.0	68.3	64.7
	Minimum	3.5	7.5	8.7
	Maximum	62.8	63.2	59.0

Table 6.5-2 Summary of desethylamiodarone pharmacokinetic parameters following 200 mg doses of amiodarone at steady state alone (Day 2) or with 30 mg tolvaptan (Day 3) or 90 mg tolvaptan (Day 4) in 16 subjects with cardiac arrhythmias

Parameter	Statistic	Day 2 alone	Day 3 +30 mg tolvaptan	Day 4 +90 mg tolvaptan
C_{max} (µg/mL)	Median	0.42	0.45	0.47
	Mean	0.53	0.57	0.55
	SD	0.23	0.28	0.26
	%CV	42.8	49.1	46.9
	Minimum	0.21	0.26	0.26
	Maximum	1.00	1.27	1.19
$C_{max,ss}$ (µg/mL)	Median	0.60	0.61	0.63
	Mean	0.73	0.73	0.72
	SD	0.33	0.30	0.29
	%CV	45.1	41.9	41.1
	Minimum	0.31	0.37	0.36
	Maximum	1.42	1.48	1.44
t_{max} (h)	Median	8.00	6.00	8.11
	Minimum	0	0	0
	Maximum	22.73	24.00	11.98
	Mean	12.6	12.8	12.0
$AUC_{0-\infty}$ (µg·h/mL)	Median	15.3	13.5	15.0
	Mean	18.9	17.6	18.5
	SD	6.9	6.9	6.5
	%CV	44.9	44.9	43.6
	Minimum	7.1	7.8	7.7
Maximum	30.4	32.7	30.6	

The geometric mean ratios (90% CI) of amiodarone and DEA are provided in the below table:

Table 6.5-4 Geometric mean ratios (±90% CI) for amiodarone and desethylamiodarone (DEA) pharmacokinetic parameters

Parameter Ratio	Amiodarone		DEA	
	+ 30 mg tolvaptan	+ 90 mg tolvaptan	+ 30 mg tolvaptan	+ 90 mg tolvaptan
C_{max}	0.97 (0.91-1.02)	1.01 (0.96-1.07)	0.97 (0.92-1.03)	0.99 (0.94-1.05)
$C_{max,ss}$	0.99 (0.90-1.09)	1.00 (0.89-1.12)	0.99 (0.92-1.05)	0.99 (0.93-1.05)
$AUC_{0-\infty}$	0.98 (0.92-1.04)	1.01 (0.94-1.07)	0.98 (0.93-1.02)	1.01 (0.96-1.04)

$C_{max,ss}$ and $AUC_{0-\infty}$ of amiodarone in the presence of tolvaptan 30 mg are 0.99 and 0.98, respectively, of the corresponding values in the absence of 30 mg tolvaptan. $C_{max,ss}$ and $AUC_{0-\infty}$ of amiodarone in the presence of 90 mg tolvaptan are 1.00 and 1.01, respectively, of the corresponding values in the absence of 90 mg tolvaptan. $C_{max,ss}$ and $AUC_{0-\infty}$ of DEA in the presence of 30 mg tolvaptan are 0.99 and 0.98, respectively, of the values in the absence of 30 mg tolvaptan. C_{max} and $AUC_{0-\infty}$ in the presence of tolvaptan 90 mg are 0.99 and 1.0, respectively, of the values in the absence of 90 mg tolvaptan. These findings indicate that single dose co-administration of tolvaptan in doses of 30 mg or 90 mg has no impact on the pharmacokinetics of amiodarone or its metabolite DEA at steady-state.

Conclusions

Co-administration of single doses of 30 mg or 90 mg tolvaptan has no impact on the apparent steady-state PK of amiodarone and DEA. The report does not state how attainment of steady-state of amiodarone was verified.

Comments

1. The report does not state how the sponsor verified that amiodarone and DEA are at steady-state at screening.
2. The usual maintenance dose of amiodarone is 400 mg

3. The statement with respect to the 4 subjects who were excluded from the data analysis because they exhibited tolvaptan concentrations in the pre-dose samples on Day 3 “the reason for this is still under investigation” should be updated.
4. Precision and accuracy during sample analysis and method validation show the same results for both amiodarone and DEA. Is this possible?

Study Report 156-01-225:” An Open-Label, Randomized, Placebo-Controlled and Cross-Over Study of the Potential Drug Interaction between Tolvaptan (OPC-41061) and Warfarin in Healthy Male and Female Subjects”

Investigator and Study Site

_____ b(4)

Objectives

Primary

To evaluate the effects of 60 mg multiple tolvaptan administration on warfarin pharmacokinetics and pharmacodynamics following a 25 mg dose of warfarin.

Secondary

To evaluate the pharmacokinetics of tolvaptan in the presence of warfarin

Investigational Drugs and Formulations

Study Drug	Lot Number	Manufacturer
OPC-41061 60 mg tablet	99E87A060	Otsuka Pharmaceutical Co., Ltd.
Placebo tablet	99D96P000	Otsuka Pharmaceutical Co., Ltd.
Warfarin sodium 10 mg tablet	308351002	Barr Laboratories
Warfarin sodium 5 mg tablet	408331039T	Barr Laboratories

Design

This was an open-label, randomized, placebo-controlled, cross-over study of the potential drug interaction between tolvaptan and warfarin in healthy male and female subjects. The study plan is shown in the below scheme:

Treatment Group ^a	Period I (Days 20 – 32)	Period II (Days 34-46)
A	tolvaptan 60 mg QD	placebo
B	placebo	tolvaptan 60 mg QD

^a A single warfarin 25 mg dose was administered on Day 1 as the priming dose, and on Day 23 for

On Day -1, eligible subjects were randomized into Group A and B. On Day 1 all subjects receive a priming dose of warfarin 25 mg. They remain in the clinic for 50 h post-dose for blood sampling. On Day 19 the subjects return to the clinic for check-in procedures.

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Period I (Days 20-32): On Days 20-32 Group A receives tolvaptan 60 mg qd and Group B receives placebo. On Day 23 the subjects of both Groups receive 25 mg warfarin. On Day 33, the subjects cross-over to the opposite treatment arm, i.e. Group A receives placebo and Group B tolvaptan 60 mg qd on Days 34-46.

Period II (Days 34-46): On Days 34-46 Group B receives 60 mg tolvaptan qd and Group A placebo for 13 days. Subjects in both groups receive 25 mg warfarin on Day 37. The subjects are discharged from the Unit on Day 37. They are contacted by phone 7 days later to assess adverse events.

Study medication was to be administered at approximately 0800 h under fasting conditions with 240 mL water on all treatment days. On dosing days, subjects were to fast from food and beverages (other than water) overnight for 11 h.

Subjects were not allowed to ingest grapefruit- or Sevilla orange containing products within 7 days prior to dosing or during the study. Use of alcohol or xanthine containing products within 1 day prior to dosing of tolvaptan and during the study was prohibited. The use of any prescription drug (including hormonal contraceptives) other than warfarin, over-the counter, or herbal medications, within 14 days prior to dosing or antibiotics within 30 days prior to dosing was prohibited.

Pharmacokinetic Profiling

Warfarin

Blood samples for the determination of warfarin plasma concentrations were collected at the following times on: Days 23 (Period I) and 37 (Period II): Pre-dose, and 2, 4, 8, 12, 24, 48, 72, 144 and 240 h post-dose. Additional blood samples for the determination of the protein binding of warfarin were collected on Days 23 and 37 pre-dose and 4 h post-dose.

Tolvaptan

Blood samples for the determination of plasma concentrations of tolvaptan were collected at the following times on:

Days 23 (Period I) and 37 (Period II): Pre-dose, and 2, 4, 8, 12, 24, 72, 144 and 240 h post dose. Additional Blood samples were collected for the determination of the plasma protein binding of tolvaptan on Days 23 and 37 pre-dose and 4 h post-dose. The assay was performed by _____

b(4)

Plasma Protein Binding

The plasma protein binding of racemic tolvaptan (160 and 600 ng/mL) and warfarin (1000 and 2000 ng/mL) was determined by equilibrium dialysis and ultrafiltration, respectively, at 37° C using ¹⁴C- warfarin and ¹⁴C- tolvaptan. The pH was kept at 7.4 during dialysis and filtration. The experiments were performed by _____

b(4)

Bioassay

Warfarin

The plasma concentrations of S- and R- warfarin were analyzed by a HPLC/MS/MS method. The assay is linear over the range 1.00 ng/mL-100.00 ng/mL for S- and R- warfarin. The mean correlation coefficient for R- and S- warfarin is 0.9978 and 0.9983, respectively. The accuracy for the assay for R-warfarin ranges between -2.6 % and -2.0 % and the precision is ≤ 9.8 %. The accuracy of the assay for S-warfarin ranges between -4.4 % and -1.7 % and the precision is ≤ 9.4 %. Information on the stability of the enantiomers in plasma exposed to freeze/thaw cycles, long term freezer storage and storage at room temperature or during process is not provided. The assay was performed by _____

b(4)

7-Hydroxywarfarin and 10-hydroxywarfarin

7-Hydroxywarfarin and 10-hydroxywarfarin plasma concentration were analyzed by a HPLC/MS/MS assay. The assay is linear ($1/y^2$) over the range between 5.00 ng/mL and 1000.00 ng/mL with a mean correlation coefficient for 7-hydroxywarfarin and 10-hydroxywarfarin of 0.9974 and 0.9968, respectively. The accuracy of the assay for 7-hydroxywarfarin ranges between -10.0 % and 0.0 % and the precision is ≤ 9.4 %. The accuracy of the assay for 10-hydroxywarfarin ranges between -4.8 % and -1.3 % and the precision is ≤ 8.3 %. Experiments showed that the warfarin metabolites were stable in plasma exposed to 3 freeze/thaw cycles and fater storage at room temperature for 5 h prior to analysis. The assay was performed by _____

b(4)

Tolvaptan

The plasma concentrations of tolvaptan were analyzed by a HPLC/UV method using an internal standard. The assay is linear ($1/y$) over the concentration range between 5.00 ng/mL and 1000.00 ng/mL with a correlation coefficient of 0.998. The accuracy of tolvaptan ranges between -2.5 % and 2.6 % and the precision is ≤ 7.0 %. Stability of the analyte was demonstrated by exposure to room temperature for 4 h and after extraction on the autosampler for 49 h. Results on the stability when exposed to freeze/thaw cycles and long term freezer conditions are not reported. The assay was performed by _____

b(4)

Pharmacodynamics

PT and aPTT

Blood samples for the determination of PT and aPTT were collected at the following times on:

Days 23 and 37: pre-dose, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h post-dose.

Pharmacokinetic Data Analysis

Warfarin

C_{max} , t_{max} , AUC_t , AUC_{∞} , V_z/F and CL/F for the R- and S-warfarin enantiomers were determined.

Tolvaptan

$C_{ss,max}$, t_{max} , $C_{ss,min}$, AUC_t , $t_{1/2z}$, V_z/F and CL/F were determined. C_{max} , $C_{max,ss}$, $C_{ss,min}$ and t_{max} were taken directly from the observed data. AUC_{tlast} and AUC_t were calculated using the linear trapezoidal rule. AUC_{∞} was determined if λ_z could be estimated. If needed AUC_t was obtained by inter- or extrapolation if t_{last} did not coincide with 24 h.

Pharmacodynamic Data Analysis

AUC_{CaPTT} , AUC_{PT} and AUC_{INR} were calculated using the linear trapezoidal rule.

Statistical Analysis

Sample Size and Power

For the exposure measures for warfarin 16 subjects were needed as estimated based on a 30 % coefficient of variation (CV%) to detect a 20% difference with 80 % power. Twenty-four subjects were planned to be enrolled in the study.

Impact of Tolvaptan on Warfarin PK and PD

The confidence interval approach with the one-sided test was used to determine the impact of tolvaptan co-administration on the PK and PD of warfarin.

Plasma Protein Binding

A paired t-test was used to analyze the mutual impact of tolvaptan and warfarin on their binding to plasma proteins.

RESULTS

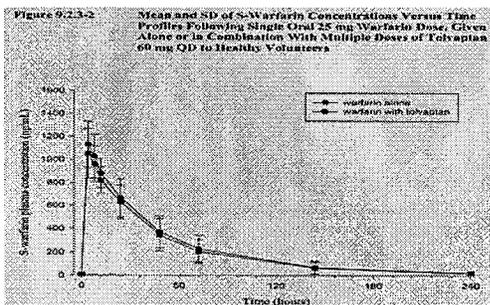
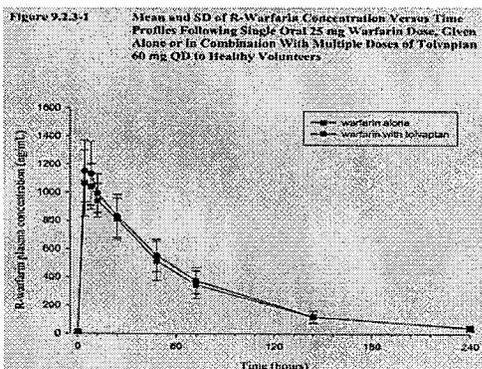
Disposition and Demographics

Twenty four subjects were enrolled and 12 randomized to Group A and 12 to Group B and 21 subjects completed the study. Three subjects were discontinued. Two subjects experienced adverse events and one subject randomized to the tolvaptan/placebo group received only warfarin and was discontinued because of protocol violation. The mean age and body weight of the subjects was 30.6 (8.5) years and 78.6 (9.6) kg, respectively. There were 4 females and 19 males.

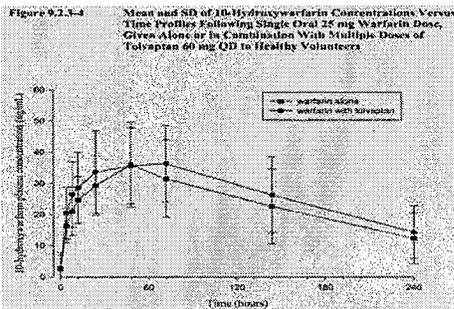
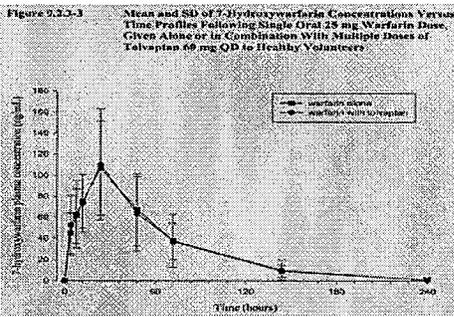
Pharmacokinetics

Warfarin

The respective mean (SD) plasma concentration time profiles of S- and R-warfarin and 7-hydroxywarfarin and 10-hydroxywarfarin after administration of 25 mg warfarin alone and after co-administration of multiple doses of 60 mg tolvaptan qd are shown in the 4 figures below:



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The plasma concentration time curves of the warfarin enantiomers and metabolites in the presence and absence of tolvaptan are similar suggesting no impact of the multiple dose 60 mg qd regimen of tolvaptan on the pharmacokinetics of the warfarin enantiomers and metabolites.

The ratios of the geometric means and confidence intervals for the warfarin enantiomers in the presence absence of tolvaptan are provided in the below table:

Analyte		C_{max}	AUC_t	AUC_{∞}	CL/F	$t_{1/2}$
R-warfarin	Ratio ^a	1.06	1.06	1.05	0.95	0.95
	90% CI ^b	1.02-1.09	1.01-1.11	1.01-1.11	0.91-1.00	0.89-1.00
S-warfarin	Ratio ^a	1.09	1.09	1.09	0.92	0.93
	90% CI ^b	1.05-1.12	1.04-1.13	1.04-1.13	0.89-0.96	0.87-1.00

^a Ratio of geometric means parameters for warfarin given with tolvaptan (test) to that for warfarin given alone (reference).

^b 90% confidence interval for the ratios of the geometric means.

The geometric means and their ratio for 7-hydroxywarfarin and 10-hydroxywarfarin in the presence and absence of tolvaptan are listed in the below table:

Geometric Mean for 7-Hydroxywarfarin and 10-Hydroxywarfarin in Presence and Absence of Tolvaptan and the Respective Ratios

	Cmax ng/mL	AUCt ng•h/mL	AUC∞ ng•h/mL	Cmax Ratio	AUC∞ Ratio
7-Hydroxywarfarin + Placebo	101.69	6140	6747		
7-Hydroxywarfarin + Tolvaptan	99.78	5980	7402	0.98	1.10
10-Hydroxywarfarin + Placebo	34.61	5193	7124		
10-Hydroxywarfarin + Tolvaptan	37.13	5967	7911	1.07	1.11

The geometric mean ratios confirm absence of an impact of tolvaptan on the pharmacokinetics of the warfarin enantiomers and metabolites.

The below tables lists the trough plasma concentrations of tolvaptan on Days 4, 5 and 7 of the 60 mg qd multiple dose regimen:

Table 9.2.3-6 Summary of Pre-dose Plasma Concentration (C_{ss,min}) (ng/mL) of Tolvaptan Following Multiple Oral Administration of Tolvaptan 60 mg QD to Healthy Volunteers on Day 4, 5 and 7^a

	Days of Pre-Dose		
	4	5	7
	Pre-dose Concentration (ng/mL)		
N	22	22	22
Median	29.20	40.79	33.60
Mean	44.38	49.92	47.34
SD	39.20	35.05	39.35
CV%	88.3	70.2	83.1
Min	0.00	9.36	0.00
Max	151.53	126.52	146.82

^a Protocol study days 23, 24 and 26 for Period I and 37, 38 and 40 for Period II respectively.

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The similarity of the mean C_{ss,min} data on consecutive days tolvaptan was steady state when its impact on the warfarin kinetics was assessed. However, even though the mean C_{ss,min} data of tolvaptan on Days 4, 5 and 7 during the co-administration phase are similar it should be noted that percent coefficient of variation about the mean values ranges between 70 % and 88 % indicating significant intersubject variation.

Plasma Protein Binding

Warfarin

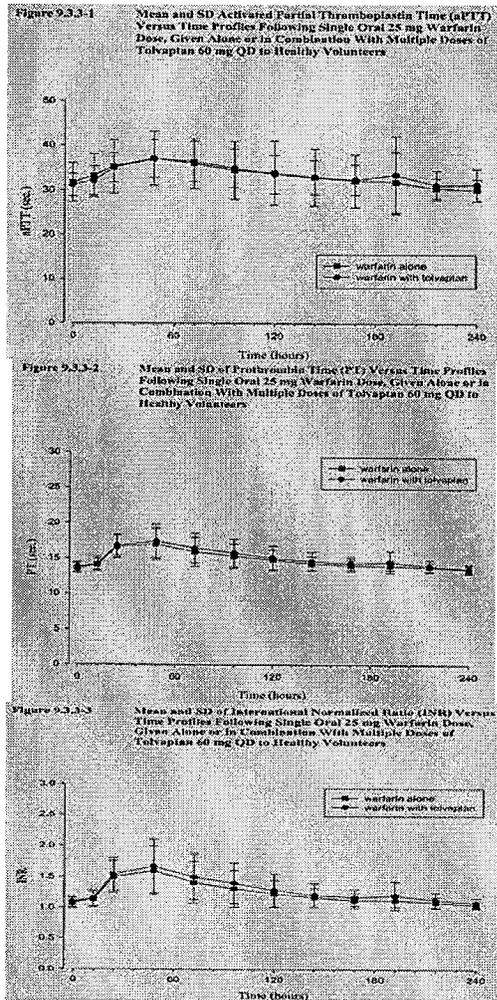
The mean (SD) percentage of unbound racemic warfarin in the presence and absence of warfarin is 1.07 (0.13) % and 1.12 (0.20) %, respectively, and not statistically significantly different. Tolvaptan does not impact the plasma protein binding of warfarin.

Tolvaptan

The mean (SD) percentage of unbound racemic tolvaptan in the presence and absence of tolvaptan is 0.83 (0.20) % and 0.76(0.27) %, respectively, and not statistically significantly different. Warfarin does not impact the plasma protein binding of tolvaptan.

Pharmacodynamics

The below figures show the mean (SD) time profiles of aPTT, PT and INR over a period of 240 h after administration of 25 mg warfarin alone or after co-administration of multiple doses of tolvaptan 60 mg qd:



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The profiles are similar suggesting no impact of a multiple dose 60 mg qd regimen on the pharmacodynamics of co-administered warfarin.

The below table tabulates the results of the statistical evaluation of the pharmacodynamic data of warfarin in the presence and absence of tolvaptan:

Table 9.3.3-2 Ratios of Geometric Means With 90% Confidence Intervals for Warfarin Pharmacodynamic Parameters

	AUC _{aPTT}	AUC _{PT}	AUC _{INR}
Ratio ^a	1.02	1.02	1.05
90% CI ^b	1.01-1.04	1.01-1.04	1.03-1.07

^a Ratio of geometric means parameters for warfarin given with tolvaftan (test) to that for warfarin given alone (reference).

^b 90% confidence interval for the ratio of the geometric means.

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The results indicate that co-administration of multiple doses of 60 mg tolvaftan qd has no impact on the pharmacodynamics of warfarin.

Conclusions

PK

Co-administration of multiple doses of 60 mg qd tolvaftan does not impact the kinetics of the warfarin enantiomers and the metabolites 7-hydroxywarfarin and 10-hydroxywarfarin. The plasma protein binding of racemic warfarin is not altered by the presence of tolvaftan. Co-administration of tolvaftan also does not affect the pharmacodynamics of warfarin, i.e. aPTT, PT and INR.

Tolvaftan's plasma protein binding is unchanged in the presence of racemic warfarin.

Comments

1. The peak effects of warfarin in the presence and absence of tolvaftan on aPTT, PT and INR should be evaluated statistically.
2. Information on the stability of the warfarin enantiomers in plasma exposed to three freeze/thaw cycles, stored at room temperature and during sample analysis is not provided.
3. The radiochemical purity of the radio-labeled warfarin and tolvaftan is not indicated.

Study Report 156-03-240 : " An Open-Label, Randomized, Two-Period, Cross-Over Study of the Pharmacokinetic Interaction between Tolvaftan and Grapefruit Juice Following Single Oral Dose Administration of 60 mg Tolvaftan Tablets to Healthy Men and Women"

Investigator and Study Site



b(4)

Objectives

To compare the pharmacokinetics of tolvaftan following oral administration in the absence and presence of grapefruit juice in healthy subjects

Investigational Drugs and Formulations

Tolvaptan 30 mg tablets (Lot No. 02C80A030B) were provided by the sponsor. Grapefruit juice was reconstituted according to package directions with room temperature tap water. The strength of the grapefruit juice is not indicated.

b(4)

Design

This was a single-center, randomized, open-label, two-period cross-over study of single doses of tolvaptan 60 mg administered with and without grapefruit juice. On Day 1 subjects were randomized to a sequence AB or BA of the following two treatments:

Treatment A: tolvaptan 60 mg administered with 240 mL room temperature water

Treatment B: tolvaptan 60 mg co-administered with 240 mL room temperature reconstituted grapefruit juice. Dosing occurred on Days 1 and 4 with a 72 h washout period between administrations. Each tolvaptan dose was administered to the subjects in the morning after an overnight fast of 10 h. Subjects abstained from food for 4 h post-dose and from drinking any liquids 2 h prior to and after dosing.

20 male and female subjects in the age between 18-45 years, nonsmoking and within ±15 % IBW were enrolled to ensure a minimum of 16 subjects to complete the study.

The scheduled study activities are provided in the below scheme:

Table 3.5-3 Schedule of Assessments - Days 1 to 7

Procedure	Study Day						
	1	2	3	4	6	7/ET	
Vital Signs	X ¹			X ²			X
Body Weight							X
Physical Examination ³							X
Resting 12-lead ECG	X ⁴			X ⁵			X
Hematology/Serum Chemistry/Urinalysis							X
Serum Pregnancy Test - Females							X
PK Blood Draws	X ⁶	X ⁶	X ⁶	X ⁶	X ⁶	X ⁶	X ⁶
Tolvaptan Dosing (60 mg)	X ⁷			X ⁷			
Concomitant Medications	X	X	X	X	X	X	X
Adverse Event Assessment	X	X	X	X	X	X	X
Meals ⁸	X ⁹	X	X	X ¹⁰	X	X	X
Randomization	X						
Begin fasting at 10 PM (2200 hours)			X				

¹ At end of treatment; ECG = electrocardiogram; PK = pharmacokinetic.
² Blood pressure, pulse, and temperature. Vital signs were to be performed pre-dose, after the subject was seated for 2-3 minutes within 30 minutes prior to dose.
³ Elbow breadth and height at screening only.
⁴ 12-lead ECG was performed pre-dose and at 2 and 4 hours post-dose. ECGs were to be performed after the subject had been recumbent and at rest for at least 10 minutes prior to the ECG.
⁵ PK blood draws: pre-dose, followed by 0.5, 1, 2, 3, 4, 6, 8, and 12 hours post-dose.
⁶ PK blood draws: 16, 24, 30, 48, and 72 hours post-dose. (Note: The Day 4 pre-dose draw was the same as the Day 1 72-hour post-dose draw and was to be taken 2 minutes prior to dosing.)
⁷ Tolvaptan dosing at approximately 0800 hours.
⁸ Meals were breakfast, morning snack, lunch, dinner, and evening snack except on Day 7 when only breakfast was given.
⁹ No meals but morning snack was given. Lunch was to be served at 4 hours post-dose and after the PK draw.

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Pharmacokinetic Profiling

Blood samples for the determination of the plasma concentrations of tolvaptan and its metabolite DM-4103 were collected at the following times on:

Days 1 and 4: Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 30, 48, and 72 h post-dose.

Bioassay

Tolvaptan and DM-4103 plasma concentration were measured by a validated HPLC/MS/MS method. The assay for tolvaptan is linear (1/y²) in the concentration range between 5.00 ng/mL and 1000 ng/mL with a mean coefficient of correlation of 0.9978. The accuracy of the assay for tolvaptan ranges between 1.63 % and 8.87 % and the precision is ≤ 7.72 %. The assay for DM-4103 is linear (1/y²) in the concentration range between 12.5 ng/mL and 2500 ng/mL with a mean coefficient of correlation of 0.9977. Freeze/thaw, stability, process stability, refrigeration and autosampler stability was demonstrated. The accuracy of the assay for DM-4103 ranges between 2.20 % and 10.7 % and the precision is ≤ 9.45 %. The measurements were performed by _____

b(4)

PK Data Analysis

C_{max} , AUC_{∞} (primary parameters) and t_{max} , AUC_t , $t_{1/2z}$ and CL/F (secondary parameters) were to be determined.

Statistical Analysis

The equivalence of test and reference treatment were assessed for the primary PK parameters of interest (C_{max} and AUC_{∞} of tolvaptan). This assessment was performed on the subjects who had C_{max} and AUC_{∞} determined for both periods.

An analysis of variance (ANOVA) was performed on natural logarithmic transformation of C_{max} and AUC_{∞} with period, treatment, subject within sequence, and sequence in the model. The difference of the least squares means of the log transformed results for the test and reference treatments, as well as the within-subject standard error (SE) were used to construct the 90% CIs corresponding to Schirrmann's two one-sided t-tests at the 0.05 significance level. The antilogs of the confidence limits were used to obtain the 90% CI for $\mu \pm 1.645$. Equivalence was concluded if the CI fell within 0.80 to 1.25 for both CIs of C_{max} and AUC_{∞} .

RESULTS

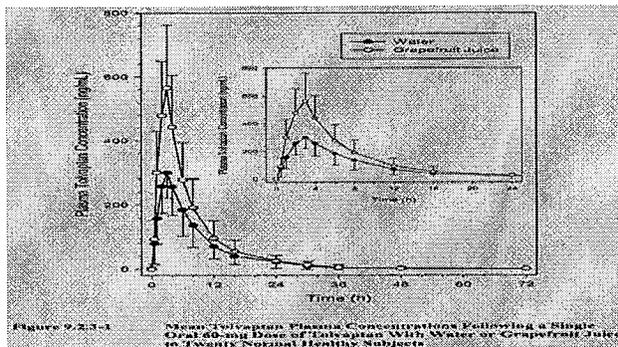
Demographics and Disposition of the Subjects

Twenty subjects, 15 males and 5 females of median age 36 years and median weight 77 kg were enrolled, and all completed the study.

Pharmacokinetics

The DM-4103 plasma concentrations of Period 2 were not analyzed because the washout phase of 3 days was considered too short given the long half-life of the metabolite.

The mean (SD) plasma concentration time profiles of tolvaptan in the presence and absence of grapefruit juice are shown in the below figure:



The mean plasma concentration time profiles of tolvaptan in the presence and absence of grapefruit juice vary significantly and indicate a relevant increase in exposure to tolvaptan caused by ingredients of grapefruit juice.

Geometric mean ratios (90% confidence intervals) of the respective treatments with tolvaptan administered with and without grapefruit juice are tabulated in the below table:

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Table 9.2.3-2 Geometric Mean Ratios (90% CI) for Tolvaptan Pharmacokinetic Parameters Following a Single 60-mg Oral Dose of Tolvaptan With Grapefruit Juice Versus Tolvaptan With Water	
Parameter	Tolvaptan + Grapefruit Juice (T) versus Tolvaptan + Water (R)
C_{max}	1.86 (1.67-2.06)
AUC ₀₋₁₆	1.56 (1.42-1.70)
AUC _∞	1.56 (1.40-1.74)

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Grapefruit juice increases peak exposure to tolvaptan 1.9 fold and average exposure to tolvaptan about 1.6 fold.

It should be noted that AUC_∞ of tolvaptan could not be determined in 5 of the 20 subjects.

The plasma concentrations of tolvaptan in the absence and presence of grapefruit juice were measurable in all subjects up to 16 h, pointing to the insufficient time interval for proper determination of λ_z and related parameters, including AUC_∞. Since the consequence of the bias in λ_z for AUC_∞ can be considered relatively small, the sponsor's values for the average exposure may serve as crude estimates.

Conclusions

Grapefruit juice increases peak exposure to tolvaptan 1.9 fold and average exposure to tolvaptan about 1.6 fold. Ingredients of grapefruit juice are known to inhibit CYP3A, the main enzyme mediating the metabolism of tolvaptan.

Comments

1. The strength of the grapefruit juice used is not indicated.
2. The sensitivity of the assay for tolvaptan is not sufficient for proper determination of λ_z and derived parameters.

Study Report 156-01-234: "An Open-Label Study of the Pharmacokinetic Interaction between Tolvaptan and Digoxin Following Multiple Oral Doses to Healthy Men and Women"

Investigator and Study Site

██████████

b(4)

Objectives

To determine the effect of tolvaptan administration on steady-state digoxin concentrations

Investigational Drugs and Formulations

Tolvaptan 60 mg tablets (Lot No. 02C80A060) were provided by the sponsor. Digoxin (Lanoxin®) 0.25 mg tablets (Lot No. 05379) were acquired by the sponsor from the manufacturer ██████████

b(4)

Design

This was a single center, open-label study of the pharmacokinetic interaction between tolvaptan and digoxin in healthy male and female volunteers. Eligible subjects were admitted to the unit on Day -2 and underwent baseline assessments on Day -1. A 17-day in-patient period ensued. The subjects received 60 mg tolvaptan qd on Days 1 and 12 to 16. A loading dose of digoxin was administered on Day 4 (0.5 mg at 8 AM, with an additional 0.25 mg at 4 PM), and digoxin 0.25 mg qd was administered on Days 5-16. Blood and urine samples were collected at serial

points on Days 1, 2, 12, and 16 for pharmacokinetic and pharmacodynamic assessments. The subjects were discharged on Day 17 and a follow-up telephone contact was performed 7 days later.

Fourteen male and female subjects in the age between 18 and 45 years and a body weight within $\pm 15\%$ of IBW were to be enrolled in the study.

The study medication was to be administered together with 240 mL water of room temperature. On non-PK sampling days the medications were given 1 h after breakfast. On the PK sampling Days 1, 11, 12, and 16, treatment was administered following a 10 h fast. Subjects were asked to continue the fast for 4 h after dosing. The subjects were to receive *nil per os* for 2 h prior to and after dosing. The subjects maintained a sitting position for 4 h after dosing.

Use of any prescription (including hormonal contraceptives), over-the-counter, or herbal medication within 14 days prior to dosing, or antibiotics within 30 days prior to dosing was prohibited. Consumption of grapefruit and Seville orange-containing products within 72 h prior to dosing was prohibited. Use of alcohol as well as food and beverages containing methyl-xanthines within 72 h prior to dosing was prohibited.

The scheduled study activities are outlined in the below scheme:

Procedure	Screening (Day -21 to -3)	Day 1 ^a	Day 2	Day 3	Days 2-3	Day 4	Days 5-8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17 ^b ET	7-Day Follow-up
Informed Consent	X ^c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inclusion/Exclusion Criteria	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Medical History	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drug and Alcohol Screen	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Admission to Clinic	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Physical Examination	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Survey Pregnancy Test (Female only)	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Randomization	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clinical Laboratories	X ^d	X	X	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Vital Signs	X ^e	X ^f	X ^g	X ^g	-	X ^g	-	-	X ^g	X ^g	X ^g	-	-	-	X ^g	X	-
12-lead ECG	X	-	X ^h	X ^h	-	X ^h	-	-	X ^h	-	X ^h	X	-				
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
Adverse Event Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
0 to 12 and 12 to 24 Hour Urine Collection	-	-	X ⁱ	X ⁱ	-	-	-	-	-	X ⁱ	X ⁱ	-	-	-	X ⁱ	X	-
Begin Fasting at 10 pm	-	-	X	-	-	-	-	-	X	X	-	-	-	X	-	-	-

Procedure	Screening (Day -21 to -3)	Day 1 ^a	Day 2	Day 3	Days 2-3	Day 4	Days 5-8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17 ^b ET	7-Day Follow-up
Tobacutin Dosing (60 mg QD)	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	-	-
Tobacutin Blood Draw	-	-	-	X ^c	X ^c	-	-	-	-	X ^c	X ^c	-	-	-	X ^c	X ^c	-
Digoxin Dosing (0.25 mg QD)	-	-	-	-	-	X ^d	-										
Digoxin Blood Draw	-	-	-	-	-	-	X ^e	-	-	-	X ^e	X ^e	-				

Source: Appendix I-1.
^a Subject admission to the study site.
^b Baseline.
^c Subject must be consented prior to any study procedures.
^d Clinical labs will also include serum hepatitis B and C, and HIV screen.
^e Including height, weight, and elbow breadth.
^f Including body weight.
^g To be performed at 8 am / postdose.
^h To be performed pre-dose, and at 2 h and 4 h postdose.
ⁱ Begin 0 to 12- and 12 to 24-hour urine output measurements at 8 AM.
^j Pre-dose, followed by 0.5, 1, 2, 3, 4, 8, and 12 h postdose.
^k Collected 24, 36 (Day 2), 48 (Day 3), and 72 (Day 4) h postdose from Day 1.
^l 24 h postdose from the previous day.
^m Subjects received 2 x 0.25 mg Lanoxin[®] tablets at 8 am, followed by one 0.25 mg tablet given 6 h after morning dose.
ⁿ Pre-dose blood collection at 8 AM only.
^o Pre-dose, followed by 0.5, 1, 1.5, 2, 3, 4, 8, and 12 h postdose.

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Pharmacokinetic Profiling

Digoxin

Blood

Blood samples for the determination of digoxin serum concentrations were collected at the following times:

On Days 9 and 10: pre-dose

On Days 11 and 16: pre-dose, and 0.5, 1, 2, 3, 4, 8, 12, and 24 h post-dose

Urine

Total urine volumes were collected on Days -1, 11, 12 and 16 in the intervals 0-12 and 12-14 h post-dose.

Tolvaptan

Blood samples for the determination of tolvaptan concentrations in plasma were collected at the following times:

On Day 1: pre-dose, and 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48, and 72 h post-dose

On Days 12 and 16: pre-dose and 0.5, 1, 2, 3, 4, 8, 12, and 24 h post-dose

Bioassay

Digoxin

Digoxin in serum and urine was analyzed using a [REDACTED] turbidimetric immunoassay. [REDACTED] instrument was calibrated per manufacturer's specification within 14 days prior to each sample run. QC samples were processed along samples with unknown digoxin concentrations. The method is linear between 0.325 ng/ml and 6.0 ng/mL digoxin. The precision of the assay is $\leq 15.1\%$. No information on accuracy and specificity of the assay and on the stability of the analyte in the matrices is provided. The assay was performed by [REDACTED]

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Tolvaptan and DM-4103

The plasma concentrations of tolvaptan and DM-4103 were measured by a validated HPLC/MS/MS method. The assay for tolvaptan is linear between 5.00 ng/ml and 1000 ng/mL. The respective correlation coefficients for OPC-41061 and DM-4103 are 0.0089 and 0.99979, respectively. Information on analyte stability was provided. The accuracy of the assay for tolvaptan ranges between -7.88 % and -3.33% and the precision is $\leq 7.63\%$. The assay for DM-4103 is linear between 12.5 ng/mL and 2500 ng/ml. The accuracy ranges between -7.47 % and -6.00 % and the precision is $\leq 11.3\%$. The assay was performed by [REDACTED]

b(4)

Pharmacodynamic Profiling

Urine volumes were collected in the following intervals:

On Days -1, 1, 11, 12, and 16: 0-12 and 12-24 h.

Pharmacokinetic Data Analysis

C_{max} , $C_{ss,max}$ (for digoxin on Days 11 and 16) and t_{max} were taken directly from the data. Where possible λ_z was estimated by log-linear regression of at least 3 non-zero concentrations. AUC_t was obtained by the linear trapezoidal rule. Standard methods were used to calculate AUC_∞, CL/F, CL_{ss}/F and Vz/F using WinNonlin Pro Version 3.1 (Pharsight Corporation, Mountain View, Ca). Renal clearance of digoxin was computed from dividing AUC₀₋₂₄ into Ae₀₋₂₄. AUC_r was obtained by applying the linear trapezoidal rule. If tlast did not coincide with 24 h, AUC_r

was obtained by interpolation or extrapolation. The accumulation index was obtained from $Rac(C_{max}) = C_{max}(\text{Day 16}) / C_{max}(\text{Day 12})$ and $Rac(AUC) = AUC(\text{Day 16}) / AUC(0-24)(\text{Day 12})$.

There was insufficient data on digoxin to determine $t_{1/2z}$ and $V_{z,ss}$.

C_{max} and AUC_{0-24} were calculated for tolvaptan on Days 1 and 12 to evaluate a possible impact of digoxin on the kinetics of tolvaptan.

Statistical Analysis

7.5 Statistics

The primary analyses were to establish the bioequivalence (BE) of C_{max} and of AUC_{0-24} for digoxin on Day 16 compared to Day 11, to establish the BE of C_{max} and of AUC_{0-24} for tolvaptan on Day 12 compared to Day 1, and to conduct significance tests on the renal clearance of digoxin on Day 16 compared to Day 11.

To establish BE for C_{max} and AUC_{0-24} for digoxin and tolvaptan on different days, 90% CIs for the difference in the means of the log-transformed data were calculated using analysis of variance (ANOVA) with subject and treatment day as factors. The width of the CI derived from the previous step would provide a 90% CI for the ratio of the geometric means for each comparison. If the 90% CI for the ratio of the geometric means of a PK variable fell in the interval [0.8, 1.25], BE was claimed for the PK variable. Due to the nature of the normal-theory confidence intervals, this approach was equivalent to carrying out two one-sided tests of the hypothesis at the 5% significance level.^{12,13}

To conduct significance tests on renal clearance of digoxin on Day 16 compared to Day 11, ANOVA with subject and treatment day as factors was applied to derive a two-sided comparison of Day 16 versus Day 11, at the 5% significance level. This approach is equivalent to a paired t-test.

Bioequivalence analysis and ANOVA were conducted using SAS Version 8.2.

All drug concentration data in plasma and all pharmacokinetic parameter estimates were summarized by dosing regimen and day of sampling using descriptive statistics performed in WinNonlin.

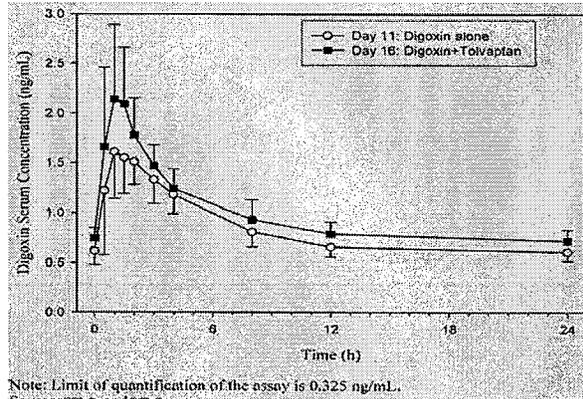
RESULTS

Demographics and Disposition of Subjects

Fourteen subjects, 9 males and 5 females of mean age 34.1 (9.0) years and mean body weight 77.0 (9.2) kg were enrolled and completed the study.

Pharmacokinetics

The mean (SD) serum concentration-time profiles of digoxin at steady-state in the presence and absence of tolvaptan are shown in the below figure:



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The steady-state serum levels of digoxin are increased in the presence of tolvaptan.

The geometric mean ratios (90 % CI) for C_{max} and AUC_τ for digoxin are listed in the below table:

Comparison ^a		C _{max}	AUC _τ
		(N=14)	(N=14)
0.25 mg digoxin + 60 mg tolvaptan (T) vs 0.25 mg digoxin alone (R)	Ratio	1.274	1.178
	90% CI	1.113-1.457 ^b	1.107-1.253 ^b

^a T is Test; R is Reference.

^b Not Equivalent.

The results indicate that peak and average exposure of digoxin at steady-state are increased 1.3 and 1.2 fold, respectively, in the presence of tolvaptan.

The below table lists the parameters obtained for digoxin in the presence and absence of tolvaptan:

Parameters	Statistics	Day 11	Day 16
t _{max} (h)	N	14	14
	Median	1.60	1.25
	Range	0.50-3.00	1.00-3.00
C _{max} (ng/mL)	N	14	14
	Geo Mean	1.76	2.55
	Mean	1.80	2.33
	CV%	19.7	29.0
AUC _τ (ng·h/mL)	N	14	14
	Geo Mean	19.7	23.2
	Mean	19.9	23.4
	CV%	14.4	13.2
A ₀₋₂₄ (ng)	N	9	13
	Mean	832.16	5176.7
	CV%	48.9	41.7
CL _r (mL/min/kg)	N	8	8
	Mean	1.05	0.43 ^b
	CV%	49.9	45.6

Geo Mean = Geometric mean.
^b Statistically significantly lower than that on Day 11, p < 0.05 (ANOVA with treatment and subject as factors)

In the presence of tolvaptan the renal clearance of digoxin is reduced statistically significantly to 41%.

The next figure shows the mean plasma concentrations of tolvaptan in the presence and absence of digoxin:

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