

4. It is unclear how the unknown metabolite (later labeled DM-4119) was identified. DM-4119 was not listed as available reference drug.
5. The $[I]/K_i$ ratios for the in vitro inhibitors should be calculated to predict the likelihood for an in vivo interaction.
6. The scheme proposed does not follow from the results of the study. The report does not discuss the unlabeled intermediary products or a rationale for OMP-21826 not to be an intermediary in the production of the OPC-41061 metabolites.
7. Linearization of enzyme data (Lineweaver-Burk, Eadie-Hofstee and Dixon-plots) may introduce bias. Software exist that uses untransformed data to determine the enzyme kinetic parameters.
8. The impact of the vehicle for OPC-41061, DMF, on the microsomes was not tested.

Study Report 011240:” Study of the Metabolism of OPC-41061 using Microsomes Derived from Human Liver”

Investigator and Study Site

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Objectives

To investigate the enzymes involved in the metabolism of OPC-41061 in human liver microsomes. The investigate the variability in produced metabolites when the concentrations of OPC-41061 in the reaction mixture is varied

Methods

Synthesized DM-4103, DM-4104, Dm4105, DM-4107-Na, DM-4110, DM-4111, OPM-21286 were available. Human livers were obtained from a commercial source. Microsomes were prepared and diluted to a concentration of 10 mg/mL. Anti-CYP1A- antiserum (rabbit anti rat CYP1A2 polyclonal antiserum), anti-CYP2C- antiserum (goat anti-rat CYP2C13 polyclonal antiserum), anti-CYP2D antiserum (rabbit antihuman CYP2D6 polyclonal antiserum), anti-CYP3A antiserum (rabbit anti-rat CYP3A2 polyclonal antiserum) and anti-CYP2E antibody (MAB-2E1 monoclonal antibody against human CYP2E1) were purchased from a commercial source.

Assays

A HPLC method with spectro-photometric detector and column switching was used to determine OPC-41061 and metabolites. An internal standard was used which was added to the reaction mixture after stopping the reaction. A HPLC method with UV detector was used for the determination of hydroxyl-testosterone.

Computation of K_m and V_{max} Values

The reaction time was established first. After pre-incubation for 10 min at 37° C the reaction system consisted of 0.1 mM pH 7.4 phosphate buffer (0.5 mg/mL), 0.05 mM EDTA, 50 μ M OPC-41061 and 1 mg/mL pooled microsomes. After addition of 1 mM NADPH and 1 mM NADH, the mixture was incubated for 5, 10, 20, 40, and 80 min at 37°C. The reaction was stopped by adding 10% TCA. After centrifugation, the supernatant was analyzed for OPC-41061 and metabolites by HPLC. In subsequent experiments the reaction mixture was incubated for 5 min. at 37°C. OPC-41061 was tested at concentrations of 3.13, 6.25, 12.5, 25 and 50 μ M. Then 1 mM NADPH and 1 mM NADH

were added and the reaction mixture incubated for 20 min. The reaction mixture was stopped and the concentrations of OPC-41061 and metabolites determined as described above. Km and Vmax were computed from the generated metabolite data in Eadie-Hofstee plots.

Identification of Involved Enzymes in Human Microsomes

The following inhibitors were used: Furafylline (CYP1A1/2), sulfaphenazole (CYP2C8/9), tranylcypromine (CYP2C19), quinidine (CYP2D6), dimethyldithiocarbamate (CYP2E1) and troleandomycin and ketoconazol (CYP3A4). The above described reaction system was used, but pre-incubation was 40 min. After addition of OPC-41061 the reaction mixture was incubated for 15 min at 37°C. The reaction was stopped and the supernatant analyzed as described above.

Studies with Antisera

The different antisera were used at a concentration of 1 mg/mL. The above reaction system was pre-incubated for 10 min at 37 °C. After addition of 10 µM OPC-41061 the reaction mixture was incubated for 15 min at 37 °C. The reaction was then stopped and analyzed as described above.

Interindividual Variability in Metabolism of OPC-41061

Replicate experiments (n=8) were performed with the reaction mixture described above with a pre-incubation of 5min. OPC-41061 was tested at 3 µM and 30 µM and an incubation time of 15 min at 37°C.

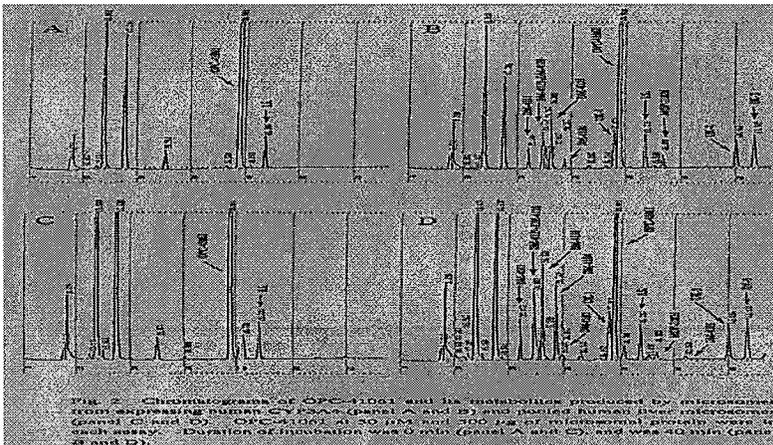
Correlation between Metabolism of OPC -41061 and Testosterone

Km and Vmax values were obtained for 6β- hydroxylation of testosterone. The optimum reaction time was determined to be 5 min with testosterone at a concentration of 50 µM in the reaction mixture.

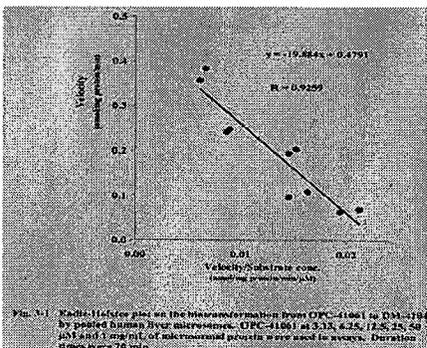
RESULTS

Calculation of Km and Vmax of biotransformation of OPC-41061 by Human Liver Microsomes

The below chromatogram indicates that DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, DM-4111, MOP-21826 and 3 unknown metabolites are formed from OPC-41061:



The apparent linearity of Eadie-Hofstee plots for the metabolites DM-4104, DM-4110 and DM-4111 generated from OPC-41061 suggests involvement of a single enzyme (only shown for DM-4104):



The calculated respective K_m and V_{max} values for the formation of the metabolites are listed below:

Table 2. K_m and V_{max} for the metabolism of OPC-41061 by human microsomes

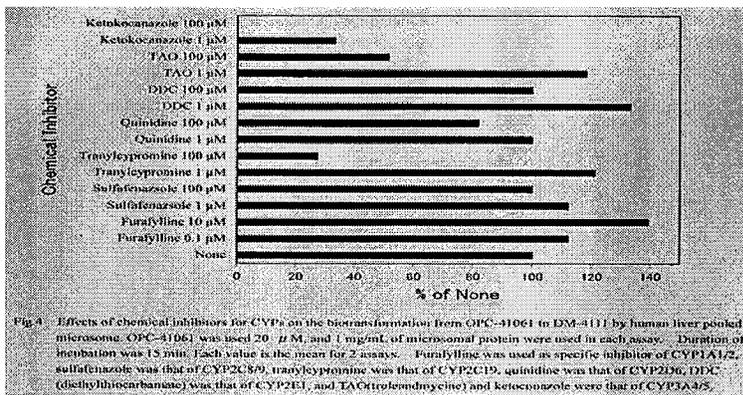
Substrate	Metabolite	K_m (μM)	V_{max} (nmol/mg/min)	V_{max}/K_m (nmol/mg/min/ μM)
OPC-41061	DM-4104	19.8	0.479	0.024
	DM-4110	19.8	1.250	0.063
	DM-4111	24.5	0.524	0.021

The reaction condition was as follows: OPC-41061 at 3.13, 6.25, 12.5, 25, 50 μM and 1 mg/ml of microsomal protein were used in each assays. Duration of incubation time was 20 min. K_m and V_{max} were calculated Eadie-Hofstee plot.

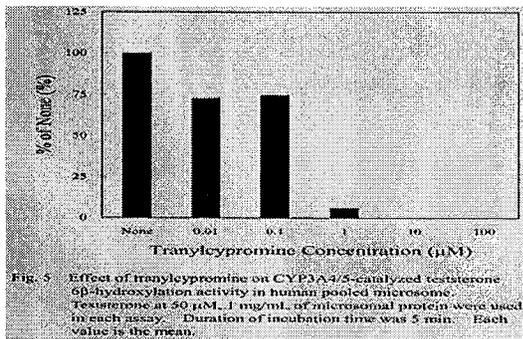
Identification of Enzyme Responsible for Biotransformation of OPC-41061

Of the tested inhibitors only the CYP3A4 inhibitors ketoconazole, troleandomycin and the CYP 2C19 inhibitor tranlycypromine inhibited the metabolism of OPC-42061 dose dependently and relevantly as shown below:

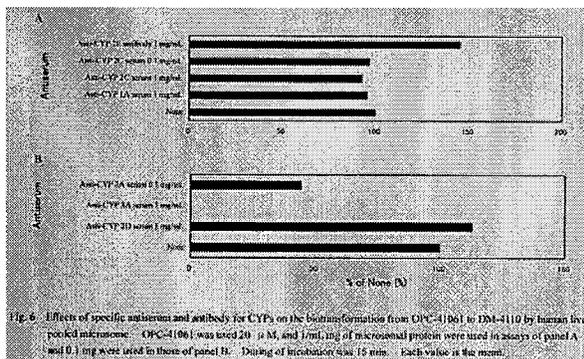
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Experiments with testosterone (β -hydroxylation), a CYP3A4 substrate, showed that tranylcyproline can also inhibit CYP3A4 as shown below:



Anti-CYP3A4 serum inhibits the CYP3A4 catalyzed formation of DM-4110 dose dependently with complete abolition of CYP3A4 activity at the higher concentration. In contrast, the anti-sera against the other CYPs had no impact on the formation of DM-4110 confirming that CYP3A4 is involved in the metabolism of OPC-41061:



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Variability of Metabolism of OPC-41061 when Studied at different Concentrations

Incubations with 3 µM OPC-41061 did not produce the metabolites MOP-21826 and DM-4107 and unknown metabolite 2 observed when 30 µM OPC-41061 was incubated with human microsomes.

Correlation between Metabolism of OPC -41061 and Testosterone

The below figure indicates the existence of a correlation between the metabolism of OPC-41061 and testosterone, a substrate of CYP3A4/5:

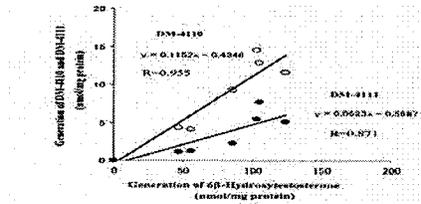


Fig. 16 Relationship between testosterone 6β-hydroxylation activity and metabolism of OPC-41061 to DSI-4110 and DSI-4111 by human microsomes. Open circle expressed the relationship for DSI-4110, closed circle expressed the relationship for DSI-4111. Duration of incubation was 15 min for the metabolism of OPC-41061, and 5 min for the metabolism of testosterone. OPC-41061 was used 30 µM, and testosterone was used at 50 µM. 500 µg of microsomal protein was used in assays.

Conclusions

The results indicate that CYP3A4 in human liver microsomes is responsible for the metabolism of OPC-41061 to its metabolites. Supporting evidence for this conclusion is the inhibition of OPC-41061 metabolism by the specific CYP3A inhibitors ketoconazole and troleanomycin, anti-CYP3A4 serum and the correlation between the metabolism of OPC-41061 and testosterone, a substrate of CYP 3A4.

Comments

1. Linearization of enzyme data (Lineweaver-Burk, Eadie-Hofstee and Dixon-plots) may introduce bias. Software exist that uses untransformed data to determine the enzyme kinetic parameters.

Study Report No. 016560:” Involvement of MDR1 in Membrane Permeation of OPC-156 and Inhibitory Effects of OPC-156 on Digoxin Transport Mediated by MDR1”

Investigator and Study Site

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Objectives

- To determine whether OPC-156 (OPC-41061) is a substrate of MDR1 expressing cells
- To determine whether OPC-156 (OPC-41061) is an inhibitor of MDR1 expressing cells

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Methods

¹⁴C-OPC-156 (radiochemical purity 97.2%), ³H-digoxin (radiochemical purity not indicated), ¹⁴C-verapamil (radiochemical purity not indicated) and ¹⁴C-mannitol (radiochemical purity not indicated) were used.

The MDR1 expressing cells were porcine kidney epithelial LLC-PK1 cells with vectors containing human MDR1 cDNA and control cells (LLC-KK1 cells with only vectors). The MDR1 expressing cells and control cells were obtained under a sublicense from ██████████. Prior to the experiments the cells were cultured in 75 cm² bottom flasks and subjected to passage every 4 to 5 days. The control and MDR1 expressing cells were added to ██████████ (polycarbonate porous filters with a pore size of 3 μm, area 0.33 cm²) and incubated in a CO₂ (5%) incubator at 37°C for 8 days to prepare cell monolayers for the determination of trans-cellular transport activity. The medium in the flasks contained 9% fetal bovine serum (50 μg/mL), gentamycin (50 μg/mL), and hygromycin (100 μg/mL).

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Transported cumulative amounts of the labeled test substance and reference compound across control and MDR1 expressing cells were determined and cleared volume was calculated from:

$$\text{Cleared volume} = A/P/C_0$$

where A is the transported amount, P is the amount of protein and C₀ is the initial concentration.

OPC-41061 as a Potential Substrate of MDR1

¹⁴C-OPC-115, ³H-digoxin and ¹⁴C-mannitol were used in a final concentration of 1 μM. ³H-Digoxin (1 μM), a known substrate of MDR1, ¹⁴C-verapamil (30 μM), a known inhibitor of MDR1 and ¹⁴C-mannitol (1 μM), which is inter-cellularly transported, were used as controls.

Digoxin transport was investigated in the presence and absence of OPC-156 in final concentrations ranging between 0.1- 50 μM. Verapamil (30 μM), a known inhibitor of MDR1 was used as active control and ¹⁴C-mannitol (1 μM/L) as passive control.

The medium in the culture insert and plate was removed by aspiration and replaced with Hanks balanced salts solution (HBSS), and the cells were pre-incubated at 37°C for 1 h. The apical side (top side of cell, 100 μL) or the basal side (base side of cell, 600 μL) was replaced with HBSS containing the test substance and the cells incubated at 37°C. After incubations of 1, 2 and 4 h, 50 μL HBSS was collected from the opposite compartment. The collected volume was supplemented by 50 μL HBSS and mixed with scintillation fluid and the concentrations of the radio-labeled compounds measured by liquid scintillation spectrometry. Trans-cellular transport activity was determined from the concentration of the test substance and model substrate (digoxin) before incubation, amounts transported and amount of cellular protein. Incubation was performed in triplicate.

To determine the protein concentration, the HBSS in the apical and basal compartments was removed by aspiration, and the cells were solubilized with 0.1 M NaOH. After neutralization with 0.1 M HCl an aliquot of the solution was placed in a 96 well plate and the protein concentration measured by a BCA protein assay kit.

When the flux ratio (cleared volume ratio) across MDR1 cells increases compared to that of control cells, OPC-156 is considered a substrate of MDR1.

OPC-41061 as a Potential Inhibitor of MDR1

¹⁴C-OPC-156 was used in concentrations of 0, 0.1, 0.5, 2, 10, and 50 μM, digoxin in a concentration of 1 μM, verapamil in a concentration of 30 μM and ¹⁴C-mannitol in a concentration of 1 μM.

The experiment used four groups:

Group A; OPC-156 (0 μM) + ³H-digoxin

Group B; OPC-156 (0.1, 0.5, 2, 10, and 50 μM) + ³H-digoxin

Group C: verapamil (30 μM) + ³H-digoxin
 Group D: ¹⁴C-mannitol

The incubations were performed at 37 °C for 1 h. Trans-cellular transport activity and protein concentration were determined as described above.

The transported amounts of the model substrate across control and MDR1 expressing cells in the presence of the test substance was determined, and trans-cellular flux computed from:

$$\text{Flux ratio}_{\text{control cell}} = \frac{\text{Flux}_{\text{basal to apical control cell}}}{\text{Flux}_{\text{apical to basal control cell}}}$$

$$\text{Flux ratio}_{\text{MDR1 expressing cell}} = \frac{\text{Flux}_{\text{basal to apical MDR1 expressing cell}}}{\text{Flux}_{\text{apical to basal MDR1 expressing cell}}}$$

Basal to apical flux of digoxin across MDR1 expressing cells in percent of control was obtained from:

$$\text{Flux (\% control)} = (C-B)/(A-B) \cdot 100$$

where A is flux in the absence of OPC-156, B is flux in the presence of verapamil, and C is flux in the presence of OPC-156.

IC₅₀ was obtained from:

$$\text{Flux (\% control)} = IC_{50}/(IC_{50} + I) \cdot 100$$

where I is the concentration of OPC-156. A least-squares regression method was used. When the flux ratio of digoxin decreases in the presence of OPC-156, OPC-156 is considered an inhibitor of MDR1.

RESULTS

1. OPC as a potential substrate of MDR1

The results are listed in the below table:

Table 6 Time profile for the transcellular transport of ¹⁴C-OPC-156, ³H-digoxin and ¹⁴C-mannitol across control cell and MDR1 expressing cell monolayers

Components	Incubation time (h)	Control cells			MDR1 expressing cells		
		Cleared volume (μl/mg protein)		Flux ratio	Cleared volume (μl/mg protein)		Flux ratio
		Apical to basal	Basal to apical		Apical to basal	Basal to apical	
¹⁴ C-OPC-156 (1 μmol/L)	1	341.0 ± 27.7	362.5 ± 8.0	1.1	176.8 ± 24.9	1216.6 ± 182.4	7.0
	2	624.9 ± 8.6	702.0 ± 30.9	1.1	361.7 ± 43.1	2311.8 ± 228.3	7.7
	4	831.6 ± 76.6	1203.0 ± 40.7	1.4	492.6 ± 37.0	3879.9 ± 377.1	7.9
³ H-Digoxin (1 μmol/L)	1	21.4 ± 1.8	46.2 ± 8.2	2.2	32.6 ± 8.4	263.7 ± 14.8	8.1
	2	41.6 ± 2.3	103.7 ± 18.4	2.4	64.6 ± 22.4	537.9 ± 24.8	8.3
	4	96.5 ± 10.8	249.2 ± 33.2	2.6	127.3 ± 15.9	1088.9 ± 52.2	8.5
¹⁴ C-Mannitol (1 μmol/L)	1	21.1 ± 21.0	21.6 ± 5.9	0.7	69.1 ± 20.6	80.1 ± 22.9	0.6
	2	26.8 ± 24.9	44.9 ± 6.2	0.5	136.2 ± 73.2	81.6 ± 9.8	0.6
	4	118.1 ± 36.9	102.2 ± 21.0	0.9	231.0 ± 73.9	188.3 ± 68.0	0.9

After incubations of 1, 2 and 4 h the flux ratio of OPC-156 across MDR1 expressing cells was 7.0, 7.7, and 7.9, respectively, about 6 to 7 times greater than in control cells. The corresponding flux ratios for the active control

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digoxin were 8.1, 8.3 and 8.5, respectively, and about 3 to 4 times greater than the flux ratios in control cells. The flux ratios of mannitol in MDR1 cells and control cells was constantly about 0.7. There was significant variation of the fluxes in both directions for OPC-156 and digoxin. It can be concluded that OPC-156 is a substrate of MDR1.

2. OCP-156 as a potential inhibitor of MDR1

The results are shown in the below table and figure:

Table 7. Effect of OPC-156 on the digoxin transport across control cell and MDR1 expressing cell monolayers.

Components	Conc. ($\mu\text{mol/L}$)	Control cells				MDR1 expressing cells			
		Flux ($\mu\text{M}/\text{mg protein/h}$)		Flux ratio	Flux ($\mu\text{M}/\text{mg protein/h}$)		Flux ratio		
		Apical to basal	Basal to apical		Apical to basal	Basal to apical			
Inhibitor (-)	0	22.9 \pm 2.5	62.5 \pm 19.5	2.7	183 ^a	250.6 \pm 22.9	13.7		
OPC-156	0.1	25.3 \pm 2.1	65.7 \pm 13.7	2.6	16.0 \pm 6.7	232.8 \pm 15.6	15.8		
	0.5	26.5 \pm 1.0	67.8 \pm 1.3	2.5	18.8 \pm 1.4	274.5 \pm 11.1	15.3		
	1	43.1 \pm 13.5	50.7 \pm 1.6	1.2	17.4 \pm 2.7	235.7 \pm 22.6	14.7		
	10	30.3 \pm 1.6	45.3 \pm 9.0	1.5	34.2 \pm 8.5	206.2 \pm 7.6	6.0		
	50	36.8 \pm 7.0	42.9 \pm 3.5	1.1	73.1 \pm 11.0	83.9 \pm 6.2	1.3		
Verapamil	30	37.8 \pm 1.3	32.0 \pm 5.6	1.0	67.0 \pm 1.1	63.9 \pm 3.9	1.1		
³ H-Mannitol	1	22.4 \pm 3.6	22.7 \pm 4.1	1.0	31.5 \pm 11.6	37.2 \pm 4.9	1.2		

Data are expressed as the mean values \pm S.D. of triplicate samples.

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The flux ratio of digoxin in the absence of OPC-156 in MDR1 expressing cells is 13.7 and about 5 times greater than in control cells. In the presence of increasing concentrations of OPC-156 the flux ratio for digoxin decreases from 13.7 to 1.3. At the highest concentration used, 50 μM , OPC-156 reduces the flux ratio to the same extent as 30 μM verapamil. The flux ratio for the passive control mannitol is about 1.1 in MDR1 expressing and control cells. The estimated IC_{50} of OPC156 is 15.9 μM . It can be concluded that OPC-156 is an inhibitor of MDR1.

Conclusion

OPC-156 is a substrate and an inhibitor of MDR1 with an estimated IC_{50} of 15.9 μM (? ng/mL).

Comments

1. The radiochemical purity of OPC should be greater than 97.2%.
2. The radiochemical purity of ^3H -digoxin, ^{14}C -verapamil and ^{14}C -mannitol should be known
3. The substrate and inhibitor properties of the individual enantiomers of OPC-156 should have been determined

Study Report 156-97-202: "A Study of the Absorption, Distribution, Metabolism, and Excretion of ^{14}C -OCP-41061 Following Oral Administration in Healthy Volunteers"

Investigator and Study Site

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Objectives

To determine the absorption, distribution, metabolism and excretion of radioactivity and intact drug following administration of a single 60 mg ¹⁴C-OPC-41061 dose (100 µCi total radioactivity) to healthy volunteers

Investigational Drugs and Formulations

Single capsule containing 60 mg OPC-41061 (100 µCi) (Lot No. 96I78SM1)

Study Design

This is a single-center, single-dose, open label study conducted in healthy Caucasian subjects. Twelve healthy subjects, aged 21 to 45 years were to be enrolled in the study. The subjects were prohibited from taking any prescription and over-the counter drugs or herbal products within 2 weeks of drug administration and during the study. Intake of alcohol and xanthine- or grapefruit containing products was also prohibited. The subjects ingested the single dose of radio-labeled OPC-41061 together with 240 mL water after a 10 h overnight fast. The subjects were in an upright position for the next 4 h. After the single dose administration of radio-labeled OPC-41061 subjects were followed for up to 14 days. Optional extra-days (>14 days) could be added for monitoring of the radioactivity in samples of blood, urine and feces until two consecutive 24 h plasma samples were less than 200 dpm/mL, two consecutive urine concentrations were less than 200 dpm/mL or ≤ three times background levels, and two consecutive fecal concentrations of radioactivity were either less than 500 dpm/g or ≤ three times background levels.

Pharmacokinetic Profiling

Blood: Samples were collected pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, and 312 h after dosing; Additional samples were collected up to 648 h after administration. An additional blood sample was collected on Day 1 for determining plasma protein binding.

Urine: Samples were to be collected pre-dose, and from 0-16, 16-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, and 288-312 h after administration. However, after 192-216 h the radioactivity in the urine samples met the exit criterion of < 200 dpm/mL.

Feces: Samples were collected predose, and during 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, and 288-312 h after administration. Additional samples were collected until 936-960 h after administration.

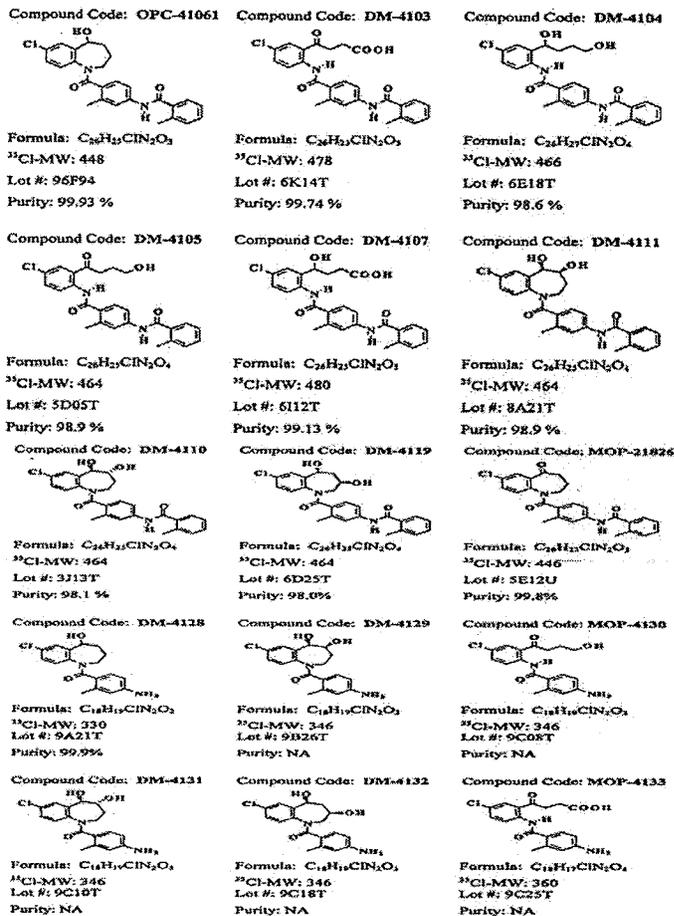
Bioassay

Total radioactivity in plasma- and urine samples was analyzed directly by liquid scintillation spectrometry. Whole blood and feces samples were combusted first and the generated ¹⁴CO₂ entrapped in Harvey Carbon-14 scintillation cocktail for liquid scintillation spectrometry. Total radioactivity measurements were performed by [REDACTED]

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The plasma and urine samples were analyzed for OPC-41061 and its metabolites using a LC/MS/MS method with an internal standard. The assay was linear in the range between 5 ng/mL and 1000 ng/mL for OPC-41061 and 6 metabolites. Metabolite DM-4103 displayed a linear calibration curve between 12.5 ng/mL and 2500 ng/mL. Selected plasma-, urine- and feces samples from 3 selected subjects were analyzed for identification and quantification of OPC-41061, its known and unknown metabolites. Synthesized OPC-41061 and the metabolites

DM-4103, DM-4104, DM-4105, DM-4107, DM-4111, DM-4110, DM-4119, MOP-21826, DM-4128, DM-4129, MOP-4130, DM-4131, DM-4132 and MOP-4133 were available as shown below:



Except for DM-4129, MOP-4130, DM-4131, DM-4132 and MOP-4133, the purity was $\geq 98\%$. Ultraviolet and radiometric detectors were used in tandem on a HPLC. Identity of peaks was established by comparing the retention times on the UV chromatograms of those of known standards injected on each day of analysis. Selected urine samples were analyzed with and without an enzymic hydrolysis (β -glucuronidase). Structures of unknown metabolites were proposed based on the results of the mass spectral data. These compounds were then synthesized. The measurements of OPC-41061 and its metabolites in the matrices plasma, urine and feces were performed by [REDACTED]

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Plasma Protein Binding

The plasma protein binding of radio-labeled OPC-41061 was investigated using equilibrium dialysis and ultra-filtration with liquid scintillation spectrometry of the concentration in the plasma, buffer or ultrafiltrate. The plasma protein binding of OPC-41061 in the presence and absence of DM-4103 was investigated. The impact of pH and possible non-specific binding was investigated in preliminary experiments. The time to dialysis equilibrium was

determined to be 8 h. Due to the finding of non-specific binding of 28% with the ultra-filtration device the definitive experiments were performed only by equilibrium dialysis. The percent free drug in plasma was calculated from:

$$\text{Free Fraction} = 100 \bullet \text{Final Dialysate dpm} / \text{Final Plasma dpm}$$

The final data were calculated without correction for volume shift. The plasma protein binding determinations were performed at _____

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PK Analysis

For each subject the ratio of radioactivity in blood to plasma was calculated. Blood and plasma total radioactivity data and OPC-41061 and metabolite concentration time data were analyzed by compartment model independent methods (WINNONLIN PROFESSIONAL). The parameters k_a , C_{max} , T_{max} , λ_z (from at least 3 quantifiable concentrations in the terminal log linear phase), AUC_T , AUC_{-INF} , CL/F and V_z/F were to be determined. The last observed measurable plasma analyte- or radioactivity concentration was used to determine AUC_{-INF} . In order to sum AUC_{-INF} for the different analytes the respective MW were used. Although stipulated by the protocol k_a could not be determined. For urine and feces data the cumulative amounts excreted over time were computed. Also, the percent radioactivity in plasma, urine and feces due to OPC-41061 and identified metabolites was calculated. All below-the-quantification-limit (BQL) values and no-peak-detected (NPD) values were replaced by zero. The designated background level was 30 dpm/mL

RESULTS

All 12 Caucasian male subjects enrolled completed the study. The mean age and body weight was 32 years and 77 kg, respectively.

Plasma Protein Binding

OPC-41061

Preliminary experiments showed that the time to dialysis equilibrium is about 8 h. Changes in pH affected the extent of binding and the free fraction decreased with increasing pH (pH 7.0: percent unbound 1.7, pH 7.8: percent unbound 0.83) suggesting that pH control during dialysis is important. The results on the plasma protein binding of OPC-41061 at 37 °C and pH 7.4 are shown below:

TABLE 4A
OPC-41061 Protein Binding
 Concentration Dependency and
 Physical Processing & Storage Effects
 K₃EDTA Human Plasma *

OPC-41061 Concentration ng/mL	Cycle	Batch	n	% Free, Non-Corrected
150	0	16	5	0.93 ± 0.05
350	0	16	5	1.02 ± 0.17
500	0	16	5	0.93 ± 0.08
1000	0	16	5	0.92 ± 0.10

* Mean ± SD.

* Single source lot (MBL).

The plasma protein binding of OPC-41061 over the concentration range of 150 ng-2000 ng/mL is constant and high with an unbound percentage ranging between 0.9% and 1.2%.

The plasma protein binding of OPC-41061 (at 500 ng/mL-1000 ng/mL) is not affected by the presence of the main metabolite DM-4103 in concentrations of 500-1000 ng/mL as shown by the results listed in the below table:

TABLE 5
OPC-41061 Protein Binding
Possible Displacement of
OPC-41061 by DM-4103

<u>Pool</u>	<u>OPC-41061</u> <u>ng/mL</u>	<u>DM-4103</u> <u>ng/mL</u>	<u>OPC-41061</u> <u>% Free*</u> <u>Non-Corrected</u>
A	1000	0	1.35 ± 0.04 *
B	1000	500	1.37 ± 0.02
C	1000	1000	1.36 ± 0.03
D	500	500	1.28 ± 0.06 *

* Mean ± SD, n = 5

* n = 4.

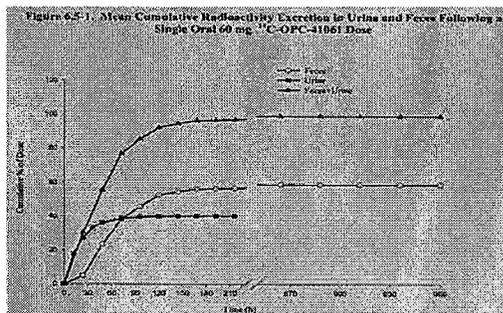
PK

Mass Balance and Primary Route of Elimination

The below table and figure show the results of the recovery of total radioactivity in feces and urine in % of the dose:

Mean (SD) Recovery of Radioactivity in Percent of Dose

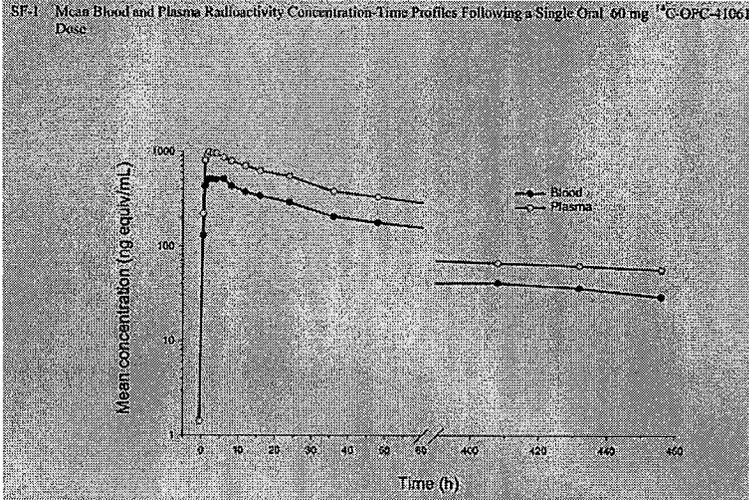
Mean Recovery, % Dose		
Total	Fecal	Urine
98.87 (8.06)	58.71 (9.12)	40.16 (8.64)



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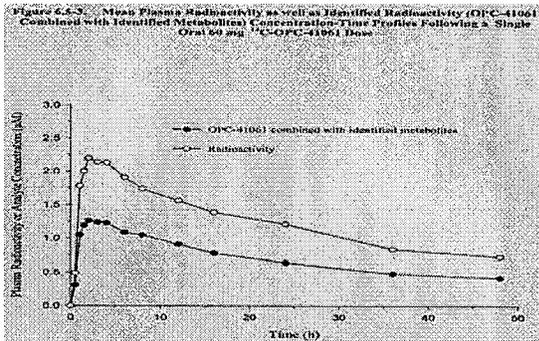
The respective recoveries in urine were measured up to 216 h and 960 h, respectively after administration. Both with urine and feces a plateau is reached and thus the collection intervals appear to be appropriate. The results indicate that after oral administration the main recovery of total radioactivity is in the feces. The 40% figure of radioactivity excreted in urine indicates that at least this percentage of the dose is absorbed as parent drug or metabolite into the systemic circulation.

The below mean plot shows the concentrations of total radioactivity in whole blood and plasma:



The data indicate that the whole blood to plasma concentration ratio of about 0.5 remains fairly constant over time. The low value of the ratio indicates that negligible amounts of radioactivity are associated with the red blood cells suggesting extensive plasma protein binding of OPC-41061 and metabolites.

The mean plasma concentrations of total radioactivity and the sum of (OPC-4106 + identified metabolites) over an interval of 48 h after administration are shown in the below plot:



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The plot shows that the levels of total radioactivity are exceeding those of the identified analytes indicating that significant concentrations of unidentified circulating metabolites exist.

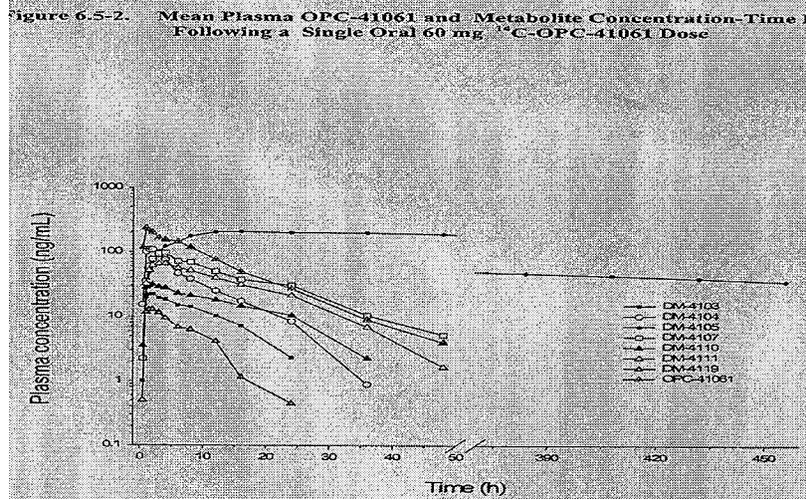
The below table lists the individual percentages that OPC-41061 and the identified seven metabolites contribute to total radioactivity in plasma:

Table 6.5-3 Mean Percent of Plasma Radioactivity AUC-INF Accounted for by OPC-41061 and Identified Metabolites

	DM-4103	DM-4104	DM-4105	DM-4107	DM-4110	DM-4111	DM-4119*	OPC-41061	Total
N	12	12	12	12	12	12	12	12	12
Mean	52.47	0.98	0.35	1.71	0.66	1.31	0.10	2.84	60.42
SD	3.28	0.25	0.07	0.44	0.22	0.45	0.07	1.29	3.34
Median	52.22	0.95	0.36	1.62	0.63	1.31	0.10	3.04	61.32

The AUC-INF of the combined, identified compounds OPC-41061, DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, DM-4111 and DM-4119 represents about 60 % of that for total radioactivity, so that about 40% of the total radioactivity in plasma is unidentified. The major circulating metabolite is DM-4103 which makes up about 52 % of the AUC-INF for total radioactivity. OPC-41061 contributes slightly less than about 3% to the AUC-INF of total radioactivity.

The next plot shows the plasma concentration time profiles of OPC-41061 and the seven identified metabolites:



The number of subjects contributing to the mean concentrations varies over time as more values are BLQ. The mean concentrations of OPC-41061 and the 7 metabolites in all 6 tested subjects can be followed for considerably shorter time periods than shown in the plot. The follow-up period of the respective profiles is shorter than the time interval needed for a reliable estimate of λ_z and the extrapolated $AUC_{t \rightarrow \infty}$. Thus, only the estimates for C_{max} and t_{max} are reliable, whereas $t_{1/2z}$ and AUC_{INF} and the derived CL/F and V/F must be considered biased.

From a comparison of the respective tmax values and the post peak decay patterns it appears that the kinetics of six of the seven metabolites is formation limited. Only with DM-4103 the kinetics are dictated by elimination.

Summaries of the mean PK parameters derived by the sponsor from the plasma data for OPC-41061 and the identified metabolites are shown below and should be interpreted with due caution:

Table 6.5-2 Summary of Plasma OPC-41061 and Metabolite Pharmacokinetics Following a Single Oral 60mg ¹⁴C-OPC-41061 Dose

		C _{max} (ng/mL)	t _{max} (h)	AUC _{0-∞} (ng·h/mL)	AUC-Inf (ng·h/mL)	t _{1/2} (h)	CL/F (mL/min/kg)	V _{Z/F} (L/kg)	f _e
OPC-41061	N	12	12	12	12	12	12	12	12
	Mean	259.44	1.7	2431.28	2574.91	9.34	6.01	4.33	1.13
	SD	81.60	1.5	917.49	965.22	4.46	2.69	2.29	0.15
	Median	247.08	1.0	2481.45	2621.38	9.02	5.47	3.53	1.06
DM-4103	N	12	12	12	12	12	12	12	12
	Mean	219.93	21.0	44470.56	54022.53	182.66	0.27	4.22	
	SD	47.52	10.0	8109.79	10854.66	33.03	0.07	1.04	
	Median	231.50	16.0	45612.97	49878.53	188.22	0.27	4.70	
DM-4104	N	12	12	12	12	12	12	12	12
	Mean	120.71	1.5	859.82	949.44	8.02	14.65	10.10	
	SD	19.42	0.6	150.61	149.43	2.68	2.40	3.83	
	Median	121.76	1.0	841.76	910.15	7.62	14.29	9.28	

Table 6.5-2 (continued) Summary of Plasma OPC-41061 and Metabolite Pharmacokinetics Following a Single Oral 60mg ¹⁴C-OPC-41061 Dose

		C _{max} (ng/mL)	t _{max} (h)	AUC _{0-∞} (ng·h/mL)	AUC-Inf (ng·h/mL)	t _{1/2} (h)	CL/F (mL/min/kg)	V _{Z/F} (L/kg)
DM-4105	N	12	12	12	12	12	12	12
	Mean	24.93	1.6	235.00	340.64	11.27	41.61	38.94
	SD	6.55	0.7	50.47	63.17	5.28	10.42	16.06
	Median	25.11	1.5	250.49	346.30	10.65	40.22	37.17
DM-4107	N	12	12	12	12	12	12	12
	Mean	100.19	3.4	1564.17	1725.23	12.44	8.51	8.91
	SD	36.61	0.7	309.48	369.29	3.27	1.95	2.61
	Median	92.00	3.5	1631.18	1750.62	12.41	8.17	8.54
DM-4110	N	12	12	12	12	12	12	12
	Mean	34.79	1.6	478.16	630.74	13.41	22.59	25.61
	SD	7.35	0.7	137.11	146.13	2.24	5.45	4.83
	Median	34.72	1.5	433.45	622.84	13.38	21.73	24.47

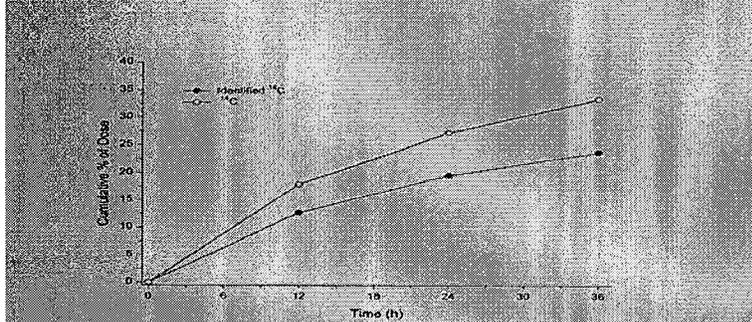
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Table 6.5-2 (continued) Summary of Plasma OPC-41061 and Metabolite Pharmacokinetics Following a Single Oral 60mg ¹⁴C-OPC-41061 Dose

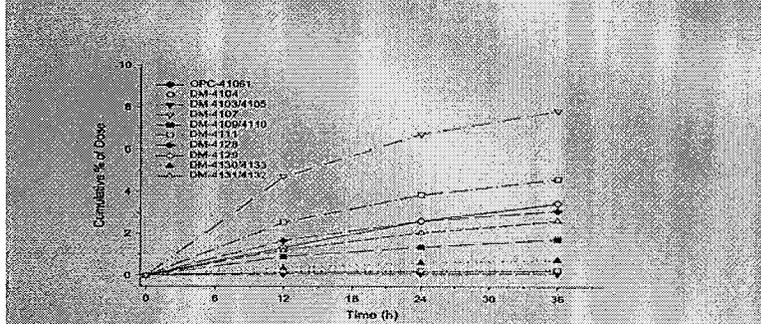
		<i>C</i> _{max} (ng/mL)	<i>t</i> _{max} (h)	AUC _{0-∞} (ng h/mL)	AUC _{0-12h} (ng h/mL)	<i>t</i> _{1/2} (h)	CL/F (mL/min/kg)	V _d /F (L/kg)
DM-4111	N	12	12	12	12	12	12	12
	Mean	68.97	3.0	1128.30	1250.78	9.89	11.34	9.53
	SD	13.76	0.9	305.42	285.80	1.96	2.69	2.33
	Median	71.36	3.0	1087.95	1197.38	9.44	11.52	9.32
DM-4119	N	12	12	12	5	5	5	5
	Mean	14.85	1.6	94.07	267.14	16.38	65.47	64.62
	SD	3.38	0.6	55.57	131.39	13.95	40.00	24.78
	Median	13.71	1.5	88.06	253.26	12.69	56.67	46.86

The below plots and tables show the cumulative amounts excreted in urine over 36 h after administration for total radioactivity, total identified compounds and individual identified compound expressed as percent of the dose:

SP-12. Mean Cumulative Urinary Excretion (% of Dose) of Radioactivity and Identified Radioactivity (OPC-41061 Combined with its Metabolites) Following a Single Oral 60 mg ¹⁴C-OPC-41061 Dose



SP-13. Mean Cumulative Urinary Excretion (% of Dose) of OPC-41061 and its Metabolites Following a Single Oral 60 mg ¹⁴C-OPC-41061 Dose



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ST-37 (Cont'd) Urine Excretion due to OPC-41061 and Metabolites Identified by HPLC with Radiometric Detection, Expressed as Percent of Dose, Following a Single Oral 60 mg (100 µCi) ¹⁴C-OPC-41061 Dose (Subjects 10-12 and Mean)

Collection Interval	OPC-41061	DM-4104	DM-4103 or DM-4105	DM-4107	DM-4119 or DM-4110	DM-4111	DM-4128	DM-4129	DM-4130 or DM-4133	DM-4132 or DM-4131	Total % of Dose as OPC-41061 plus metabolites	% of Dose as ¹⁴ C	% of ¹⁴ C Identified
MEAN													
0-12	0.163	0.194	0.055	4.710	0.897	2.543	1.673	1.351	0.383	1.218	12.663	17.814	71.032
12-24	0.045	0.058	0.026	2.000	0.487	1.312	0.950	1.265	0.258	0.832	6.841	9.338	73.476
24-36	0.026	0.034	0.014	1.123	0.353	0.749	0.498	0.839	0.106	0.569	4.198	6.114	68.027
Total % of dose	0.194	0.257	0.045	7.833	1.737	4.604	3.101	3.348	0.565	2.517	23.701	33.267	71.229
Total % of ¹⁴ C	0.584	0.834	0.130	23.277	5.384	14.085	9.270	9.957	1.537	7.595	-	-	72.653

About 33% of the total radioactivity administered is excreted in urine 36 h after dosing. About 72% of the total radioactivity excreted in urine over the same period is identified tentatively or definitively leaving about 28 % of unidentified radioactivity. Five metabolites in urine are definitively identified. DM-4107 (about 8% of dose) and DM-4111 (about 5 % of dose) are the most abundant metabolites excreted in urine. OPC-41061 excretion in urine appears to be negligible. Also, the excretion in urine of the most abundant metabolite in plasma, DM-4103, appears to be small up to 36 h after administration, but will increase if followed for a more prolonged interval. There was no evidence for the formation of glucuronides. It should be noted the 36 h time interval is small relative to the protracted rate of elimination of total radioactivity in urine with measurable total radioactivity up to 216 h post-dose.

The below table shows the cumulative amounts excreted in feces over 36 h of total radioactivity, total identified compounds and individual identified compound expressed as percent of the dose:

ST-38 Fecal Excretion due to OPC-41061 and Metabolites Identified by HPLC with Radiometric Detection, Expressed as Percent of Dose, Following a Single Oral 60 mg (100 µCi) ¹⁴C-OPC-41061 Dose

Collection Interval	OPC-41061	DM-4104	DM-4103/4105	DM-4107	DM-4119/4110	DM-4111	DM-4128	DM-4129	DM-4130/4133	DM-4132/4131	Total % of Dose as OPC-41061 Plus Metabolites	% of Dose as ¹⁴ C	% of ¹⁴ C Identified
MEAN													
Total % of dose	13.70	0.56	0.68	9.02	2.75	4.93	-	0.22	-	0.65	32.43	43.54	74.66
Total as % of ¹⁴ C	31.91	1.29	1.56	20.55	6.29	11.23	-	0.51	-	1.50	-	-	74.84

About 44% of the total radioactivity administered was recovered in the feces up to 36 h after dosing. Of the recovered radioactivity in the feces about 75% is identified tentatively or definitively leaving about 25% unidentified. Four metabolites are definitively identified. The most abundant compound in the feces is OPC-41061 (about 14% of dose) followed by DM-4107 (about 9 % of dose). The time interval of 36 h is small on consideration of the protracted elimination of total radioactivity in the feces with total radioactivity measurable up to 960 h after administration. As in urine the recovery of DM-4103 in the feces is likely underestimated.

Conclusions

The mean total recovery of the radioactivity administered is about 99 % with about 40 % excreted in urine and about 59 % in the feces. An extended period of collection is necessary to capture the protracted elimination of total radioactivity and DM-4103. The main route of total radioactivity is non-renal. The absorption of OPC-41061 as parent drug or metabolites is ≥ 40 %. OCP-41061 is mainly eliminated by non-renal pathways. The data suggest that more of OPC-41061 is metabolized than excreted unchanged into the bile. In plasma, urine and feces 7, 5 and 4 metabolites, respectively, are definitively identified. About 60 % of the total radioactivity circulating in plasma is definitively or tentatively identified. DM-4103 is the most abundant circulating metabolite in plasma with a partial

AUC equivalent to about 52% of the circulating total radioactivity. OPC-4161 is a minor constituent in plasma. About 73% of the total radioactivity excreted in urine is definitively or tentatively identified. The most abundant compound excreted in urine is DM-4107 (about 8% of dose). OPC-41061 is negligibly excreted in urine. About 75% of total radioactivity excreted in the feces is definitively or tentatively identified. The cumulative amounts of OPC-4161 fecally excreted represent about 14% of the radioactivity administered representing unabsorbed and/or absorbed and subsequently with the bile excreted parent drug. DM-4107 is the most abundant metabolite excreted in the feces (about 9% of dose).

Comments

1. A significant percentage of radioactivity in plasma, urine and feces remains unidentified or not definitively identified.
2. Identification of parent drug and metabolites in the excreta was carried out only over a period of 36 h after administration. This is a short interval considering the protracted elimination of total radioactivity observed. Therefore, the ratios of identified compounds to total radioactivity in the matrices may be biased.
3. The estimates for $t_{1/2z}$, AUC INF, CL/F and Vz/F for OPC-41061 are biased. Vz/F depends on the elimination rate constant and thus is not a true distribution parameter.

Study Report 156-05-254: "An Open-Label, Sequential Study to Determine the Absolute Bioavailability of Tolvaptan Following Administration as 30 mg Oral Tolvaptan Tablets in Normal, Healthy Subjects"

Investigator and Study Site

b(4)

Objectives

The primary objective of the study was to evaluate the absolute bioavailability of tolvaptan following administration as the 30 mg oral tablet formulation. Secondary objectives were to compare urine volume, fluid intake, _____ and urine osmolality following intravenous and oral administration of tolvaptan and to determine the safety of tolvaptan following intravenous and oral administration.

b(4)

Investigational Drugs and Formulations

The study medication was provided to the Clinical Unit by the sponsor. Intravenous tolvaptan (Lot No. 0554961) and intravenous placebo (Lot No. 0654970) were manufactured by _____ and supplied as sterile solutions in 10 mg glass vials at a concentration of 0.1 mg/mL tolvaptan. The oral tolvaptan 30 mg tablets (Lot No. 04C77A030A) were manufactured by the sponsor.

b(4)

Design

This was a single-center, open-label, sequential administration study of 1 mg tolvaptan given as a 1 h infusion and a single dose of a 30 mg tablet in healthy male and female subjects. Fourteen (14) subjects were to receive on Day-2 an infusion of placebo, on Day 1 an infusion of tolvaptan and on Day 8 a single dose of a 30 mg tolvaptan tablet in that order. The 1 h placebo infusion consisted of 250 ml 5% dextrose in water. The 1 h infusion of tolvaptan 1 mg used 10 mL 0.1 mg/ml tolvaptan diluted with 250 mL 5% dextrose in water. The single 30 mg tolvaptan tablet was given to the subjects with 250 mL water. No additional water was to be given to the subjects. On Days -3 and -1 the subjects were to consume a minimum of 500 mL water prior to the evening snack. The doses on Days -2, 1 and 8 were administered in the fasting state. Subjects were to fast for 4 h after start of the intravenous infusion or

administration of the oral tablet. Subjects were to receive *nothing per os* for 2 h prior to and 2 h after each dose or start of the infusion. Subjects were to remain seated for the first 4 h after dosing. The use of any prescription medication including hormonal contraceptives, over-the-counter medication, herbal medication, or vitamin supplement within 14 days prior to dosing and during the study or antibiotics within 30 days prior to dosing and during the study was prohibited. Additionally, the consumption of grapefruit- or Seville orange containing products, alcohol and xanthine containing products was prohibited. Fluid intake was noted throughout Days-2, 1 and 8, including liquids served with meals.

Safety

Physical examinations and laboratory tests were performed and vital signs and 12 Lead ECGs recorded.

Pharmacokinetic Profiling

Blood

Blood samples for the determination of tolvaptan and metabolites DM-4103 and DM-4107 were taken pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 24, 30, 36 and 48 h post-dose with the intravenous infusion treatment and pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, and 48 h post-dose for the tablet treatment.

Urine

Urine volume was measured on Day -2, 1 and 8 during the following intervals: 0-2, 2-4, 4-6, 6-8, 8-12, 12-16 and 16-24 h post-dose.

Bioassay

The plasma concentrations of tolvaptan, DM 4103 and DM-4107 were measured by a HPLC/MS/MS using an internal standard. The calibration curve is linear from 5.00 ng/mL to 1000 ng/mL for tolvaptan and from 12.5 ng/mL and 2500 ng/mL for the metabolites DM-4103 and DM-4107. The coefficient of correlation of the linear regressions (weighted $1/y^2$) is ≥ 0.9980 for OPC-41061, ≥ 0.9964 for DM-4103 and ≥ 0.9976 for DM-4107. With the QC samples the overall inter-day precision is $\leq 7.69\%$ and the accuracy ranges between 2.38 % and 4.00 % for OPC-41061. For DM-4103 the inter-day precision is $\leq 7.42\%$ and the accuracy ranges between -7.00 % and -2.80 %. For DM-4107 the inter-day precision is $\leq 7.82\%$ and the precision ranges between 3.55% and 5.50%. Benchtop stability for 19.5 h at room temperature, stability after 3 freeze-thaw cycles and stability after long term refrigeration (8 days at -70°C) is guaranteed for OPC-41061, DM-4103 and DM-4107 in plasma samples.

The assay was revalidated enabling measurement of lower concentrations of OPC-41061 and DM-4103. The respective calibration curves are linear between 1.0 ng/mL and 200 ng/mL for tolvaptan and 2.5 ng/mL and 500 ng/mL for DM-4103. The concentrations of tolvaptan and DM-4103 after intravenous infusion were determined in all 14 subjects using the assay with the higher sensitivity. The assay sensitivity for DM-4107 after intravenous infusion of tolvaptan was found to be BQL, and no data were reported in the subjects on DM-4107 after intravenous infusion of OPC-41061. The measurements were conducted by _____

b(4)

Pharmacokinetic Data Analysis

C_{max} , t_{max} , AUC_t and λ_z , if possible, were determined for tolvaptan. $AUC_{0-24\text{ h}}$. For the metabolites C_{max} , t_{max} and AUC_t were determined. C_{max} and t_{max} were taken directly from the data. AUC_{0-24} and AUC_t was determined by the linear trapezoidal rule. Non-compartmental methods were applied using WinNonlin Pro. Absolute bioavailability of tolvaptan was determined from:

$$F = \frac{AUC_{\infty,po}}{AUC_{\infty,iv}} \cdot \frac{\text{Dose}_{iv}}{\text{Dose}_{po}}$$

Pharmacodynamics

24 h urine volume was measured. Urine osmolality was integrated over the 0-24 h time interval by summing up the product of urine osmolality and duration of the collection interval.

Changes from baseline (values obtained after placebo iv administration) were computed.

b(4)

Pharmacodynamic/Pharmacokinetic Correlations

Linear regressions were performed.

Statistical Analysis

No statistical analysis was planned.

RESULTS

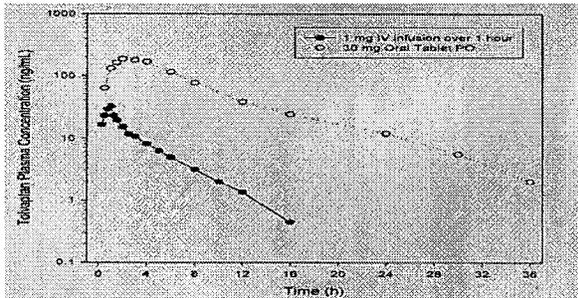
Fourteen subjects, 7 females and 7 males, of median age 25 (range 19-44) years and body weight 74 (range 56-105) kg enrolled and completed the study. Six (6) subjects each were Caucasian and Hispanic and 2 subjects were of Black origin.

Safety

No serious or severe adverse events were recorded. The only drug related adverse event observed was thirst.

Pharmacokinetics

The below semi-logarithmic plot shows the respective plasma concentration time profiles of tolvaptan after a 1 h intravenous infusion of 1 mg and oral administration of 30 mg, and the below table lists the corresponding parameters:



Notes: Lower limit of quantitation of tolvaptan is 1.00 ng/ml.
Source: PDR, 2014.
Figure 9.2.3.1-1 Mean Tolvaptan Plasma Concentrations Following a 1-Hour Constant Rate Intravenous Infusion of Tolvaptan 1 mg or a Single Oral Tablet Dose of Tolvaptan 30 mg to 14 Normal Healthy Subjects

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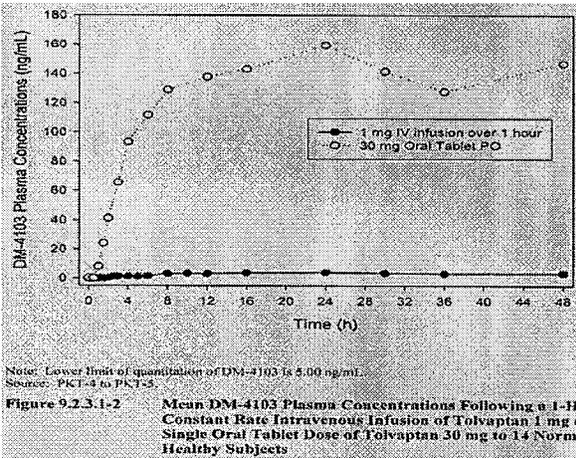
Table 9.2.3.2-1 Mean (SD) Plasma Pharmacokinetic Parameters for Tolvaptan Following a 1-Hour Constant Rate Intravenous Infusion of 1 mg Tolvaptan or a Single Oral Tablet Dose of Tolvaptan 30 mg to Normal Healthy Subjects

Parameter	Tolvaptan 1 mg IV (N = 14)	Tolvaptan 30 mg PO (N = 14)
C _{max} (ng/mL)	35.7 (6.98)	231 (81.3)
t _{max} (h) ^a	1.00 (0.75 - 1.00)	2.00 (1.50 - 6.00)
AUC _{0-24h} (ng·h/mL)	105 (29) ^b	1557 (623)
ATTC _t (ng·h/mL)	96 (28)	1611 (693)
t _{1/2z} (h)	3.4 (1.0) ^b	6.7 (2.6) ^c
AUC _∞ (ng·h/mL)	106 (30) ^b	(73) (698) ^c
CL or CL/F (mL·min/kg)	2.34 (6.78) ^b	4.46 (1.34) ^c
F (%)	—	56 (10) ^d

^a Values are median (range).
^b N = 11.
^c N = 13.
^d N = 10.
Source: PKT-8 to PKT-9.

The sensitivity of the LC/MS/MS assay (with and without increased sensitivity) does not allow a satisfactory characterization of the disposition of tolvaptan after intravenous infusion of 1 mg or oral administration of 30 mg. After intravenous administration, the plasma concentrations are only measurable up to 6 h post-dose in all subjects. After oral administration the plasma concentrations are only measurable up to 16 h post dose in all subjects. The observable respective time intervals for tolvaptan and the metabolites DM-4103 and DM-4107 are too short for a reliable determination of λ_z, and derived parameters. The decline of the plasma concentrations of OPC-41061 in the observable interval after intravenous infusion is steeper than after oral administration. The 56 % estimate for absolute bioavailability of tolvaptan by the sponsor is not reliable.

The below figure and table show the plasma concentration time curve and the parameters for the metabolite DM-4103, respectively, after intravenous infusion and oral administration of tolvaptan:



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Table 9.2.3.2-2 Mean (SD) Plasma Pharmacokinetic Parameters for DM-4103 Following a 1-Hour Constant Rate Intravenous Infusion of Tolvaptan 1 mg or a Single Oral Tablet Dose of Tolvaptan 30 mg to Normal Healthy Subjects

Parameter	Tolvaptan 1 mg IV (N = 14)	Tolvaptan 30 mg PO (N = 14)
C _{max} (ng/mL)	3.34 (1.29)	162 (38.5)
t _{max} (h) ^a	27.09 (8.00 - 48.00)	24.00 (12.18 - 48.00)
AUC _t (ng·h/mL) ^b	132 (74)	6266 (1445)

^a Values are median (range).
^b t = 48 hours for all subjects.
 Source: PKT-11 and PKT-12.

The plasma concentration profile of DM-4103 after intravenous administration is very flat suggesting a small and slow generation of the metabolite after intravenous administration of tolvaptan. After oral administration of tolvaptan the mean t_{max} of DM-4103 is 24 h. Since the plasma concentrations of DM-4103 were only followed for 48 h the terminal disposition phase of DM-4103 cannot be characterized. Much larger plasma concentrations of DM-4103 are seen after oral administration of tolvaptan, but it should be considered that the oral dose was 30 times greater than the intravenous dose administered.

The below figure and table show the plasma concentration time curve and the parameters for the metabolite DM-4107, respectively, after oral administration of 30 mg tolvaptan:

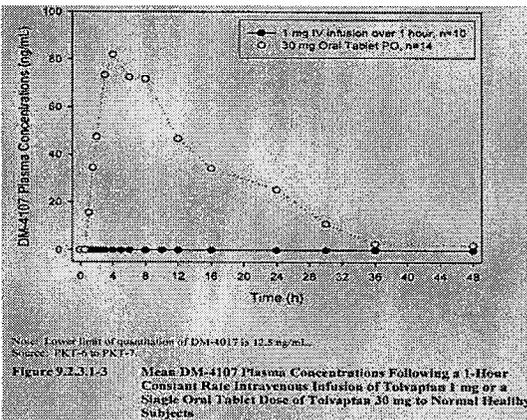


Table 9.2.3.2-3 Mean (SD) Plasma Pharmacokinetic Parameters for DM-4107 Following a Single Oral Tablet Dose of Tolvaptan 30 mg to Normal Healthy Subjects

Parameter	Tolvaptan 30 mg PO (N = 14)
C _{max} (ng/mL)	93.2 (24.1)
t _{max} (h) ^a	4.00 (3.00 - 8.00)
AUC _t (ng·h/mL)	1242 (572)

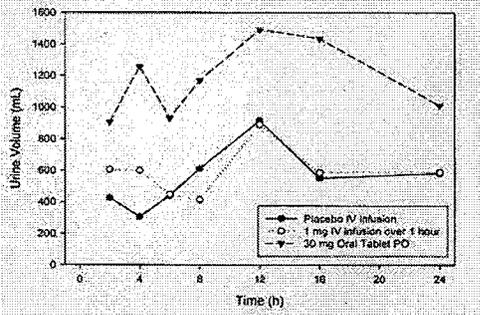
^a Values are median (range).
 Source: PKT-13.

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The plasma concentrations of DM-4107 after intravenous administration were BLQ. After oral administration the mean plasma concentration of the metabolite DM-4107 peaks 4 h after tolvaptan administration.

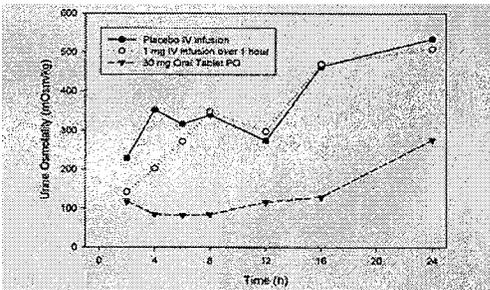
Pharmacodynamics and Pharmacodynamic-Pharmacokinetic Correlation

The below figures show linear time profiles of urine osmolality and urine volume after intravenous administration of placebo, 1 mg intravenous tolvaptan and 30 mg oral tolvaptan 30 mg tolvaptan:



Source: CT-15.2.1.
Figure 9.3.3.2-1 Mean Urine Volume Plotted at the End-time of the Collection Interval Following a 1-Hour Constant Rate Intravenous Infusion of Placebo or Tolvaptan 1 mg or a Single Oral Tablet Dose of Tolvaptan 30 mg to 14 Normal Healthy Subjects

After intravenous administration of 1 mg tolvaptan the urine volumes exceed those after placebo for 4 h post-dose. After oral administration of 30 mg tolvaptan the urine volumes exceeds those after placebo for 24 h post-dose.



Source: CT-15.1.1.
Figure 9.3.3.1-1 Mean Urine Osmolality Plotted at the End-time of the Collection Interval Following a 1-Hour Constant Rate Intravenous Infusion of Placebo or Tolvaptan 1 mg or a Single Oral Tablet Dose of Tolvaptan 30 mg to 14 Normal Healthy Subjects

Urine osmolality after 1 mg tolvaptan is lower than that after placebo for 6 h post-dose. After oral administration of 30 mg tolvaptan urine osmolality is decreased for 24 h compared to placebo administration.

The below table lists the median urine volumes after intravenous and oral administration of tolvaptan and intravenous administration of placebo:

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Median 24 h Urine Volume Excreted after Intravenous Infusion of 1 mg Tolvaptan, Placebo or Oral Administration of 30 mg Tolvaptan

Test Medication	Urine Volume Excreted ^a mL		
	Placebo	Tolvaptan 1 mg iv	Tolvaptan 30 mg po
Collection Interval, h			
0-2	390	605	875
2-4	283	640	1210
4-6	380	243	965
6-8	355	250	1195
8-12	625	685	1540
12-16	290	225	1335
16-24	535	430	940
Sum	3370	2855	7735

The below table lists the median urine excretion rates after intravenous administration of 1 mg tolvaptan iv or administration of 30 mg tolvaptan po:

Median Urine Excretion Rate after Intravenous Administration of 1 mg Tolvaptan or Oral Administration of 30 mg Tolvaptan and Corresponding Median Plasma Concentrations at Mid Time

Treatment	Median Urine Excretion Rate, ^a mL/min		Median Plasma Concentration, ng/mL	
	1 mg iv	30 mg po	1 mg iv	30 mg po
Urine Collection Interval h				
0-2	1.8	4.0	34	136
2-4	3.0	7.7	10	165
4-6	-1.1	4.9	6	126
6-8	-0.88	7.0	BLQ	79
8-12	0.13	3.8	BLQ	46
12-16	-0.27	4.4	BLQ	26
16-24	-0.14	0.84	BLQ	15

^a Placebo corrected median urine excretion rate BLQ= below limit of quantitation

iv Infusion

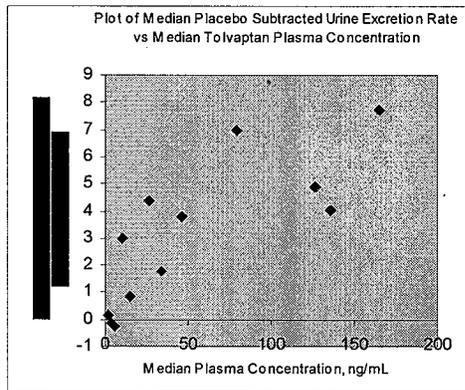
The median placebo subtracted urine excretion rate during the first 0-2 h collection interval is 1.8 mL/min indicating a swift onset of action of tolvaptan. A peak rate of 3.0 mL/min is attained in the 2-4 h collection interval, i.e. about 2 h later than C_{max} suggesting a possible lagging of the effect behind the plasma concentration of tolvaptan. During the subsequent period from 4 h to 24 h after infusion start the median net excretion rate ranges between -1.1 mL and 0.13 mL/min suggesting a small rebound with slightly increased water retention compared to placebo. The aquaretic activity of 1 mg tolvaptan administered by a 1 h infusion appears to last about 4 h.

Oral Administration

The onset of the aquaretic effect of tolvaptan after oral administration of 30 mg occurs during the first urine collection period of 0-2 h. The median peak plasma concentration of tolvaptan after oral administration is observed at about 3 h. The median net urine excretion rate attains a maximum value of 7.7 mL/min in the 2-4 h collection interval indicating no significant lagging of the aquaretic effect behind the plasma concentrations after oral administration. Subsequently, the median net urine excretion rate declines, but compared to placebo, remains

elevated up to 24 h after dosing. The aquaretic activity of 30 mg tolvaptan administered orally lasts at least 24 h. The comparison of the effect size and duration of the aquaretic activity of 1 mg and 30 mg tolvaptan administered intravenously and orally, respectively, indicates a dose dependency. The peak aquaretic effect appears to lag behind the peak plasma concentration of tolvaptan after both modes of administration.

In the below plot the median net urine excretion rate is plotted against the median plasma concentration of tolvaptan after intravenous and oral administration:



The results indicate a nonlinear relationship between plasma concentration and aquaretic effect of tolvaptan.

Conclusions

PK

The limitation introduced by the assay sensitivity resulting in too short observation periods for tolvaptan and the metabolites DM-4103 and 4107 handicapped the interpretation of the results on the absolute bioavailability study. An unbiased estimate for absolute bioavailability of tolvaptan after administration of a 30 mg tablet cannot be derived from the available data. The sponsor's estimate of 54% for absolute bioavailability is not reliable.

PD

The onset of the aquaretic effect of tolvaptan is swift after intravenous administration of 1 mg and oral administration of 30 mg. After intravenous administration of tolvaptan 1 mg the net peak excretion rate (3.0 mL/min) occurs about 3 h after start of the infusion and the aquaretic effect appears to last about 4 h. After oral administration of 30 mg the net peak excretion rate (7.7 mL/min) occurs also about 3 h post-dose and the aquaretic effect lasts at least 24 h.

PK-PD

The relationship between plasma concentration of tolvaptan and aquaretic effect is saturable.

Comments

1. The sensitivity of the LC/MS/MS assay for tolvaptan and the metabolites DM-4103 and DM-4107, the respective intervals and time points for collection of blood samples after intravenous and oral administration of tolvaptan are insufficient for a reliable determination of absolute bioavailability.

2. The report should state the individual values for t used in determining AUCt for tolvaptan after intravenous administration.
3. The report does not provide data in support of validation of the more sensitive assay for tolvaptan that the sponsor also used. The plasma concentrations measured by the more sensitive method are also not provided.

Study Report No. 156-01-233: "Open-Label, Randomized Crossover Study to Assess Dose Strength Equivalence among 15, 30, and 60 mg Strength Oral Tablets of Tolvaptan"

Investigator and Study Site

~~_____~~

b(4)

Objectives

The primary objective was to assess dose-strength equivalence among 15, 30 and 60 mg strength tablets of OPC-41061. The secondary objective was to confirm the results of the interaction between OPC-41061 and lovastatin observed in a previous study.

Investigational Drugs and Formulations

Tolvaptan 15 mg tablets (Lot No. 99E87A015), tolvaptan 30 mg tablets (Lot No. 99E87A030A), tolvaptan 60 mg tablets (Lot No.: 99E87A060) and lovastatin 40 mg tablets (Lot No. L1200) _____ were provided by the sponsor.

b(4)

Design

This was a single-center, randomized, crossover study to assess dose-strength equivalence among 15, 30 and 60 mg strength oral tablets of OPC-41061, as well as a combination treatment of OPC-41061 60 mg (single dose administration of 60 mg tablet) with lovastatin 80 mg (single dose administration of two 40 mg tablets). Approximately 30 healthy male and female subjects were to be enrolled. The screened subjects entered the study site on Day -2 and remained institutionalized for a total of 19 days. On Day-1 the subjects were randomized to one of six treatment sequences as shown in the below scheme:

Treatment Plan				
Treatment Sequence	Day 1 (Period 1)	Day 5 (Period 2)	Day 9 (Period 3)	Day 13 (Period 4)
1. ABCD	15 mg x 4	30 mg x 2	60 mg x 1	60 mg x 1 + 40 mg x 2 lovastatin
2. BACD	30 mg x 2	15 mg x 4	60 mg x 1	60 mg x 1 + 40 mg x 2 lovastatin
3. CABD	60 mg x 1	15 mg x 4	30 mg x 2	60 mg x 1 + 40 mg x 2 lovastatin
4. ACBD	15 mg x 4	60 mg x 1	30 mg x 2	60 mg x 1 + 40 mg x 2 lovastatin
5. BCAD	30 mg x 2	60 mg x 1	15 mg x 4	60 mg x 1 + 40 mg x 2 lovastatin
6. CBAD	60 mg x 1	30 mg x 2	15 mg x 4	60 mg x 1 + 40 mg x 2 lovastatin

On Days 1, 5 and 9 (Periods 1, 2 and 3, respectively), each group of 5 subjects received Treatments A (4 x15 mg tolvaptan tablets), B (2 x 30 mg tolvaptan tablets, Treatment C (1 x 60 tolvaptan tablet) in a 3-period, crossover manner. Doses were separated by a 96 h wash-out period. After treatment in the first 3 periods, subjects received Treatment D, a 60 mg oral tolvaptan tablet co-administered with 2 x 40 mg lovastatin tablets on Day 13 (Period 4). The total treatment duration was 4 days. Each study medication was administered with 240 mL room temperature water to the subjects in the fasted state (10 h overnight fast). The subjects remained fasted for 4 h after administration. No fluid intake was permitted from 2 h before to 2 h after dosing.

The use of prescription- or over-the counter medication or herbal medicines taken within 14 days prior to dosing and during the study was prohibited. The use of antibiotics within 30 days prior to dosing and during the study was also prohibited. Consumption of grapefruit-, Seville orange- or xanthine containing products or alcohol within 72 h of dosing and during the study was not permitted.

Pharmacokinetic Profiling

Tolvaptan

Blood

Samples for the determination of plasma concentrations of tolvaptan were collected at the following times: pre-dose, and 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48, and 72 h after dosing on Days 1, 5, 9 and 13 of Periods 1, 2, 3 and 4, respectively. An additional blood sample was collected at 96 h after dosing in Period 4. A blood sample was also to be collected at early termination of the study.

Lovastatin

Blood

Samples were collected for the determination of lovastatin and lovastatin β -hydroxyacid at pre-dose, and 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48, 72, and 96 h after administration on Day 13 of Period 4.

Bioassay

OPC-41061 and DM-4103

The plasma concentrations of OPC-41061 and its metabolite DM-4103 were determined by a LC/MS/MS method that used an internal standard. The method is linear over the range of 5.00 ng/mL to 1000 ng/mL for OPC-41061 and 12.5 ng/mL to 2500 ng/mL for DM-4103. The respective correlation coefficients are ≥ 0.9978 for OPC-41061 and ≥ 0.9964 for DM-4103. Using QC samples the precision is $\leq 6.64\%$ and the accuracy ranges between -1.95% and -6.91% for OPC-41061. The precision is $\leq 10.06\%$ and the accuracy ranges between -2.30% and 10.05% for DM-4103. OPC-41061 and DM-4103 in plasma over a period of 24 h at room temperature, exposed to three freeze/thaw cycles and stored at -70°C for up to 22 days are stable. OPC-41061 and DM-4103 are also stable in whole blood for at least 1 h at 0°C or room temperature prior to processing for plasma. The measurements were performed at

b(4)

Lovastatin and Lovastatin β -Hydroxyacid

The plasma concentrations of lovastatin and lovastatin β -hydroxyacid were determined by a LC/MS/MS method that used deuterated lovastatin and lovastatin β -hydroxyacid as internal standards. The method is linear (weight $1/y^2$) for both analytes between 0.10 ng/mL and 10.00 ng/mL. The respective mean correlation coefficients are 0.9978 and 0.9970 for lovastatin and lovastatin β -hydroxyacid. Using QC samples the precision is $\leq 11.55\%$ and the accuracy ranges between -3.69% and 1.00% for lovastatin. For lovastatin β -hydroxyacid the precision is $\leq 15.11\%$ and the accuracy ranges between -4.00% and 3.67% .

Lovastatin and lovastatin β -hydroxyacid are stable in plasma stored at -70°C for a period of at least 57 weeks, exposed to three freeze/thaw cycles, or temperatures of $2-8^\circ\text{C}$ for 23 h. At ambient temperature lovastatin in plasma

is stable for 14 h. In contrast, lovastatin β -hydroxyacid in plasma is only stable for 3 h. Thus, exposure of the samples at room temperature is to be limited to 3 h. The measurements were performed at _____

b(4)

PK Data Analysis

The following parameters were determined using non-compartmental methods (WinNonlin Version 3.1, Pharsight Corporation, Mountain View, CA): AUC_t, AUC_∞, t_{1/2,z}, CL/F, Vz/F and C_{max} and t_{max}. C_{max} and t_{max} were read off the data listings. AUC_t was determined using the linear trapezoidal rule. The λ_z was estimated from a log linear regression of at least 3 non-zero concentrations against time. AUC_t was obtained by the linear trapezoidal rule.

Pharmacodynamic Profiling

Urine output was measured during the following intervals: 0-24, 24-48, 48-72 and 72-96 h after administration of tolvaptan after dosing on Days 1, 5, 9 and 13. The baseline of urine output was defined as the volume excreted during the 24 h interval prior to dosing on Day 1. Fluid intake at each time interval was also recorded.

Statistical Analysis

Sample Size and Power

The crossover design was to have an 80% power to reject the null hypothesis that the ratio between the means of C_{max} and AUC_t from different treatments were less or equal to 0.8 or greater than or equal to 1.25, assuming that the expected ratio is 1.00, the between-subject coefficient of variation is 0.30 for C_{max} and AUC_t and the intra-subject coefficient of variation is 0.18. The respective coefficients had been determined in previous studies.

Statistical Methods

To demonstrate equivalence in each comparison, the 90% CI for the ratio test means and reference means of C_{max} and AUC_t from OPC-41061 had to lie in the interval 0.80-1.25. AUC_∞, t_{1/2,z}, CL/F, and Vz/F were assessed for treatment differences; t_{max} was not included in the analysis as it was not considered to be normally distributed. An analysis of variance (ANOVA) for a 3-period, randomized, crossover design was performed on natural logarithmic transformed C_{max} and AUC_t with model terms of sequence, subjects within sequence, period, treatment, and first order carryover. The difference of the least squares means of the log transformed results for the test and reference treatments, as well as the corresponding within subject standard error, was used to construct the 90% CI corresponding to the 2 one-sided t-tests at the 0.0166 significance level (0.05/3, for 3 comparisons). The anti-logs of the confidence limits were used to obtain the 90% CI for the ratio of the geometric means of the test and reference treatments. For Period 4 data a paired t-test was used to compare C_{max} and AUC_t of OPC-41061 given alone and when co-administered with lovastatin.

RESULTS

Thirty (30) subjects were enrolled in the study and 27 completed the study as shown in the table below:

Subjects	OPC-41061 15 mg (15 mg x 4)	OPC-41061 30 mg (30 mg x 2)	OPC-41061 60 mg (60 mg x 1)	OPC-41061 150 mg (150 mg x 1)	Total
Screened					95
Randomized					30
Treated in Period 1	10	10	10	0	30
Treated in Period 2	10	10	10	0	30
Treated in Period 3	9	10	10	0	29
Treated in Period 4	0	0	0	27	27
Completed ^a	29	30	27	27	113
Safety analysis ^b	29	30	20	27	106

^a Subject completed respective study period for total group; subject completed all study periods.
^b Subject received study medication during respective study periods for total group; subject received at least one dose of study medication.

Two subjects withdrew consent for personal reasons after they had completed all tolvaptan alone treatments. The sponsor discontinued one subject from the study because the subject would bite and break the tablets before swallowing.

The mean (SD) age of the subjects was 28 (9) years and the mean body weight was 73 (9) kg. Seventy-seven (77) % of the subjects were male and 67% were Caucasian.

Bioequivalence of 15 mg, 30 mg and 60 mg Tablet Strengths

Linear plots of the plasma concentration time profiles of tolvaptan after administration of the 15, 30 and 60 mg strength tablets and the PK parameters and statistics are shown in the below figure and tables, respectively:

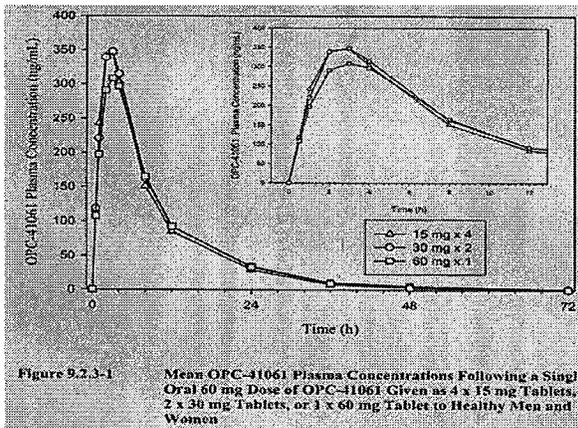


Figure 9.2.3-1 Mean OPC-41061 Plasma Concentrations Following a Single Oral 60 mg Dose of OPC-41061 Given as 4 x 15 mg Tablets, 2 x 30 mg Tablets, or 1 x 60 mg Tablets to Healthy Men and Women

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Group	t_{max} (h)		C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC ₀₋₂₄ (ng·h/mL)	AUC _∞ (ng·h/mL)	CL/F (mL/min/kg)
4 x 15 mg	29	N	29	29	29	29	29
	2.00	Mean	380	8.4	3401	3515	4.27
	0.50	SD	111	3.9	949	962	1.22
	4.00	%CV	29.2	47.0	27.9	27.4	28.4
		Geo. Mean ^b	366	7.7	3287	3402	4.11
2 x 30 mg	29	N	29	29	29	29	29
	2.00	Mean	379	8.91	3371	3710	4.11
	1.00	SD	103	3.69	1144	1185	1.15
	8.00	%CV	27.1	63.9	32.0	31.9	26.1
		Geo. Mean ^b	363	7.73	3422	3557	3.93
1 x 60 mg	29	N	29	27	28	27	27
	2.00	Mean	351	8.8	3261	3421	4.33
	1.00	SD	96.3	4.0	1088	1120	1.57
	8.00	%CV	27.4	45.5	33.4	32.7	34.7
		Geo. Mean ^b	339	8.2	3101	3259	4.27

a. Values for t_{max} are N, median, minimum, and maximum.
b. Geo = geometric.

Comparison		C_{max}	AUC _∞
2 x 30 mg (T) vs. 4 x 15 mg (R)	Ratio	1.031	1.045
	90% CI	0.939 - 1.133	0.978 - 1.117
1 x 60 mg (T) vs. 4 x 15 mg (R)	Ratio	0.961	0.960
	90% CI	0.876 - 1.054	0.899 - 1.024
1 x 60 mg (T) vs. 2 x 30 mg (R)	Ratio	0.932	0.918
	90% CI	0.849 - 1.023	0.860 - 0.980

The respective plasma concentration time profiles after single dose administration of 60 mg tolvaptan as 4•15 mg, 2•30 mg and 1•60 mg tablets are similar. However, it should be noted that the concentrations of OPC-41061 were measurable only up to 24 h post-dose. It is unclear whether at 24 h post-dose the true terminal disposition phase is attained and hence λ_z and the derived parameters $t_{1/2z}$, AUC_{∞} , CL/F and V_z/F may be biased. The relationship between AUC_t and AUC_{∞} is not known. However, it is not likely that the bias in AUC_{∞} is major and thus the average exposure measures computed by the sponsor may be used as crude estimates. The inter-subject variation measured as coefficient of variation about mean C_{max} ranges between 27.1 % and 29.2 %, respectively, for the tablets of different strength.

Linear plots of the plasma concentration profiles for the metabolite DM-4103 after administration of equivalent doses of the 15, 30 and 60 mg tablets and the corresponding PK parameters are shown in the below figure and table, respectively:

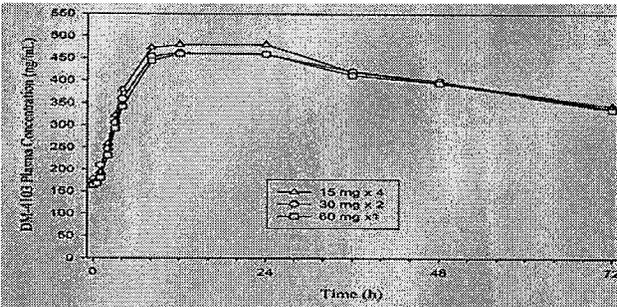


Figure 9.2.3-2 Mean DM-4103 Plasma Concentrations Following a Single Oral 60 mg Dose of OPC-41061 Given as 4 x 15 mg Tablets, 2 x 30 mg Tablets, or 1 x 60 mg Tablet to Healthy Men and Women

Table 9.2.3-2 Summary of DM-4103 Pharmacokinetic Parameters Following a Single Oral 60 mg Dose of OPC-41061 Given as 4 x 15 mg Tablets, 2 x 30 mg Tablets, and 1 x 60 mg Tablet to Healthy Men and Women

Group	Parameter	n	Mean (ng/mL)	AUC ₀₋₇₂ (ng·h/mL)
4 x 15 mg	N	29	29	29
	Median	15.00	456	24634
	Mean	ND ^a	515	26487
	SD	ND ^a	227	13718
	MCV	ND ^a	44.3	46.4
	Min	4.00	180	10839
	Max	68.00	1031	51812
Geo Mean ^b	13.58	468	26831	
2 x 30 mg	N	29	29	29
	Median	12.00	515	27439
	Mean	ND ^a	508	29238
	SD	ND ^a	183	11126
	MCV	ND ^a	36.6	37.9
	Min	1.00	233	14678
	Max	48.00	999	62264
Geo Mean ^b	14.35	477	27493	
1 x 60 mg	N	29	29	29
	Median	24.00	470	25000
	Mean	ND ^a	494	28101
	SD	ND ^a	163	10252
	MCV	ND ^a	33.0	36.5
	Min	4.00	274	11956
	Max	72.00	821	50673
Geo Mean ^b	16.64	458	26353	

^a ND = not determined.
^b Geo = geometric.

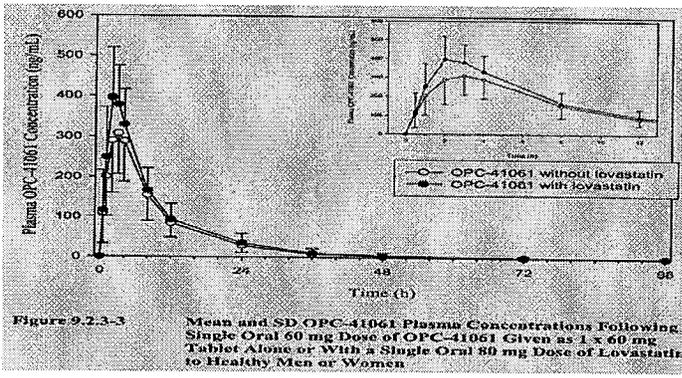
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The mean plasma concentrations of the metabolite truncated to 72 h post-dose appear to be similar with all three treatments. Given that t_{1/2z} of the DM-4103 exceeds 72 h, the observation interval is too small for estimating apparent F for the metabolite. In addition, the washout of 96 h between treatments was too short resulting in mean plasma concentrations at time zero of about 170 ng/mL for DM-4103 with all three strength tablets. With the randomized, crossover design used, the plasma concentrations of DM-4103 do not represent concentrations of the metabolite after single dose administrations of tolvaptan, but rather an average of the concentrations of the metabolite after one, two and three doses of tolvaptan. Thus, the geometric means of C_{max} and AUC_t of the 3 treatments are not reliable and their comparison is not meaningful.

Lovastatin-Tolvaptan Interaction

Impact of Lovastatin on Tolvaptan

Linear plots of the plasma concentration time curves of tolvaptan in the presence and absence of lovastatin are shown in the below figure:



The PK parameters obtained for tolvaptan (60 mg) in the presence and absence of lovastatin 80 mg are shown in the below table:

Summary of OPC-41061 Plasma Pharmacokinetic Parameters Following a Single Oral 60 mg Dose of OPC-41061 Alone or with 80 mg Lovastatin in Healthy Subjects							
Group	t_{max} ^a (h)		C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC_0-t (ng·h/mL)	AUC_{∞} (ng·h/mL)	CL/F (mL/min/kg)
OPC-41061 alone	27	N	27	25	27	25	25
	2.00	Mean	355	8.7	3253	3414	4.58
	1.00	SD	98	4.1	1117	1155	1.62
	8.00	%CV	27.5	46.4	34.3	33.8	35.4
		Geo Mean ^b	343	8.12	3085	3243	4.31
OPC-41061 + lovastatin	27	N	27	27	27	27	27
	2.00	Mean	425	11.6	3877	4050	3.87
	1.00	SD	106	11.6	1251	1354	1.40
	4.00	%CV	24.9	100.1	32.3	33.4	36.2
		Geo Mean ^b	411	9.0	3700	3858	3.64

^a Values for t_{max} are N, median, minimum, and maximum.
^b Geo = geometric.

The above mentioned limitations hold also true for the interpretation of the mean exposure measure of OPC-41061 in the presence and absence of lovastatin. However, the values for exposure by the sponsor can serve as crude estimates.

The geometric mean of C_{max} and AUC_{∞} for tolvaptan in the presence of lovastatin is increased by the same factor of about 1.2, indicating a clinically irrelevant increase in exposure to tolvaptan in the presence of lovastatin.

Linear plots of the plasma concentration time profiles of the inactive metabolite DM-4103 in the presence and absence of lovastatin and the resulting parameters are shown in the below figure and table, respectively:

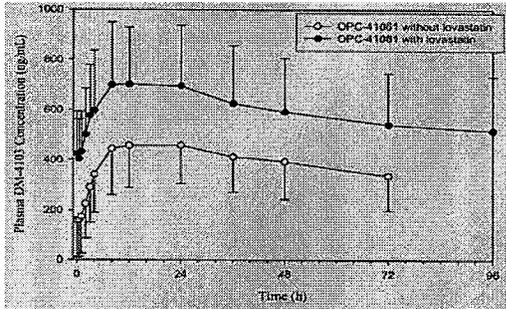


Figure 9.2.3-4 Mean and SD DM-4103 Plasma Concentrations Following a Single Oral 60 mg Dose of OPC-41061 Given as 1 x 60 mg Tablet Alone or With a Single Oral 80 mg Dose of Lovastatin to Healthy Men or Women

Table 9.2.3-5 Summary of DM-4103 Pharmacokinetic Parameters Following a Single Oral 60 mg Dose of OPC-41061 Given as 1 x 60 mg Tablet, With or Without Single Oral Dose of Lovastatin 80 mg to Healthy Men and Women

Group		$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC ₀₋₉₆ (ng·h/mL)
OPC-41061 alone	N	27	27	27
	Median	24.00	470	26054
	Mean	ND	492	28566
	SD	ND	168	10450
	%CV	ND	34.2	36.6
	Min	4.00	214	11936
	Max	72.00	821	50673
	Geo Mean ^a	16.62	464	26711
OPC-41061 with lovastatin	N	27	27	27
	Median	12.00	691	54598
	Mean	ND	734	57231
	SD	ND	246	20538
	%CV	ND	33.6	35.9
	Min	4.00	417	33523
	Max	72.00	1314	106297
	Geo Mean ^a	13.66	698	54103

^a ND = not determined.
^b Geo = geometric.

The ratio of the geometric mean C_{max} is 1.50.

The usefulness of AUC estimate by the sponsor for the metabolite DM-4103 is limited, because it is unknown whether tlast is in the terminal log linear phase of the metabolite.

Lovastatin and Lovastatin β-Hydroxyacid PK in Presence of Tolvaptan

Linear plots of the mean plasma concentration time profile of lovastatin and lovastatin β-hydroxyacid and the corresponding PK parameters in the presence of tolvaptan are shown in the below figures and tables:

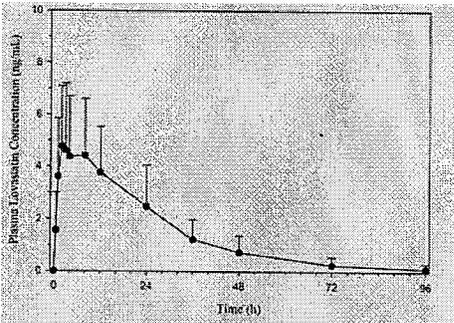


Figure 9.2.3-5 Mean and SD of Lovastatin Plasma Concentration Versus Time Profile Following a Single Oral 80 mg Dose of Lovastatin With a Single Dose of OPC-41061 60 mg as 1 x 60 mg Tablet in Healthy Men and Women

Table 9.2.3-6 Summary of Lovastatin Pharmacokinetic Parameters Following a Single Oral 80 mg Dose of Lovastatin Concurrently Administered With a Single Oral 60 mg Dose of OPC-41061 Given as 1 x 60 mg Tablet to Healthy Men and Women

	t_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC _{0-∞} (ng·h/mL)	AUC ₀₋₂₄ (ng·h/mL)	CL/F (mL/min/kg)	V _d /F (L/kg)
N	27	37	23	27	23	23	23
Median	4.00	5.22	14.1	120	128	165	174
Mean	ND ^a	6.20	21.6	135	147	167	123
SD	ND ^a	2.66	19.6	66.2	76.5	83.4	45.4
%CV	ND ^a	43.0	90.8	49.1	52.2	30.0	140.6
Min	1.00	3.17	6.73	46.6	48.2	54.4	30.7
Max	24.0	15.0	92.7	284	320	359	2162
Geo Mean ^b	4.07	5.75	16.5	121	129	148	269

^a ND = not determined.
^b Geo = geometric.

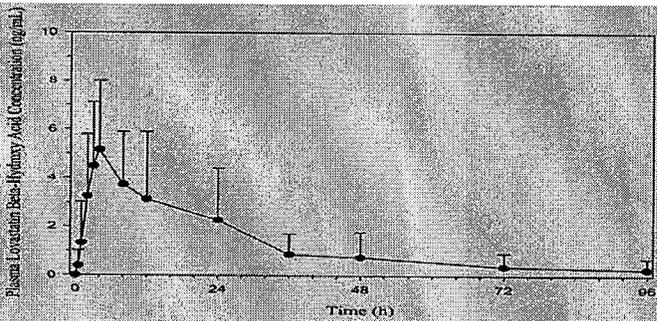


Figure 9.2.3-6 Mean and SD of Lovastatin beta-Hydroxy Acid Plasma Concentration Versus Time Profile Following a Single Oral 80 mg Dose of Lovastatin With a Single Dose of OPC-41061 60 mg as 1 x 60 mg Tablet in Healthy Men and Women

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Table 9.2.3-7 Summary of Lovastatin beta-Hydroxy Acid Pharmacokinetic Parameters Following a Single Oral Dose of 80 mg Lovastatin Concurrently Administered With a Single Oral Dose of OPC-41061 Given as 1 x 60 mg Tablet to Healthy Men and Women

	t_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC ₀₋₂₄ (ng·h/mL)	AUC _{0-∞} (ng·h/mL)
N	27	27	9	27	9
Median	4.00	4.45	23.4	94.5	105
Mean	ND ^a	5.67	26.4	123	130
SD	ND ^a	3.09	15.1	103	71.3
%CV	ND ^a	54.5	57.2	83.0	54.9
Min	3.00	2.37	6.1	40.5	45.5
Max	12.00	14.4	59.8	445	301
Geo Mean ^b	5.00	5.05	22.8	101	116

^a ND = not determined.
^b Geo = geometric.

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The reported plasma concentrations and parameter values for lovastatin and its metabolite are only useful to document that the test subjects were compliant. The plasma concentrations of lovastatin and lovastatin β-hydroxyacid were not measured in the absence of tolvaptan, and a possible impact of tolvaptan on lovastatin and its metabolite cannot be excluded.

PD

The below table summarizes the results of treatments with 60 mg tolvaptan given as 4•15 mg tablets, 2•30 mg tablets and 1•60 mg tablets:

Mean (CV) 24 h Urine Volumes and Excretion Rates Measured after Treatments of Young, Healthy Male and Female Subjects with a Single Dose of 60 mg Tolvaptan Administered as 4•15 mg Tablets, 2•30 mg Tablets and 1•60 mg Tablets

	Single Dose Tolvaptan, mg					
	4•15	2•30	1•60	4•15	2•30	1•60
	Mean (CV) 24 h Urine Volume, ml			Mean 24 h Urine Excretion Rate, mL/min		
Baseline	2270 (48)	2285 (47)	2285 (47)	1.6	1.6	1.6
Day 1	9235 (22)	9067 (20)	9082 (26)	6.4	6.3	6.3
Day 2	2661 (31)	2623 (36)	2697 (33)	1.8	1.8	1.9
Day 3	1765 (54)	1806 (51)	1640 (53)	1.2	1.3	1.1
Day 4	2185 (54)	1987 (50)	2026 (47)	1.5	1.4	1.4
Baseline Corrected						
Day 1	6965 (37)	6782 (32)	6797 (41)	4.8	4.7	4.7
Day 2	390 (277)	338 (314)	412 (270)	0.27	0.23	0.29
Day 3	-505 (nd)	-478 (nd)	-631 (nd)	-0.35	-0.33	-0.44
Day 4	-85 (nd)	-298 (nd)	-291 (nd)	-0.059	-0.21	-0.20

nd = not determined

Compared to baseline the mean 24 h urine volume/excretion rate on Day 1 is increased 4.7 fold with all three treatments. The mean 24 h urine volumes/excretion rates in the subjects receiving the 4•15 mg, 2•30 mg and 1•60 mg tablets are remarkably similar. The percent coefficient of variation about the mean net 24 h urine volume/excretion rate ranges between 32% and 41% on Day 1. On Day 2 the net increase in the mean 24 h urine volume/excretion rate is only 1.2 fold and on Days 3 and 4 the mean 24 h excretion volume/excretion rate is reduced by 4%-28% compared to baseline indicating a small rebound effect. The data suggest that the aquaretic effect of 60

mg tolvaptan lasts about 36 h. The baseline corrected and uncorrected mean 24 urine volumes on Days 1 and 2 are remarkably similar with the three treatments.

Conclusions

PK

The exposure to 60 mg tolvaptan treatments with 4•15 mg, 2•30 mg and 1•60 mg tablets appears to be similar. It is possible that the tlast used in determining AUCt is not in the terminal log linear phase. Since the relationship between AUCt and AUC∞ at the 60 mg dose level is not established for tolvaptan a bias in the sponsor's estimate for F based on AUCt obtained in the 4 treatments is possible. However, based on the information available from other studies with higher doses the bias is likely relatively minor. The inter-subject variation of Cmax of tolvaptan ranges between 27 % and 29 %. The highest recommended dose of lovastatin 80 mg increases peak and average exposure to tolvaptan (60 mg) by a factor of about 1.2. The sponsor's estimates for the exposures measures for the metabolite are not helpful.

PD

Tolvaptan 60 mg increases the mean net 24 h urine volume/excretion rate about 5 fold on Day 1. The inter-subject variation about the mean net 24 h urine volume/excretion rate ranges between 32% and 41% on Day 1. The aquaretic effect of 60 mg tolvaptan lasts about 36 h. There is evidence for a small rebound effect on Days 3 and 4 after a single dose of 60 mg tolvaptan.

Comments

1. The sensitivity of the assay method for OPC-41061 and DM-4103 is not appropriate.
2. The wash-out phase between the treatments is too short hindering a proper evaluation of the DM-4103 data.

Study Report No. 156-05-256: "An Open-Label, Randomized Study of the Effect of Food (Standard FDA High-Fat Breakfast) on Tolvaptan Pharmacokinetics in Normal Subjects Following Administration of 60 mg Oral Tolvaptan Tablets"

Investigator and Study Site



b(4)

Objectives

Primary: To determine the effect of food (FDA high-fat breakfast) on the PK of tolvaptan and its metabolites DM-4103 and DM-4107 following oral administration of 60 mg tolvaptan

Secondary: To determine the safety of tolvaptan following oral administration of tolvaptan with and without food and to compare urine volumes following oral tolvaptan administration with and without food

Investigational Drugs and Formulations

Tolvaptan 60 mg oral tablets (Lot No. 04L93A060) were provided to the study site by the sponsor.

Design

Non-compartmental methods were used (WinNonlin Pro, Version 4.0, Pharsight Corporation). C_{max} and t_{max} were taken directly from the observed values. AUC_t was determined by using the linear trapezoidal rule. Where possible λ_z was estimated from a log linear regression of at least 3 non-zero concentrations on time for tolvaptan. AUC₀₋₂₄ was estimated applying the linear trapezoidal rule if a) the 24 h sample was measurable or b) with extrapolation using λ_z from the time of the last measurable time, t_{last}, if t_{last} < 24 h or c) or using interpolation to 24 h if t_{last} was > 24 h.

PD Profiling

Urine volume was measured on Days 1 and 5 from the start of the meal through 24 h post-dose. Fluid intake was measured on Days 1 and 5 from 0 to 24 h post-dose and included liquids served with meals and used for dose administration. The total volume for the 24 h interval was recorded.

PD Data Analysis

The primary outcome variable is the 24 h urine volume

Statistical Analysis

Sample Size and Power

With a sample size of 12, a cross-over design will have 80% power to reject both the null hypothesis that the ratio of the high fat food state mean to the fasted state mean is outside the interval [0.8-1.25], (i.e. that the high-fat food and fasted states are not equivalent), in favor of the alternative hypothesis that the means of the two states are equivalent. This assumes that the expected ratio of mean AUC_∞ for tolvaptan is 1.20; the crossover analysis of variance (ANOVA) root mean square error (MSE), in the natural log scale, is 0.116; that the data will be analyzed in a natural log scale using t-tests for differences in means; and that each t-test is performed at the 0.05 level.

RESULTS

Fourteen subjects, 5 females and 9 males, all Caucasians, were enrolled and completed the study. Their median age was 24.5 (range 18-45) years and their median body weight 75.8 (range 62.5- 104.3) kg.

Safety

No serious or severe adverse events were recorded. No clinically relevant changes in physical examination, vital signs, ECGs or laboratory values were recorded.

PK

Linear plots of the mean plasma concentrations of tolvaptan in subjects in the fed or fasted state and corresponding parameters are shown in the figure and tables below:

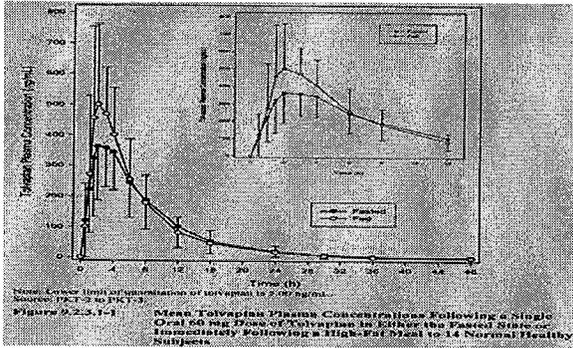


Table 9.2.3.2-1 Mean (SD) Tolvaptan Plasma Pharmacokinetic Parameters Following a Single Oral 60 mg Dose of Tolvaptan in Either the Fasted State or Immediately Following a High-Fat Meal to Normal Healthy Subjects

Parameter	Fasted (n = 14)	Fed (n = 14)
C _{max} (ng/mL)	430 (150)	605 (223)
t _{max} (h) ^a	2.00 (1.50 - 4.00)	2.00 (1.00 - 4.00)
AUC _{0-24h} (ng·h/mL)	3470 (1260)	3590 (1340)
AUC _t (ng·h/mL)	3500 (1440)	3670 (1440)
t _{1/2z} (h)	7.1 (2.6) ^b	4.3 (1.3) ^c
AUC _∞ (ng·h/mL)	3980 (1290) ^b	3880 (1750) ^c
CL/F (mL/min/kg)	3.35 (1.0) ^b	4.23 (2.72) ^c

^a Values are median (range).
^b N=7.
^c N=9.

Table 9.2.3.2-2 Estimated Geometric Mean Ratios (Test/Reference) and 90% CI for Tolvaptan Pharmacokinetic Parameters

Comparison		C _{max} (N = 14)	AUC _t (N = 14)
60 mg tolvaptan Fed (test) versus 60 mg tolvaptan Fasted (reference)	Ratio	1.40	1.06
	90% CI	1.17 - 1.67 ^a	0.97 - 1.16

^a Not equivalent, 90% CI outside the interval of 0.80-1.25.
Source: CT-5.

The mean plasma concentration time profiles suggest an effect of food on C_{max}. The plasma concentrations of tolvaptan are measurable in all subjects only for 16 h. Thus, the observation interval for estimating λ_z is importantly shorter than 3•t_{1/2z} + 2•t_{max} and the estimates for λ_z and derived parameters including t_{1/2z}, AUC_∞ and CL/F for tolvaptan may be biased. The sponsor also evaluated mean exposure based on AUC_t, measured up to the last measurable plasma concentration. However, the relationship between AUC_t and AUC_∞ is not known with tolvaptan. However, it is anticipated that the bias in AUC_∞ is relatively minor so that the sponsor's value for average exposure can be considered a crude estimate.

Food increases C_{max} of tolvaptan by a factor of 1.40, indicating bio-inequivalence, but the food induced increase in peak exposure is too small to be of clinical relevance. The food induced increase in AUC_t is even smaller (about 1.1 fold). Mean t_{max} for tolvaptan is 2.0 h after administration with or without food indicating that food does not importantly increase the rate of absorption of tolvaptan at the 60 mg dose level tested.

Linear plots of the mean plasma concentration time profiles and corresponding parameters of DM-4103 after administration of tolvaptan to the subjects in the fed and fasted state on Day 1 are shown in the below figure and table:

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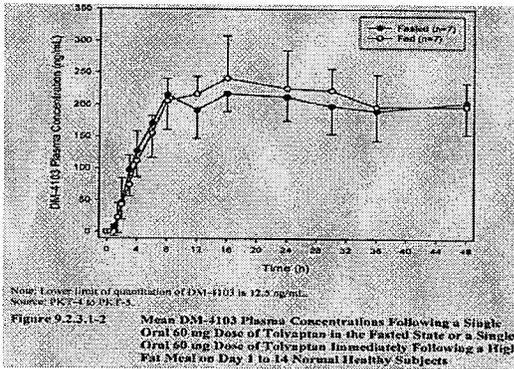


Table 9.2.3.2-3 Mean (SD) Plasma Pharmacokinetic Parameters for DM-4103 Following a Single Oral 60 mg Dose of Tolvaptan in the Fasted State or a Single Oral 60 mg Dose of Tolvaptan Immediately Following a High-Fat Meal on Day 1 to Normal Healthy Subjects

Parameter	Fasted (n = 7)	Fed (n = 7)
C_{max} (ng/mL)	241 (38.3)	253 (57.4)
t_{max} (h) ^a	16.00 (8.00 - 48.00)	16.00 (8.00 - 30.00)
AUC _t (ng·h/mL) ^b	9020 (1710)	9290 (1810)

^a Values are median (range).
^b $t_1 = 48$ hours for all subjects.

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The mean plasma concentrations of DM-4103 truncated to 48 h post-dose are slightly increased in the presence of food compared to the fasted state.

The plasma concentrations of DM-4103 were followed for 48 h in all subjects. Given the long $t_{1/2}$ of the metabolite this is a too short a follow-up time for estimating λ_z and derived parameters. The usefulness of the AUC_t estimates for DM-4103 provided by the sponsor is doubtful. Because the wash-out phase observed in the study was too short for the slowly eliminated DM-4103, only the data from subjects who were fasted or fed in the first treatment period could be used. The mean C_{max} value for DM-4103 in the fed state is slightly greater than in fed state in the subjects. Peak concentrations of DM-4103 are observed on average 16 h after tolvaptan administration with or without food.

Linear plots of the mean plasma concentration time profiles and corresponding parameters of DM-4107 after administration of tolvaptan to the subjects in the fed and fasted state on Day 1 are shown in the below figure and table:

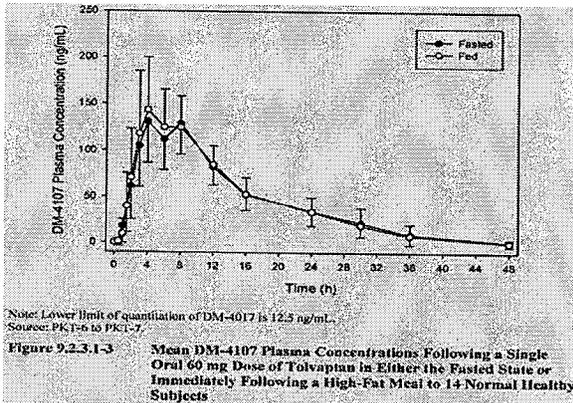


Table 9.2.3.2-4 Mean (SD) Plasma Pharmacokinetic Parameters for DM-4107 Following a Single Oral 60 mg Dose of Tolvaptan in Either the Fasted State or Immediately Following a High-Fat Meal to Normal Healthy Subjects

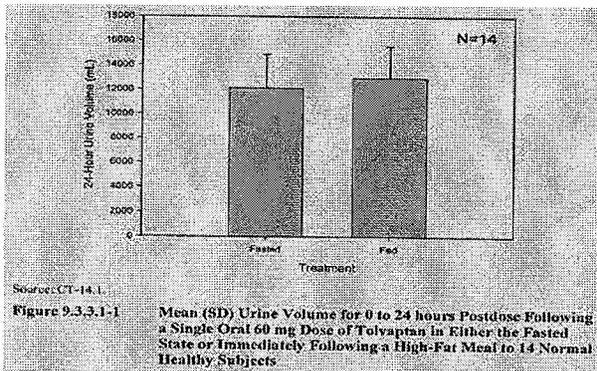
Parameter	Fasted (n = 14)	Fed (n = 14)
C_{max} (ng/mL)	146 (38.9)	157 (48.0)
t_{max} (h) ^a	4.00 (3.00 - 8.00)	4.00 (3.00 - 16.00)
AUC_0-t (ng·h/mL) ^b	2030 (441)	2050 (564)

^a Values are median (range).
^b t = 48 hours for all subjects.

The mean plasma concentrations of DM-4107 show the same trend for a small increase in the peak concentration in the fed state. The plasma concentrations of DM-4107 are measurable for 24 h in all subjects. The same limitations in the interpretability of the results on exposure to this metabolite exist that were mentioned above for tolvaptan and DM-4103. The results show slightly greater mean C_{max} values with the subjects in the fed state than in the fasted state. Peak concentrations of DM-4107 are observed in either state on average 4 h after tolvaptan administration.

PD

The respective 24 h urine volumes excreted by the subjects receiving tolvaptan when they were in the fed or in the fasted state is shown in the below figure:



Source: CT-14.1

Figure 9.3.3.1-1 Mean (SD) Urine Volume for 0 to 24 hours Postdose Following a Single Oral 60 mg Dose of Tolvaptan in Either the Fasted State or Immediately Following a High-Fat Meal to 14 Normal Healthy Subjects

The respective mean (SD) 24 h urine volume in the fed and fasted state was 12121 (2771) mL and 12977 (2585) mL, respectively, corresponding to mean 24 h urine excretion rates of 8.4 mL/min and 9.0 mL/min.

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Conclusions

PK

Food increases mean C_{max} of tolvaptan 1.4 fold and AUC_∞ about 1.1 fold. The impact of food on exposure to tolvaptan is not clinically relevant. Mean C_{max} for DM-4103 and DM-4107 in the fed state are only marginally greater than in the fasted state. The observed impact of food on peak exposure to the pharmacologically inactive metabolites is not considered relevant.

PD

The mean 24 urine volume excreted by the subjects in the fed and fasted state are compatible with comparable exposure to tolvaptan in the fed and fasted state.

Comments

1. Given the protracted elimination of DM-4103 known from previous studies it was foreseeable that a wash-out of 96 h between treatments as well as a follow-up of 48 h is inadequate.
2. The respective sensitivities of the LC/MS/MS assay for tolvaptan and DM-4107 are not sufficient to determine λ_z and $t_{1/2z}$ and derived parameters reliably.

Study Report 156-98-202: "An Open-Label, Single/Multiple Dose Study of the Safety, Pharmacokinetics and Pharmacodynamics of Orally Administered OPC-41061 Tablets in Healthy Adult and Elderly Male and Female Subjects"

Investigator and Site

b(4)

Objectives

The objectives of the study were to determine the effects of age and gender on the safety, PK and PD of OPC-41061 after single and multiple dose administrations.

Primary Outcome Variables:	
•	Area under the concentration-time curve (AUC)
•	Peak plasma concentration (C_{max})
•	Time of peak concentration (t_{max})
•	Elimination half-life ($t_{1/2}$)
•	Apparent volume of distribution (V_d/F)
•	Apparent clearance (CL/F)
•	Plasma protein binding of OPC-41061
•	Renal clearance (CL_r)

Secondary Outcome Variables:	
•	Baseline and post-treatment physical examination
•	Vital signs
•	Routine 12-lead electrocardiogram
•	Adverse experiences
•	Laboratory evaluations (hematology, chemistry, urinalysis)
•	Urine volume excreted
•	Plasma renin activity
•	Urine osmolality
•	Plasma AVP concentration

Investigational Drugs and Formulations

The study medication consisted of a single 60 mg dose of OPC-41061 (four 15 mg tablets (Lot Nos. 97B94A015 and 98D82A015A)).

Design

This was an open-label, single dose and 7 day multiple dose study of OPC-41061 conducted in healthy adults (18-45 years) and elderly (age ≥ 65 years) male and female subjects. A minimum of 48 subjects (24 for each age group) was to be enrolled in the study. Each subjects received under fasting conditions, a single 60 mg dose of OPC-41061 (four 15 mg tablets) on Day 1 and on Days 4 through 10 for PK and PD measurements. Safety evaluations were based on physical examinations, recording of adverse events, clinical laboratory tests, vital signs, and 12- Lead ECGs.

Inclusion and exclusion criteria are listed below:

TABLE 4.2-1 INCLUSION CRITERIA	
1.	Healthy male and female subjects 18 to 45 years of age or ≥ 65 years of age.
2.	Body weight was within $\pm 20\%$ of ideal body weight.
3.	No clinically significant diseases or clinically significant abnormal laboratory values as assessed during the screening medical history, physical examination, or pre-treatment laboratory evaluations.
4.	Normal 12-lead electrocardiogram, or one with abnormalities considered to be clinically insignificant by the investigator.
5.	The dosage of any chronic medications had to have remained constant for 30 days prior to the screening visit with the exception of those noted in Concomitant Medications.
6.	Females of childbearing potential had to test negative for pregnancy using a pregnancy test detecting the beta subunit of the hCG molecule and had to be using acceptable methods of contraception (diaphragm and condom with spermicide). Females of non-child bearing potential are defined as those who are post-menopausal (amenorrhetic for at least one year) or are surgically sterile.
7.	Able to communicate effectively with study personnel.
8.	Able to be confined to the clinical research unit for the duration of the inpatient period of the study and be able to return to the units at times noted during the outpatient portion of the study.
9.	Eligible subjects had to sign an informed consent prior to initiation of any study procedures. In the event that the subject was re-screened for study participation, a new informed consent form had to be signed.

Source: Appendix J-1 (Protocol)

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TABLE 4.2-2 EXCLUSION CRITERIA

1.	Any clinically significant abnormality in past medical history or on prescreen physical that in the investigator's or sponsor's opinion might place the volunteer at risk or interfere with outcome variables of the study including absorption, distribution, metabolism, and excretion of a drug. This included but was not limited to concurrent or history of cardiac, hepatic, renal, neurologic, gastrointestinal, respiratory, hematologic, and immunologic disease, glaucoma, and alcohol or drug abuse. In addition, subjects must have no clinically significant abnormal laboratory values as assessed during the screening medical history, physical examination, or pretreatment laboratory evaluations.
2.	Current use of any recreational drugs or a history of drug addiction within one year.
3.	History of alcoholism or of moderate (≥ 4 drinks per day) daily alcohol use or use of alcohol within 48 hours of enrollment into the study and for the duration of the study.
4.	Use of xanthine-containing food or drinks (eg, caffeine), grapefruit and grapefruit juice within 48 hours prior to enrollment in the study and for the duration of the study.
5.	Use of tobacco or tobacco products within 6 months prior to the initiation of the study.
6.	History of hypersensitivity and/or idiosyncratic reaction to benzazepine derivatives such as benazepril (Lotensin) or other clinically significant drug allergies.
TABLE 4.2-2 EXCLUSION CRITERIA (Continued)	
7.	Any history of significant bleeding or hemorrhagic tendencies.
8.	Volunteers having taken an investigational drug within the four weeks before study entry.
9.	A history of difficulty in donating blood.
10.	The donation of blood or plasma within 30 days prior to or during the study.
11.	History of or current hepatitis or carriers of hepatitis B surface antigen (HBsAg), hepatitis C antibodies (anti-HCV) or HIV.
12.	Volunteers who had participated in any OPC-41061 clinical trial.
13.	Use of any drug known to stimulate or inhibit drug metabolism within 30 days of dosing.
14.	Use of hormonal contraceptives or hormone replacement therapy (females only) within 45 days of dosing.
15.	Use of any over-the-counter, prescription, or herbal products (not including herbal teas) within two weeks of dosing, except for chronic medications that were judged by the clinical site investigator and sponsor to not interfere with OPC-41061 pharmacokinetics and that had remained at a constant dosage for 30 days prior to screening, with the exception of insulin, topical, inhaled, and intranasal medications.

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The subjects received the 60 mg oral dose of OPC-41061 on Days 1 and 4 through 10 with approximately 240 mL of room temperature tap water. On Days 1 and 10 the subjects were to stay in the sitting position for the first 4 h after administration. Fluid intake was ad lib. The following prior or concomitant medications were prohibited: an investigational drug (within 4 weeks before study entry), any recreational drugs in current use; any drug known to stimulate or inhibit drug metabolism (within 30 days of dosing); hormonal contraceptives or hormonal replacement therapy (females only) within 45 days of dosing; and any over the counter-, prescription-, or herbal products (not including herbal teas) (within 2 weeks of dosing), except for chronic medications that were judged by the investigator or sponsor to not interfere with OPC-41061 PK and that had remained at a constant dosage for 30 days before screening, with the exception of insulin, topical, inhaled, and intranasal medications. The subjects were allowed to continue their chronic medications during the study. However, they could not take their oral chronic medications within about 32 h of dosing on Days 1 and 10 and within approximately 1 h of dosing on Days 4 to 9.

During the study the subjects abstained from alcohol-, tobacco-, and xanthine- and grapefruit containing products for 48 h before admission and for the duration of the study.

Pharmacokinetic Profiling

Blood

Samples for the determination of plasma concentrations of OPC-41061 were collected on Days 1 and 10 pre-dose, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h post-dose. Additional samples were to be collected at 36, 48 and 72 h post-dose. An additional blood sample was to be collected 30 minutes before dosing and 2 h after dosing for determination of the plasma protein binding of OPC-41061.

Urine

Total urine volumes were to be collected at pre-dose, and the following intervals: 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 h post dose on Days 1 and 10. The volume excreted, sodium and potassium concentrations, pH, OPC-41061 and metabolite concentrations, and osmolality were to be measured. All urine produced on Day-1 (beginning with the morning void at approximately 24 h prior to dosing on Day 1) was collected and the volume excreted measured. The morning void on Day 1 was defined as the pre-dose sample for that study day.

from the ANOVA was used as the error term (reported in SAS output only; Appendix III-4).

After completion of the above analyses, supplemental statistical evaluations were performed. An analysis of covariance, which employed the factors from the previous model together with weight as a covariate, was performed for C_{max} , V_d/P (L), CL/P (mL/min), and $t_{1/2}$. A test for equality of slopes was also performed. Type III sums of squares were used, except for the covariate, where Type I sums of squares were used. The LSMEAN statement from a one-way ANOVA on gender from the PROC GLM procedure was used to obtain the estimated least square means for each gender group, and the ESTIMATE statement was used to obtain the difference between the least square means and the standard error of the difference.

All plasma concentration data below quantification limits (BQL) were assigned a value of zero for the analyses and presentation. All plasma OPC-41061 and metabolite concentration data listed as below quantification limit (BQL) and ND (not detectable) were assigned a value of zero for the analyses and presentation. All plasma OPC-41061 and metabolite concentration data listed as above quantification limit (AQL) were identified as a missing sample "" for the analyses and presentation. All pharmacokinetic statistical analyses were performed on non-rounded parameter estimates. For presentation, plasma OPC-41061 and metabolite concentrations were rounded to the nearest tenth (i.e., a maximum of 4 to 5 significant figures). All other pharmacokinetic parameter estimates were rounded to either 3 or 4 significant figures for presentation. All statistical output is presented in Appendix III-4.

The means under the curve values for AUC, plasma renin activity, and urine osmolality were analyzed with a two-way ANOVA, after log-transformation. Separate analyses were performed for each of the two study days (Day 1 and Day 10). The model for ANOVA included age group and gender effects and their interaction. In addition, pairwise comparisons (young male vs. young female, young male vs. elderly male, young male vs. elderly female, young female vs. elderly male, young female vs. elderly female) were done with the CONTRAST statement. Pairwise comparisons were also done on the baseline values (pre-dose values on Day 1 and Day 10) for AUC, plasma renin activity, and urine osmolality, since the respective baselines could have influenced AUC values.

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RESULTS

Fifty-one (51) subjects were enrolled in the study and completed the Day-1 treatment. Forty-seven (47) completed the entire study. The demographics of the subjects are shown in the below table:

Characteristics	M (18-45 y) N = 13	F (18-45 y) N = 13	M (≥ 65 y) N = 13	F (≥ 65 y) N = 12
Age (years)				
Mean ± SD	26.4 ± 7.6	28.6 ± 9.7	67.7 ± 2.7	72.0 ± 6.6
Range	18 - 42	18 - 45	65 - 74	66 - 91
Weight (kg)				
Mean ± SD	75.7 ± 13.2	58.3 ± 6.4	87.4 ± 11.2	65.9 ± 10.0
Range	59.3 - 94.7	49.8 - 67.5	64.3 - 102.4	45.3 - 80.2
Race, n (%)				
Caucasian	12 (92.3)	13 (100)	13 (100)	12 (100)
Asian	1 (7.7)	0	0	0
Frame size, n (%)				
Medium	12 (92.3)	10 (76.9)	5 (38.5)	1 (8.3)
Large	1 (7.7)	3 (23.1)	8 (61.5)	11 (91.7)
Smoking status, n (%)				
Never	13 (100)	13 (100)	13 (100)	12 (100)
Drinking status, n (%)				
Never	6 (46.2)	4 (30.8)	5 (38.5)	10 (83.3)
Drinker	7 (53.8)	9 (69.2)	8 (61.5)	2 (16.7)

Females in both age categories had a significantly smaller mean body weight than males. Alcohol intake was smaller in the elderly women than in males and younger women.

Safety

No serious or severe adverse events occurred. The most common treatment emerge events were thirst, asthenia and headache.

Pharmacokinetics

OPC-41061

Semi-logarithmic plots of the plasma concentration time profiles of OPC-41061 for all females and males on Day 1, all females and males on Day 10, all young and elderly subjects on Day 1 and all young and elderly subjects on Day 10 are shown in the below figures:

Figure 1. Mean (SD) Plasma OPC-41061 Concentration-Time Profiles for all Male and all Female Subjects on Day 1 Following Oral Administration of 60 mg OPC-41061 (Log-Linear Coordinates).

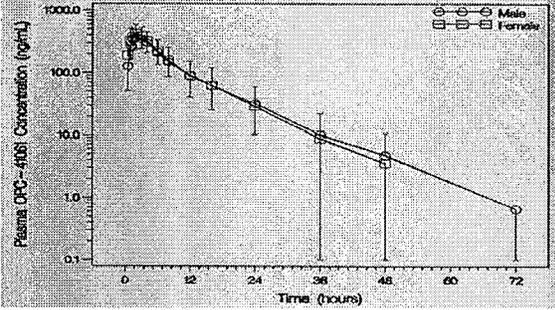


Figure 2. Mean (SD) Plasma OPC-41061 Concentration-Time Profiles for all Male and all Female Subjects on Day 10 Following Oral Administration of 60 mg OPC-41061 (Log-Linear Coordinates).

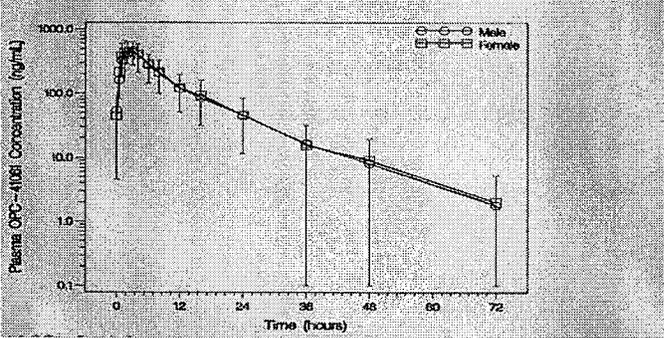
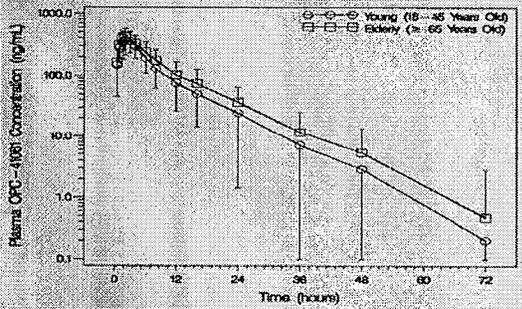
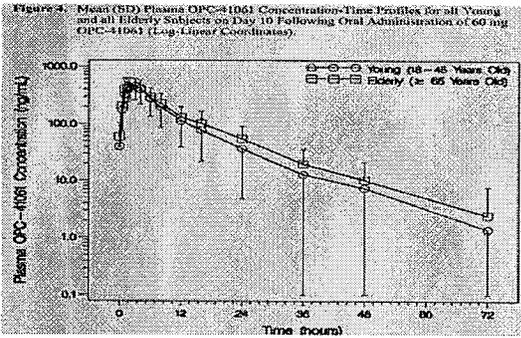


Figure 3. Mean (SD) Plasma OPC-41061 Concentration-Time Profiles for all Young and all Elderly Subjects on Day 1 Following Oral Administration of 60 mg OPC-41061 (Log-Linear Coordinates).



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The plasma concentration time profiles of OPC-41061 on Days 1 and 10 for females and males appear similar. The plasma concentrations in the elderly appear to be greater on Days 1 and 10 than in the young to middle aged subjects. The above plots show data from all subjects with measurable concentrations. However, it should be noted that the time of the last measurable concentration for OPC-41061 varies importantly among the individual subjects in each group and among the groups. OPC-41061 is only measurable up to 24 h after dosing in all subjects of the four groups.

The PK parameters of OPC-41061 for the 4 groups as estimated by the sponsor are listed in the 2 tables below:

Summary [Mean (SD)] and Statistical Comparison of OPC-41061 Pharmacokinetic Parameters on Day 1 Following Single-Dose Oral Administration of 60 mg OPC-41061 as Tablets (4 x 15 mg) in Young and Elderly, Male and Female Subjects

	C _{max} (ng/mL)	t _{max} (h)	AUC-INF (ng•h/mL)	V _z /F (L/kg)	CL/F (mL/min/kg)	t _{1/2} (h)
Young Males n=13	366 (138)	2.58 (0.89)	3488 (1606)	3.31 (1.68)	4.848 (2.637)	8.63 (3.07)
Young Females n=13	429 (134)	1.89 (0.72)	3037 (1213)	3.1 (1.02)	6.604 (2.586)	5.86 (1.81)
Elderly Males n=13	405 (101)	1.89 (0.83)	3746 (1186)	2.32 (1.02)	3.484 (1.507)	8.4 (4.75)
Elderly Females n=12	507 (190)	2.25 (0.84)	4574 (2389)	2.77 (1.36)	4.252 (2.254)	7.96 (1.98)
p-values: Age	0.151	0.593	0.058	0.077	0.006*	0.296
Gender	0.045*	0.619	0.686	0.751	0.055	0.076
Age*Gender	0.63	---	0.174	0.374	0.446	0.193

p values for t_{max} determined using Cochran-Mantel-Haenszel Tests.
 * Significant difference, p<0.05.
 --- No sample or not determined.

Summary [Mean (SD)] and Statistical Comparison of OPC-41061 Pharmacokinetic Parameters on Day 10 Following Multiple-Dose Oral Administration of 60 mg OPC-41061 as Tablets (4 x 15 mg) Daily From Days 4 to 10 in Young and Elderly, Male and Female Subjects

	C _{max} (ng/mL)	t _{max} (h)	AUC _r (ng•h/mL)	CL/F (mL/min/kg)	t _{1/2} (h)	Rac (C _{max})	Rac (AUC)
Young Males n=12	525 (230)	2.46 (0.92)	4356 (2375)	4.212 (3.001)	8.20 (3.00)	1.455 (0.576)	1.477 (0.677)
Young Females n=12	507 (155)	1.96 (1.16)	3773 (1444)	3.447 (2.495)	8.40 (5.09)	1.223 (0.324)	1.383 (0.345)
Elderly Males n=11	474 (140)	2.32 (0.84)	4047 (1224)	3.247 (1.335)	10.78 (8.71)	1.192 (0.133)	1.280 (0.175)
Elderly Females n=12	538 (223)	2.65 (1.14)	5015 (2512)	3.762 (1.846)	10.49 (4.99)	1.082 (0.254)	1.276 (0.211)
p-values: Age	0.859	0.310	0.426	0.053	0.171		
Gender	0.685	0.659	0.741	0.195	0.978		
Age*Gender	0.466	---	0.188	0.591	0.882		

p values for t_{max} determined using Cochran-Mantel-Haenszel Tests.
 --- No sample or not determined.

The respective observation intervals during which the plasma concentration time profiles of OPC-41061 can be followed are too short ($<3 \cdot t_{1/2z} + 2 \cdot t_{max}$) for a reliable determination of λ_z and the derived AUC₂₄-INF and AUCINF. The extent of the bias in the sponsor's estimates can be evaluated by comparing AUCINF (Day 1) with AUC τ (Day 10). For a drug exhibiting first order kinetics AUCINF and AUC τ should be identical provided AUC τ is obtained at steady-state. However, the sponsor's AUCINF (Day 1) is smaller than AUC τ in all four groups. The bias in the sponsor's estimated extrapolated AUC on Day 1 for tolvaptan can be quantified as shown in the below table:

Extent of Bias in Sponsor's Extrapolated AUC₂₄-INF of Tolvaptan

Group	Median					
	AUC ₀₋₂₄ (Day1)	AUCINF(Day1)	AUC ₂₄ -INF(Day1) ^a	AUC τ (Day10)	True AUC ₂₄ -INF(Day1) ^b	Bias ^c
18-45 y M	2571	2833	262	3925	1354	0.19
18-45 y F	2710	2826	116	3721	1011	0.11
≥ 65 y M	3427	3722	295	3854	427	0.69
≥ 65 y F	3516	3885	369	4391	857	0.43

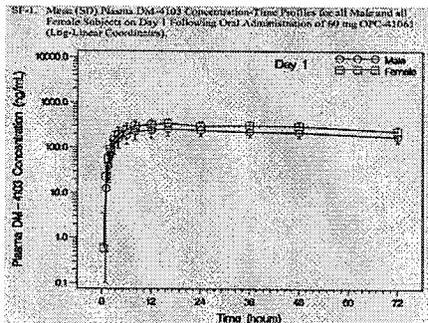
^aObtained from the difference between AUC ∞ (Day1) and AUC₀₋₂₄(Day1) ^bobtained from the difference between AUC τ (Day 10) and AUC₀₋₂₄(Day 1) ^cobtained from ratio of AUC₂₄-INF(Day 1) and true AUC₂₄-INF(Day 1)

The results show that the sponsor systematically and significantly underestimates the extrapolated AUC by between 89 % and 31 % resulting in significantly biased estimates of λ_z of OPC-41061 in all 4 groups. Because the extrapolated AUC is relatively small compared to the actually measured AUC the sponsor's AUCINF (Day 1) values underestimate true AUCINF by up to about 30% in the young to middle aged subjects.

Metabolites

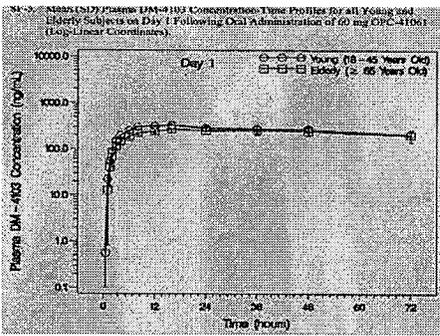
Semi-logarithmic plots of the plasma concentration profiles of the metabolites on Day 1 are shown for the 7 measured metabolites in the below figures:

DM-4103: All Male and Female Subjects

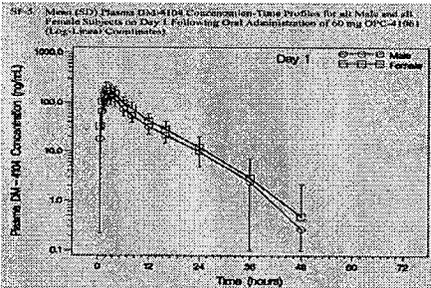


DM-4103: All Young and Elderly Subjects

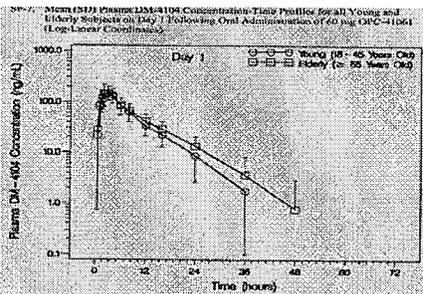
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DM-4104: All Male and Female Subjects

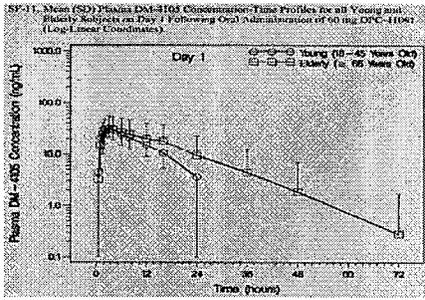


4104: All Young and Elderly Subjects

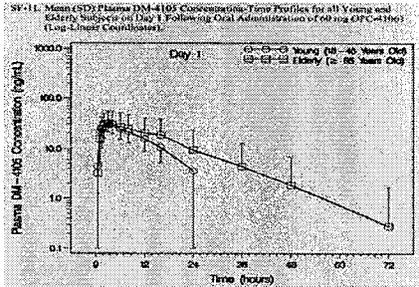


4105: All Male and Female Subjects

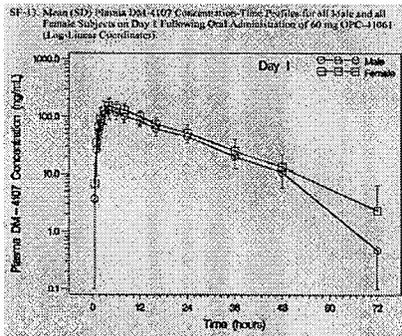
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4-105: All Young and Elderly Subjects

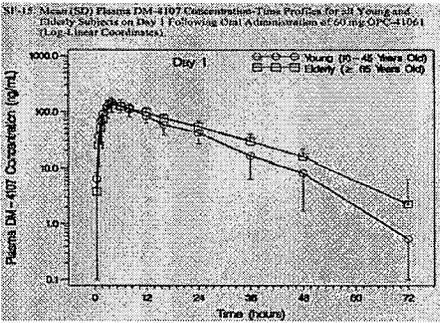


DM-4107: All Male and Female Subjects

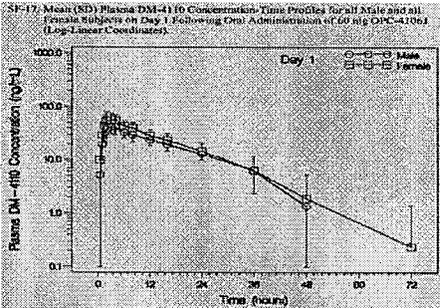


DM-4107: All Young and Elderly Subjects

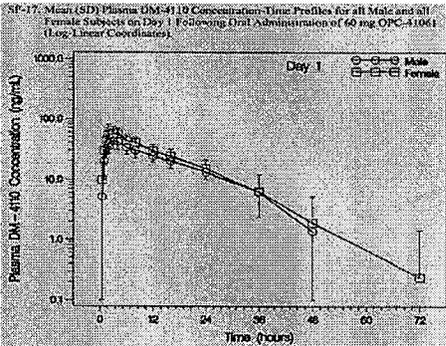
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DM-4110: All Male and Female Subjects

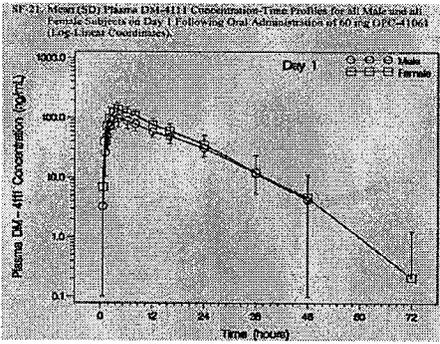


DM-4110: All Young and Elderly Subjects

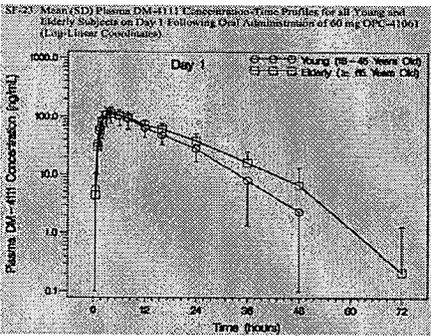


DM-4111: All Male and Female Subjects

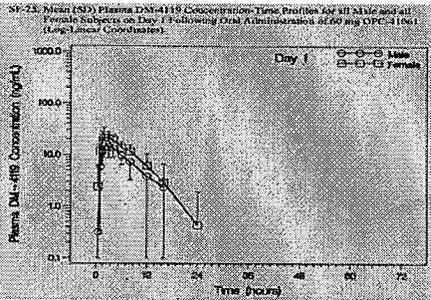
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DM-4111: All Young and Elderly Subjects



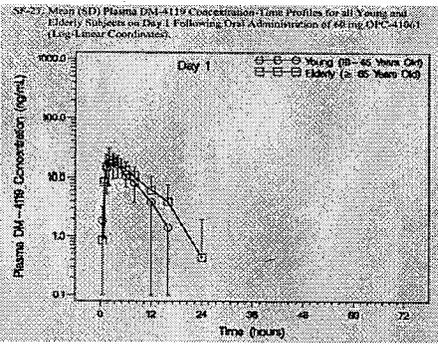
DM-4119: All Male and Female Subjects



DM-4119: All Young and Elderly Subjects

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The respective observation intervals during which the plasma concentration time profiles of the metabolites can be followed are also too short ($<3 \cdot t_{1/2z} + 2 \cdot t_{max}$) for a reliable determination of λ_z , AUC_{24-INF} and AUC_{INF} . The bias in the sponsor's estimates for the extrapolated area and hence λ_z can be quantified for the metabolites DM-4107 (bias ranges between 1.2-2.0) and DM-4111 (bias ranges between 0.44-0.82) in the four groups. The estimates for $AUC_{INF}(\text{Day}1)$ are less affected than $t_{1/2z}$ for the metabolites. Estimating the bias in the sponsor's parameters for the metabolite DM-4103 is not possible. In contrast to OPC-41061 and the other metabolites, the slowly eliminated DM-4103 is not at steady-state by Day 10. For the metabolites DM-4104, DM-4105, DM-4110 and DM-4119 reliable estimate for AUC_{0-24} are not available so that the bias in the estimated parameters cannot be assessed.

Reliable PK Parameters for OPC-41061

C_{max} and t_{max} on Days 1 and 10, AUC_{0-24} (Day 1), AUC_{τ} (Day10) and the derived CL/F on Day 10 and $RAUC$ can be considered reliable for OPC-41061 and the metabolites DM-4107 and DM-4111 in the four groups tested.

The below tables list median parameter estimates for OPC-41061 and some of the metabolites that can be considered reliable:

Median AUC_{0-24} (ng•h/mL) on Day 1 and AUC_{τ} (ng•h/mL) on Day 10 of OPC-41061

Compounds	M, 18-45 y		F, 18-45 y		M \geq 65 y		F \geq 65 y	
	1	10	1	10	1	10	1	10
OPC-41061	2571	3925	2710	3721	3427	3854	3516	4391
DM-4103	5288	24482	7682	40863	4016	29169	5755	37621
DM-4104	nr	1093	nr	1327	1152	1180	1297	1430
DM-4107	1674	2053	2110	2381	1783	2505	2241	3172
DM-4110	574	892	641	906	559	900	708	1192
DM-4111	1316	1773	1649	2044	1442	1935	1906	2704

nr= not reliable

Median C_{max} (ng/mL) on Days 1 and 10

Compound	M, 18-45 y		F, 18-45 y		M \geq 65 y		F \geq 65 y	
	1	10	1	10	1	10	1	10
OPC-41061	339	478	398	500	402	469	473	497
DM-4103	284	1114	413	1954	245	1283	312	1713

DM-4104	130	134	195	184	135	133	151	174
DM-4105	32	30	38	41	28	29	24	37
DM-4107	129	155	170	190	116	162	145	220
DM-4110	41	67	59	73	45	69	62	86
DM-4111	92	122	145	158	98	125	124	169
DM-4119	13	19	22	21	17	21	22	30

Median tmax (h) on Days 1 and 10

Compound	M, 18-45 y		F, 18-45 y		M ≥ 65 y		F ≥ 65 y	
	1	10	1	10	1	10	1	10
OPC-41061	3.0	2.0	1.5	1.8	1.5	2.0	2.0	3.0
DM-4103	16	8.0	16	12	24	6.0	30	10
DM-4104	3.0	2.5	2.0	2.0	3.0	3.0	2.0	3.5
DM-4105	3.0	2.0	2.0	1.8	3.0	3.0	2.5	3.5
DM-4107	4.0	4.0	4.0	4.0	4.0	4.0	5.0	4.0
DM-4110	3.0	3.0	3.0	3.0	3.0	3.0	3.5	4.0
DM-4111	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
DM-4119	3.0	3.5	3.0	3.0	2.0	3.0	2.5	3.0

Median CL (mL/min•kg) on Day 10

Metabolite	M, 18-45 y	F, 18-45 y	M ≥ 65 y	F ≥ 65 y
OPC-41061	3.6	4.7	2.8	3.5

Median RAUC (AUC_τ/AUC₀₋₂₄)

Metabolite	M, 18-45 y	F, 18-45 y	M ≥ 65 y	F ≥ 65 y
OPC-41061	1.3	1.4	1.2	1.2
DM-4107	1.2	1.2	1.3	1.5
DM-4111	1.4	1.3	1.3	1.3

OPC-41061

C_{max} of OPC-41061 is attained between 1.5 and 3.0 h after administration in the four groups tested. Body weight is the only significant covariate for CL/F of OPC-41061 (Day 10). Age and sex have no impact on the kinetics of OPC. The mean CL/F ranges between 2.8 and 4.7 mL/min/kg and classifies OPC-41061 as a low extraction drug. OPC-41061 administered qd accumulates by a factor between 1.2 and 1.4 in the four groups.

Metabolites

The mean plasma concentrations of the metabolites tend to be greater in the elderly subjects compared to their younger counterparts. The median AUC_τ on Day 10 in the four groups is greatest for DM-4103 followed by OPC-41061, DM-4107, DM-4111, DM-4104, DM-4110, DM-4105, and DM-4119. The relative magnitudes of the C_{max} values on Day 10 for OPC-41061 and the metabolites are in the same order as AUC_τ. The exposure to DM-4103 on Day 10 before attaining steady-state is about 8 times greater than the exposure to OPC-41061. The t_{max} of DM-

4103 on Day 10 is smaller than on Day 1 indicative for the precession on multiple dosing of an accumulating compound. The accumulation of OPC-41061 is much smaller. A comparison of tmax and RAUC among the metabolites suggests that the PK of DM-4104, DM-4105, DM-4107, DM 4110, DM-4111 and DM-4119 are formation dependent, where as those of DM-4103 are elimination dependent.

Pharmacodynamics

The below table compares the mean urine excretion rates on Days 1-3 and 10-12:

Mean Change from Baseline in 24 h Urine Excretion Rate on Days 1-3 after a Single Dose of 60 mg OPC-41061 and on Days 10-12 after Multiple Doses of 60 mg OPC-41061 in Healthy Adult and Elderly Males and Females:

Subjects n=12	Mean Change from Baseline in 24 h Urine Excretion Rate, mL/min					
	Day 1	Day 2	Day 3	Day 10	Day 11	Day 12
Male 18-45	2.8	-0.69	-0.32	1.9	0.023	-0.12
Female 18-45	2.1	-0.73	-0.24	1.4	-0.49	-0.13
Male ≥65	2.3	-0.86	-0.47	1.1	-0.61	-0.31
Female ≥ 65	1.6	-0.70	-0.16	1.2	-0.44	-0.11

Subject	“Rebound” Effect ^a
Male 18-45 y	68
Female 18-45 y	66
Male ≥65 y	49
Female ≥ 65 y	75

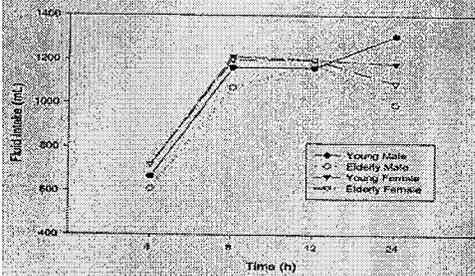
^a Rebound effect is defined as the mean change from baseline in 24 h urine excretion rate on Day 10 in percent of Day 1

A single dose of 60 mg OPC-4106 increases the mean 24 h urine excretion rate on Day 1 by 2.8, 2.3, 2.1 and 1.6 mL/min in young to middle aged males, elderly males, young to middle aged females and elderly females, respectively. Males display greater mean 24 h urine excretion rates on Day 1 than females. The coefficient of variation about the mean net 24 h urine excretion rate is small and ranges between 13% and 20%. On Days 2 and 3 the mean 24 h urine excretion rates are 5%-43% smaller than at baseline in the four groups suggesting a modest rebound with fluid retention. The diuretic effect size of OPC-41061 is reduced by 25-41 % on Day 10 compared to Day 1 in the populations studied even though the exposure to OPC-41061 on Day 10 of OPC-41061, the only aquaretic moiety, is expected to be slightly greater than on Day 1. This finding confirms that over time the rebound effect increases. The onset of the aquaretic effect of OPC-41061 is evident in the young and elderly subjects during the first 0-4 h collection interval after the first dose on Day 1. The mean net 24 h urine excretion rates on Days 2 and 11 are smaller than the baseline values suggesting that the aquaretic effect of single and multiple doses of 60 mg OPC-41061 does not last longer than about 24 h in the tested subjects.

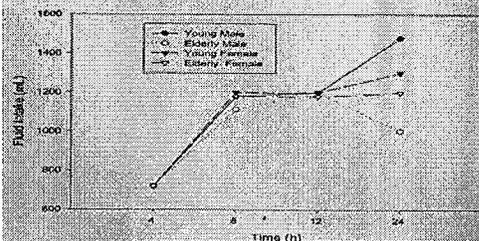
Mean Fluid Intake

The below 2 figures show the fluid intake of the subjects on Days 1 and 10 of the OPC-41061 treatments:

SF-93 Mean Fluid Intake Values vs. Time on Day 1 Following Oral Administration of 60 mg of OPC-41061



SF-94 Mean Fluid Intake Values vs. Time on Day 10 Following Daily Oral Administration of 60 mg of OPC-41061

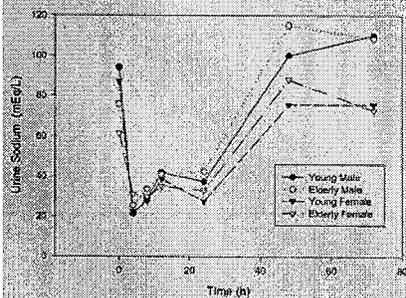


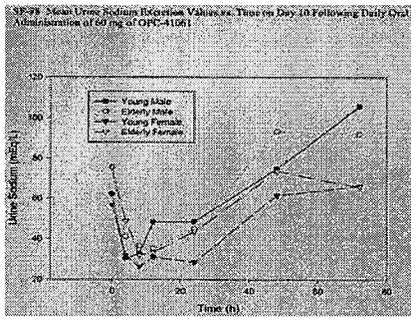
The plots show fluid intake in intervals of different length on Days 1 and 10. Plots of the ingestion rate would be more adequate. The figures show that the fluid intake up to 12 h after administration of OPC-41061 appears to be similar in all groups. In the 12-24 h interval the young to middle age subjects ingest more than their elderly counterparts and this may be responsible for the observed age related difference in the mean net 24 h urine excretion rate. Fluid intake on Days 1 and 10 appears not to be significantly different.

The below 2 figures show the mean excretion of Na⁺ in urine on Days 1 and 10 of the OPC-41061 treatments :

Urine Sodium

SF-97 Mean Urine Sodium Excretion Values vs. Time on Day 1 Following Oral Administration of 60 mg of OPC-41061

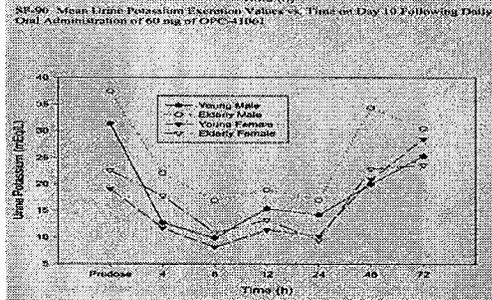
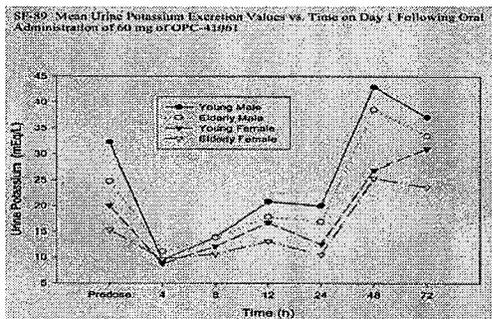




It should be noted that the figures show Na^+ excretion in urine, not the excretion rate of Na^+ in urine. The baseline values vary among the 4 groups. The plots suggest an apparent decrease in Na^+ urine excretion in the 4 groups on Days 1 and 10 following single and multiple dose treatments with OPC-41061, respectively. However, in the absence of placebo controls it is not possible to separate OPC-41061 effects from other confounding factors including circadian rhythm.

The below 2 figures show the mean excretion of K^+ in urine on Days 1 and 10 of the OPC-41061 treatments:

Urine Potassium

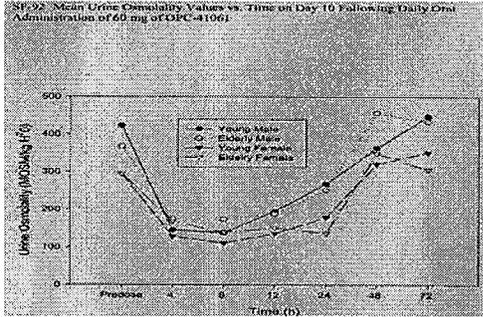


It should be noted that the figures show K^+ excretion in urine, not the excretion rate of K^+ in urine. The baseline values vary among the 4 groups. The plots suggest an apparent decrease in K^+ urine excretion in the 4 groups on Days 1 and 10 following single and multiple dose treatments with OPC-41061, respectively. However, in the

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absence of placebo controls it is not possible to separate OPC-41061 effects from other confounding factors including circadian rhythm.

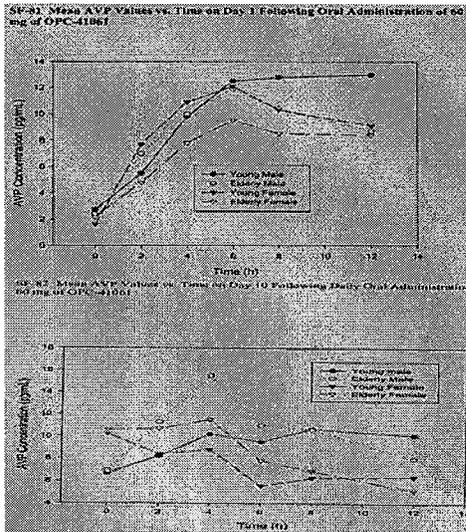
Urine Osmolality



The plots show the mean excretion of osmolality in urine, not the excretion rate of osmolality in urine. The baseline values vary among the four groups. The plots suggest an apparent decrease in urine osmolality in the 4 groups on Days 1 and 10 following single and multiple dose treatments with OPC-41061, respectively. However, in the absence of placebo controls it is not possible to separate OPC-41061 effects from other confounding factors including circadian rhythm.

Plasma AVP

The below figures show linear plots of the AVP levels on Days 1 and 10 of the OPC-41061 treatments:



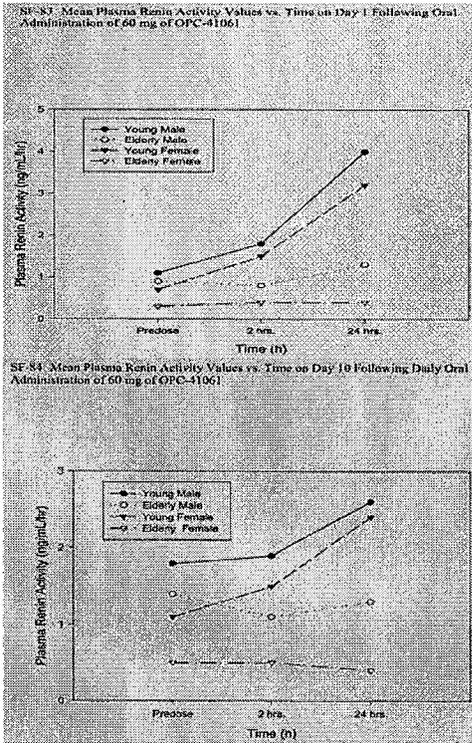
The baseline values of AVP differ among the four groups. On Day 1 starting at 2 h post-dose the mean AVP levels appear to increase during the 12 h observation interval. On Day 10 the AVP levels are still elevated in the four groups compared to the baseline values and remain relatively constant during the 12 h observation period. Single

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and multiple doses of 60 mg OPC-412061 administered qd appear to increase the AVP levels. However, the body position and activity level of the subjects were not controlled at the time the samples for the measurement of AVP were taken.

PRA in Plasma

The below figures show linear plots of mean PRA on Days 1 and 10:



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The baseline values among the four groups vary. Compared to baseline mean PRA on Day 1 at 2 h and 24 h after administration of OPC-41061 in the young to middle aged subjects appear elevated. Compared to pre-dose levels mean PRA on Day 10 at 2 h and 24 h after OPC-41061 administration is elevated in the young to middle aged subjects. In contrast, the elderly subjects on both days do not exhibit much of a change in PRA. The mean levels in elderly males appear to be greater than in elderly females. In the absence of a control group the impact of OPC-41061 on the observed changes in PRA levels is difficult to assess.

PK-PD

Population	Mean AUC _τ (Day 10)	Mean Net 24 h Urine Excretion Rate Day 10
	ng • h/mL	mL/min
Young males	4356	1.9
Young Females	3773	1.4
Elderly Males	4047	1.1

Elderly Females	5015	1.2
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The mean data appear to indicate that the exposure-response relationship in healthy elderly is different from that in healthy young to middle aged subjects in both sexes. More exposure is needed in the elderly to obtain the same aquaretic effect than in the young to middle age subjects.

Conclusions

PK

Due to the inadequate sensitivity of the assay the respective observation intervals necessary to estimate reliably λz and the derived parameters $t_{1/2z}$, AUCINF and V/F for OPC-41061 and the metabolites are too short.

The oral clearance of OPC-41061 ranges between 2.8 and 3.5 mL/min/kg in the four groups. Based on these values OPC-41061 can be classified as a drug with low hepatic extraction. Body weight is the only covariate. Age and sex do not impact the kinetics of OPC-41061. OPC-41061 accumulates by a factor of 1.2-1.4 when administered qd.

DM-4103 displays a very protracted elimination resulting in significant accumulation. In contrast DM-4104, DM-4107, DM-4110, and DM-4111 appear to exhibit formation limited kinetics. The major circulating moiety in plasma is DM-4103, however OCP-41061 is the only moiety in plasma with aquaretic activity.

PD

A single dose of 60 mg OPC-41061 increases the mean 24 h excretion rate on Day 1 by 1.6, 2.1, 2.3 and 2.8 mL/min in elderly females, young to middle aged females, elderly males and young to middle aged males, respectively. After a seven day treatment with 60 mg OPC-41061 qd the above mean net 24 urine excretion rates are reduced to 75 %, 66 %, 49 % and 68 %, respectively, indicating more retention and/or less fluid ingestion. The inter-subject variation of the aquaretic effect is small. Fluid intake increases in all four groups to compensate for aquaretic loss.

PK-PD

The exposure-response relationship in healthy elderly appears to be different from that in healthy young to middle age subjects of both sexes. More exposure is needed in the elderly to obtain the same aquaretic effect than in the young to middle aged subjects

Comments

1. The sponsor should report the findings of the analysis of the plasma concentration-response relationship of OPC-41061 and the impact of age and sex on the pharmacodynamic endpoints.
2. To appropriately evaluate the pharmacodynamics of OPC-41061 including covariates, placebo groups should have been included in the study.
3. AVP and PRA levels are known to be impacted by body position and activity. The protocol prescribed that the subjects were to be in the sitting position for the first 4 h post-dose. However, samples for PRA and AVP were collected at additional times so that a possible effect of OPC-41061 is confounded by the impact of body position and activity at these other times.
4. The observation interval for OPC-41061 and the metabolites is too short for a reliable determination of λz and derived parameters including $t_{1/2z}$, AUCINF, and Vz/F .
5. The report does not describe methods and results of the plasma protein binding experiments to be performed with the blood samples collected 3 h after administration.