CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-275

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO

NDA: 22-275, [redacted] b(4)

Submission receipt date: October 23, 2007
Drug: tolvaptan
Sponsor: Otsuka Pharmaceutical Company
Indication: 22-275: treatment of patients with hypervolemic and euvolemic hyponatremia

[redacted] b(4)

Reviewing Division: Division of Cardiovascular and Renal Products

Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for tolvaptan to be sufficient to support the use of up to a 60 mg daily dose in the above indications.

The sponsor and reviewer proposed pregnancy category C for the labeling. Embryofetal studies revealed some embryotoxicity and malformations at high maternally toxic doses. These doses provided large margins compared to the human dose based on mg/m^2 comparisons. Therefore, while the risk to human fetuses is likely to be minimal, it is still appropriate for this drug to be labeled with a pregnancy category of C. The wording as proposed by the pharm/tox reviewer is acceptable.

No carcinogenicity signal was detected in two-year rat and mouse studies. The wording for this section of the labeling as proposed by the pharm/tox reviewer is acceptable.

The reviewer recommended that section 13.1 of the labeling as proposed by the sponsor be deleted since it does not add useful information. I agree.

Conclusion:
I concur with the Division pharm/tox conclusion that the nonclinical data support approval of these NDAs.

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/s/

Paul Brown
8/22/2008 09:58:57 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-275
DATE RECEIVED BY CENTER:	10/23/2007
PRODUCT:	Samska™ Tablets (Tolvaptan)
INTENDED CLINICAL POPULATION:	Congestive heart failure patients with [redacted] patients with hyponatremia associated with euvolemic and hypervolemic states
SPONSOR:	Otsuka Pharmaceutical Company, Ltd Rockville, MD
REVIEW DIVISION:	Division of Cardiovascular and Renal Products
PHARM/TOX REVIEWER:	Xavier Joseph
PHARM/TOX SUPERVISOR:	Charles A. Resnick
DIVISION DIRECTOR:	Norman Stockbridge
PROJECT MANAGER:	Dan Brum

b(4)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

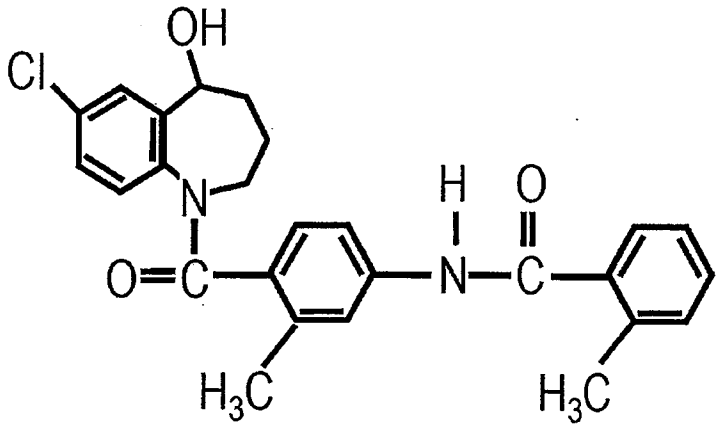
Xavier Joseph, D.V.M.
June 23, 2008

ORIGINAL NDA DATED: October 22, 2007
CENTER RECEIPT DATE: October 23, 2007
REVIEWER RECEIPT DATE: October 26, 2007

SPONSOR: Otsuka Pharmaceutical Company, Ltd
2440 Research Boulevard
Rockville, MD 20850

DRUG PRODUCT: Trade name - Samska™ Tablets
DRUG SUBSTANCE: Generic name – Tolvaptan
Code names – OPC-41061 and OPC-156

Chemical Structure



MW 448.94

FORMULATION: Samska™ immediate release tablets are formulated to contain 15 or 30 mg tolvaptan with the following inactive ingredients: hydroxypropyl cellulose, [redacted], lactose monohydrate, corn starch, microcystlline cellulose, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, FD&C Blue No. 2, magnesium stearate and [redacted]

b(4)

[redacted] b(4)

PHARMACOLOGICAL CLASS: Non-peptide vasopressin V_2-receptor antagonist

PROPOSED INDICATIONS: 1) For the short-term treatment of the signs and symptoms of worsening heart failure beyond that achieved with standard of care and 2) for the treatment of hypervolemic and euvolemic hyponatremia and for the prevention of worsening hyponatremia.

PROPOSED DOSAGE REGIMEN: The recommended dose of Samska for the worsening heart failure indication is 30 mg/day. For hyponatremia, the recommended starting dose is 15 mg/day; the dose may be increased, at intervals of at least 24 hours, to 30 mg/day and to a maximum of 60 mg/day, as tolerated, to achieve the desired level of serum sodium.

INDs UNDER WHICH CLINICAL TRIALS WERE CONDUCTED: [redacted] IND 54,200 b(4)
(for the treatment of hyponatremia [redacted]

RELATED NDAs: Approved NDA 21-697 for conivaptan hydrochloride injection (Vaprisol®) for the treatment of euvolemic and hypervolemic hyponatremia in hospitalized patients [redacted] b(4)

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On Original

EXECUTIVE SUMMARY

Samska™ (Tolvaptan), a benzazepine derivative, has been developed for the treatment of [redacted] hyponatremia. Tolvaptan is a vasopressin antagonist that blocks the binding of arginine vasopressin (AVP) at the V_2 receptors of the distal portions of the nephron, thereby preventing water reabsorption, and inducing water diuresis (aquaresis) without the depletion of electrolytes. (AVP causes fluid retention and hyponatremia.) Samska™ is the first oral vasopressin V_2 receptor antagonist submitted for FDA approval. b(4)

I. Recommendations

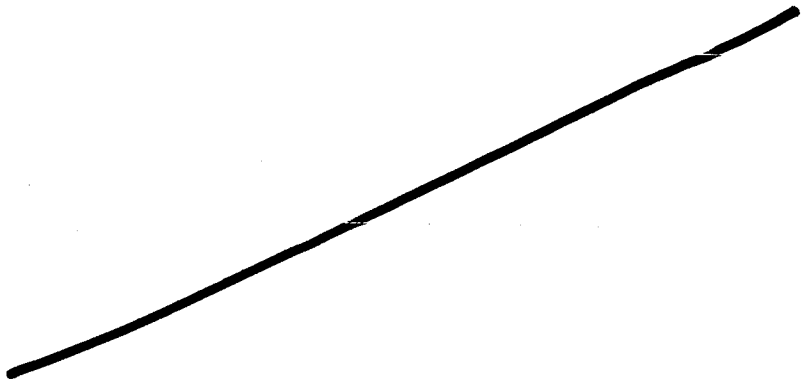
A. Recommendation on Approvability

Samska™ is approvable from a nonclinical perspective.

B. Recommendations for Additional Nonclinical Studies

None

C. Recommendations on Labeling



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1 Page(s) Withheld

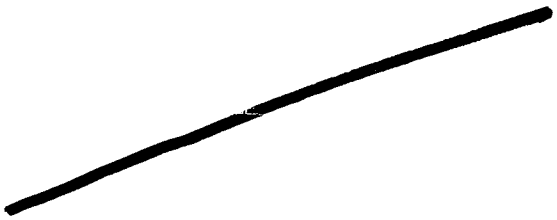
_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 8



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II. Summary of Nonclinical Findings

A. Pharmacodynamic Activity

Tolvaptan inhibits arginine vasopressin (AVP) induced water reabsorption at the renal collecting ducts by competitively blocking the binding of AVP to V_2 receptors, which results in increased water excretion without any change in electrolyte excretion. In receptor binding studies using human AVP receptors expressed in HeLa cells, tolvaptan blocked the binding of labeled AVP to human V_2 and V_{1a} receptors (V_{1a} receptors in vascular smooth muscle mediate the vasoconstrictor effect of vasopressin) but not to human V_{1b} receptors (V_{1b} receptors in the anterior pituitary mediate ACTH secretion). Tolvaptan, concentration dependently, inhibited labeled AVP binding to rat kidney V_2 and rat liver V_{1a} receptors and inhibited AVP binding to canine kidney V_2 and platelet V_{1a} receptors. These binding studies demonstrated 29, 61 and 259 times higher affinity for V_2 receptors than V_{1a} receptors in human, canine and rat species, respectively. It was shown that tolvaptan also inhibited AVP-induced cAMP production.

There were no differences in human V_2 and V_{1a} receptor antagonism between the 2 optical isomers [(R)-(+)-OPC-41061 and (S)-(-)-OPC-41061] and tolvaptan (racemic form). The metabolites of tolvaptan showed no activity at human V_2 and V_{1a} receptors or weak antagonistic activity compared to tolvaptan. Neither optical isomers nor metabolites of tolvaptan showed any antagonistic activity at human V_{1b} receptors.

Tolvaptan increased urine excretion and decreased urine osmolality in the dose range of 0.3 to 10 mg/kg, po in mice, rats, rabbits and dogs. It elevated free water clearance in mice and rats at 10 mg/kg, in rabbits at 3 mg/kg and higher, and in dogs at 1 mg/kg and above. Tolvaptan slightly increased urinary sodium excretion in mice, rats and rabbits but not in dogs. Serum sodium concentration was elevated at 4 hours postdose in rats, rabbits and dogs due to decreased body fluid volume resulting from the water diuresis induced by tolvaptan. Serum AVP concentration was increased in rats and dogs following tolvaptan administration, since tolvaptan increased serum osmolality after aquaresis.

The effects of tolvaptan on hemodynamic and renal function were evaluated in sedated conscious dogs with pacing-induced heart failure. A single oral administration of tolvaptan at 10 mg/kg produced increased water clearance with a significant increase in

serum sodium, accompanied by a decrease in cardiac preload without affecting cardiac afterload or renal function. The results of this study indicated that tolvaptan may be useful for the treatment of the volume overload state of congestive heart failure without any adverse effects on renal function.

The effects of tolvaptan on hyponatremia were evaluated in a rat model of acute and chronic hyponatremia. A combination of 1-deamino-8-D-arginine vasopressin (DDAVP) infusion and water loading produced acute progressive hyponatremia with decreased plasma sodium concentration associated with high mortality. Oral administration of tolvaptan (1, 3 and 10 mg/kg for 3 days) produced dose-dependent aquaresis with concurrent increase in plasma sodium concentration and the prevention of death. Oral treatment with tolvaptan (0.25 to 8 mg/kg for 10 days) normalized hyponatremia and improved brain edema in rats with chronic hyponatremia induced by DDAVP sc infusion and liquid diet.

Tolvaptan was shown to produce an inhibitory effect on the development of polycystic kidney disease in PCK rats (an animal model for human autosomal recessive polycystic kidney disease), by decreasing intracellular levels of cAMP which is shown to play a major role in cyst formation.

Tolvaptan inhibited AVP-induced (mediated via V_{1a} receptors), but not adenosine diphosphate (ADP)-induced, platelet aggregation with an IC_{50} of 1.28 μM, confirming its action as a V_{1a} receptor antagonist.

Tolvaptan showed a very low affinity (Ki of 431 nM) for oxytocin receptors (oxytocin, a hormone structurally similar to AVP, is also synthesized in the hypothalamus and released from the posterior pituitary). Tolvaptan and its major metabolites in humans (DM-4103 and DM-4107) showed no notable affinity for acetylcholine, adenosine, adrenergic, angiotensin II, bradykinin, dopamine, endothelin, histamine, opioid, serotonin, vasoactive intestinal peptide receptors and calcium, potassium and sodium channels. Safety pharmacology studies showed that tolvaptan had no effects on nervous, muscular (smooth muscle), respiratory, cardiovascular and gastrointestinal systems. The major metabolites in humans also had no effects on the nervous, respiratory and cardiovascular systems.

B. Pharmacokinetics and Metabolism

In vitro and *in vivo* studies were carried out to investigate the metabolism and pharmacokinetics of tolvaptan. Following the administration of single oral doses of tolvaptan to rats, rabbits and dogs, the C_{max} and AUC values increased dose-dependently. The exposure to tolvaptan was generally higher in female than in male rats, and there were no gender differences for exposure in dogs. A comparison of pharmacokinetics of the jet-milled and [redacted] formulations of tolvaptan showed that both the C_{max} and AUC values were higher for the [redacted] formulation (formulation used for clinical trials) in both rats and dogs. The absolute oral bioavailability of jet-milled tolvaptan in rats and dogs was determined to be 0.63 and 2.0%, respectively, while the absolute oral

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bioavailability of [redacted] tolvaptan in rats and dogs was 16.0 and 14.6%, respectively. In bile duct-cannulated rats, about 60% of drug-related material was recovered in the bile. The low oral bioavailability may be due to extensive presystemic metabolism. b(4)

Following a single oral administration of ^{14}C labeled tolvaptan (30 mg/kg) to male and female rats and male dogs, the serum concentration of radioactivity peaked at 2 to 4 hours post-dose with C_{max} values of 4.4 and 7.3 μg eq/mL in male and female rats, respectively, and 6.2 μg eq/mL in male dogs. Elimination half-life values were 4.4, 6.4 and 4.8 hours for male and female rats, and male dogs, respectively. The volume of distribution of tolvaptan in rats and dogs was substantially greater than the volume of total body water, suggesting extensive extravascular distribution of the drug and/or preferential binding to tissue proteins. Tolvaptan binds extensively to plasma proteins (97.2% or higher) *in vitro* in mouse, rat, rabbit, dog and human plasma as determined by the ultrafiltration method. The extent of binding was independent of drug concentration. The *ex vivo* protein binding of tolvaptan in rat and dog plasma was 93% or higher and was similar to protein binding determined by ultrafiltration *in vitro*, indicating that the presence of metabolites in the plasma did not affect the protein binding of tolvaptan. The major human metabolites DM-4103 and DM-4107 were also extensively bound (≥ 98.5%) to human plasma proteins. Tissue concentrations of radioactivity were determined in male rats following single oral administration of ^{14}C labeled tolvaptan (30 mg/kg). Radioactivity concentrations in the liver, stomach, small intestine, kidneys and adrenal glands were higher than in serum. The radioactivity concentrations in the central nervous system were very low.

The overall biotransformation profiles of tolvaptan were qualitatively similar across different animal species studied and in humans. In mice, rats, rabbits, dogs and humans, tolvaptan is metabolized primarily by 3 major biotransformation pathways: hydroxylation to form DM-4110, DM-4111 and DM-4119; dehydrogenation to form MOP-21826; deamidation to form DM-4128; and hydroxylation after cleavage of the benzazepine ring to form DM-4104. MOP-21826 was further converted to DM-4105 and DM-4103. DM-4104 was further converted to DM-4107.

Based on i*n vitro* studies with recombinant human cytochrome P450 isoforms, CYP3A4 was identified as responsible for catalyzing the primary metabolic reactions. In addition, CYP1A1 was found to catalyze the formation of DM-4128 from tolvaptan. Other CYP isoforms were not involved in the metabolism of tolvaptan. Tolvaptan did not inhibit CYP isoforms *in vitro* or induce the drug metabolizing enzymes *in vivo* at clinically relevant concentrations. Following oral administration of ^{14}C labeled tolvaptan to rats and dogs, most of the administered radioactivity was excreted in the feces in both species. It was shown that tolvaptan-derived radioactivity was secreted in the milk of lactating rats and was also distributed to the fetal tissues in pregnant rats, suggesting a potential for fetal and neonatal exposure to tolvaptan if administered to pregnant or lactating women.

C. Toxicology Findings

The following toxicity studies were performed using the ——— formulation of tolvaptan (formulation used for clinical trials and the intended formulation for marketing). b(4)

In a 52-week oral toxicity study in dogs (30, 100 and 1000 mg/kg/day), two high dose animals (one male and one female) in week 5 and another high dose female in week 13 were sacrificed due to their declining condition associated with decreases in body weight and food consumption. There were no gross or histopathological findings in any of the above dogs. No treatment-related adverse effects were seen in this study except for reduced adrenocorticocyte vacuolation and/or increased cortical width in the adrenal glands of mid and high dose animals. These adrenocortical findings were attributed to a physiological adaptive response to stress (chronic marked diuresis). A NOAEL was not established for the adrenocortical findings in dogs since increased cortical width was also noted at the low dose (30 mg/kg/day) in a supplementary dog study.

In a 26-week study in rats (30, 100 and 1000 mg/ kg/day), mortality was observed in high dose females after the first few doses of tolvaptan, which was attributed to drug-related increased urine output resulting in dehydration. After additional water supply was made available to the animals, no further mortality occurred in the study. Administration of tolvaptan to rats did not reveal any drug-related adverse effects. Expected increases in water consumption and increased urine volume with associated decrease in urine specific gravity and osmolality were observed throughout the study. The NOAELs were determined to be 1000 mg/kg/day for males and 100 mg/kg/day for females. [In a 4-week oral toxicity study with tolvaptan in rats, both prothrombin time (PT) and activated partial thromboplastin time (APTT) were prolonged at doses of 300 mg/kg/day and above. This prolongation in PT and APTT was attributed to depletion of vitamin K-dependent coagulation factors (II, VII, IX and X). A mechanistic study in rats given 1000 mg/kg of tolvaptan showed that the prolongation of PT and APTT occurred only under fasting conditions and was reversed by vitamin K supplementation.]

Two years of oral administration of tolvaptan (100, 300 and 1000 mg/kg/day in males and 30, 100, 300 and 1000 mg/kg/day in females) did not increase the incidence of tumors in the rat. The highest dose employed in that study is about 160 times the maximum recommended human dose (MRHD) of 60 mg/day, on a mg/m^2 basis. In the mouse, two years of oral administration of tolvaptan (10, 30 and 60 mg/kg/day in males and 10, 30 and 100 mg/kg/day in females) also did not produce an increased incidence of tumors. The highest doses employed in male and female mice were about 5 and 8 times the MRHD, respectively, on a mg/m^2 basis.

In a fertility study in rats, oral administration of tolvaptan (100, 300 and 1000 mg/ kg/day) to male rats for 9 weeks and to female rats for 2 weeks prior to mating, during mating and up to gestational day 7 was associated with reductions in body weight gain and food consumption (both sexes). The mean numbers of corpora lutea and implants at 1000 mg/kg/day were significantly lower than control. The drug treatment did not result

in any grossly observable fetal abnormalities. Oral administration of tolvaptan at 10, 100 and 1000 mg/kg/day to pregnant rats during organogenesis was associated with a reduction in maternal body weight gain and food consumption at 100 and 1000 mg/kg/day, reduced fetal weight and delayed ossification of fetuses at 1000 mg/kg/day. Lower doses did not produce any significant adverse effect on the fetus. Oral administration of tolvaptan (100, 300 and 1000 mg/kg/day) to pregnant rabbits from implantation to closure of the hard palate was associated with dose-related maternal toxicity (reduction in body weight gain and food consumption at all doses, and abortion at mid and high doses). At 1000 mg/kg/day, increased incidences of post-implantation loss, fetal microphthalmia, open eyelids, cleft palate, brachymelia and skeletal malformations were observed. Lower doses did not produce any adverse effects on the fetus. In a study in which rats were dosed with tolvaptan (10, 100 and 1000 mg/kg/day) from day 7 of gestation through day 21 postpartum, the treatment was associated with reductions in maternal body weight gain (100 and 1000 mg/kg/day) and food consumption (10 or more mg/kg/day). One dam died at the high dose. Increased perinatal death and suppressed body weight gain of offspring were noted at this same dosage level. F0 maternal drug treatment, at doses up to 1000 mg/kg/day, had no significant effect on the physical development, reflex functions, learning ability or reproductive performance of the F1 progeny.

Tolvaptan tested negative in *in vitro* (bacterial reverse mutation assay and chromosomal aberration test in Chinese hamster lung fibroblast cells) and *in vivo* (micronucleus assay in rat bone marrow erythrocytes) test systems.

No phototoxicity was observed in rabbits (at doses up to 1000 mg tolvaptan/kg) or guinea pigs (at doses up to 2000 mg/kg) although in an *in vitro* test system using a mouse embryonic cell line, tolvaptan was found to be 'probably phototoxic'.

Single dose toxicity studies (rats) and *in vitro* genotoxicity studies with DM-4103 and DM-4107, the major human metabolites of tolvaptan, revealed no notable toxicity or genotoxic potential for these metabolites.

D. Nonclinical Safety Issues Relevant to Clinical Use

Chronic toxicity studies conducted in both rats and dogs did not reveal any notable toxicity in either species. Prolongation of PT and APTT observed in rat studies at high doses were not seen in the dog. Dose limiting clinical signs (dehydration and reduced food consumption and body weight) observed in female rats and male and female dogs at 1000 mg/kg/day were considered to be the consequence of an exaggerated pharmacologic action of the drug. Nonclinical studies have shown no genotoxic or carcinogenic potential. The apparently drug-related effects on fertility and embryo/fetal development in rats and rabbits occurred at relatively high doses (about 160 times the MRHD on a mg/m^2 basis in rats and 324 times the MRHD in rabbits), and may have been secondary to maternal toxicity at these doses. We do not consider the reproductive toxicity findings observed at the very high multiples of the MRHD to constitute an approvability issue.

However, since tolvaptan was shown to be secreted in the milk of lactating rats, it is recommended that women receiving tolvaptan should not breast feed.

In conclusion, there are no approvability issues for tolvaptan based on the non-clinical toxicity–testing program.

A. Reviewer signature: _____

B. Supervisor signature: Concurrence _____
Charles A. Resnick, Ph.D.

TABLE OF CONTENTS – PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY

[Sponsor's summaries of pharmacodynamic studies are provided below.]

2.6.2.2 Primary Pharmacodynamics

2.6.2.2.1 Vasopressin Receptor Antagonism

2.6.2.2.1.1 Binding Affinity to AVP Receptors

In vitro antagonistic effects of tolvaptan were investigated in binding experiments using a human endocervical carcinoma cell line (HeLa cells) expressing human AVP receptor subtypes (V_{1a}, V_{1b}, and V_2) (Table 2.6.2.7-1).[1,2,3] Tolvaptan inhibited [^{3}H]AVP binding to the V_2-receptors in a concentration-dependent manner, with an inhibition constant (K_i) of 0.43 ± 0.06 nM, which was approximately 1.8 times higher than that of AVP (K_i = 0.78 ± 0.08 nM). Tolvaptan also inhibited [^{3}H]AVP binding to the V_{1a}-receptors with a K_i of 12.3 ± 0.8 nM, but the affinity is approximately 29 times weaker than that for V_2-receptors. On the other hand, tolvaptan did not inhibit [^{3}H]AVP binding to the V_{1b}-receptors even at 1×10^{-4}M. These results indicated that tolvaptan is a competitive AVP antagonist with high selectivity for the V_2-receptors.

The antagonistic effects of nonpeptide vasopressin antagonists YM-087 (conivaptan, V_{1a} + V_2 nonselective), SR-121463A (V_2 selective), and VPA-985 (lixivaptan, V_2 selective), which were launched or developed by other companies, were evaluated on [^{3}H]AVP binding to human V_{1a}- and V_2-receptors expressed in HeLa cells (Table 2.6.2.7-1).[4,5,6] These compounds inhibited the binding to human V_2-receptors with K_i of 3.10 ± 0.27 nM (YM-087), 2.46 ± 0.18 nM (SR-121463A), and 1.79 ± 0.14 nM (VPA-985), but the affinities were not higher than that of tolvaptan. While YM-087 showed high affinities

for not only human V_2-receptors but also to human V_{1a}-receptors (K_i = 1.81 ± 0.45 nM), SR-121463A and VPA-985 displaced the binding of [^{3}H]AVP to human V_{1a}-receptors at high concentrations (K_i of 1908 ± 303 nM and 494 ± 128 nM, respectively).

The affinities of tolvaptan for rat and canine AVP receptors were investigated by measuring the inhibition of [^{3}H]AVP binding to membrane preparations prepared from rat liver (V_{1a}), canine platelet (V_{1a}), and rat and canine kidney (V_2).[7,8,9] Tolvaptan concentration-dependently inhibited [^{3}H]AVP binding to rat AVP V_{1a}- and V_2-receptors with K_i of 345 ± 54 nM and 1.33 ± 0.26 nM, and to canine AVP V_{1a}- and V_2-receptors with K_i of 40.3 ± 12.0 nM and 0.66 ± 0.09 nM, respectively. Thus tolvaptan was approximately 259- and 61-times more selective for V_2-receptors than for V_{1a}-receptors in rats and dogs.

2.6.2.2.1.2 Cyclic AMP Production

The in vitro antagonistic effects of tolvaptan were investigated using the HeLa cells expressing human AVP V_2-receptors[1]. In the HeLa cells expressing human AVP V_2-receptors, cAMP (an intracellular mediator released by the activation of V_2-receptors) production increased at 1×10^{-12}M AVP and reached near maximum levels at 1×10^{-8}M AVP. Tolvaptan inhibited cAMP production by AVP at 1×10^{-9}M in a concentration-dependent manner and the concentration that produced 50% inhibition of the maximal response (IC_{50}) was found to be 8.0 ± 2.7 nM. On the other hand, tolvaptan did not stimulate cAMP production even at a high concentration of 1×10^{-6}M.[10] These results demonstrated that tolvaptan is a potent AVP antagonist without possessing any agonistic activity against AVP V_2-receptors.

2.6.2.2.1.3 Vasopressin Antagonism: In Vivo Studies

The inhibitory effect of V_2-receptor antagonist tolvaptan on AVP-induced antidiuresis was evaluated in water-loaded, alcohol-anesthetized rats.[11] Sprague-Dawley rats were loaded with water (5% body weight) and anesthetized with 13% ethanol orally. After constant urine flow was established, AVP and/or tolvaptan were injected intravenously. AVP-induced (approximately 0.01 mU/kg/min) decline in urine volume and increased urinary osmolality were reversed by an intravenous injection of tolvaptan in a dose-related manner with a dose that inhibited AVP-induced antidiuretic activity by 50% (ED_{50}) of 13 ± 3 μg/kg. Tolvaptan, 0.01 to 1 mg/kg, exerted no AVP-like activity (anti-diuresis), suggesting a lack of intrinsic agonistic activity. In summary, tolvaptan by the

intravenous route exhibited AVP antagonistic activity without antidiuretic activity in water-loaded, alcohol-anesthetized rats.

2.6.2.2.2 Aquaretic Action in Several Animal Species

2.6.2.2.2.1 Healthy Animals

Aquaretic effect of orally administered tolvaptan was investigated in conscious $B6C3F_1$ mice at doses of 0.3, 1, 3 and 10 mg/kg.[12] Tolvaptan increased urine volume and decreased urine osmolality during 0 to 4 hours postdosing in a dose-dependent manner. At 10 mg/kg the drug increased free water clearance to a positive value, indicating that tolvaptan has a significant aquaretic action in $B6C3F_1$ mice. Tolvaptan increased urinary excretion of sodium, creatinine and urea nitrogen also in a dose-related manner, while no differences were found in the serum concentration of each parameter at 4 hours postdosing. These results suggested that tolvaptan has a significant aquaretic action in $B6C3F_1$ mice.

Aquaretic effect of orally administered tolvaptan was investigated in conscious male Sprague-Dawley rats at doses of 0.3, 1, 3 and 10 mg/kg.[13] Tolvaptan increased urine volume and decreased urine osmolality during 0 to 4 hours postdosing in a dose-dependent manner. Urinary excretion of electrolytes (Na, K, and Cl), creatinine and urea nitrogen for the 4 hours postdosing increased in association with the increase in urine volume, but the net excretions for the 24 hours postdosing were comparable to those in the control. Serum osmolality and serum sodium and creatinine concentrations for the 4 hours postdosing were elevated by the decreased body fluid volume resulting from the water diuresis (aquaresis) induced by tolvaptan. When tolvaptan ([redacted]) was administered to conscious male Sprague-Dawley rats at doses of 1, 3, and 10 mg/kg,[14] tolvaptan exerted an aquaretic effect that was similar to the result obtained with [redacted] tolvaptan.[13] b(4)

Aquaretic effect of orally administered tolvaptan was investigated in conscious male New Zealand White rabbits at doses of 0.3, 1, 3 and 10 mg/kg.[15] Tolvaptan increased urine volume and decreased urine osmolality during 0 to 4 hours postdosing in a dose-dependent manner. Tolvaptan elevated free-water clearance to positive values at 3 and 10 mg/kg indicating aquaresis also in male rabbits as expected. As a result of potent aquaretic action, serum sodium and chloride concentrations as well as serum osmolality were significantly increased at the highest dose of tolvaptan.

The differences in the diuretic effects between tolvaptan and furosemide were investigated in conscious male beagle dogs.[16] Tolvaptan at doses of 0.3 to 10 mg/kg dose-dependently increased urine volume and decreased urine osmolality during 0 to 6 hours postdosing (Figure 2.6.2.7-1). While furosemide, a loop diuretic, significantly increased urine volume according to the increase in urinary sodium excretion, tolvaptan increased urine volume without increasing urinary sodium excretion, resulting in the elevation of free water clearance to a positive value at doses of 1 mg/kg and higher. Although tolvaptan and furosemide increased urine volume to a similar extent, several differences in the effects on neurohormonal factors were observed between the 2 treatments. Tolvaptan increased plasma AVP concentration, probably due to the elevation of plasma osmolality, but did not stimulate either the sympathetic or renin-angiotensin-aldosterone system, in spite of its potent aquaretic effect. In contrast, furosemide increased plasma renin activity (PRA), aldosterone and epinephrine concentrations and reduced atrial natriuretic peptide (ANP) level. When tolvaptan (non-[redacted] form: [redacted] was administered to conscious male beagle dogs at doses of 0.3, 1 and 3 mg/kg,[17] tolvaptan exerted an aquaretic effect that was similar to the result obtained with [redacted] tolvaptan.[16] b(4)

Aquaretic effect of tolvaptan was examined on serum AVP, pituitary AVP and the number of AVP receptors in the liver and in the kidney in male Sprague-Dawley rats.[18] Tolvaptan was administered orally once daily for 28 days at 1 and 10 mg/kg/day. Over the course of the study, tolvaptan increased urine volume and decreased urine osmolality during 0 to 4 hours postdosing. Increase in urine volume during 0 to 4 hours postdosing at 1 mg/kg did not change throughout the 4-week study period, but tended to decrease slightly at the 10-mg/kg dose. Urine osmolality showed significant differences between the tolvaptan (1- and 10-mg/kg) groups and the control group throughout the study period, suggesting that the repeated administration for 28 days did not alter the aquaretic activity of tolvaptan. Tolvaptan at 10 mg/kg significantly increased urinary excretion of sodium, creatinine and urea nitrogen during 0 to 4 hours postdosing but there were no differences between the treated and the control groups in these parameters for 0 to 24 hours postdosing. Tolvaptan dose-dependently and significantly increased the urinary excretion of AVP during 0 to 4 hours postdosing and 0 to 24 hours postdosing. The increase in urinary excretion of AVP remained almost the same throughout the study period. The treated and the control groups showed no differences in serum osmolality or serum sodium, creatinine and urea nitrogen at 24 hours postdosing throughout the study period. There were also little differences between the tolvaptan groups and the control

group in serum AVP concentration, pituitary AVP level, or AVP affinity and the number of AVP receptors in the liver and kidney at 24 hours postdosing throughout the study period. Repeated administration of tolvaptan for 28 days decreased serum renin activity and significantly lowered serum aldosterone concentration.

2.6.2.2.2 Animal Models of Heart Failure

Aquaretic effect of tolvaptan was investigated in a conscious, pacing-induced heart failure model of dogs after single oral administration at doses of 0.3, 1, 3 and 10 mg/kg.[19] Tolvaptan significantly increased urine volume and decreased urine osmolality during 0 to 6 hours postdosing in a dose-dependent manner, together with an increase in free water clearance. Tolvaptan did not significantly increase urinary sodium, chloride or creatinine excretion during 0 to 6 hours postdosing. Tolvaptan did not significantly change PRA or the plasma ANP, norepinephrine, and epinephrine levels.

b(4)

2.6.2.2.3 Animal Models of Hyponatremia

Various studies have suggested that hyponatremia is a leading cause of death in acutely and chronically hospitalized patients. The effects of tolvaptan on hyponatremia were evaluated in rat acute and chronic models of hyponatremia. It is well known that acute hyponatremia produces brain edema, which can lead to neurological symptoms or even death, and therefore requires rapid treatment to restore plasma sodium to only mildly hyponatremic levels. On the other hand, chronic hyponatremia is generally less symptomatic than acute hyponatremia, and rapid correction of chronic hyponatremia may produce central nervous system injuries such as central pontine myelinolysis, so that it is critical to control the rate and extent of plasma sodium rise.

Tolvaptan was evaluated to determine if it could correct a water metabolism disorder in a rat model of acute hyponatremia.[20] In a rat model of acutely progressive hyponatremia induced by 1-deamino-8-D-arginine vasopressin (DDAVP) infusion and water loading, plasma sodium concentrations were progressively decreased, resulting in severe hyponatremia associated with a high mortality rate (47%). When tolvaptan was administered at 1, 3, and 10 mg/kg, a clear dose-dependent aquaresis was produced throughout the dosing period, resulting in a gradual increase in plasma sodium concentration. Because of the correction of plasma sodium concentrations, no deaths were observed among animals given 3- or 10-mg/kg doses of tolvaptan.

Tolvaptan was evaluated to determine if it could correct a water metabolism disorder in a rat model of chronic hyponatremia.[21] In a model of chronic hyponatremia induced by DDAVP infusion and liquid diet, plasma sodium concentrations decreased to about 110 mEq/L, which was maintained thereafter without any deaths. Oral dose escalation of tolvaptan from 0.25 to 8 mg/kg gradually increased plasma sodium concentrations to normal levels with a mean daily increase in plasma sodium concentration of less than 10 mEq/L (Figure 2.6.2.7-2). Rats treated with tolvaptan showed no neurological symptoms associated with central pontine myelinolysis, and improved water content in the brain and heart. Throughout the dosing of tolvaptan, urine volume increased and osmolality decreased. Urinary sodium excretion was lower in the tolvaptan-treated group than in the vehicle-treated group. The experiment demonstrated that tolvaptan treatment improved severe hyponatremia and caused selective aquaresis as expected.

Hyponatremia and volume overload is often found in congestive heart failure patients. Hyponatremia is also a known risk factor for worsening prognosis and increased mortality in patients with congestive heart failure and the incidence of myocardial infarction. This study was undertaken to determine if tolvaptan would prevent the aggravation of myocardial infarction in a rat model of chronic hyponatremia.[22] Hyponatremia was induced by the similar method described above (DDAVP infusion and liquid diet).[21] From Day 4, escalating doses of tolvaptan from 0.25 to 10 mg/kg were administered orally. On Day 14, animals were subjected to 20 minutes of coronary ligation followed by 3 hours of reperfusion under pentobarbital anesthesia. Results indicated that hyponatremia and hypo-osmolality were associated with an increase of myocardial infarction after ischemia/reperfusion. Tolvaptan effectively normalized plasma sodium level and osmolality and reduced myocardial infarction in these animals. Since congestive heart failure patients often have underlying coronary artery disease, this study highlights the importance of correcting hyponatremia and volume overload in patients with congestive heart failure and myocardial infarction, and supports the clinical use of tolvaptan.

2.6.2.2.4 Other Actions

2.6.2.2.4.1 Hemodynamics and Renal Function

In order to further understand the mechanism of diuresis, the aquaretic effect of tolvaptan was compared with a well-known saluretic drug, furosemide on the hemodynamics and renal functions in 2 groups of conscious dogs: group 1 with normal dogs and group 2 with dogs in which congestive heart failure was induced by rapid ventricular pacing.[23] Tolvaptan (10 mg/kg), furosemide (also 10 mg/kg) or the vehicle

(hydroxypropylcellulose) was orally administered to both groups of sedated beagle dogs. Hemodynamics, renal function, urinary electrolyte excretion, serum electrolyte concentrations and plasma hormone levels were determined during 0 to 6 hours postdosing. Tolvaptan showed clear aquaresis with an increase in free water clearance, resulting in a significant increase in serum sodium, osmolality and a decrease in cardiac preload in both normal and congestive heart failure dogs. Tolvaptan, however, did not affect cardiac afterload or renal function in either group. In contrast, furosemide showed clear saluresis with an increase in urinary electrolyte excretion, resulting in a significant decrease in serum potassium and cardiac preload. Furosemide also did not affect cardiac afterload or renal function in either group. The reduction of ventricular preload corresponded to the decrease in the cumulative water balance by each drug in both groups. In both normal and congestive heart failure dogs, tolvaptan increased plasma AVP concentrations, but did not affect PRA or plasma norepinephrine, epinephrine or ANP concentrations. On the other hand, furosemide activated PRA and plasma norepinephrine and epinephrine concentrations. Thus the reduction of cardiac preload by furosemide and tolvaptan was comparable, as reflected by the equal volume of excreted urine following each drug. Tolvaptan increased plasma AVP concentrations through the compensatory mechanism, but this did not cause an increase in peripheral vascular resistance or the aggravation of cardiac afterload or renal functions in either group. Furthermore, in contrast to furosemide, tolvaptan did not stimulate the sympathetic nerves or the renin-angiotensisn-aldosterone system, nor cause a decrease in serum potassium concentration. Consequently, tolvaptan will be useful for the treatment of the volume over-load states of congestive heart failure without undesirable effects on renal function, systemic hemodynamics or circulating neurohormones.

2.6.2.2.4.2 Binding Affinity to Other Receptors and Channels

Although tolvaptan has been shown to be a V_2-receptor selective antagonist with a high degree of affinity, it was decided to determine if tolvaptan and its major metabolites, DM-4103 and DM-4107, had affinity also for other receptors and/or ion channels. Consequently, the affinity of tolvaptan and DM-4103 and DM-4107 were investigated for the following receptors and ion channels: acetylcholine and adenosine receptors, adrenergic receptors, angiotensin II, bradykinin, calcitonin gene-related peptide, dopamine, endothelin, epidermal growth factor, histamine, opioid, serotonin, somatostatin and vasoactive intestinal peptide receptors, and calcium, potassium and sodium channels.[24] Tolvaptan, DM-4103 and DM-4107 did not inhibit the binding between each radioligand and receptors or channels more than 50% at a concentration of

1×10^{-5}M. In a similar binding study conducted in another laboratory using tolvaptan (1×10^{-5}M) and DM-4103 (0.94×10^{-5}M), neither tolvaptan nor DM-4103 showed any notable affinity for various receptors.[25]

Vasopressin and oxytocin are neurohypophysial hormones synthesized in the hypothalamus and released from the pituitary. Although both are similar in structure they perform distinct biological functions. In view of the similarity in structures and of the origin in biosynthesis, it was pertinent to determine if tolvaptan also had any affinity for human oxytocin receptors. Thus, tolvaptan and DM-4103 (its major metabolite) were tested in the binding of [^{3}H]-oxytocin to human oxytocin receptors expressed to HeLa cells.[26] The results showed that tolvaptan concentration-dependently inhibited the specific binding of [^{3}H]-oxytocin to the human oxytocin receptors. The K_i for tolvaptan was 431 ± 63 nM which was approximately 1000 times higher than that for the human AVP V_2-receptors.[1] DM-4103 showed little affinity for the oxytocin receptors.

2.6.2.2.4.3 Platelet Aggregation

Tolvaptan demonstrated significantly higher affinity for the V_2-receptors than the V_{1a}-receptors. Therefore, the effects of tolvaptan on human platelet aggregation were investigated as a functional analysis for V_{1a}-receptors.[27] Platelets were prepared from the blood of healthy volunteers and incubated with AVP. The aggregation response was measured using an aggregometer. AVP (5 to 320 nM) produced concentration-dependent aggregation of the platelets with a submaximal dose of 80 nM. Tolvaptan inhibited AVP (80 nM)-induced but not adenosine diphosphate (ADP) (4 μM)-induced platelet aggregation in a dose-dependent manner. The IC_{50} was found to be 1.28 ± 0.20 μM. Tolvaptan alone, at 1 and 10 μM, did not induce any change in the shape of the platelets nor did it induce any aggregation of the platelets. These results suggest that tolvaptan possesses the antagonistic activity for human V_{1a}-receptors.

2.6.2.2.5 Primary Pharmacology of Tolvaptan Optical Isomers and Metabolites

2.6.2.2.5.1 Binding Affinity to AVP Receptors

The affinities of optical isomers of tolvaptan were evaluated on AVP binding to human, rat, and canine AVP subtypes. R-(+)-OPC-41061 and S-(-)-OPC-41061, which are the optical isomers of tolvaptan, inhibited [^{3}H]AVP binding to human V_{1a}- and V_2-receptors in a concentration-dependent manner with K_i of 9.89 ± 1.36 nM and 15.55 ± 2.28 nM for V_{1a}-receptors and K_i of 0.47 ± 0.10 nM and 0.47 ± 0.07 nM for V_2-receptors,

respectively.[1,28] There was no difference in the antagonistic activity against AVP to human AVP receptors between the 2 isomers or between each isomer and the racemate (tolvaptan). The optical isomers of tolvaptan did not show any affinity for human V_{1b}-receptors.[29] For rat AVP receptor subtypes,[7] R-(+)-OPC-41061 and S-(-)-OPC-41061 displaced [^{3}H]AVP binding to rat V_2-receptors with a K_i of 1.55 ± 0.43 nM and 0.77 ± 0.12 nM, which suggests that the affinity of S-(-)-OPC-41061 was approximately double as potent as that of R-(+)-OPC-41061. The K_i for antagonizing the binding to rat V_{1a}-receptors was 734 ± 120 nM for R-(+)-OPC-41061 and 259 ± 55 nM for S-(-)-OPC-41061, indicating that R-(+)-OPC-41061 and S-(-)-OPC-41061 were 474 and 336 times more selective for rat V_2-receptors, respectively. For canine AVP receptor subtypes,[30] R-(+)-OPC-41061 and S-(-)-OPC-41061 concentration-dependently inhibited [^{3}H]AVP binding to canine AVP V_{1a}- and V_2-receptors with a K_i of 40.4 ± 5.8 nM and 94.4 ± 19.8 nM for V_{1a}-receptors, and 0.94 ± 0.13 nM and 0.86 ± 0.10 nM for V_2-receptors, respectively.

DM-4103 and DM-4107 are the major human metabolites of tolvaptan. Furthermore, the other 12 metabolites (DM-4104, DM-4105, DM-4110, DM-4111, DM-4119, MOP-21826, DM-4128, DM-4129, DM-4130, DM-4131, DM-4132, and DM-4133) were identified in human serum or urine. The affinities of 14 metabolites of tolvaptan were evaluated on AVP binding to human,[1,31,32] rat,[7,33,34] and canine[30] AVP V_2-receptors (Table 2.6.2.7-2). The binding experiments were performed using HeLa cells expressing human AVP V_2-receptors and rat and canine kidney membrane preparations. DM-4110, DM-4111, DM-4119, and MOP-21826 inhibited [^{3}H]AVP binding to human V_2-receptors with K_i of 0.98 ± 0.13 nM, 1.66 ± 0.16 nM, 1.78 ± 0.06 nM, and 1.46 ± 0.17 nM, whose affinities were a little weaker than that of tolvaptan (K_i = 0.43 ± 0.06 nM). DM-4107, DM-4128, DM-4129, DM-4131, and DM-4132 also inhibited the binding in a concentration-dependent manner with K_i of 1933 ± 120 nM, 89.7 ± 21.5 nM, 676 ± 222 nM, 885 ± 278 nM, and 2294 ± 575 nM, respectively, but the affinities were much weaker. DM-4103, DM-4104, DM-4105, DM-4130, and DM-4133 did not inhibit the binding even in a concentration of 10^{-5}M. For rat and canine AVP V_2-receptors, the affinities of all metabolites were found to be weaker than tolvaptan, as for human AVP V_2-receptors.

The affinities of 14 metabolites of tolvaptan were evaluated in the inhibition of [^{3}H]AVP binding using HeLa cells expressing human AVP V_{1a}-receptors[28,35] and V_{1b}-

receptors.[29,36] DM-4110, DM-4111, DM-4119, MOP-21826, DM-4128, and DM-4131 inhibited [^{3}H]AVP binding to human V_{1a}-receptors with K_i of 35.02 ± 6.97 nM, 18.4 ± 2.1 nM, 400 ± 31 nM, 37.4 ± 6.5 nM, 315 ± 11 nM and 1100 ± 27 nM. On the other hand, DM-4103, DM-4104, DM-4105, DM-4107, DM-4129, DM-4130, DM-4132 and DM-4133 did not inhibit the binding even at a concentration of 10^{-5}M. For human V_{1b}-receptors, none of all metabolites have an affinity at a concentration of 10^{-5}M.

Tolvaptan and 14 of its metabolites were evaluated by determining their inhibitory effect on [^{3}H]AVP binding to rat[7,33,34] and canine[30] AVP V_{1a}-receptors. For rat and canine AVP V_{1a}-receptors, the affinities of all 14 metabolites were found to be weaker than those of tolvaptan.

2.6.2.2.5.2 Cyclic AMP Production

The optical isomers (R-(+)-OPC-41061 and S-(-)-OPC-41061) and metabolites (DM-4110, DM-4111, and MOP-21826) of tolvaptan were investigated to determine their antagonistic activity against AVP-induced cAMP formation in HeLa cells expressing human AVP V_2-receptors.[1] R-(+)-OPC-41061 and S-(-)-OPC-41061 inhibited cAMP production by AVP at 1×10^{-9}M in a concentration-dependent manner and the IC_{50} was found to be 10.1 ± 5.7 nM and 8.3 ± 4.2 nM, respectively. There was no difference in the antagonistic activity against cAMP production between the 2 isomers. DM-4110, DM-4111, and MOP-21826 also inhibited cAMP production and the IC_{50} was found to be 78.4 ± 53.5 nM, 40.4 ± 20.3 nM, and 122.1 ± 77.9 nM, respectively. On the other hand, neither tolvaptan nor any of its some metabolites (DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, DM-4111, DM-4113, DM-4116, DM-4119 and MOP-21826) stimulated cAMP production even at a high concentration of 1×10^{-6}M.[10] These results demonstrated that tolvaptan and its metabolites did not possess any agonistic activity against AVP V_2-receptors.

2.6.2.3 Secondary Pharmacodynamics

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2.6.2.4 Safety Pharmacology

Tolvaptan was investigated in ICR mice to determine its effects on central and somatic nervous systems, and the gastrointestinal system. Mice were administered jet-milled tolvaptan up to 1000 mg/kg. The serum concentration of unchanged compound was determined following a single oral administration of jet-milled tolvaptan to ICR mice.[42] The maximum concentration (C_{max}) following administration of jet-milled tolvaptan at 1000 mg/kg to ICR mice in a fasted state was 3.973 μg/mL.

The effect of oral doses of 0 (vehicle control), 100, 300 and 1000 mg/kg tolvaptan on body temperature was examined.[43] Predosing rectal temperatures were measured twice in male ICR mice and the second measurement was regarded as the initial temperature. After oral administration of tolvaptan, postdosing temperatures were obtained. The results showed that tolvaptan had no adverse effect on the body temperatures for up to 6 hours postdosing (the final timepoint of measurement).

The effects of oral doses of 0 (vehicle control), 100, 300 and 1000 mg/kg of tolvaptan were investigated in male ICR mice on the general condition and behavior of the animals.[44] The only drug-related change found was a dose-dependent increase in urine volume. The drug had no adverse effect on the general condition and behavior of the test animals.

The effects of oral doses of tolvaptan on the spontaneous motor activity of mice were investigated.[45] Tolvaptan was orally administered at 0 (vehicle control), 100, 300 and 1000 mg/kg doses to male ICR mice weighing approximately 31 to 41 gm. The mice, 10 per group, were fasted for 16 to 18 hours before dosing. The spontaneous motor activities of each animal were measured for 5 minutes, twice before dosing and at 0.5, 1, 2, 4 and 6 hours postdosing. Although the spontaneous activity was somewhat higher in all of the tolvaptan-treated groups from 0.5 to 2 hours postdosing, these increases were not statistically significant from the control group and were thought to be related to increased urination.

The effects of oral doses of 0 (vehicle control), 100, 300 and 1000 mg/kg of tolvaptan were investigated in male ICR mice to determine if tolvaptan had any effect on motor coordination (rota-rod method) or muscle relaxation (traction method) using diazepam as a positive control.[46] The positive control suppressed motor coordination and decreased muscle tension at 10 mg/kg while tolvaptan had no adverse effects even at the highest tested dose.

A general pharmacology study using male ICR mice investigated the effects of tolvaptan on hexobarbital-induced hypnosis and elucidated whether the compound has an anesthetic effect. Tolvaptan at oral doses of 100, 300, and 1000 mg/kg did not affect the righting reflex for one hour postdosing, suggesting the test drug has no anesthetic effect and did not affect latency time or hypnosis time.[47]

Tolvaptan did not suppress writhing in the acetic acid-writhing method at a single oral dose of 100, 300 and 1000 mg/kg in male ICR mice and therefore had no analgesic effect. Aminopyrine, the positive control substance, at an oral dose of 100 mg/kg exhibited an analgesic effect.[48]

The effects of oral doses of 0 (vehicle control), 100, 300 and 1000 mg/kg of tolvaptan were investigated in male ICR mice to determine if tolvaptan could produce any analgesic effect in the modified Haffner's assay.[49] Results showed that a single oral dose of tolvaptan at 100, 300, and 1000 mg/kg did not have an analgesic effect in mice. Morphine, the positive control, at an oral dose of 30 mg/kg exhibited an analgesic effect.

The effects of oral doses of 0 (vehicle control), 100, 300 and 1000 mg/kg of tolvaptan were investigated in fasted male ICR mice to determine if tolvaptan could induce convulsions or augment convulsions induced by pentetrazol and strychnine administered intraperitoneally and by minimum electric shock.[50] Results showed that tolvaptan did not induce or augment convulsions at the doses tested.

The effects of oral doses of 0 (vehicle control), 100, 300 and 1000 mg/kg of tolvaptan were investigated in male ICR mice to determine if the test drug had any effect on gastrointestinal propulsion of a charcoal meal in comparison with the positive control atropine.[51] Tolvaptan did not affect gastrointestinal propulsion at any dose tested. Atropine, the positive control, at an oral dose of 30 mg/kg inhibited gastrointestinal propulsion.

The effect of intravenous doses of tolvaptan on the amplitude of gastric motility and the tonus of gastric muscle in anesthetized rats were investigated.[52] Tolvaptan was administered intravenously at 0 (vehicle control), 1, 3 and 10 mg/kg into the femoral vein of fasted male Wistar rats. The animals were surgically prepared with a clamp attached to the gastric pylorus from which a thread extended and attached to an isometric transducer which recorded spontaneous motility of the stomach via a distortion pressure amplifier. Tolvaptan had no effect on gastrointestinal motility or tone at 1 and 3 mg/kg but at 10 mg/kg decreased the amplitude of gastric motility at 5 minutes postdose. The same high

dose of 10 mg/kg also decreased the tone of the gastric muscle at 1 and 5 minutes postdose.

The effect of tolvaptan on gastric secretion was investigated in pylorus-ligated male Sprague-Dawley rats after intraduodenal administration of 0 (vehicle control), 30, 100, 300 and 1000 mg/kg of compound.[53] Tolvaptan at 300 mg/kg tended to decrease gastric juice volume, acid concentration and acid output. The compound at 1000 mg/kg decreased gastric juice volume, acid concentration, and acid output and increased gastric juice pH. Tolvaptan at intraduodenal doses of 30 and 100 mg/kg did not affect gastric juice volume, gastric juice pH, acid concentration, or acid output.

The effect of tolvaptan was investigated on the contractions of guinea pig ileum produced by acetylcholine and histamine and on contractions of isolated ileal longitudinal muscle produced by barium chloride.[54] Tolvaptan at 1×10^{-6} and 3×10^{-6}M did not affect the isolated ileum directly but at $1 \times 10^{-5}$and 3×10^{-5}M it augmented spontaneous motility slightly and caused a slight, transient increase in resting tension which tended to decrease soon after. Tolvaptan did not directly affect the ileal longitudinal muscle. Tolvaptan did not affect acetylcholine and histamine-induced contractions at 1×10^{-6} and 3×10^{-6}M but suppressed them at higher concentrations of 1×10^{-5} and 3×10^{-5}M. Similarly, barium-chloride-induced contractions were unaffected by tolvaptan at 1×10^{-6}, 3×10^{-6} and 1×10^{-5}M but suppressed contractions at a higher concentration of 3×10^{-5}M.

Tolvaptan was tested to find out if there was any effect on the action potential parameters of isolated guinea-pig papillary muscles.[55] Five papillary muscle preparations were exposed to the compound at 1×10^{-6}, 1×10^{-5}, and at 3×10^{-5}M. The other 5 preparations were cumulatively exposed to the vehicle equivalent to the concentrations of tolvaptan before exposure to 1×10^{-5}M haloperidol. The action potential parameters were recorded at cycle lengths of 2000, 1000, and 500 msec at 37°C. Tolvaptan at 1×10^{-6}, 1×10^{-5}, and 3×10^{-5}M showed no effects on any action potential parameters such as resting membrane potential (RMP), action potential amplitude (APA), the maximum upstroke velocity of the action potential (V_{max}) and the action potential duration at 30%, 60% and 90% of repolarization (APD_{30}, APD_{60}, and APD_{90}, respectively). Afterdepolarizations were never observed with tolvaptan at these concentrations. Thus, the study provided conclusive evidence that tolvaptan did not affect the action potential parameters at any of the tested dosages.

The effects of intravenous tolvaptan on the respiratory and cardiovascular systems were investigated in beagle dogs.[56] Pentobarbital-anesthetized animals were maintained on stable anesthesia for at least one hour for hemodynamic stability before intravenous administration of tolvaptan at 0 (vehicle control), 0.3, 1, 3 and 10 mg/kg. Blood pressure, heart rate, femoral artery blood flow and electrocardiogram (ECG) were measured just prior to dosing and subsequently at 0.5, 1, 3, 5, 10, 20 and 30 minutes postdosing and then at 60 minutes in the high dose group only. The respiration rate was counted at 3-minute intervals before dosing and at 0, 3, 8, 18 and 27 minutes postdosing and then at 57 minutes in the high-dose group only. The ECG was used to obtain PR, QRS, QT intervals, ST segment and T-wave amplitudes. Effect on respiration: tolvaptan did not influence respiration at 0.3, 1 and 3 mg/kg but at 10 mg/kg dose the respiration had shallowed transiently with an increase in rate. The respiration rate, nevertheless, was restored to normal within 60 minutes. Effect on cardiovascular system; heart rate: at all the tested doses of tolvaptan, as well as the vehicle, heart rate transiently increased and then gradually decreased. In the 10 mg/kg group, the gradual decrease was followed by a second increase which began at 3 minutes postdosing. Although the increase of heart rate in 1- and 3- mg/kg dose groups were statistically significant ($p < 0.01$) at 20 and 30 minutes postdosing, they were not dose-related and therefore the change in the heart rate was considered unlikely to be drug-related. Effect on cardiovascular system; blood pressure: although the blood pressure decreased transiently in the 0.3-, 1- and 3- mg/kg groups, a similar decrease was also apparent in the control group which consisted of vehicle administration. Blood pressure was significantly lower in the high dose (10 mg/kg) group at 1 and 3 minutes postdosing. Effect on cardiovascular system; femoral artery blood flow: a transient increase in femoral artery blood flow in the 0.3-, 1- and 3-mg/kg groups was similar to that found in the control group and therefore the increase was regarded considered unrelated to tolvaptan. The high dose of 10 mg/kg decreased femoral artery blood flow but the decreases disappeared within several minutes. Effect on cardiovascular system; ECG: there were no discernable differences between the vehicle and all the 3 doses of tolvaptan on QRS, QT intervals or ST segments. However, the T-wave amplitude decreased at 30 seconds and 1 minute postdosing at 3 and 10 mg/kg doses. Also, at 3 mg/kg, PR intervals were shortened at 1 minute postdosing compared to the baseline and 1 animal had a ventricular premature beat. The changes in PR intervals were not dose dependent and the ventricular premature beat was not observed in the 10 mg/kg group. Therefore, none of these changes were considered drug related.

The purpose of this safety study was to investigate the effects of tolvaptan on the respiratory and cardiovascular systems in conscious dogs.[57] Oral doses of ____ 0 (vehicle control), 10, 100, and 1000 mg/kg of tolvaptan were sequentially administered to each animal at intervals of 7 to 14 days. Relevant endpoints such as the general condition, respiratory rate, heart rate and mean blood pressure and of electrocardiogram parameters such as ST segment, T-wave amplitude, PR interval, QRS width, QT interval, and QTc (QT interval corrected by Van de water's formula) were monitored for 5 hours postdosing. Oral tolvaptan at 1000 mg/kg significantly decreased the T-wave amplitude and at 100 and 1000 mg/kg significantly shortened the PR interval with no significant changes in respiratory rate, heart rate, mean blood pressure, ST segment, QRS width, QT interval and/or QTc when compared with the same parameters of the control group. Restlessness was apparent in the treated groups. The C_{max} of the unchanged compound were 0.15, 0.46, and 2.83 μg/mL at the 10, 100 and 1000 mg/kg dosages of tolvaptan, respectively. Tolvaptan at 10, 100 and 1000 mg/kg markedly increased the urine volume and significantly increased plasma sodium and chloride concentrations. The changes in the plasma electrolytes and urine volume suggested selective excretion of free water and concentration of the blood by the compound. These observations suggested that tolvaptan had no influence on the respiratory and cardiovascular systems at up to serum concentration of 0.15 μg/mL, but it did shorten the PR interval at 0.46 μg/mL or higher and decreased the T-wave amplitude at 2.83 μg/mL in conscious dogs which were not allowed free access to water.

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The effect of tolvaptan was investigated on hERG (human ether-a-go-go related gene) channel current in CHO-K1 cells stably expressing the transfected gene.[58] Tolvaptan was tested at 0 (vehicle), 1×10^{-6}, and 2×10^{-6}M (preliminary experiments indicated maximum testable level at 2×10^{-6}M in dimethylsulfoxide (DMSO)-containing HEPES-buffered Tyrode's solution[59]). The vehicle group received 0.5% DMSO. Five cells were tested at each concentration of tolvaptan. The positive control, identified as E-4031, was tested simultaneously at 1×10^{-6}M in 3 cells. Ion currents were recorded at 23.0 to 24.3°C using the whole-cell patch-clamp technique. The test pulses were applied to the cells every 15 seconds and the peak value of the tail current was analyzed as a parameter for the hERG channel current. The vehicle and tolvaptan at 1×10^{-6}M and 2×10^{-6}M reduced the hERG channel current to 94.2%, 92.3% and 90.2% of the pretreatment value, respectively. Thus the test drug at 1×10^{-6}M and 2×10^{-6}M had no significant effects on the hERG channel current in comparison with the vehicle control. On the other hand, the

positive control E-4031 significantly decreased the hERG channel current to 3.9% of the pretreatment value. In conclusion, tolvaptan did not affect the hERG channel current at the tested concentrations up to 2 x 10^{-6}M, which was the solubility limit.

Tolvaptan metabolites:

DM-4103 and DM-4107 have been identified as the major metabolites of tolvaptan occurring in man.

The effect of DM-4103 was investigated on the behavior and general symptoms of mice.[60] DM-4103 was administered intravenously at doses of 0 (vehicle), 1 and 10 mg/kg to 8 male ICR mice per group under non-fasting conditions. General symptoms and behavior were observed as per Irwin's comprehensive assessments prior to and subsequent to the administration of DM-4103 at 5 and 30 minutes and at 2, 4, and 6 hours after administration. In the vehicle, 1, and 10 mg/kg groups, no abnormalities were observed up to 6 hours after administration. The mean serum concentrations of DM-4103 in the 1- and 10-mg/kg groups at 5 minutes after administration were 0.47 and 12.64 μg/mL, respectively. The experiment was conclusive that the metabolite DM-4103 has no adverse effect on the behavior and general symptoms of mice at serum concentrations up to 12.46 μg/mL.

The effects of DM-4103 were investigated on the respiratory and cardiovascular system of conscious dogs.[61] DM-4103 was tested at 0 (vehicle), 1 and 10 mg/kg (a preliminary study indicated that the serum concentration of DM-4103 after a single intravenous administration of DM-4103 at 10 mg/kg was 136 μg/mL which exceeded the level achieved in the clinical study[62]). DM-4103 was sequentially administered to each animal at an interval of 1 week. The vehicle group received 0.05 mL/kg of DMSO. Four animals were acclimatized and the respiratory rate, heart rate, mean blood pressure and ECG parameters ST segment (J point), T-wave amplitude, PR interval, QRS width, QT interval, QTc (QT interval corrected by Van de Water's formula) as well as the general condition were continuously observed by acute instrumentation methods until 4 hours after administration. The serum concentration of DM-4103 was also measured at 1 minute and 4 hours after the administration. DM-4103 showed no significant changes in the respiratory rate, heart rate, mean blood pressure, ST segment, PR interval, QRS width, QT interval and QTc when compared with the vehicle-treated group. However, DM-4103 at 1 mg/kg significantly increased the T-wave amplitude but only at 2 hours after administration. This effect was relatively small and was not confirmed at the high dose of 10 mg/kg thus supporting the fact that the T-wave amplitude change was not attributed

to DM-4103. In addition, DM-4103 did not have any adverse effect on general condition. The mean serum concentrations of DM-4103 in the 1- and 10-mg/kg groups at 1 minute of administration were 11.66 and 59.43 μg/mL, respectively. In conclusion, these findings suggested that DM-4103 had no adverse effects on any of the tested respiratory or ECG parameters at serum concentrations up to 59.43 μg/mL in conscious dogs.

The effect of the major metabolite DM-4103 of tolvaptan was investigated on hERG potassium currents in stably transfected CHO-K1 cells.[63] DM-4103 was tested at 0 (vehicle), 1×10^{-5}, 3×10^{-5} and 1×10^{-4}M (preliminary experiments indicated maximum applicable concentration was 1×10^{-4}M in DMSO-containing HEPES-buffered Tyrode's solution in terms of solubility[64]). The vehicle group received 0.5% DMSO. Five cells were tested at each concentration of DM-4103. The positive control, identified as E-4031, was tested simultaneously at 1×10^{-6}M in 3 cells. The hERG current was recorded using the whole-cell patch-clamp technique. The test pulses were applied to the cells every 15 seconds and the peak value of the tail current was analyzed as a parameter for the hERG current. DM-4103 showed no significant effects on the hERG current when tested at 1×10^{-5}M. However, DM-4103 at 3×10^{-5} and at 1×10^{-4}M hERG current was significantly decreased to 89.9% and 77.3% of the pretreatment value, respectively. The positive control, E-4031, at 1×10^{-6}M significantly decreased the current to 7.7% of the pretreatment value. In conclusion, DM-4103 did not affect the hERG current at tested concentrations up to 1×10^{-5}M.

The effect of intravenous DM-4107 was investigated at 0 (vehicle), 1 and 10 mg/kg in male ICR mice.[65] The general symptoms and behavior were recorded before the administration of the metabolite and at 5 minutes, 30 minutes, and at 2, 4 and 6 hours after administration. The mean serum concentrations of DM-4107 at 5 minutes after administration in the 1 and 10 mg/kg groups were 0.30 and 2.64 μg/mL, respectively. No abnormalities were observed and the data suggested that DM-4107 has no adverse effect on general symptoms and behavior up to a serum concentration of 2.64 μg/mL in mice.

The effects of DM-4107 were investigated on the respiratory and cardiovascular systems of conscious dogs.[66] Intravenous doses of 0 (vehicle), 1 and 10 mg/kg of DM-4107 were sequentially administered to 4 animals. Respiratory rate, heart rate, mean blood pressure and ECG (to obtain PR interval, QRS width, QT intervals, QTc [QT interval corrected by Van de Water's formula], ST segment and T-wave amplitudes) were continuously monitored for 4 hours through acute instrumentation methods. General condition was

monitored and the serum concentration of DM-4107 was determined at 1 minute and at 4 hours after administration. DM-4107 did not show any significant changes in the respiratory or cardiovascular parameters in comparison with the vehicle-treated group except for the ST segment that was significantly elevated at the 1 and 10 mg/kg dose at 1 minute and at 1 and 15 minutes respectively. Although the QTc was significantly prolonged at 1 minute after a dose of 1 mg/kg, the higher dose did not result in any prolongation indicating that the effect was not attributable to DM-4107. In addition, DM-4107 did not show abnormalities in the general condition of the animals. The mean serum concentration of DM-4107 was found to be 9.08 and 70.10 μg/mL at dosages of 1- and 10 mg/kg, respectively. The data supported the conclusion that DM-4107 had no adverse effect on the respiratory and cardiovascular parameters except for elevated ST segment at concentrations up to 70.10 μg/mL in conscious dogs.

The effect of the major metabolite DM-4107 was investigated on hERG potassium currents in stably transfected CHO-K1 cells.[67] DM-4107 was tested at 0 (vehicle), 1×10^{-5}, and 1×10^{-4} M (preliminary experiments indicated maximum applicable concentration was 1×10^{-4} M in DMSO-containing HEPES-buffered Tyrode's solution in terms of solubility[64]). The vehicle group received 0.1% DMSO. Five cells were tested at each concentration of DM-4107. The positive control, E-4031, was tested simultaneously at 1×10^{-6}M in 3 cells. The hERG current was recorded using the whole-cell patch-clamp technique. The test pulses were applied to the cells every 15 seconds and the peak value of the tail current was analyzed as a parameter for the hERG current. DM-4107 showed no significant effects on the hERG current when tested at 1×10^{-5} and 1×10^{-4}M. The positive control article E-4031 at 1×10^{-6}M significantly decreased the hERG current to 4.2% of the pretreatment value. Thus the experiment indicated that DM-4107 had no effect on the hERG current up to a concentration of 1×10^{-4}M.

2.6.2.5 Pharmacodynamic Drug Interactions

The effects of oral administration of tolvaptan (1 and 10 mg/kg) in combination with furosemide (10 and 100 mg/kg) on diuresis, serum parameters and hormone levels were investigated in conscious male Sprague-Dawley rats.[68] Compared to the administration of furosemide alone, tolvaptan administered in combination with furosemide dose-dependently increased urine volume and decreased urine osmolality during 0 to 4 hours postdosing. Free water clearance remained negative after administration of furosemide alone, but was elevated to a positive value after combination with tolvaptan. Although the administration of furosemide alone tended to decrease serum sodium concentrations

at 4 hours postdosing, tolvaptan in combination with furosemide increased serum sodium concentrations in a dose-dependent manner. Tolvaptan administered in combination with furosemide dose-dependently increased serum AVP concentration but did not change serum renin activity and aldosterone concentration. These results suggested that tolvaptan exerted its aquaretic action even in combination with furosemide in rats.

The effects of oral administration of tolvaptan (1 and 3 mg/kg) in combination with furosemide (1 mg/kg) on diuresis, serum parameters and hormone levels were investigated in conscious male beagle dogs.[16] Compared to the administration of furosemide alone, tolvaptan administered in combination with furosemide dose-dependently increased urine volume and decreased urine osmolality during 0 to 6 hours postdosing. Free water clearance remained negative after administration of furosemide alone, but was elevated to a positive value after combination with tolvaptan. Electrolyte excretion slightly increased, but the increase was smaller than that after administration of furosemide alone at the dose which induced an equivalent urine volume. The decreases in serum osmolality and sodium concentration observed after administration of furosemide alone were significantly inhibited by combined administration. There was no difference in serum AVP level or serum renin activity between the group treated with furosemide alone and the groups that received combined administration. These results suggested that tolvaptan exerted its aquaretic action even in combination with furosemide in dogs, similar to the results obtained in rats.

The effects of oral administration of tolvaptan (3 mg/kg) in combination with furosemide (1 mg/kg) on diuresis, serum parameters and hormone levels were investigated in conscious male beagle dogs with congestive heart failure induced by ventricular rapid pacing.[69] Oral administration of tolvaptan alone and furosemide significantly increased urine volume and decreased urine osmolality. The combination of tolvaptan and furosemide increased urine volume more than each drug alone. Urine osmolality was lower than plasma osmolality in both the tolvaptan alone group and the combination group. Free water clearance was elevated to positive, showing that tolvaptan, even when administered in combination with furosemide, selectively increased the urinary excretion of free water. Urinary sodium and potassium excretion were slightly but significantly increased by tolvaptan alone, although these increases were much less than those produced by furosemide alone. Tolvaptan in combination with furosemide further increased urinary sodium and potassium excretion. Plasma sodium concentrations were significantly increased in the tolvaptan group in comparison with the control group. Tolvaptan did not significantly change plasma AVP, ANP, norepinephrine, or

epinephrine levels or PRA, but furosemide significantly increased PRA and epinephrine levels. The combination of both drugs produced no additional increases.

Table 2.6.2.7-1 Binding Affinities of Tolvaptan for Human AVP Receptor Subtypes

	K_i (nM)		
	V_2	V_{1a}	V_{1b}
Tolvaptan	0.43 ± 0.06 (5)	12.3 ± 0.8 (6)	NC (4)
YM-087 (Conivaptan)	3.10 ± 0.27 (6)	1.81 ± 0.45 (6)	—
SR-121463A	2.46 ± 0.18 (6)	1908 ± 303 (6)	—
VPA-985 (Lixivaptan)	1.79 ± 0.14 (6)	494 ± 128 (6)	—
AVP	0.78 ± 0.08 (5)	0.84 ± 0.08 (6)	0.59 ± 0.05 (4)

Values are expressed as means ± SE. Numbers of experiments are shown in parenthesis. NC = K_i values were not calculated because more than 50% inhibition was not seen at tested maximal concentrations.

Source: Otsuka Report Nos. 009827, 011324, 011622, 012103, 012383

Table 2.6.2.7-2 Binding Affinities of Optical Isomers and Metabolites of Tolvaptan for Human, Rat, and Canine AVP V2-Receptors

	K_i (nM)		
	Human	Rat	Dog
Tolvaptan	0.43 ± 0.06 (5)	1.33 ± 0.26 (4)	0.66 ± 0.09 (4)
R-(+)-OPC-41061	0.47 ± 0.10 (5)	1.55 ± 0.43 (5)	0.94 ± 0.13 (5)
S-(-)-OPC-41061	0.47 ± 0.07 (5)	0.77 ± 0.12 (5)	0.86 ± 0.10 (5)
DM-4103	—	13535 ± 1951 (4)	—
DM-4104	—	—	—
DM-4105	—	—	—
DM-4107	1993 ± 120 (5)	21330 ± 6974 (4)	—
DM-4110	0.98 ± 0.13 (5)	5.32 ± 0.53 (4)	4.39 ± 0.43 (4)
DM-4111	1.66 ± 0.16 (5)	5.59 ± 0.61 (4)	7.37 ± 0.73 (4)
DM-4119	1.78 ± 0.06 (4)	14.55 ± 3.69 (4)	6.88 ± 0.78 (4)
MOP-21826	1.46 ± 0.17 (5)	74.61 ± 13.19 (4)	21.9 ± 3.3 (4)

Table 2.6.2.7-2	Binding Affinities of Optical Isomers and Metabolites of Tolvaptan for Human, Rat, and Canine AVP V2-Receptors		
	K_i (nM)		
	Human	Rat	Dog
DM-4128	89.7 ± 21.5 (4)	360 ± 12 (4)	792 ± 60 (4)
DM-4129	676 ± 222 (4)	—	—
DM-4130	—	—	—
DM-4131	885 ± 278 (4)	3298 ± 183 (4)	—
DM-4132	2294 ± 575 (4)	—	—
DM-4133	—	—	—

Values are expressed as means ± SE. Numbers of experiments are shown in parenthesis. —= K_i values of some metabolites were not calculated because more than 50% inhibition was not seen at tested maximal concentrations.
Source: Otsuka Report Nos. 009827, 009867, 011360, 012382, 013854, 015477, 015668, 017961

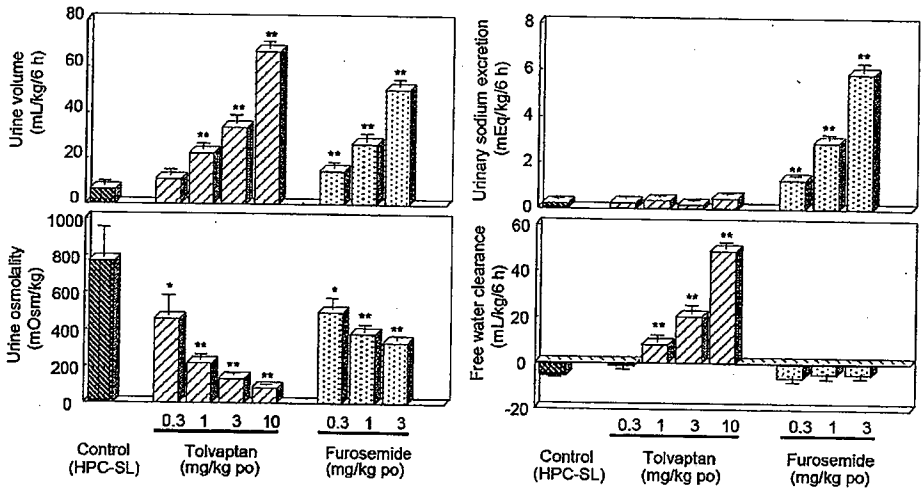

Figure 2.6.2.7-1 Diuretic Effects of Single Oral Administration of Tolvaptan and Furosemide During 6 Hours Postdosing in Male Conscious Beagle Dogs

Each dog (n=6) received vehicle (hydroxypropylcellulose, HPC-SL) and all doses of tolvaptan and furosemide at about 1-week intervals. Values are expressed as the mean ± S.E. Differences between the tolvaptan or furosemide treatments and the HPC-SL treatment (control) were analyzed by ANOVA with a randomized block design, followed by a two-tailed Dunnett's multiple-comparison test. *P < 0.05, **P < 0.01 vs control.
Source: Otsuka Report No. 010842

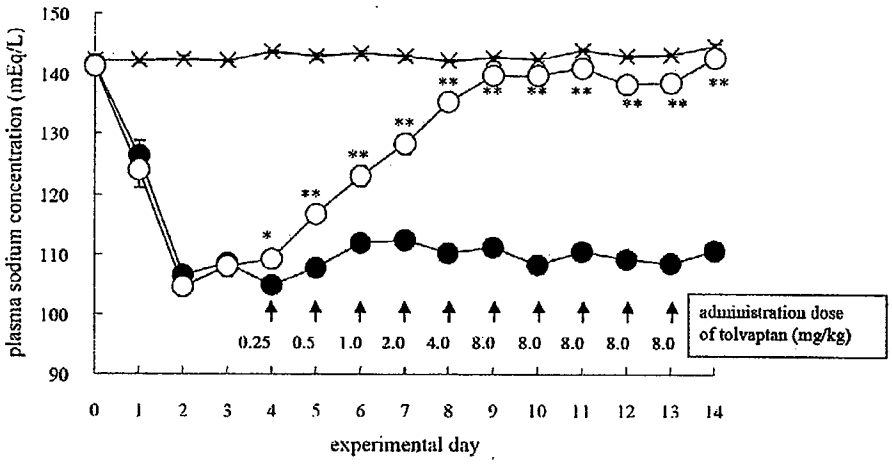

Figure 2.6.2.7-2 Effects of Tolvaptan on Plasma Sodium Levels in Chronic Hyponatremia Rats

Tolvaptan or vehicle were administered from Day 4 to Day 13. The differences between the vehicle- and tolvaptan-treated groups were analyzed by repeated measures ANOVA, followed by a two-tailed t test at each time point. *P < 0.05 **P < 0.01 vs. vehicle. Symbols = saline-infused (×), vehicle (•), and tolvaptan (○).

Source: Otsuka Report No. 015404

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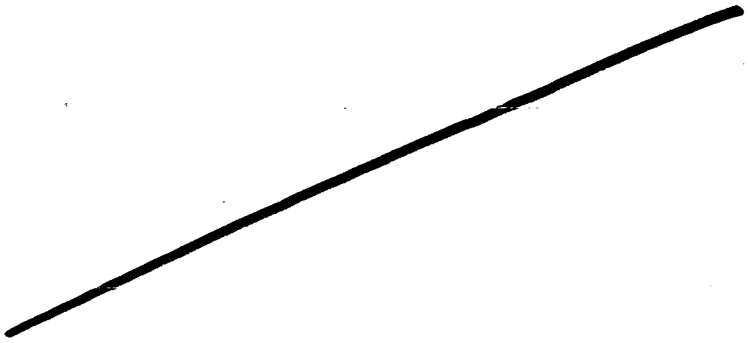

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PHARMACOKINETICS/TOXICOKINETICS

[Sponsor's summaries of pharmacokinetic/toxicokinetic studies are provided below.]

2.4.3.1 Absorption

Jet-milled tolvaptan was initially used for pharmacokinetic studies in Sprague-Dawley rats,[32,33] New Zealand White rabbits,[34] and beagle dogs.[35,36] The time courses of the serum concentration of the unchanged compound were determined after a single oral dose at 1 to 1000 mg/kg to male and female rats, male rabbits, and male and female dogs. The compound was rapidly absorbed with a time to reach maximum concentration (t_{max}) of 4 hours or less. The maximum concentration (C_{max}) and area under the concentration-time curve (AUC) increased with dose increment. The serum concentration of tolvaptan was the highest in female rats, similar among male and female dogs and male rabbits, and lowest in male rats.

In comparison, a fine-granule formulation of [redacted] tolvaptan was used for pharmacokinetic studies in rats and dogs.[37,38,39,40] In male and female rats, the C_{max} and AUC increased dose-dependently, and serum concentration of tolvaptan was higher in females than in males. The results from pharmacokinetic studies demonstrated that [redacted] tolvaptan exhibited higher exposure than jet-milled tolvaptan after an oral administration in rats. To compare the pharmacokinetics of the [redacted] formulation of tolvaptan with the jet-milled formulation in dogs, the serum concentration of tolvaptan was determined after single oral administration of [redacted] tolvaptan at 0.67, 2, 6.7,

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and 20 mg/kg to fasted male beagle dogs. The C_{max} and AUC increased dose-dependently in the range of 0.022 to 0.326 μg/mL and 0.072 to 1.517 μg·h/mL, respectively. Both C_{max} and AUC showed higher values for the [redacted] formulation in male dogs. The results thus demonstrated that the exposure of tolvaptan was higher when administered in [redacted] formulation in both rats and dogs.

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The absolute oral bioavailability of tolvaptan determined after administration of 30 mg/kg of jet-milled and [redacted] formulations in male rats was 0.63% and 16%.[38,41] The bioavailability of jet-milled tolvaptan and [redacted] tolvaptan was compared after single oral administration of 1000 mg/kg to fasted male and female beagle dogs.[40] The C_{max} and AUC of [redacted] tolvaptan were 1.91- and 6.90-times higher than the respective values of jet-milled tolvaptan. Further studies showed that the absolute bioavailability of [redacted] tolvaptan and jet-milled tolvaptan in fasted male dogs were 14.6% and 2.0%, respectively.[35,39,42]

The effect of food on absorption of tolvaptan was investigated by oral administration of tolvaptan to male rats[43] and male dogs.[35] The serum concentration of tolvaptan in non-fasted male rats after single oral administration of jet-milled tolvaptan at 30, 100, 300, and 1000 mg/kg was lower than that in fasted rats.[32] The serum concentrations of tolvaptan were also determined after single oral administration of jet-milled tolvaptan at 30 mg/kg in non-fasted male beagle dogs.[35] The exposure of tolvaptan was about 2.5-times higher in non-fasted dogs than in fasted dogs. Following 14-day repeated oral doses of tolvaptan at 30 mg/kg/day to male beagle dogs,[44] the daily time-course of the serum concentration of the unchanged compound showed similar patterns throughout the dosing period, indicating no changes in the serum pharmacokinetics of tolvaptan after repeated oral doses.

After single intravenous administration of 1, 3, 10, and 30 mg/kg to male rats,[41] the time courses of the serum concentrations of tolvaptan were evaluated. The elimination half-life was 0.50 to 0.81 hour and total body clearance ranged from 4163 to 6369 mL/kg/h. The AUC increased with dose increment ranging from 0.214 to 7.207 μg·h/mL.

Pharmacokinetic studies using [^{14}C]tolvaptan after a single oral dose of 30 mg/kg to male and female rats[45,46] and male dogs[47] showed that the serum concentration of radioactivity peaked at 2 to 4 hours postdose with C_{max} values of 4.441 and 7.739 μg eq/mL, respectively, in male and female rats and 6.193 μg eq/mL in male dogs. The values of elimination half-life were 4.4 and 6.4 hours, respectively, for male and female rats and

4.8 hours for male dogs. The ratio of serum concentration of unchanged compound to that of the radioactivity was the highest in female rats, followed in descending order by male dogs and male rats. Following 14-day repeated oral doses of [^{14}C]tolvaptan at 30 mg/kg/day to male rats,[48] the blood concentration of radioactivity gradually increased, reaching a plateau on the 12th dosing day. The concentration decreased gradually after the final dosing.

2.4.3.2 Distribution

The volume of distribution in male rats following a single intravenous dose of tolvaptan at 1 to 30 mg/kg[41] ranged from 3400 to 5002 mL/kg, suggesting extensive extravascular distribution of the unchanged compound in rats. The volume of distribution value in dogs (3526 mL/kg) was also high.[42] Distribution of tolvaptan in the kidney was studied following single oral administration of the compound at 10 and 100 mg/kg in female rats.[49] The renal concentration of tolvaptan increased dose-dependently. The changes of tolvaptan concentration in the kidney and serum with time were similar. The data showed that tolvaptan in the kidney was about 4-times higher than that in serum.

After a single oral dose of [^{14}C]tolvaptan at 30 mg/kg to male and female rats,[50,51] the radioactivity was high in the liver, stomach, small intestine, adrenals, large intestine, and kidneys in both male and female rats, and was also high in the pituitary gland, Harder's gland, mandibular gland, heart, lung, and pancreas in female rats. In pregnant female rats, the concentration of radioactivity was low in the fetus and amniotic fluid on Day 18 of gestation.[52] Following 14-day repeated oral doses of [^{14}C]tolvaptan at 30 mg/kg/day to male rats,[53] although the concentration of radioactivity showed distribution patterns similar to those in the single dose study in rats,[50] the elimination of radioactivity from the tissues was slower than in the single dose study. The transfer rate of radioactivity to the blood cells was 32.3% or lower after a single oral dose of [^{14}C]tolvaptan at 30 mg/kg to male and female rats[45,46] as well as to male dogs.[47]

Tolvaptan binds extensively to plasma proteins (97.2% or higher) in vitro in mouse,[54] rat,[55] rabbit,[54] dog,[56] and human[57] plasma as determined by the ultrafiltration method. The extent of binding was independent of drug concentration in test species. The ex vivo protein binding of tolvaptan in rats[55] and dogs[56] was 93.0% or higher, and was similar to the protein binding determined by ultrafiltration in vitro, indicating that the presence of metabolites in the plasma did not affect the protein binding of tolvaptan. The metabolites DM-4103 and DM-4107 were also extensively bound (≥ 98.5%) to human plasma

proteins.[58] Tolvaptan and its metabolites DM-4103 and DM-4107 bound to human plasma proteins remained high (≥ 98.3%) in the presence of concomitant drugs, furosemide (1 μg/mL), spironolactone (0.5 μg/mL), propranolol (0.05 μg/mL), dispyramide (2 μg/mL), lidocaine (5 μg/mL), and warfarin (10 μg/mL). These data suggest that a potential for drug-drug interactions via competitive displacement from protein binding site(s) was low. Furthermore, no effect of tolvaptan and DM-4103 on protein binding of propranolol, lidocaine, or spironolactone in human plasma was found.[59]

2.4.3.3 Metabolism

In mice,[60] rats,[61,62,63] rabbits,[63,64] dogs,[65] and humans,[66,67,68,69,70,71] tolvaptan is metabolized primarily by 3 major biotransformation pathways: dehydrogenation, hydroxylation, and deamidation. These pathways generate a number of metabolites that have been identified in both in vitro and in vivo biotransformation studies. Structural characterization of the metabolites of tolvaptan from major metabolic reactions revealed one hydroxide of the benzazepine ring (DM-4110) and its isomers (DM-4111 and DM-4119), one oxide of the hydroxy group at the 5' position (MOP-21826), one metabolite (DM-4128) resulting from deamidation, and 4 metabolites with the benzazepine ring cleaved (DM-4103, DM-4104, DM-4105, and DM-4107). Proposed metabolic pathways of tolvaptan in both animals and humans are shown in Figure 2.4.3.3-1. Based on in vitro studies with recombinant human cytochrome P450 isoforms, CYP3A4 has been identified to be responsible for the primary metabolic reactions.[66,69] In addition, CYP1A1 is also found to catalyze the formation of DM-4128 from tolvaptan.[69] Other CYP isoforms, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CY2E1 are not involved in tolvaptan metabolism. Tolvaptan and its major metabolites in humans, DM-4103 and DM-4107, do not significantly inhibit CYP1A2, CYP2D6, CYP3A4, CYP2C19, and CYP2E1 in vitro,[72] or induce drug metabolizing enzymes in vivo[73] at clinically relevant concentrations.

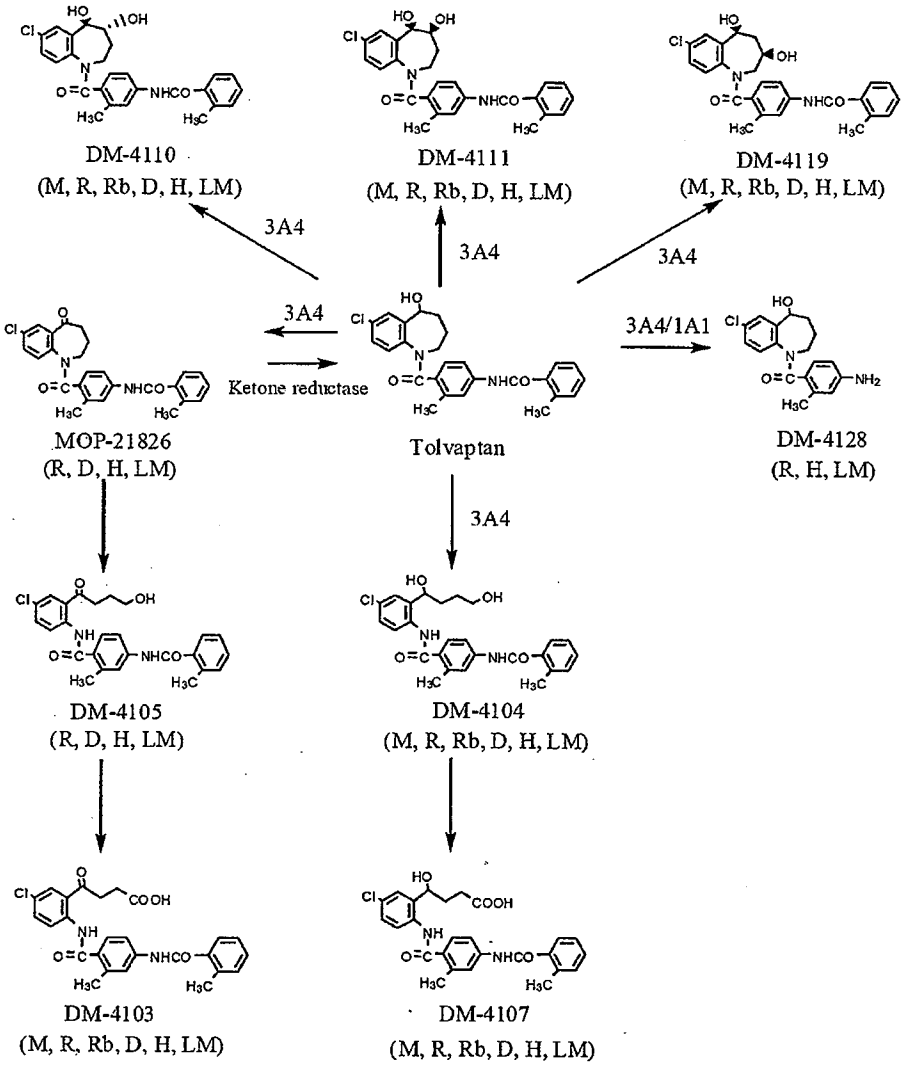

Figure 2.4.3.3-1 Proposed Metabolic Pathways of Tolvaptan in Animals and Humans

D = dog; H = human. LM = liver microsomes. M = mouse, R = rat. Rb = rabbit

Source: Otsuka Report Numbers 010659, 010760, 011240, 011691, 014276, 016298, 016882, 016940, 016951, 016953, 017062, and 156-97-202

In a single oral dose study in rats (30 mg/kg), the metabolites DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, DM-4111, and MOP-21826 were found in the serum.[74] In male rats, the serum concentrations of metabolites DM-4103, DM-4107, and DM-4110 were higher than that of the unchanged compound, but in female rats, the serum concentration of the unchanged compound was higher than that of all metabolites, indicating a sex difference in the metabolism of tolvaptan in rats. Metabolites seen in the serum of rabbits[63] and dogs[75] after a single oral dose of tolvaptan (30 mg/kg) were similar to those found in rats.[63]

After single oral administration of tolvaptan in dogs (30 mg/kg), 6 metabolites (DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, and DM-4111) were detected in the serum.[75] When compared with the unchanged compound, DM-4104 (180%) showed a higher serum maximum concentration (C_{max}), and DM-4111 (100%) and DM-4110 combined with an unknown metabolite (94%, identified later as DM-4119) showed similar serum concentrations. The C_{max} of DM-4103, DM-4105, and DM-4107 were lower than that of the unchanged compound. The area under the serum concentration-time curve from 0 to 24 hours (AUC_{0-24h}) of DM-4104, DM-4110 (combined with an unknown metabolite, identified later as DM-4119), DM-4111, and DM-4105, was 256%, 174%, 172%, and 130% of the unchanged compound, respectively, while the AUC_{0-24h} values of DM-4103 and DM-4107 were lower than that of the unchanged compound.

In single oral dose studies with [^{14}C]tolvaptan (30 mg/kg) in male mice, rats, rabbits and dogs, serum concentrations of tolvaptan and its metabolites were evaluated. At 2 hours after administration, the rank order of the serum radioactivity concentration in mice was DM-4110 > DM-4111 > DM-4103 > DM-4139 (4-*O*-glucuronide of DM-4110) > DM-4121 (3-hydroxy-5-oxobenzazepine derivatives) > DM-4119 > tolvaptan, followed by 4 metabolites.[60] The percent of the serum radioactivity of the identified metabolites was 75.5%. Each unknown metabolite represented less than 3.56% of the total serum radioactivity. The rank order of the serum radioactivity concentration at 2 hours in rats was DM-4103 > tolvaptan > DM-4121 > DM-4119 > DM-4139, followed by 16 metabolites.[62] The percent of the serum radioactivity of tolvaptan and the identified metabolites was 85.3%. Each unknown metabolite accounted for less than 1.87% of the total serum radioactivity. The rank order of the serum radioactivity concentration at 2 hours in rabbits was DM-4103 > DM-4107 > DM-4119 > unknown-4 > DM-4139 > tolvaptan, followed by 12 metabolites.[64] The percent of the serum radioactivity of the identified metabolites was 57.6%. Each unknown metabolite represented less than 5.34%

of the total serum radioactivity. At 2 hours after administration, the rank order of the serum radioactivity concentration in dogs was tolvaptan > DM-4104 > DM-4111 > DM-4103 > DM-4119, followed by 9 metabolites.[65] The percent of the serum radioactivity of the identified metabolites was 58.1%. Each unknown metabolite represented less than 4.07% of the total serum radioactivity. The biotransformation profile of tolvaptan and its metabolites in rat bile was evaluated after single oral administration of 30 mg/kg [^{14}C]tolvaptan to fasted male rats.[62] The radioactivity of tolvaptan and the identified metabolites in the bile accounted for 39.1%, while each unknown metabolite represented less than 10.1% of the total bile radioactivity.

The pharmacokinetics of isomers of (R)-(+)- and (S)-(-)-OPC-41061 was investigated in rats,[76] rabbits,[77] and dogs.[78,79] Following single oral doses of [redacted] tolvaptan at 10 and 1000 mg/kg to male and female rats and at 30 mg/kg to male rabbits, the serum concentration of (R)-(+)-OPC-41061 was higher than that of (S)-(-)-OPC-41061. Following single oral doses of 10 and 1000 mg/kg tolvaptan to dogs, the serum concentration of (S)-(-)-OPC-41061 was higher than that of (R)-(+)-OPC-41061. The transformation of (R)-(+)-OPC-41061 to (S)-(-)-OPC-41061 was observed to a slight degree, but transformation of (S)-isomer to (R)-isomer was not observed.

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In metabolism studies in humans following a single dose of 60 mg [^{14}C]tolvaptan,[71] tolvaptan and 7 of its metabolites (DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, DM-4111 and DM-4119) were detected in the plasma. Tolvaptan and these metabolites accounted for 60.42% of the plasma radioactivity (based on mean AUC). DM-4103 alone accounted for 52.47% of the plasma radioactivity, whereas unchanged tolvaptan accounted for 2.84% and the other metabolites combined accounted for 5.11%. Tolvaptan and identified metabolites accounted for 71.23% of the radioactivity excreted in urine for up to 36 hours. The major metabolite in urine was DM-4107, which accounted for 23.28% of the radioactivity, followed by DM-4111, which accounted for another 14.09%. Tolvaptan and the other metabolites together accounted for 35.29% of the radioactivity. Hydrolysis experiments did not detect the presence of conjugates. The balance of radioactivity excreted in urine was due to the presence of other minor unidentified metabolite(s), none of which alone accounted for more than 5% of the radioactivity. No glucuronide conjugates of the parent compound or the identified metabolites were detected. Tolvaptan and identified metabolites accounted for 74.66% of the radioactivity excreted in the feces, based on specimens collected during the first 96 hours postdose. Tolvaptan accounted for 31.91% of radioactivity, followed by DM-4107, which accounted for another 20.55%, whereas the other metabolites combined accounted

for 22.38% of the radioactivity. The balance of radioactivity excreted in feces was due to the presence of other unidentified minor metabolite(s), none of which alone accounted for more than 2% of the radioactivity.

2.4.3.4 Excretion

Tolvaptan was mainly eliminated via metabolic clearance in both animals and humans as determined by the excretion of the metabolites as well as the unchanged compound. Following single oral doses of jet-milled tolvaptan at 10 to 100 mg/kg to male rats[80] and male dogs,[35] the unchanged compound in the urine accounted for less than 0.1% of the administered dose at all doses tested. Following a single oral dose of [^{14}C]tolvaptan at 30 mg/kg to male and female rats[81] and male dogs,[82] 91.7% to 96.6% of the administered dose of radioactivity was excreted in the feces, and 3.9% to 8.1% was excreted in the urine. Approximately 98.9% to 100.4% of radioactivity was excreted in total in both rats and dogs. In rats, 51.4% to 58.1% of the administered dose of radioactivity was excreted via the bile, of which 39.5% underwent enterohepatic circulation. After 14-day repeated oral administration of [^{14}C]tolvaptan at 30 mg/kg/day to male rats,[83] 90.9% of the administered dose of radioactivity was excreted in the feces and 6.5% in the urine. In a single oral dose study of [^{14}C]tolvaptan (60 mg) in humans,[71] a mean of 98.87% of the administered radioactivity was recovered in urine (40.16%) and feces (58.71%). The primary route of excretion was feces, although urinary radioactivity excretion was also substantial. About 80% of the cumulative urine ^{14}C excretion occurred during the first 36 hours. Detectable quantities of radioactivity were excreted in the feces for up to 960 hours postdose. However, about 65% of the cumulative ^{14}C excretion in feces occurred within the first 72 hours.

2.4.3.5 Toxicokinetics

Dose-related systemic exposures (area under the concentration-time curve calculated to the last observable concentration at time t [$AUC_{[t]}$ and C_{max}) to tolvaptan and its principal human metabolites, DM-4103 and DM-4107, were established in species for nonclinical studies (see Table 2.6.7.3 in the Tabulated Summary of Toxicokinetics: Overview of Toxicokinetics Data).

Exposure to tolvaptan in mice after 4 weeks of daily dosing at up to 100 mg/kg increased with dose increment, but was not directly proportional to dose.[84] The exposure was generally higher in male mice than female mice. The concentrations of DM-4103 and DM-4107 were calculated only for the 100 mg/kg/day dose group in both sexes and were

generally higher in female mice than in male mice. The levels of exposure to tolvaptan increased proportionally to dose in male rats in the range of 30 and 100 mg/kg/day, but sub-proportionally for 1000 mg/kg/day in Week 4, while in female rats the exposure was in a sub-proportional increase with dose.[85,86] Exposure to DM-4103 and DM-4107 in rats increased with dose. The levels of exposure to tolvaptan were generally higher in female rats than in male rats, while exposure to the principal metabolites of tolvaptan was higher in male rats than in female rats. In a 52-week oral toxicity study,[87] dogs were exposed to tolvaptan at 30, 100 and 1000 mg/kg and the serum concentrations of tolvaptan and its metabolites in humans, DM-4103 and DM-4107, were evaluated. Exposure to tolvaptan and its metabolites in Week 52 increased with dose increment in both male and female dogs. There were no significant gender-related differences for exposure to tolvaptan, DM-4103, and DM-4107 in dogs.

Overall, the animal species used for toxicology studies were generally exposed to tolvaptan and the principal human metabolites to a greater extent than humans given the anticipated maximum recommended human dose (MRHD) of 60 mg.

[Systemic exposure (AUC) values, obtained in toxicology studies, are given in the Table below.]

Table 2.6.7.3-2 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: Tolvaptan

Method of Administration	Daily Dose (mg/kg)	Sampling Time (Week)	AUC (μg·h/mL) of OPC-41061									
			Mice		Rats		Dogs		Rabbits	Monkeys	Humans	Report No.
			M	F	M	F	M	F	F	M & F	M & F	
Oral (gavage)	1000 JM	4			1.221	13.033						009965
Oral (gavage)	2000 JM	1 (Day 3)			6.554	38.672						009965
Oral (gavage)	3	4	0.282	NC								013773
Oral (gavage)	10	4	1.008	0.236								013773
Oral (gavage)	10	4	0.428	0.181								014252
Oral (gavage)	30	4	1.379	0.895								014252
Oral (gavage)	30	4	3.506	1.380								013773
Oral (gavage)	30	4			2.210	15.904						013774
Oral (gelatin capsule)	30	52					9.79	6.78				012707
Oral (gavage)	60	4	2.860									014252
Oral (gavage)	100	2 (Day 11)[a]				28.671						012903
Oral (gavage)	100	2 (Day 13)							6.480			009894
Oral (gavage)	100	2 (Day 13)[b]							3.882			012779

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Table 2.6.7.3-2 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: Tolvaptan

Method of Administration	Daily Dose (mg/kg)	Sampling Time (Week)	AUC (μg·h/mL) of OPC-41061									
			Mice		Rats		Dogs		Rabbits	Monkeys	Humans	Report No.
			M	F	M	F	M	F	F	M & F	M & F	
Oral (gavage)	100	4	8.304	9.308								013773
Oral (gavage)	100	4		4.332								014252
Oral (gavage)	100	4			7.764	20.700						013774
Oral (gelatin capsule)	100	52					31.45	42.35				012707
Oral (gavage)	300	1 (Day 3)[c]							17.019			013578
Oral (gavage)	300	2 (Day 11)[a]				63.778						012903
Oral (gavage)	300	2 (Day 13)							14.150			009894
Oral (gavage)	300	2 (Day 13)[b]							8.117			012779
Oral (gavage)	300	3			15.373	23.618						010255
Oral (gavage)	300	4			7.775	24.669						014253
Oral (gavage)	1000	1 (Day 3)[c]							48.343			013578
Oral (gavage)	1000	2 (Day 11)[a]				113.779						012903
Oral (gavage)	1000	2 (Day 13)							18.268			009894
Oral (gavage)	1000	2 (Day 13)[b]							16.924			012779

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Table 2.6.7.3-2 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: Tolvaptan

Method of Administration	Daily Dose (mg/kg)	Sampling Time (Week)	AUC (μg·h/mL) of OPC-41061									
			Mice		Rats		Dogs		Rabbits	Monkeys	Humans	Report No.
			M	F	M	F	M	F	F	M & F	M & F	
Oral (gavage)	1000 [redacted]	3			19.605	15.937						010255
Oral (gavage)	1000 [redacted]	4			12.716	33.449						013774
Oral (gelatin capsule)	1000 [redacted]	52					270.57	227.87				012707
Oral (gavage)	500[d] [redacted]	Single dose				373.680[e]						017325
Oral (gavage)	500[f] [redacted]	Single dose				179.230[e]						017325
Oral (gavage)	500 [redacted]	Single dose				69.605						017325
Oral (gavage)	2000 [redacted]	Single dose				254.084						017325
Tablets	60 mg	2 (Day 10)									4.356 (M, Young)	156-98-202
Tablets	60 mg	2 (Day 10)									3.773 (F, Young)	156-98-202
Tablets	60 mg	2 (Day 10)									4.047 (M, Elderly)	156-98-202
Tablets	60 mg	2 (Day 10)									5.015 (F, Elderly)	156-98-202
Intra-venous	0.0385	Single dose								0.031 (M)		017580

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Table 2.6.7.3-2 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: Tolvaptan

Method of Administration	Daily Dose (mg/kg)	Sampling Time (Week)	AUC (μg·h/mL) of OPC-41061									
			Mice		Rats		Dogs		Rabbits	Monkeys	Humans	Report No.
			M	F	M	F	M	F	F	M & F	M & F	
Intra-venous	0.0385	Single dose								0.026 (F)		017580
Intra-venous	0.111	Single dose								0.091 (M)		017580
Intra-venous	0.111	Single dose								0.092 (F)		017580
Intra-venous[g]	0.12	Single dose			0.009	0.015						017265
Intra-venous[g]	0.33	Single dose			0.072	0.141						017265
Intra-venous	0.333	Single dose								0.301 (M)		017580
Intra-venous	0.333	Single dose								0.222 (F)		017580
Intra-venous[g]	1.0	Single dose			0.303	0.557						017265

AUC values reported generally as 0-24 h values. Elderly = healthy male and female subjects ages 65 or older; JM = Jet-milled formulation; [redacted] formulation; Young = healthy male and female subjects ages 18 to 45 years old.

[a] On gestation Day 17; rats were dosed from gestation Day 7 to 17.

[b] On Day 13 of dosing; rabbits were dosed from gestation Day 6 to 18.

[c] On gestation Day 11; rabbits were dosed from gestation Day 9 to 11.

[d] (R)-(+)-OPC-41061 500 mg/kg

[e] The value was the sum of the concentrations of (R)-(+)-OPC-41061 and (S)-(-)-OPC-41061.

[f] (S)-(-)-OPC-41061 500 mg/kg

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GENERAL TOXICOLOGY

Acute Oral Toxicity Studies

Single dose oral administration of tolvaptan to Sprague-Dawley rats or beagle dogs at doses up to 2000 mg/kg resulted in no treatment-related mortalities, clinical signs or macroscopic findings (13- or 14-day observation period).

Chronic Oral Toxicity Studies

Repeat dose oral toxicity studies were conducted in dogs and rats for up to 52 and 26 weeks, respectively.

Fifty-two Week Oral Toxicity Study in the Dog With 5-Week Recovery Period

Testing Facility: [redacted] b(4)

Study Number: Main study - 275/88 / [redacted]
Supplementary study – 275/106 [redacted]

Report Number: 012707 (Sponsor's #)

Study Dates: Main study – Initiation of treatment – June 14, 1996
Completion of necropsy – June 15, 1997 &
July 18, 1997 (recovery group)

Supplementary study – Initiation of treatment – September 20, 1996
Completion of necropsy – September 19, 1997

GLP Compliance: The study was performed in accordance with GLP regulations. (Final quality assured study report submitted.)

Animals: Purebred beagle dogs, 4 (low and mid dose groups) or 6 (control and high dose groups)/sex/group were obtained from [redacted]. Two dogs/sex from the control and high dose groups were maintained for a 5-week recovery period following 52 weeks of dosing. b(4)

It is stated that due to "significant reduction in food consumption and a more than 20% decrease in body weight during Weeks 1 and 2, one high dose female (animal # 35) was removed from dosing in Week 3", and was replaced by another female (# 41) at the beginning of Week 5 for 52 weeks of treatment.

Due to the deaths of 2 low dose females (#s 27 & 29) during dosing in Week 7, a supplementary 52-week study with 2 groups (control and low dose) of females (4/group) was initiated, to support the data obtained for the two remaining low dose females in the main study.

The dogs were housed singly during the day and, where possible, animals of the same group and sex were housed together overnight. The animals, fed daily with about 400 g of SQC Diet A with ad libitum drinking water, were about 7-9 months old (males weighed 6.65 to 10.15 kg and females 5.40 to 9.60 kg) at the onset of treatment.

Dose Levels and Mode of Administration: 0, 30, 100 and 1000 mg/kg/day for 52 weeks.

(It is stated that "the high dose was intended to produce target organ toxicity or overt toxicity and the intermediate dose a dose response effect. The low dose was to produce no toxicity.")

The test article, a [redacted] powder, (Batch numbers 6D73SD, 6G79SD and 6H99SD; each batch of the test article contained 66.7% OPC-156 and 33.3% amorphous hydroxypropyl cellulose) was administered orally, once daily, in gelatin capsules. Control animals received empty gelatin capsules. (It is stated that post-dosing vomiting seen on day 1, caused by the large number of capsules administered to control and high dose animals, was controlled by administering the capsules in 2 lots, with a suitable short gap between dosing, starting day 2 of the study.) b(4)

It is stated that the test article was stable throughout the study period.

Observations/Measurements: All animals were observed daily for signs of ill health or overt toxicity. Clinical signs were observed before dosing, immediately after dosing, at mid-day and at the end of the working day. All animals were examined for morbidity and mortality at the beginning and end of the working day. Each animal was given a detailed physical examination at weekly intervals. Body weight and food consumption were recorded weekly. Weekly water consumption was determined pre-test and during study weeks 12, 25, 51 and 56 (recovery period). Ophthalmoscopic examinations were performed on all animals pre-treatment and during weeks 13, 26 and 52. EKG and body temperature were recorded on all animals pre-test and in weeks 12, 25 and 51. Auditory response was measured pre-test and in weeks 13, 26 and 52.

Blood samples were collected on day 1 at 4 and 24 hours post-dose, and in week 52, pre-dose and at 2, 4, 8 and 24 hours post-dose for toxicokinetic evaluations.

Hematology [RBC, WBC (total and differential), platelet and reticulocyte counts, hemoglobin, hematocrit, MCV, MCH, MCHC, prothrombin time and activated partial thromboplastin time] and clinical chemistry (AST, ALT, alkaline phosphatase, gamma glutamyl transferase, lactate dehydrogenase, sodium, potassium, calcium, inorganic phosphorus, chloride, total protein, albumin, globulin, protein fractions, total cholesterol, triglycerides, phospholipids, glucose, urea, total bilirubin and creatinine) evaluations were performed on all animals pretest and during study weeks 13, 26, 52 and 57. Urinalysis (including quantitative determinations of urine sodium, potassium, calcium, chloride and creatinine) was performed on all animals pretest and during weeks 12, 25, 51 and 56.

Complete necropsies were performed on all animals including those that died or were sacrificed in extremis. Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, spleen, testes, epididymides, thymus, thyroids and parathyroids and uterus were weighed. Samples of the following tissues and all gross lesions were fixed in 10% neutral buffered formalin, with the exception of eyes and optic nerves which were fixed in Davidson's fluid: adrenals, aorta, brain, cecum, colon, duodenum, eyes and optic nerves, esophagus, femur with marrow and articular surface, gall bladder,

heart, ileum, jejunum, kidneys, lacrimal glands, liver, lungs, mammary glands, mandibular and mesenteric lymph nodes, muscle, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerves, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes and epididymides, thymus, thyroids and parathyriods, tongue, treacha, urinary bladder, uterus and vagina. All the above tissues were processed for light microscopy.

Bone marrow smears were prepared but not examined.

Data on body weight, clinical chemistry, hematology and urine parameters were analyzed using two-way analysis of variance (ANOVA). Pairwise comparisons, for each sex, were made using Dunnett's test. A regression test was performed to determine whether there was a linear relationship between increasing dose and response. Levene's test for equality of variances across groups, between sexes and for any interaction was also performed. When Levene's test showed evidence of group effects or a sex-group interaction, the data were reanalyzed after applying a log-transformation or using non-parametric methods. The non-parametric methods employed were the Kruskal-Wallis ANOVA, the Terpstra-Jonckheere test for a dose-related trend and the Wilcoxon rank-sum test for pairwise comparisons. Organ weights were analyzed using Analysis of Covariance and Dunnett's test, using the necropsy body weight as covariate.

Supplementary study data were analyzed using a two-sample t-test.

Results: Two low dose females (#s 27F and 29F) died during dosing (due to choking on the capsule*) in study week 7. Two high dose animals (#s 17M and 35F) in week 5 and another high dose female (# 41F) in week 13 were sacrificed due to marked reduction in body weights (more than 20% decrease from the initial weight) and decreased food consumption. According to the sponsor, "there were no macroscopic or microscopic findings in any of the dogs suggestive of specific target organ toxicity likely to be the primary cause of morbidity." Animal #s 17 and 41 had inflammation of the lower intestinal tract.

[* It is stated that the size of the capsules was reduced after the choking accident (correspondence dated August 1, 2001).]

There were no treatment-related clinical signs.

Mean body weight data are presented graphically in Figure 1. Reductions in mean body weights, compared to pre-test body weights, were noted in high dose males (up to 9% reduction) from weeks 1 to 15 of treatment, and in high dose females (up to 7%) from weeks 1 to 27 of treatment. Both groups of animals started to gain weight after the above time periods. At the end of the 52-week treatment period, the mean body weights of high dose male and female groups were 15 and 5% higher, respectively, and the mean body weights of control male and female groups were 10 and 7% higher, respectively, than the corresponding pre-test values.

There were no significant treatment-related effects on body weight in other treatment groups (main and supplementary studies).

At the end of the recovery period, the body weight gains were comparable in the high dose and control groups.

Food consumption of high dose animals was reduced during weeks 1 to 4. Throughout the remainder of the period, except for some individual cases of reduced food consumption, the food consumption of high dose animals was generally comparable to pre-treatment levels. There were no treatment-related effects on food consumption in other groups.

FIGURE 1

Group mean body weight (kg)

Males

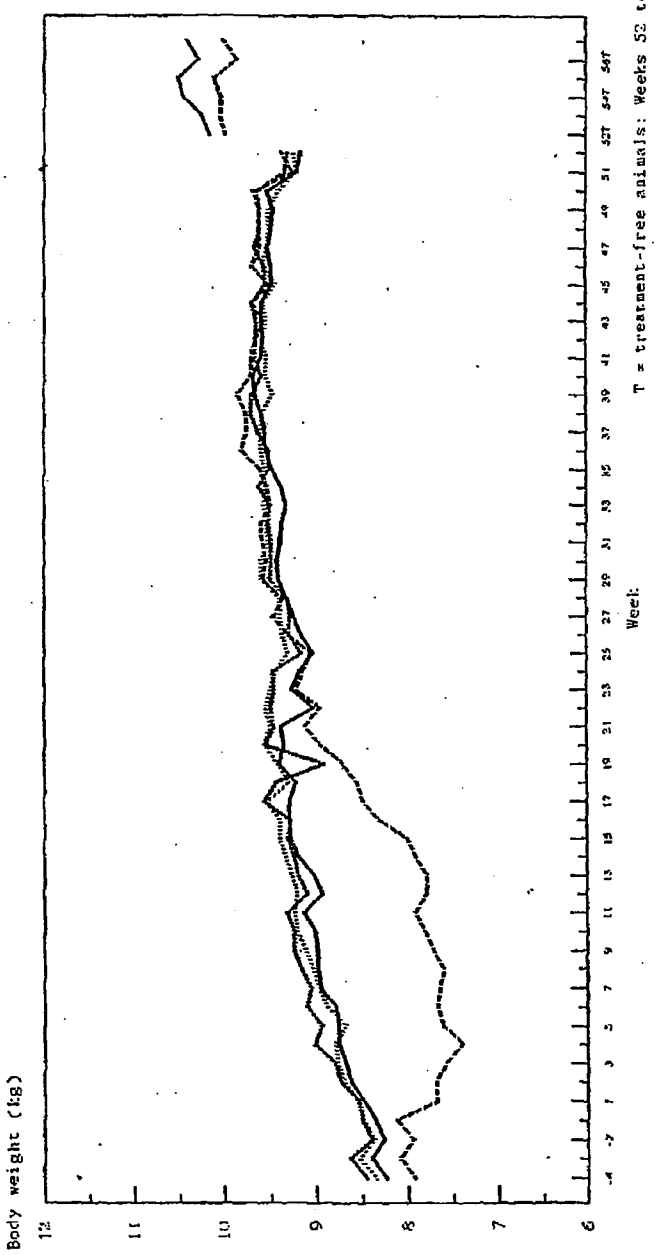
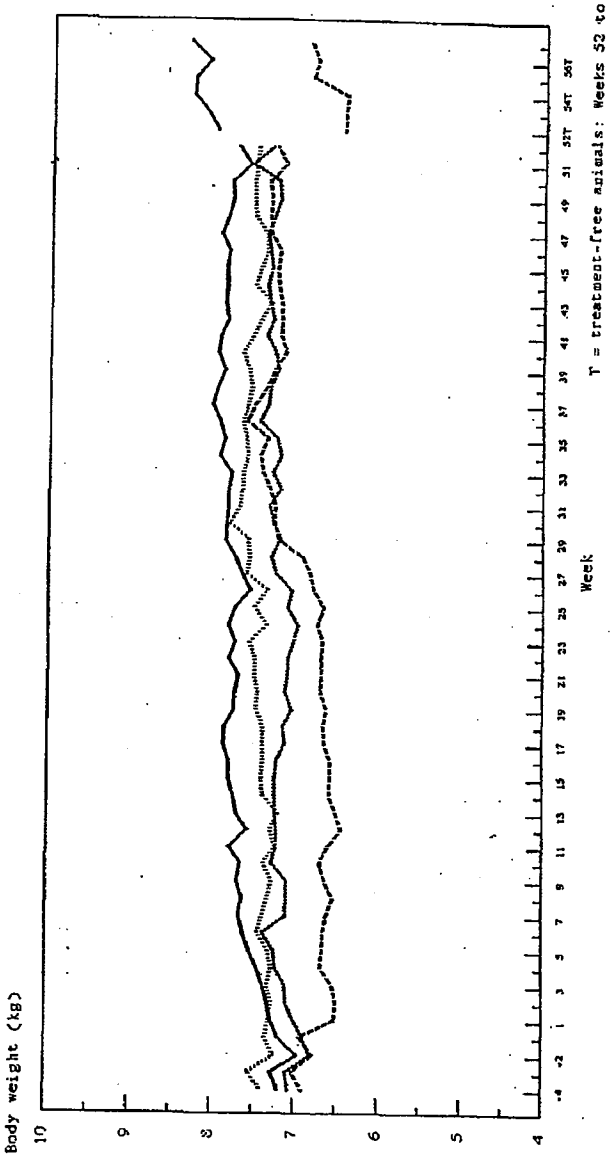


FIGURE 1 (continued)

Group mean body weight (kg)

Females



Water consumption was increased (2 to 4 fold) in all treatment groups throughout the dosing period compared to control or pre-dose values (dose dependent in males but not females). During the recovery period, the high dose water consumption was comparable to control water consumption.

There were no treatment-related effects on auditory response, body temperature, heart rate, EKG and ophthalmoscopic parameters.

Statistically significant reductions in mean hemoglobin concentration (12%), red blood cell count (16%) and packed cell volume (13%), compared to control, were noted in the high dose male group during study week 13. At week 26, although the values for the above parameters remained lower than control values, the differences were not statistically significant except for the red blood cell count. At week 52, the values were comparable to control values. For the high dose female group, significant reductions (compared to control) in mean hemoglobin concentration (14%), RBC count (17%) and packed cell volume (14%) were noted in week 26. At week 52, and also at the end of the recovery period, the values for the above parameters for the high dose female group were lower than control, but the differences did not attain statistical significance. The mean cell volume values for the high dose males were increased at all time points.

Mean alkaline phosphatase levels for the high dose male and female groups were significantly higher than control levels (2 to 4 fold) during treatment weeks 13, 26 and 52. The high dose recovery group values were comparable to control values. The mean total cholesterol, triglycerides and phospholipid levels in high dose male and female groups were increased, compared to control, at all time intervals (weeks 13, 26 and 52). Again the high dose recovery group values were comparable to control values. Total protein and albumin levels for high dose animals were lower than control levels only in week 13.

Significant dose-related increased production of urine (with dose-related reduction in specific gravity) was noted in animals of all treated groups. Individual electrolyte excretion was variable. During week 12, excretion of total potassium, chloride and calcium was generally increased while total sodium excretion was decreased especially in high dose animals. During the recovery period, the volume and specific gravity of urine produced by high dose animals were comparable to that of controls and there were no significant differences in the total electrolyte excretion between these groups.

Decreases in mean heart weight (18-20%), compared to control, were noted in high dose male and female groups, the difference from control being statistically significant ($p<0.01$) for the male group. Dose-dependent increases in mean adrenal weight, compared to control, were seen in mid (36%; $p>0.05$) and high (63%; $p<0.01$) dose female groups. In males, statistically non-significant increases in adrenal weights (20-21%) were seen in mid and high dose groups. After the recovery period, no significant differences in organ weights were seen.

There were no remarkable macroscopic findings in the study.

There were no significant treatment-related microscopic findings except for dose-related reduced adrenocorticocyte vacuolation and/or increased cortical width that were seen in the adrenal gland of mid and high dose males and females. The incidence and the severity of the above adrenocortical findings are given below.

Group incidence of selected adrenal cortical findings

		Terminal kill										Treatment-free			
		Males				Females						Males		Females	
Organ & finding		1M	2M	3M	4M	1F	1F*	2F	2F*	3F	4F	1M	4M	1F	4F
Adrenal	No Exam	4	4	4	3	4	4	2	4	4	3	2	2	2	2
reduced cortical vacuolation	Grade -	4	4	3	2	4	4	2	4	2	0	2	1	2	2
	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	2	0	0	0	1	0	0	0	0	2	3	0	0	0	0
increased cortical width	Grade -	4	4	2	0	4	4	2	1	2	0	2	2	2	2
	1	0	0	2	2	0	0	0	1	2	0	0	0	0	0
	2	0	0	0	1	0	0	0	2	0	3	0	0	0	0

Key: Grade - = finding not present, 1 = minimal, 2 = slight.
* = ancillary study

There were no adrenocortical findings in the low dose animals of the main study; however, increased cortical width was noted in low dose females (3/4) of the supplementary study.

In the high dose recovery group, one male had minimal reduced cortical vacuolation (no other adrenocortical lesions seen), indicating that the adrenocortical effects were mostly reversed after the recovery period.

According to the sponsor, "the no toxic effect level of OPC in this study was 100 mg/kg/ day."

The toxicokinetic parameters (week 52) are summarized below.

Summary of toxicokinetic parameters of OPC-156 in dog serum (CLE 275/88): Week 52 males

Group (Dose)	$AUC_{(0-24h)}$ (ng.h/mL)	T_{max} (h)	C_{max} (ng/mL)	$t_{½}$ (h)
2 (30 mg/kg/day)	9794	4	1551	2.4
3 (100 mg/kg/day)	31451	3	5463	4.4
4 (1000 mg/kg/day)	270568	6	22655	2.8

Summary of toxicokinetic parameters of OPC-156 in dog serum (CLE 275/88 and 275/106): Week 52 females

Group (Dose)	$AUC_{(0-24h)}$ (ng.h/mL)	T_{max} (h)	C_{max} (ng/mL)	$t_{½}$ (h)
2 (30 mg/kg/day)	6777	4	1211	3.4
3 (100 mg/kg/day)	42346	4	6045	2.9
4 (1000 mg/kg/day)	227867	5	20031	2.8

In general, increases in AUC values were proportional with dose in male and female groups except in the mid dose female group, where the AUC value was about twice the value expected. The increases in C_{max} values in both males and females did not appear to be proportional to dose especially at the high dose level. T_{max} and terminal serum elimination half-lives did not seem to vary appreciably with dose or gender.

There were no apparent differences between serum concentrations in Week 1 and Week 52 except the week 1 serum levels in the high dose males and females at 24 hours were more than 10-fold higher than 24 hr serum levels seen in Week 52.

Twenty-six Week Oral Toxicity Study in the Rat With a 5-Week Recovery Period

[This study was reviewed earlier by Dr. Sidney J. Stolzenberg and his review is provided below (review dated July 14, 1998).]

26-Week Oral Toxicity Study in Rats (Study No. 275/87): The study was performed at [redacted] (dosing instituted 5/23/96) in accordance with the following design. b(4)

Group Number	Dose mg/kg/day	Main Study Animals/group Male	Female	Satellite Animals/group Male	Female
1	0	15	15	0	0
2	30	15	15	27	27
3	100	15	15	27	27
4	1000	15	15	27	27

Five additional animals/sex in control and high dose groups were retained for a 5 week treatment-free reversibility evaluation. The stated objective of the study was to test OPC-41061 for toxicity, but the experiment also serves as a dose range finding study to establish dose levels for the carcinogenicity test. The diet employed, strain of rat used and the method of drug administration (oral gavage) were the same as that indicated in the protocol for the carcinogenicity study. The test substance consisted of 66.7% [redacted] OPC-41061 (Batch nos. 5174 [redacted] 6G79[redacted] and 6H99[redacted] and 33.3% amorphous hydroxypropyl cellulose. Dose volume for all 4 groups was 10 ml/kg. Rationale for the doses selected was that "the high dose was intended to produce target organ toxicity or overt toxicity", the mid dose to establish a dose response effect and the low dose was expected to produce no toxicity. b(4)

The rats were housed 5 per cage, and were 7 to 8 weeks of age at initiation of dosing. Body weights of the main study animals ranged between 264.2 and 304.8 g for males and between 187.7 and 217.1 g for females. Main study parameters included twice daily inspections for mortality and daily inspections for clinical signs. Body weights and food consumption (satellite groups included) were obtained weekly up to week 13, then biweekly thereafter; water intake was measured during weeks 12, 25 and 30; ophthalmoscopy was performed on all animals pre-treatment, but on only 5/sex/main study group at week 25. Hematology and clinical

chemistry measurements were determined from orbital sinus blood from 10 rats/sex/group at weeks 13 and 26; urinalyses were conducted on 24 hour collections (started immediately following dosing) from 5 rats/sex/group (food but not water was withheld) in weeks 12 and 25. Gross pathology examinations were performed on all animals (main study and satellite) found dead or in a moribund state. At the week 26 necropsy, for all surviving main study animals on test, organ weights were determined for liver, spleen, adrenals, kidneys, thymus, prostate, testes and epididymides, ovaries, uterus, heart, lungs, thymus thyroid/parathyroids, pituitary and brain. Tissues were collected and preserved from all main study animals. Histopathologic examinations were performed on control, high dose and decedent animals of the main study. Bone marrow smears were collected from all main study animals at necropsy but were not examined. Liver and kidney samples from 2 animals/sex/group were prepared for electron microscopy but there was no data to indicate that they were examined. Toxicokinetics were derived from serum of orbital sinus blood collected from animals in the satellite groups on Day 1 and after 4 and 26 weeks; on Day 1 and in Week 26, at 4 and 24 hours after dosing; in Week 4, immediately prior to dosing and 2, 4, 6 and 24 hours after dosing. Animals in satellite groups that survived were discarded without necropsy.

In the main study, the following parameters were evaluated:

Hematology

- Hemoglobin
- Hematocrit
- Red blood cell count
- Platelet count
- Mean cell volume
- Mean cell hemoglobin
- Mean cell hemoglobin concentration
- Total and differential white blood cell count

Coagulation

- Prothrombin time
- Activated partial thromboplastin time

Clinical Chemistry

A/G ratio
Albumin
Alkaline phosphatase
Aspartate aminotransferase
Alanine aminotransferase
Creatinine
Calcium
Chloride
Cholesterol (total)
Creatine
Gamma glutamyl transferase
Glucose
Globulin
Lactate dehydrogenase
Phosphorus (inorganic)
Potassium
Protein fractions
Phospholipids
Sodium
Total bilirubin
Total protein
Triglycerides
Urea

Histopathology

Adrenals
Aorta
Brain
Cecum
Colon
Duodenum
Epididymides
Hardarian glands
Head
Lachrymal gland
Esophagus
Eyes with optic nerve
Femur (with articular surface & marrow)
Heart
Lymph nodes
Lachrymal glands
Ileum
Jejunum
Kidneys
Larynx
Liver
Lungs (with mainstem bronchus)
Mammary gland
Mesenteric lymph node
Nasopharynx
Nasal turbinates
Optic Nerve
Ovaries
Pituitary gland
Prostate
Rectum
Salivary gland
Sciatic nerve
Seminal vesicles
Skin
Spinal cord (cervical, midthoracic, & lumbar)
Spleen
Sternum (with bone marrow)
Stomach
Testes
Thymus
Thyroid/parathyroid
Tongue
Trachea
Urinary bladder
Uterus
Vagina
Zymbal glands
Pancreas
All gross lesions

Results

Three main study animals died or were killed because of poor condition: a control male (found dead at week 7), a control female (found dead at week 17) and a high dose female (killed in moribund condition during week 6 to prevent autolysis). For these animals sponsor notes that "there was no morphological evidence of specific target organ toxicity likely to cause death".

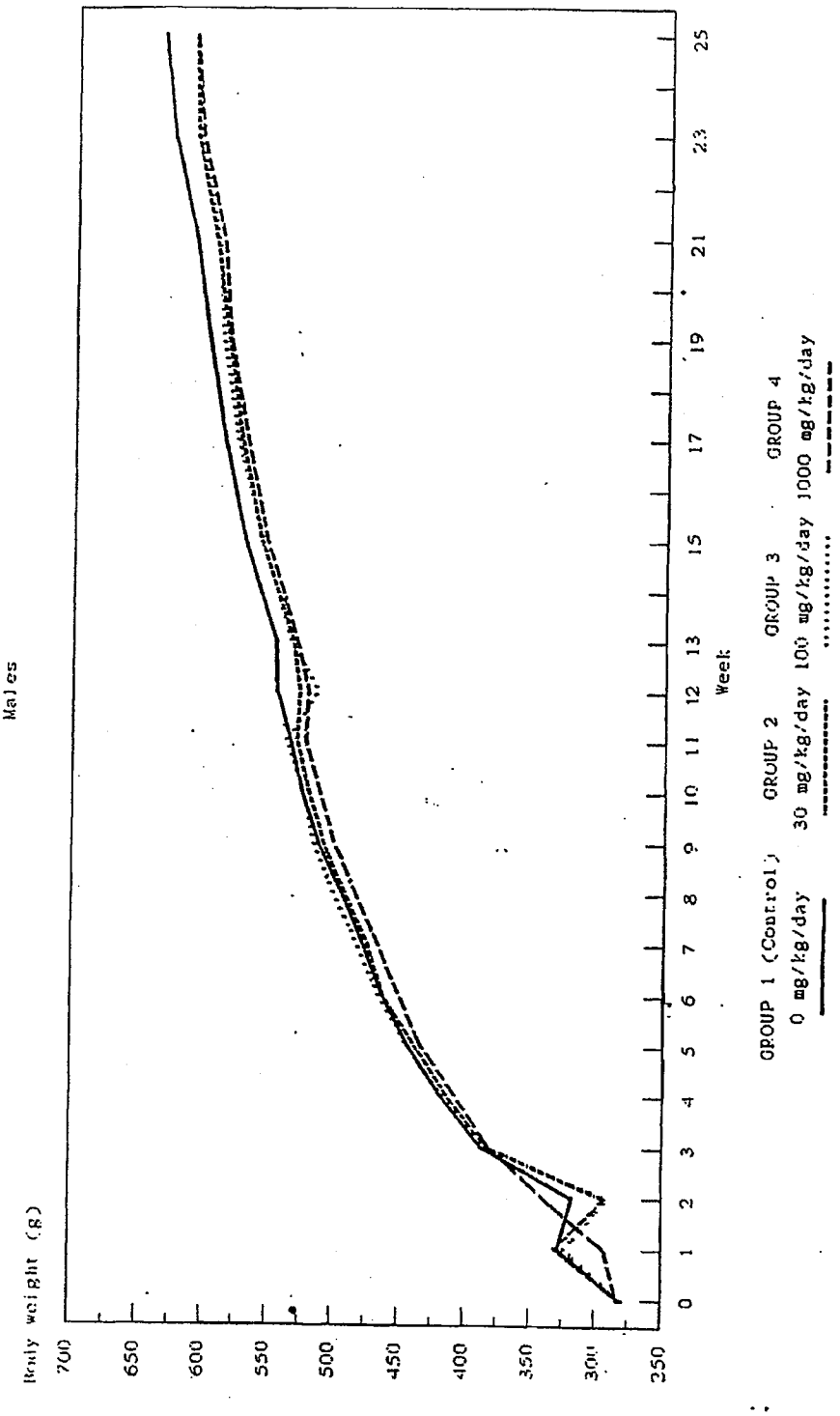
A number of high dose females which showed a deterioration in clinical condition during the first days of dosing were removed from the study and replaced. These animals (3 main study and 6 satellite) appeared pinched around the abdomen, hunched and slow to respond to stimuli; one had several convulsive episodes. All treated animals were excreting very large amounts of urine, compared to control and the cause of the deteriorating condition of the high dose females was suggested to be dehydration. To improve access to water (animals housed 5/cage), the automatic watering system was augmented by the addition of two water bottles to each cage of high dose animals. Animals remained in good health after this modification and water bottles were later added to all cages.

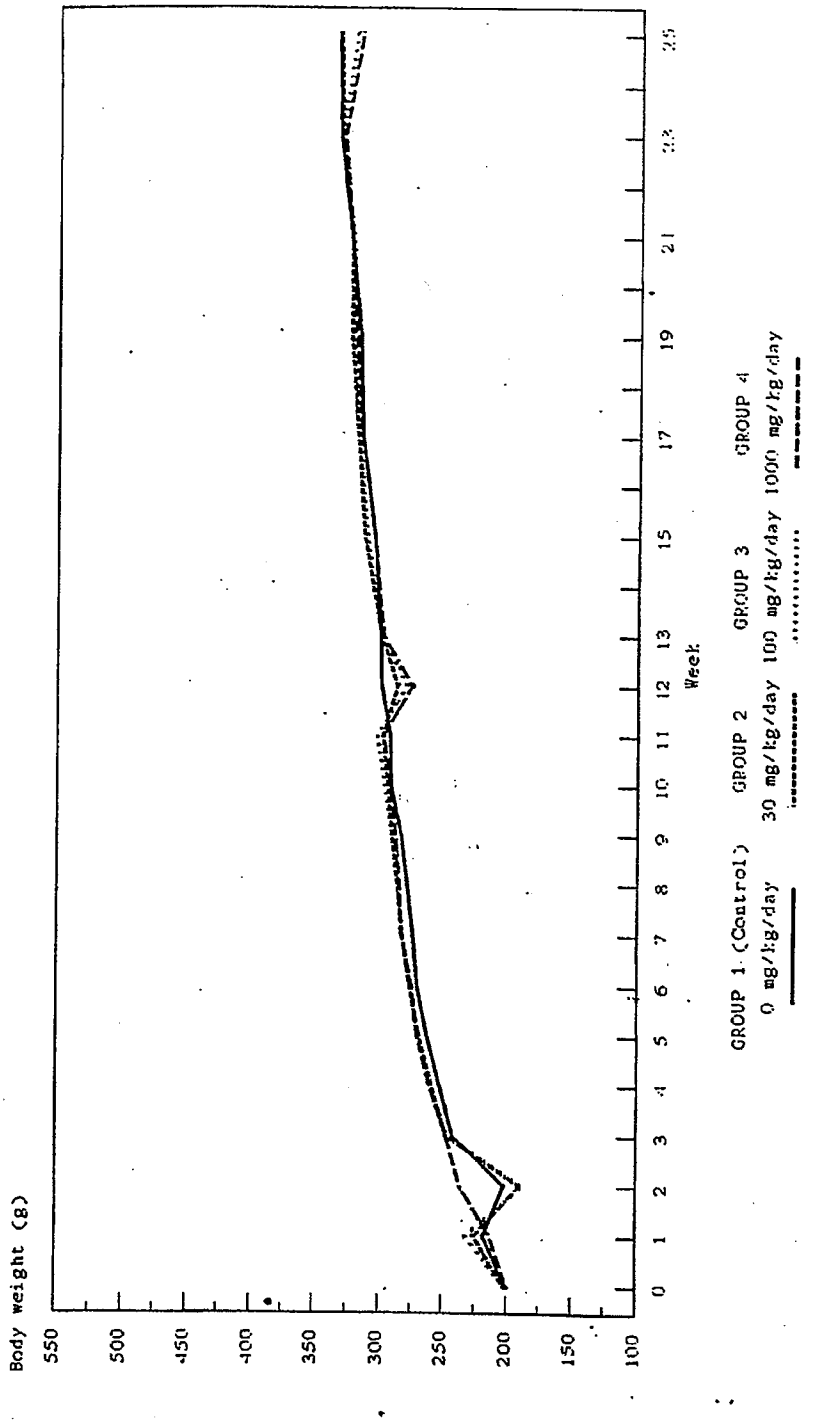
Mean body weights and body weight gains of the treated males were slightly but not significantly ($P>0.05$) lower than control during most of the treatment period. The decrement in body weight gain did not exceed 10% and was virtually the same for all 3 treated groups. Mean body weights and weight gains of the treated females were comparable to control throughout most of the study, but there was a significant decrease in weight gain in mid ($P<0.05$) and high ($P<0.01$) dose groups during the later two weeks of treatment. The amount of water consumed and urine produced were greater in treated groups (generally dose related) and specific gravity and osmolality of urine correspondingly decreased. Sponsor noted that there was a high degree of individual variability in electrolyte excretion in urine of control and treated animals, but generally the mean urinary total excretion of chloride and calcium were higher, whereas sodium was lower than control.

During week 13, mean Hb concentration, RBC and PCV were slightly and generally significantly lower than control in mid and high dose males, PT and APTT were longer than control in high dose males ($P<0.001$), and platelet count tended to be higher than control in high dose males ($P>0.05$). In high dose females, red cell parameters, RBC and PCV, were lower than control ($P<0.01$ and $P<0.05$, respectively), whereas MCH and platelet count were

higher ($P<0.01$ and $P<0.05$, respectively), and PT was slightly lower ($P<0.05$). Only the increase in APTT in high dose males, and the increases in platelet count in females (dose related in treated groups) persisted into week 26. During weeks 13 and 26, potassium concentrations were slightly but consistently lower than control in male and female treated groups, whereas total bilirubin was higher in both sexes; creatinine was higher in males. Inorganic phosphorus was slightly higher in treated groups, whereas calcium was lower in treated males only during week 13. Glucose levels were higher in treated females only during week 26.

All hematology and clinical chemistry changes were considered small (values obtained within historical control for this species and strain at the facility where the study was conducted) and "considered to be of little or no biological or toxicological significance".


Group mean body weight (g)
Males
Body weight (g)
700
650
600
550
500
450
400
350
300
250
0 1 2 3 4 5 6 7 8 9 10 11 12 13 15 17 19 21 23 25
Week
GROUP 1 (Control)
0 mg/kg/day
GROUP 2
30 mg/kg/day
GROUP 3
100 mg/kg/day
GROUP 4
1000 mg/kg/day


Group mean body weight (g)
Females
Body weight (g)
550
500
450
400
350
300
250
200
150
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 15 17 19 21
Week
GROUP 1 (Control)
0 mg/kg/day
GROUP 2
30 mg/kg/day
GROUP 3
100 mg/kg/day
GROUP 4
1000 mg/kg/day

Mean liver and kidney weights were higher than control for mid and high dose groups of both sexes. In high dose females, adrenal, uterine and pituitary weights were higher than control, whereas thymus weight was lower than control.

The incidences of renal hydronephrosis (usually minor in severity and unilateral) and small clusters of foamy histiocytes in the lung alveoli, both of which are claimed to be common microscopic findings in the strain used, were slightly higher than control in the high dose male and female groups and were considered to be of no toxicological significance. There was no other compound related histopathology noted.

After the 5 week treatment-free recovery period, there were no differences in hematology, clinical chemistry or organ weights between the high dose treated and control groups except for slightly higher levels of serum urea in treated males ($P<0.01$) and females ($P<0.05$). Water consumption and quantity of urine excreted returned to control levels, and there was a rebound increase in body weight gain ($P<0.05$).

Summary of Compound Related Effects Observed at Autopsy in Males

Dose (mg/kg/day)	Control	30	100	1000
Organ weights[1]				
↑ Liver	-	1.2	11.4	14.8**
↑ Kidney	-	2.7	6.1	10.9*
Gross Necropsy				
Enlarged liver	1/14	0/15	2/15	2/15
Pelvic dilatation	0/14	2/15	4/15	5/15
Histopathology				
Renal hydronephrosis	4/14	2/15	5/15	7/15
Foamy histiocytes (lung)	6/14	1/15	2/15	11/15

[1] Percent higher than control (relative to body weight)
* $P<0.05$
** $P<0.01$

Summary of Compound Related Effects Observed at Autopsy in Females

Dose (mg/kg/day)	Control	30	100	1000
Organ Weights[1]				
↑ Liver	-	5.3	7.9	26.5***
↑ Kidney	-	1.7	6.1	17.2***
↑ Adrenals	-	0.0	8.6	29.0***
↑ Uterus	-	13.8	19.5	32.2*
↓ Thymus	-	18.2	5.9	28.6*
↑ Pituitary gland	-	20.0	20.0	33.3**
Gross Necropsy				
Enlarged liver	0/14	0/15	0/15	2/14
Pelvic dilatation	1/14	0/15	4/15	4/14
Histopathology				
Renal hydronephrosis	3/14	0/15	4/15	7/15
Foamy histiocytes (lung)	4/14	2/15	2/15	7/15

[1] Percent higher or lower than control (relative to body weight)
* P<0.05
** P<0.01
*** P<0.001

Toxicokinetics:

Serum samples from animals in the satellite groups were analyzed for concentrations of OPC-41061 on Day 1 and after 4 and 26 weeks. Basic pharmacokinetic parameters (C_{max}, T_{max}, $t_{1/2}$ and AUC_{0-24}) were derived from the week 4 data. Values < LLOQ were originally set at 5 ng/ml (half the LLOQ). Sponsor was asked by this reviewer to reduce these values to 0. The following is a summary of mean corrected serum concentrations found on Day 1 and after 4 and 26 weeks (N=3/time point for each dose).

	Day 1				Week 26			
Dose mg/kg	Males (ng/ml)		Females (ng/ml)		Males (ng/ml)		Females (ng/ml)	
	4 Hr	24 Hr	4 Hr	24 hr	4 Hr	24 Hr	4 Hr	24 Hr
30	291	ND	3097	610	116	ND	1845	ND
100	1317	39	11451	34	564	ND	6262	ND
1000	7262	183	29710	190	1408	ND	25333	54

Week 4

Dose mg/kg	0 hr** (ng/ml)		2 hr (ng/ml)		4 hr (ng/ml)		6 hr (ng/ml)		24 hr (ng/ml)	
	Females*		Males	Females	Males	Females	Males	Females	Males	Females
30	ND		519	2894	278	1229	61	759	ND	ND
100	36*		1132	3422	755	2859	394	806	ND	ND
1000	945		1088	2915	1369	2294	751	2178	32	34

* Mean based on only 2 animals because 1 of 3 was LLOQ or problem in analysis
** Not detectable in males at 0 hour

Sponsor recalculated the AUCs using a non-compartmental analysis with the linear/log trapezoidal method. The following is a summary of pharmacokinetic parameters based on corrected serum concentrations found after 4 weeks.

Dose mg/kg	$AUC_{(0-6\ h)}$ (ng.hr/ml)		$AUC_{(0-\infty)}$ (ng.hr/ml)		C_{max} (ng/ml)		T_{max} (hr)		$t_{1/2}$ (hr)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
30	1655	9005	1768	11273	519	2894	2	2	3.9	3.1
100	4168	13404	5661	15597	1132	3422	2	2	3.1	2.7
1000	12712[1]	33449[1]	12886	33604	1369	2915	4	2	3.8	3.2

[1] $AUC_{(0-24\ h)}$

The serum AUC_{0-24} values at week 4 originally calculated (prior to correction) were 2258, 7794 and 12720 for males and 15961, 20756 and 33449 for females, for the 30, 100 and 1000 mg/kg/day doses, respectively.

Toxicokinetic data derived from this study was not used as a basis for selection of dose levels for the proposed rat carcinogenicty test. However, data obtained between 2 and 6

hours confirm that remarkably higher concentrations are found in the serum of female than in the serum of male rats. There was a decline in serum concentration of OPC-41061 in both males and females (serum level at 4 hours) after treatment for 4 weeks (compared to Day 1 serum concentrations) which was more pronounced in females than in males. In males, by 26 weeks, there were further, but smaller declines in serum concentrations of OPC-41061 after treatment with 30 and 100 mg/kg/day, but no further decline with treatment at 1000 mg/kg. Surprisingly, in females, there was a large rebound in serum OPC-41061 concentrations by 26 weeks of treatment, compared to 4 weeks, at all 3 doses. Sponsor confirmed that at week 26 there were increases (rather than the expected decreases) in serum OPC-41061 concentrations of all 3 female treated groups, but serum concentrations of the two chief metabolic products (DM-4103 and DM 4107) were claimed to be correspondingly decreased.

CARCINOGENICITY STUDIES

104-Week Oral (Gavage) Carcinogenicity Study in the Mouse

Key Findings: Two-years of daily oral administration of tolvaptan at 0, 10, 30 and 60 mg/kg/day in males and 0, 10, 30 and 100 mg/kg/day in females did not increase the incidence of tumors in the mouse.

Testing Facility: [redacted] b(4)

Study Numbers: Otsuka study number - 013783 (Report No.014252)
Contract lab's study number – 4283 (163-024)

Study Dates: Initiation of Dosing – January 28, 1999
Termination of Dosing – January 24-29, 2001

GLP Compliance: with Guidances and Ordinances of the Ministry of Health and Welfare, Japan

QA Report: yes

Drug Lot # and % Purity: 97I93[redacted] & 66.7% b(4)

Species/Strain: Mouse/B6C3F1 (SPF), 4-week-old, obtained from [redacted] b(4)

Number, Age and Weight at Start of Study: 55/sex/main study group; additional 30/sex/treatment group for toxicokinetic determinations; 5 weeks of age; males 18.0 – 21.2 g and females 15.0 – 17.8 g

Animal Housing: Animals were housed individually in aluminum cages with stainless steel wire mesh bottom and had free access to the diet (Modified NIH Open Formula Rat and Mouse Ration – Oriental Yeast Co., Ltd.) and tap water.

Formulation: The test drug was suspended weekly in 1% hydroxypropyl methylcellulose at appropriate concentrations.

Drug Stability: The dose formulations were found to be stable for 8 days when stored in a refrigerator shielded from light.

Methods

Doses: 0, 10, 30 and 60 mg/kg/day for males and
0, 10, 30 and 100 mg/kg/day for females

Basis of dose selection: In a 13-week toxicity study, deaths were observed in males at 100 mg/kg/day. In a 12-day dose range finding study, deaths were seen in females at 300 mg/kg/day. Hence, the doses for the 2-year bioassay were set at 10, 30 and 60 mg/kg/day for males and 10, 30 and 100 mg/kg/day for females. (Exec. CAC concurrence on July 14, 1998)

Route of administration: Oral (gavage)

Frequency of drug administration: Once daily for 104 weeks

Interim sacrifices: None

Deviations from original study protocol: None

Statistical methods: The data on body weight, food consumption, food efficiency, hematological values and organ weight were statistically analyzed using the Dunnett's multiple comparison test. The survival data and the incidence of tumors were analyzed by the Log rank test and Fisher's exact test, respectively. Additionally, the incidence of tumors was also analyzed using the Cochran-Armitage test for determination of dose dependency. When the survival ratio was considered statistically significant between groups, it was then analyzed by Peto's test. The numbers of benign, malignant and total tumors were analyzed by Dunnett's multiple comparison and Steel tests.

Observations and Measurements

Clinical signs and mortality: twice daily (before dosing and within 3 hours postdose) or more frequently during early days of dosing; palpation was performed weekly for the presence of neoplastic lesions.

Body weight and food consumption: weekly

Hematology: Evaluations were performed on all surviving animals at the end of the treatment period. [parameters evaluated – RBC, WBC (total and differential) and platelet counts, hemoglobin, hematocrit, MCV, MCH and MCHC]

Clinical chemistry: not performed

Organ weights: Brain, heart, lungs, liver, kidneys, spleen, adrenals, testes and ovaries were weighed.

Gross pathology: At necropsy, all organs were examined macroscopically and gross findings were recorded.

The following organs/tissues were fixed in 10% neutral buffered formalin: skin, mammary glands, lymph nodes (mesenteric and mandibular), sublingual, mandibular and

Zymbal's gland, sternum, femur, bone marrow (sternum and femur), thymus, trachea, lungs, heart, thyroid, parathyroid, tongue, pharynx, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, gallbladder, pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles, prostate glands, testes, epididymis, ovaries, uterus, eyeballs (including optic nerve), Harderian gland, lacrymal gland, brain, pituitary, spinal cord, skeletal muscle, sciatic nerve, aorta and gross lesions.

Histopathology: All tissues (except Zymbal's gland, pharynx and lachrymal gland) from all control and high dose animals, from dead/moribund animals from all groups, all gross lesions, and lymph nodes from low and middle dose group male animals were examined microscopically.

Toxicokinetics: Blood was collected from drug treated animals (3 animals/sex/group/ time point) at 2 time points (1 and 2 hr post-dose) on day 1 and week 26, and 6 time points (pre-dose and 1, 2, 4, 6 & 24 hr post-dose) in week 4 for the determination of the parent and metabolite (DM-4103 and DM-4107) concentrations.

Results

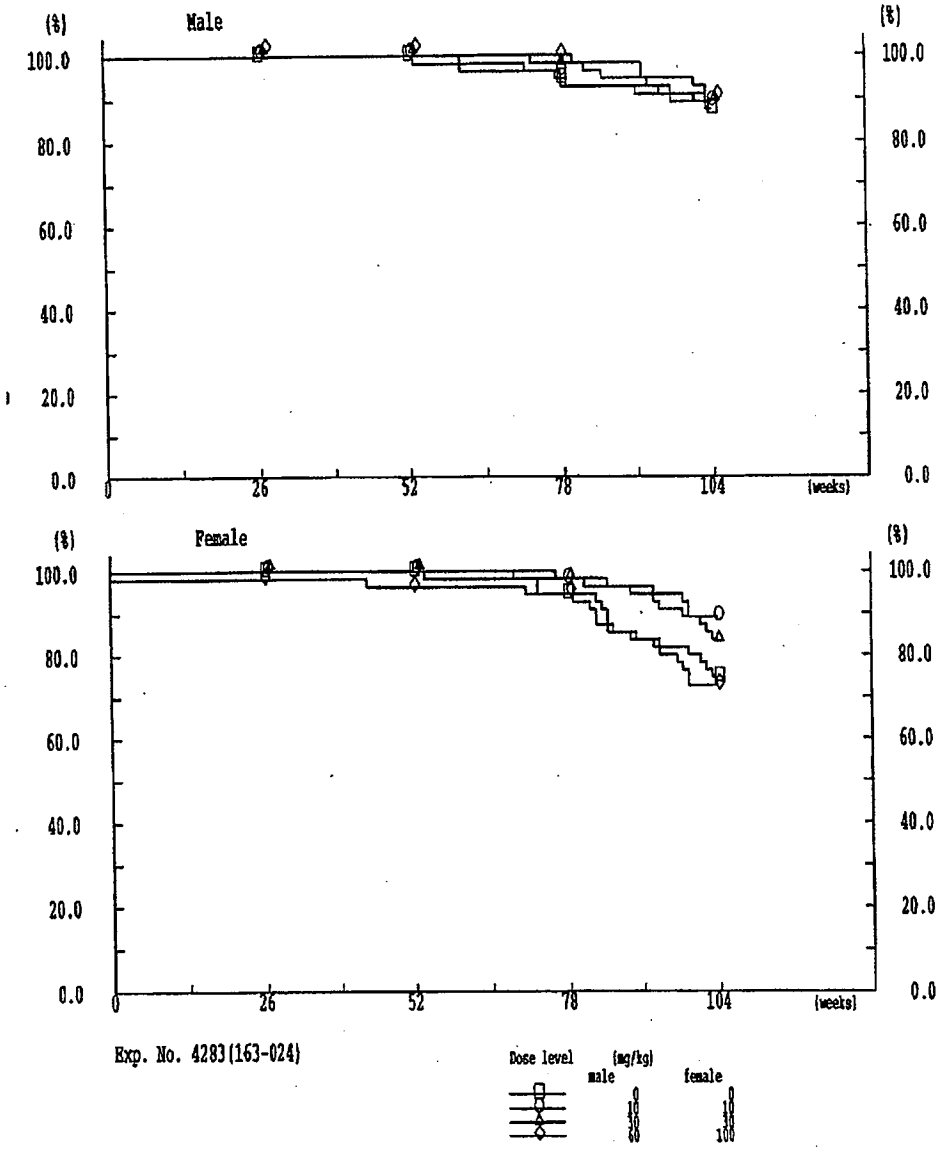
Mortality: The survival data over the course of the study is presented graphically in Figure 2. The percent mortalities at weeks 78 and 104 are given below.

	Males				Females			
	Mortality (%)							
Dose level (mg/kg/day)	0	10	30	60	0	10	30	100
Week 78	5.5	3.6	1.8	0.0	5.5	1.8	1.8	5.5
Week 104	12.7	10.9	10.9	10.9	25.9	10.9	16.4	27.3

There were no statistically significant differences in the mortality rates between control and treated groups of either sex.

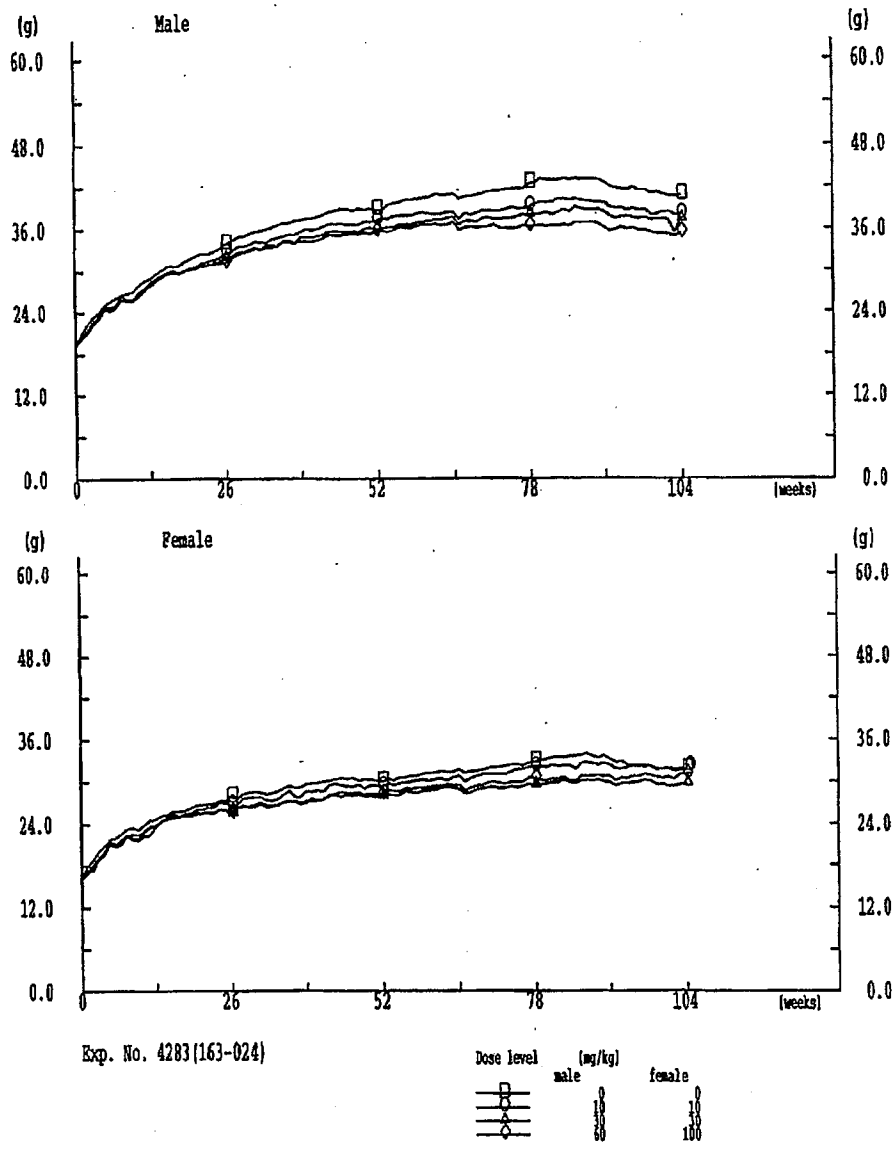
Clinical signs: No treatment-related clinical signs were noted. Clinical signs observed both in control and treatment groups, mainly beginning from study week 53, included wasting, piloerection, loss of teeth, subnormal temperature, abdominal and subcutaneous tissue masses, tachypnea, decreased motor activity and swelling of orbit in both sexes; prolapse of penis, urogenital organ nodules and prone position in males, and abdominal distention, swelling of the head and urogenital hemorrhage in females were noted.

Figure 2.



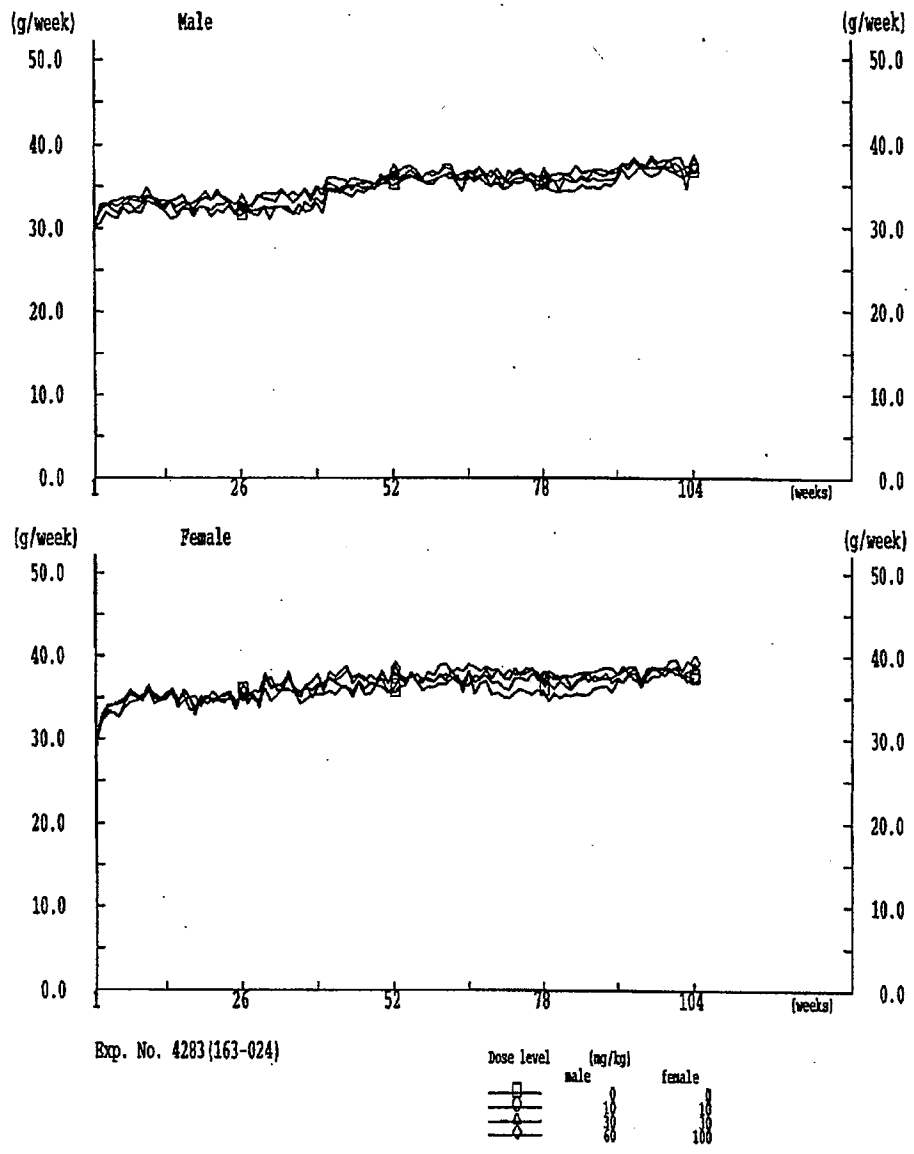
Survival ratio

Figure 3.



Body weight

Figure 4.



Food consumption

Body weights: Body weight data are presented graphically in Figure 3 and the body weight gain data at different treatment intervals are given below:

	Body Weight Gain (g)							
	Males				Females			
Dose levels (mg/kg/day)	0	10	30	60	0	10	30	100
Treatment intervals (weeks)								
0 - 13	9.7	8.5**	9.0*	9.2	8.6	7.7**	7.5**	7.7**
0 – 26	14.7	13.3*	12.7**	12.0**	11.9	10.7*	9.4**	9.6**
0 – 52	19.4	18.0	16.8**	16.4**	13.9	13.2	11.8**	12.1**
0 – 78	23.2	19.8**	18.8**	17.1**	16.7	16.0	13.3**	14.3**
0 – 104	21.4	18.8	18.0**	16.2**	15.6	15.6	13.5*	14.8

* = $p \leq 0.05$ ** = $p \leq 0.01$ (significant difference from the control group)

The body weight gains for mid and high dose males and females were statistically significantly lower than respective control values for most treatment intervals. The body weight gain to week 78 and (although not statistically significant) week 104 for the low dose male animals was also lower than control.

At the termination of the study, the mean body weights at low, mid and high dose levels were 6.6, 8.4 and 13.3% lower than control for males, and 0.0, 6.3 and 2.5% lower than control for females.

Food consumption: The food consumption data is presented graphically in Fig 4. For males, the food consumption was generally higher than control at mid and high dose levels throughout the study and, for females, it was also higher in mid and high dose groups beginning about week 39 and continuing till the end of the study.

Hematology: There were no significant hematological findings.

Organ weights: The absolute heart weights were lower for mid and high dose males and females, with lower relative heart weight observed only in high dose females. A reduction in absolute kidney weights in high dose females and a dose-related increase in relative brain weights in treated males were also observed.

Gross pathology: There were no treatment-related increased incidences of gross lesions in mice that were sacrificed terminally. However, dose-related decreased incidences,

compared to control, were noted for the following gross findings in treated females: enlargement of the spleen, nodules of the liver, mass in the abdominal cavity and white patch/zone in the stomach.

In animals found dead or killed in moribund condition, increased incidences of enlarged lymph nodes, compared to control, in treated males and decreased incidences of thymus atrophy and thoracic vertebra nodules in treated females were observed.

Histopathology: Neoplastic findings: The incidences of neoplastic findings observed in all control and high dose animals (dead and sacrificed terminally and *in extremis*) are presented in Table 1. Sponsor's analysis, using Fisher's exact test, showed no statistically significant increased incidence of tumors in the high dose group of either sex. However, Peto's test revealed an increased incidence for malignant lymphoma (lymph node) in high dose males (incidence = control 1/55 vs HD 5/55; p = 0.045). Hence, histological examination of lymph nodes from low- and mid- dose males was also performed. The incidences of malignant lymphoma (control 1/55, LD 4/55, MD 1/55 and HD 5/55) in males were analyzed by Cochran Armitage test, and the test result indicated no dosage dependence (Table 2).

Hepatocellular adenoma in females showed a decreased incidence in the high dose group compared to control (C 10/55 vs HD 3/55). Other tumors with a high but not treatment-related incidence included alveolar/bronchiolar adenoma, hepatocellular carcinoma, histiocytic sarcoma in the uterus and adenoma in the Harderian gland.

Hepatocellular carcinoma, histiocytic sarcoma and malignant lymphoma were considered to be the primary cause of death in both males and females during the study.

The FDA statistical analyses of the mouse tumor data showed no statistically significant dose-response relationship for any of the tested tumor types. Also, none of the pairwise comparisons between the control and high dose groups were considered to be statistically significant. However, a significant increased incidence of malignant lymphoma (lymph node) was noted in the low dose male group compared to control (p = 0.004).

Non-neoplastic findings: In terminally sacrificed animals, the incidence of hyaline casts in kidneys was increased in high dose females. Lesions which decreased in incidence in the high dose group included vacuolar degeneration of pancreas (males and females), microgranuloma of the liver (males), basiophilic tubules of the kidneys and pigment deposit in adrenal glands

In animals found dead or killed in moribund condition, no dose-related increased incidences in non-neoplastic findings were noted. The incidences of bone marrow pigment deposit and exostosis of the thoracic vertebra were decreased in treated females.

Table 1.

Summary of neoplastic findings with statistical analysis
(104 Weeks experiment) Exp. No. 42

Dose level (mg/kg)	Male animals		Female animals	
No. of animals necropsied	Am 55	Bm 55	Af 55	Bf 55
Organ / Findings				
HEMATOPOIETIC SYSTEM				
bone marrow				
=hemangioma	1	2	1	1
spleen				
=hemangioma	0	1	3	0
#hemangiosarcoma	3	1	0	1
#histiocytic sarcoma	0	0	1	0
#malignant lymphoma	0	0	0	1
lymph node				
#hemangiosarcoma	1	0	0	0
#malignant lymphoma	1	5	8	4
RESPIRATORY SYSTEM				
lung				
=alveolar/bronchiolar adenoma	6	8	4	6
#alveolar/bronchiolar carcinoma	2	3	0	0
DIGESTIVE SYSTEM				
stomach				
=squamous cell papilloma	1	0	1	2
#leiomyosarcoma	0	0	0	1
duodenum				
#adenocarcinoma	0	1	0	0
jejunum				
#malignant lymphoma	0	1	0	0
liver				
=hepatocellular adenoma	18	13	10	3*
=hemangioma	0	0	0	1
#hepatocellular carcinoma	8	7	3	2
#hemangiosarcoma	0	0	1	0
#histiocytic sarcoma	1	0	0	2
mandibular gland				
=mastocytoma	1	0	0	0

Am: 0 Bm: 60
Af: 0 Bf: 100
=: benign #: malignant
Significant difference from control group by Fisher's exact test; * : $P \leq 0.05$ ** : $P \leq 0.01$

Table 1 continued

-continued Summary of neoplastic findings with statistical analysis (104 Weeks experiment) Exp. No.

Dose level (mg/kg) / No. of animals necropsied / Organ — Findings	Male animals Am 55	Bm 55	Female animals Af 55	Bf 55
URINARY SYSTEM				
kidney				
#adenocarcinoma	1	0	0	0
REPRODUCTIVE SYSTEM				
mammary gland				
#adenocarcinoma	0	0	0	1
testis				
=interstitial cell tumor	0	1	-	-
ovary				
=cystadenoma	-	-	2	1
uterus				
=endometrial stromal polyp	-	-	1	1
#endometrial sarcoma	-	-	2	0
#histiocytic sarcoma	-	-	3	4
vagina				
#histiocytic sarcoma	-	-	0	1
ENDOCRINE SYSTEM				
pituitary gland				
=adenoma	0	0	4	0
#adenocarcinoma	0	0	0	1
thyroid gland				
=follicular cell adenoma	3	0	3	0
pancreatic islet				
=adenoma	0	1	0	0
SPECIAL SENSE SYSTEM				
Harderian gland				
=adenoma	4	1	1	4
INTEGUMENTARY SYSTEM				
subcutaneous tissue				
=hemangioma	1	0	0	0

Am: 0 Bm: 60
Af: 0 Bf: 100
=: benign #: malignant
Significant difference from control group by Fisher's exact test; * : $P \leq 0.05$ ** : $P \leq 0.01$

Table 2.

Summary of neoplastic findings of lymph node with statistical analysis (104 Weeks experiment) Exp. No. 4283 (163

Dose level (mg/kg)	Male animals			
No. of animals necropsied	Am 55	Bm 55	Cm 55	Dm 55
Organ Findings				
HEMATOPOIETIC SYSTEM				
lymph node				
#hemangiosarcoma	1	0	0	0
#malignant lymphoma	1	4	1	5

Am: 0 Bm: 10 Cm: 30 Dm: 60
=: benign #: malignant
Significant difference from control group by Fisher's exact test; * : P ≤ 0.05 ** : P ≤ 0.01
Malignant lymphoma of lymph node in male is analyzed by Cochran Armitage test; $: P ≤ 0.05 $$: P ≤ 0.01

Concentrations of OPC-156 and its metabolites in serum: Mean Cmax values on day 1 and at weeks 4 and 26, and Cmax and $AUC_{0\text{-}24hr}$ values at week 4 are given in Tables 3 and 4, respectively. On day 1, Cmax values of the parent compound and its metabolites were increased in a dose-related manner in both sexes. While there was no notable difference between sexes in the Cmax values of the parent compound, these values for metabolites were higher in females than in males at the same dose level. There was no marked accumulation of parent or metabolites with repeated dosing. In week 4, the Cmax and $AUC_{0\text{-}24hr}$ values of the parent compound in males were higher than in females, and these parameters for metabolites in males were lower than in females for the same dosage.

Table 3.

Mean Cmax(μg/mL) in male mice

Dose (mg/kg)	Analytes	Male		
		Day 1	Week 4	Week 26
10	OPC-156	0.1107	0.3056	0.1364
	DM-4103	0.0608	0.0875	0.0526
	DM-4107	0.0430	0.0496	0.0328
30	OPC-156	0.4570	1.0744	0.7116
	DM-4103	0.1378	0.3361	0.4224
	DM-4107	0.0754	0.2317	0.1399
60	OPC-156	1.0938	1.1906	1.2146
	DM-4103	0.4120	0.5757	0.7334
	DM-4107	0.2977	0.2731	0.3559

Mean Cmax(μg/mL) in female mice

Dose (mg/kg)	Analytes	Female		
		Day 1	Week 4	Week 26
10	OPC-156	0.0808	0.0938	0.1062
	DM-4103	0.1110	0.1690	0.1182
	DM-4107	0.0604	0.0851	0.1092
30	OPC-156	0.5290	0.4247	0.4497
	DM-4103	0.4116	1.3724	0.7805
	DM-4107	0.2870	0.4527	0.5201
100	OPC-156	1.7534	0.9563	1.4211
	DM-4103	1.6737	5.9461	2.5815
	DM-4107	1.0471	1.0723	2.1199

Table 4.

Toxicokinetic parameters of OPC-156, DM-4103 and DM-4107 in serum after 4 weeks oral administration in male mice

Dose (mg/kg)	Analytes	Tmax (hr)	Cmax (μg/mL)	$AUC_{0\text{-}24hr}$ (μg·hr/mL)
10	OPC-156	1	0.3056	0.4282
	DM-4103	4	0.0875	0.5889
	DM-4107	1	0.0496	0.1375
30	OPC-156	1	1.0744	1.3792
	DM-4103	1	0.3361	1.4294
	DM-4107	1	0.2317	0.4186
60	OPC-156	1	1.1906	2.8595
	DM-4103	2	0.5757	6.0671
	DM-4107	1	0.2731	1.2203

Toxicokinetic parameters of OPC-156, DM-4103 and DM-4107 in serum after 4 weeks oral administration in female mice

Dose (mg/kg)	Analytes	Tmax (hr)	Cmax (μg/mL)	$AUC_{0\text{-}24hr}$ (μg·hr/mL)
10	OPC-156	1	0.0938	0.1810
	DM-4103	2	0.1690	1.0319
	DM-4107	1	0.0851	0.3031
30	OPC-156	1	0.4247	0.8952
	DM-4103	2	1.3724	4.4120
	DM-4107	2	0.4527	1.5084
100	OPC-156	1	0.9563	4.3317
	DM-4103	6	5.9461	72.9211
	DM-4107	2	1.0723	11.7367

The study appeared to be adequately performed using doses previously recommended by the ECAC. The highest doses employed in males (60 mg/kg/day) and females (100 mg/kg/day) were about 5 and 8 times the maximum recommended human dose (MRHD), respectively, on a mg/m^2 basis; these highest doses resulted in systemic exposures that were just lower (males) or about the same (females) as the exposures in humans at steady state at the MRHD of 60 mg/day. At least 48 males and 40 females in each group survived to scheduled termination.