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NON-CLINICAL REVIEW(S)



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND
EVALUATION

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Product: Tolvaptan (Jynarque®)
Indication: To slow progressive kidney disease in adults
with autosomal dominant polycystic kidney
disease (ADPKD)
Applicant: Otsuka Pharmaceutical Development &
Commercialization, Inc., Rockville, Maryland,
USA
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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	5
1.1	INTRODUCTION (AND CLINICAL RATIONALE)	5
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	6
1.3	RECOMMENDATIONS	9
1.3.1	Approvability	9
1.3.3	Labeling	9
2	DRUG INFORMATION	10
2.1	DRUG	10
2.2	RELEVANT INDs, NDAs, BLAs AND DMFs.....	10
2.3	DRUG FORMULATION	10
2.4	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	11
2.5	REGULATORY BACKGROUND	11
3	PHARMACOLOGY	12
3.1	PRIMARY PHARMACOLOGY	12
3.1.1	In vivo studies	12
3.1.1.1	Comparison of the effects of OPC-41061 and furosemide in Nagase ... albuminemic rats and SD rats.....	12
3.1.1.2	Effects of tolvaptan on the portal vein pressure in rats	20
4	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	22
4.1	Nine-week repeat dose toxicity study in 4-day old juvenile rats.....	22
5	OTHER TOXICITY STUDIES	36
5.1	Toxicogenetic analysis of tolvaptan using collaborative cross mice	36
5.2	Stress response pathways initiated in human hepatocytes exposed to tolvaptan.....	43
5.3	Tolvaptan in the DILIsym® software platform.....	51
6.	INTEGRATED SUMMARY AND SAFETY EVALUATION	53

Table of Tables

Table 1. Experimental design for PD and PK studies (similar doses but differs in size/group)	13
Table 2. Experimental design for in vitro protein binding assay	13
Table 3. Pharmacokinetics parameters after the administration of OPC-41061 in SD rats and NAR.....	17
Table 4. Pharmacokinetics parameters after the administration of furosemide in SD rats and NAR.....	17
Table 5. Serum protein binding of OPC-41061 in SD rats and NAR	18
Table 6. Serum protein binding of furosemide in SD rats and NAR	18
Table 7. Pharmacokinetic parameters of OPC-41061 in plasma after single intravenous administration at 1 mg/kg in male rats.....	19
Table 8. Study design and group allocation of animals	23
Table 9. Subject animals and time points of blood collection (day 1 of administration) .	25
Table 10. Subject animals and time points of blood collection (days 32 and 63 of administration).....	25
Table 11. Tissues/organs sampled for histopathological examination	27
Table 12. Body weight and food consumption.....	29
Table 13. Water consumption	29
Table 14. Summary of urinalysis (week 9 of administration)	30
Table 15. Summary of blood chemistry (end of administration period)	31
Table 16. Summary of organ weight (end of administration period)	32
Table 17. Summary of necropsy findings (end of administration period).....	33
Table 18. Summary of histopathology findings (end of administration period)	34
Table 19. Summary of histopathology findings (end of recovery period).....	34
Table 20. The toxicokinetic parameters of tolvaptan and its two metabolites in the treated groups	35
Table 21. Pathway-level changes associated with tolvaptan exposure in primary human hepatocytes, and proposed biomarkers to measure pathway activation in vivo	48

Table of Figures

Figure 1. Time course of urine volume after the administration of OPC-41061 and furosemide in SD rats and NAR.	15
Figure 2. Effects on urine volume by the administration of OPC-41061 and furosemide in SD rats and NAR.	16
Figure 3. Average ALT(top) and AST (bottom) fold change (tolvaptan-treated animal over vehicle-treated paired control) for each strain in order by increasing average fold change from left to right.....	37
Figure 4. Average total bilirubin (top) and miR-122 (bottom) fold change (tolvaptan-treated animal over vehicle-treated paired control) for each strain in order by increasing average fold change from left to right.	38
Figure 5. Average plasma tolvaptan concentrations at (A) 2 h and (B) 24 h by strain...	39
Figure 6. QTL mapping using ALT fold change	40
Figure 7. Pathway enrichment analysis of genes associated with tolvaptan treatment .	41
Figure 8. Pathway enrichment analysis of genes correlated with ALT fold change	41
Figure 9. Left: Average fold change (relative to control) in % hepatocytes with both small and textured nuclei.....	44
Figure 10. Right: Average fold change (relative to control) in % of hepatocytes with high cytoplasmic cytochrome c.	44
Figure 11. Average fold change (relative to control) in ALT (left) and miR-122 (right) release into culture medium.	45
Figure 12. Top 10 (A) pathways and (B) toxicity lists enriched among genes significantly differentially expressed with 20 μ M tolvaptan at 72 h.	46
Figure 13. Top 10 (A) pathways and (B) toxicity lists enriched among genes significantly differentially expressed with 50 μ M tolvaptan at 72 hr.....	47
Figure 14. GoFigure Map of GO biological processes enriched among the genes differentially expressed with 50 μ M tolvaptan at 72 h.....	49
Figure 15. Hypothesis for the pathogenesis of tolvaptan-induced liver injury.....	50

1 Executive Summary

1.1 Introduction (and Clinical Rationale)

Polycystic kidney disease (PKD) is an inherited renal disease that exhibits profound morphological disorganization characterized by massive enlargement of fluid-filled renal tubular and/or collecting duct cysts.^{1,2} Progressive cystic enlargement mediated by increased and persistent epithelial cell proliferation compromise normal renal parenchyma, often leading to renal failure. In the human, the most clinically significant type of PKD is inherited as an autosomal dominant polycystic kidney disease (ADPKD). Approximately 85% of ADPKD is caused by a mutation in the PKD1 gene and 15% by mutation in the PKD2 gene.³ Biochemically, cystic enlargement is mediated by the mitogenic action of epidermal growth factor stimulated tyrosine kinase and vasopressin-stimulated cAMP second messenger. In PKD, renal intracellular levels of cAMP are higher than are in the normal kidney due to hyperactivation of V2 receptors that are mainly expressed in the basolateral membrane of renal collecting ducts.⁴ Active secretion of fluid into the lumen is mediated by apically located Na-K-ATPase and chloride channels.⁵ Additionally, a reduction in steady-state calcium levels contributes to cyst formation.⁶

Tolvaptan, a selective vasopressin V2 receptor antagonist, exerts a diuretic action by inhibiting water reabsorption in the collecting duct of the kidney. It was approved by the FDA in May 2009 for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (NDA 22-275). Tolvaptan was also approved for ADPKD in various European countries. Otsuka Pharma has previously submitted an NDA for tolvaptan for the treatment of ADPKD (NDA 204441 SN0000) on March 1, 2013; however, the advisory committee did not recommend approval of the application on August 5, 2013 for concerns on insufficient efficacy of the drug. While fully addressing the Complete Response Letter from the Division, through the current NDA re-submission, Otsuka is seeking approval of tolvaptan for the treatment of polycystic kidney disease. The maximum human recommended dose (MRHD) for PKD is 120 mg/day, while the MRHD for hyponatremia is 60 mg/kg/day.

The nonclinical testing for tolvaptan for PKD is supported in part on the nonclinical results from previous studies conducted to support the indication for clinically significant hypervolemic and euvolemic hyponatremia (NDA 22-275). This NDA contained a majority of the nonclinical studies conducted to support the hyponatremia

¹ Takiar V, Caplan MJ. Polycystic kidney disease: Pathogenesis and potential therapies. *Biochim Biophys Acta*. 2011; 1812:1337-1343.

² Sun Y, et al. Drug discovery for polycystic kidney disease. *Acta Pharmacol Sinica* 2011; 32:805-816.

³ Ward CJ, et al. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor like protein. *Nat Genet* 2002; 30(3):259-269.

⁴ Robben JH, et al. Regulation of the vasopressin V2 receptor by vasopressin in polarized renal collecting duct cells. *Mol Biol Cell*. 2004;15: 5693–5699.

⁵ Wilson PD. Mouse models of polycystic kidney disease. *Curr Topics Develop Med*. 2008; 84:311-349.

⁶ Wallace DP. Cyclic AMP-mediated cyst expansion. *Biochim Biophys Acta* 2011; 1812: 1291-1300.

indication. Additional pharmacology, pharmacokinetics, and toxicology studies for the PKD NDA were conducted after the NDA 22-275 submission, and these additional studies were described in the previous NDA 204441 submission. Both NDAs were previously reviewed by Dr. Xavier Joseph (see Appendix for his review on NDA 204,441, original submission). In the current NDA re-submission, the sponsor has submitted a few nonclinical studies, especially the mechanistic studies of tolvaptan-induced hepatotoxicity that were completed after the submission of previous NDA 204441.

1.2 Brief Discussion of Nonclinical Findings

[Part of it is extracted from the review of Dr. Xavier Joseph on NDA 204441 (see Appendix for details)]

Chronic oral toxicity studies conducted in rats (26 weeks) and dogs (52 weeks), at doses up to 1000 mg/kg/day did not reveal any liver toxicity. 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 the consequence of an excessive pharmacologic action of the drug. No increased incidence of tumors was seen after two years of oral administration of tolvaptan to male and female rats at doses up to 1000 mg/kg/day (1.9 to 5.1 times the human exposure at the 90/30 mg dose based on AUC), to male mice at doses up to 60 mg/kg/day (0.4 times the human exposure at the 90/30 mg dose based on AUC) or to female mice at doses up to 100 mg/kg/day (0.7 times the human exposure at the 90/30 mg dose based on AUC). Tolvaptan tested negative for genotoxicity in in vitro and in vivo test systems.

In a fertility study in which male and female rats were orally administered tolvaptan at doses of 100, 300 or 1000 mg/kg/day, the highest dose level was associated with statistically significantly fewer corpora lutea and implants than control. Maternal toxicity (reduced body weight gain and food consumption) was noted at 100 mg/kg/day (4.4 times the human exposure at the 90/30 mg dose based on AUC). Oral administration of tolvaptan in pregnant rats at 10, 100 and 1000 mg/kg/day during organogenesis was associated with reduced fetal weight and delayed ossification of fetuses at 1000 mg/kg/day (17 times the human exposure at the 90/30 mg dose based on AUC). In pregnant rabbits, oral administration of tolvaptan at 100, 300 and 1000 mg/kg/day during organogenesis was associated with abortions (mid and high doses) and increased incidences of embryo-fetal death, fetal microphthalmia, brachymelia and skeletal malformations at the maternally toxic highest dose (2.6 times the human exposure at the 90/30 mg dose based on AUC). In a prenatal and postnatal study in rats, increased perinatal death and decreased body weight of the offspring during the lactation period and after weaning were observed at the maternally toxic dose of 1000 mg/kg/day (17 times the human exposure at the 90/30 mg dose based on AUC). 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 and lactating women. The level of activity in milk ranged 1.5- to 15.8-fold higher than those in blood over the

period of 72 hr post-dose. Thus, it is recommended that women receiving tolvaptan should not breast feed.

In juvenile rat toxicity studies, 4-, 7- and 25-day old male and female pups were treated with tolvaptan for a period ranging from 1 week to 9 weeks. The maximum tolerated dose in 25 day old pups was 1000 mg/kg/day. Treatment-related changes noted in 25-day old rats were congestion and pigmentation of the spleen red pulp, hypertrophy of adrenal cortical cells and thyroid follicular cells and centrilobular hypertrophy of hepatocytes. These changes were reversible after 4 weeks of drug discontinuation. The maximum tolerated dose in 4- and 7-day old pups was 100 mg/kg/day as all pups receiving 1000 mg/kg/day died or euthanized on day 2. In 4-day old pups, the principal organs of toxicity were kidneys and lungs. Focal hemorrhage in lungs, renal pelvis dilatation, atrophy in the thymus and decreased hematopoiesis in bone marrow in the femur and in the spleen, were observed at ≥ 30 mg/kg/day. Most of them were still present at the end of 4 week recovery but at reduced incidence and severity.

In the current NDA resubmission, additional information has been included in the toxicity section on tolvaptan-induced hepatotoxicity that were completed after the submission of previous NDA 22275 and NDA 204441. These studies investigated a mechanistic understanding of tolvaptan-induced hepatotoxicity seen during studies in ADPKD patients.

Assessment of tolvaptan-induced hepatotoxicity based on mechanistic studies

During clinical studies in ADPKD subjects, multiple serious adverse event reports describing liver-related events were submitted to the Agency. A similar signal had not been seen in previous studies conducted in healthy volunteers and non-ADPKD patients and in preclinical species. A higher incidence of liver enzyme elevations was observed in the tolvaptan relative to placebo arm and several cases meeting Hy's Law criteria (bilirubin $>2x$ ULN and ALT $>3x$ ULN) were identified in ADPKD patients. It is noted that the ADPKD studies used a dose (120 mg/day) higher than that currently approved for the treatment of hyponatremia (60 mg/day). A hepatic adjudication committee (submitted to the Agency in November 2012) concluded that "in patients with ADPKD tolvaptan has the potential to cause liver injury capable of progression to liver failure" (for details see Nhi Beasley memo dated 3/20/2013, TSI 1332). Dr. John Senior in his review on the hepatic safety of tolvaptan (TSI 1332, 3/20/2013) writes that "the form of idiosyncratic DILI seen in humans both before and after marketing tends to be rare, dependent more on individual characteristics of the people being treated (therefore "**idiosyncratic**") rather than on the dose. This is due to the increased susceptibility of a few people to show liver injury at doses well tolerated by most people, for reasons not yet known. We cannot at present identify those individuals likely to show initial susceptibility to liver injury or who cannot adapt to repeated exposure. Thus, special susceptibility to tolvaptan-induced liver injury must be considered **idiosyncratic**."

Patients with ADPKD are different from others because of a genetic inheritance of PKD1 or PKD2 genes that lead to renal tubular cyst development and slow growth. It is

not known whether this might also confer some increased risk of hepatocellular injury (apart from the also associated development of liver cysts). As John Senior states “the sponsor should be tasked to investigate whether PKD1 or PKD2 are associated with an increased chance of hepatocellular injury in patients with the disease being treated ADPKD.” There is no biomarker to predict or diagnose DILI, only careful clinical observation can serve to protect patients from potentially severe harm from liver failure. In concluding the report, Dr. Senior calls for genome-wide testing for increased susceptibility to liver injury.

Consistent with this report, the sponsor has conducted 3 nonclinical mechanistic studies in 2014-2015 (for details see sections 5.1 to 5.3 of this review) that investigated possible mechanisms in tolvaptan-induced hepatotoxicity and guided biomarker identification/qualification.

In Collaborative Cross (CC) mice in vivo study, tolvaptan when given as a single 100 mg/kg dose (4X MRHD based on surface area, 24 hr sacrifice) produced elevated ALT levels in 3 of 45 CC strains (#7, 34, and 44), which are genetically sensitive to tolvaptan-induced liver response than other CC strains or traditional rodents. AST, total bilirubin, and miR-122 (a specific marker for liver injury) were all significantly correlated with the fold changes in ALT. However, tolvaptan did not produce histologic changes in the liver. Although such elevation in ALT (which may be transient and reversible) and total miR-122 was not observed in isolated human hepatocytes exposed to tolvaptan, exosomal release of miR-122 and declined urea release into the culture medium suggested leaky hepatocytes with decreased cellular function. This is supported from a statistically significant increase in percentage of hepatocytes with both small and textured nuclei, which was indicative of cells undergoing programmed cell death (PCD). Additional observations showed that PCD that was not **apoptosis** was induced with the translocation of cytochrome c from the mitochondria to the cytoplasm suggesting tolvaptan induced **mitochondrial dysfunction**. The hepatocellular injury causes some dysfunction of the whole liver with reduced ability to clear the plasma of bilirubin conjugate it and excrete it into bile (i.e., **bile acid transport**). Tolvaptan and its metabolites inhibit several human hepatic transporters involved in bile acid transport, thus affecting bile acid homeostasis. In susceptible patients, the cellular stress, apoptosis, and release of extracellular vesicle observed is hypothesized to provoke an innate immune response that in combination with adaptive immune attack results in liver injury. The in-silico simulation (DILIsym®) results have also identified mitochondrial toxicity as one of the potential keys to tolvaptan susceptibility. Increased tolvaptan exposure (high dose in ADPKD patients) might reveal other more predictive patient susceptibility factors related to mitochondrial function and bile acid transport.

Genetic mapping in CC mice identified a locus on chromosome 14 associated with tolvaptan-induced liver response. Microarray analysis in both in vivo and in vitro studies identified gene expression changes linked to nuclear receptor pathways that are associated with liver injury. The top pathways enriched among genes affected with treatment were oxidative stress, superoxide radicals degradation, mitochondrial dysfunction, and altered bile acid homeostasis. It is possible that the liver response observed in these studies could have mechanistic similarities to the early hepatocellular

events that may have occurred in the ADPKD patients. The results suggested to the sponsor that the liver injury that begins at the hepatocyte level is susceptible or sensitive to genetic and non-genetic factors, that may be unique to ADPKD patients and potentiates the toxic response to tolvaptan.

The occurrence of hepatotoxicity in many DILIsym® simulated individuals could differentiate tolvaptan responders from non-responders. A change in dose and dose regimen suggested a potential contribution of liver exposure resulted in an increase in simulated hepatotoxicity. Analysis of DILIsym® simulations indicated that while the increased exposure to tolvaptan (and influenced by active metabolite DM-4103) is a risk factor, exposure alone is not a strong predictor of patient susceptibility. Thus, qualification of novel genetic and non-genetic biomarkers might be required that could be predictive of delayed tolvaptan hepatotoxicity. Clinical DILI cases in ADPKD patients were delayed in onset presenting between 3 and 14 months of treatment and exhibited a relatively prompt recurrence upon rechallenge. The delayed clinical representation of liver safety signals in the tolvaptan ADPKD trials has led to the suggestion that liver toxicity may be mediated by an adaptive immune response to a newly exposed neoantigen in susceptible individuals. Sponsor contends this supports involvement of **adaptive immune system** in the final critical events underlying liver injury. It was hypothesized that even if an adaptive immune response is the final insult responsible for eliciting overt liver injury, events that are necessary, but not sufficient, for the liver injury begin at the hepatocyte level, and may require genetic and non-genetic susceptibility factors as well. However, DILIsym® software platform does not yet include the adaptive immune response pathways.

Toxicity is mechanistically multifactorial in nature, with contributions from both bile acid accumulation and mitochondrial electron transport chain (ETC) inhibition. Identification of pathways and demonstration of key signaling mechanisms may provide biological plausibility for genetic and/or non-genetic biomarkers. In this regard, the sponsor suggested **six non-invasive biomarkers** (e.g., tolvaptan exposure, bile acids, miR-122, acylcarnitines, etc.) associated with the activation of nuclear receptor pathways (see Table 21) would provide ways to test hepatocellular stress and liver injury. This could potentially identify patients susceptible to liver injury, particularly after initiating therapy with tolvaptan.

1.3 Recommendations

1.3.1 Approvability

There are no approvability issues for tolvaptan based on nonclinical toxicity testing program.

1.3.3 Labeling

Recommendations and edits were made on the labeling document on SharePoint and presented at the Division labeling meetings.

2 Drug Information

2.1 Drug

CAS Registry Number: 68521-88-0 (acetate salt)
4474-91-3 (free base)

Generic Name: Tolvaptan

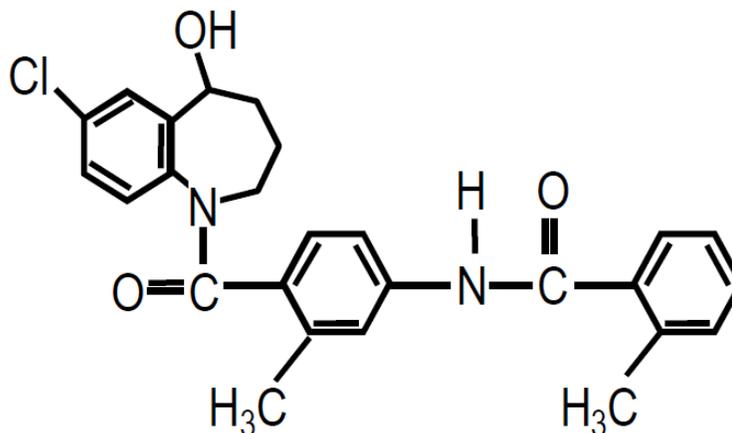
Code Name: OPC-41061; OPC-156

Trade name: Jynarque®

Chemical Name: (±)-4'-[(7-chloro-2,3,4,5-tetrahydro-5-hydroxy-1*H*-1-benzazepin-1-yl) carbonyl]-*o*-tolu-*m*-toluidide.

Molecular Formula/Molecular Weight: C₅₀H₇₁N₁₃O₁₂, 448.94 gm/mol

Structure and Biochemical Description:



Pharmacologic Class: Vasopressin V2-receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 72,975; NDA 22,275 - Samsca® tablets (for the treatment of hypervolemic and euvolemic hyponatremia).

2.3 Drug Formulation

Jynarque® immediate release tablets for oral administration are available in 15, 30, 45, 60 and 90 mg strengths of tolvaptan. Inactive ingredients include corn starch,

hydroxypropyl cellulose, lactose monohydrate, low – substituted hydroxypropyl cellulose, magnesium stearate and microcrystalline cellulose and FD & C Blue No.2 Aluminum Lake as colorant.

2.4 Proposed Clinical Population and Dosing Regimen

Adults at risk of rapidly progressing autosomal dominant polycystic kidney disease (ADPKD). Oral tablet taken twice daily as a split-dose (30/15 mg, 45/15 mg, 60/30 mg, or 90/30 mg) with titration as tolerated to a maximum daily dose of 120 mg.

2.5 Regulatory Background

Tolvaptan was approved by the FDA in 2009 for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (NDA 22,275) in May 2009. On November 15, 2012, the sponsor submitted an NDA (204441) for tolvaptan for the treatment of autosomal dominant polycystic kidney disease (ADPKD). A Cardiovascular and Renal Drugs Advisory Committee meeting held on August 5, 2013 did not recommend its approval. A complete response letter issued by the FDA on August 28, 2013 stated for a need of an additional efficacy trial if the drug to be approved for the stated indication. As advised by the FDA, the sponsor has resubmitted the current NDA (on October 24, 2017) seeking approval for the treatment of ADPKD.

3 Pharmacology

3.1 Primary Pharmacology

3.1.1. *In vivo studies*

3.1.1.1 Comparison of the effects of OPC-41061 and furosemide in Nagase analbuminemic rats and SD rats

This non-GLP study (#037333, Report No. 031027) was conducted at Frontier Sciences Unit, Department of Medical Innovations, New Drug Research Division, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima, Japan. The study was initiated on September 12, 2014 and completed on July 25, 2016. The objective of the study was to determine the diuretic effects of OPC-41061 and furosemide in Nagase analbuminemic rat (NAR), which is established from Sprague–Dawley rats. NAR is deficient in the production of albumin due to mutation in its gene. Furosemide is a loop diuretic for the treatment of edematous symptoms in cardiac, renal and hepatic failure.

Methods

OPC-41061- (b) (4) (lot #140228-1) and furosemide were weighed and suspended in a 1% hydroxypropyl methylcellulose solution in distilled water.

Animals used: a) male rat/Sprague-Dawley [Cri:CD(SD)], 7 to 9 weeks old
 b) male rat/ Nagase Analbuminemia [NAR/Slc], 7 to 9 weeks old

Rats were allocated for 3 kinds of experiments: diuretic response (n = 5/group), PK (n = 3/group) and protein binding (n = 3/group) (Tables 1 and 2).

PD study: Each rat was weighed before administration of drugs (oral, 5 ml/kg). After the administration of each compound, the rats were individually placed in metabolic cages with free access to water and food. Urine sample was collected over periods of 0 to 2, 2 to 4 and 4 to 6 hours. The urine volume collected at each interval was measured by its weight and subsequently analyzed. At the end of 6 hr, each rat was weighed again and then euthanized.

PK study: Blood samples were collected from the tail vein at 0.5, 1, 2, 4 and 6 hr after oral administration of each compound (5 ml/kg). Rats were euthanized at the end of the study.

In vitro protein binding assay: Blood samples were collected from abdominal vein under anesthesia. OPC-41061 or furosemide was dissolved in methanol. Protein binding was determined by equilibrium dialysis method.

Table 1. Experimental design for PD and PK studies (similar doses but differs in size/group)

Number	Group Name	Strain	Test Compound and Dose	Number per group	
				PD	PK
1	SD-Control	SD	1% HPMC solution 5 mL/kg PO	5	3
2	SD-O-1		1 mg/kg OPC-41061 PO	5	3
3	SD-O-3		3 mg/kg OPC-41061 PO	5	3
4	SD-O-10		10 mg/kg OPC-41061 PO	5	3
5	SD-F-10		10 mg/kg Furosemide PO	5	3
6	SD-F-30		30 mg/kg Furosemide PO	5	3
7	SD-F-100		100 mg/kg Furosemide PO	5	3
8	NAR-Control	NAR	1% HPMC solution 5 mL/kg PO	5	3
9	NAR-O-1		1 mg/kg OPC-41061 PO	5	3
10	NAR-O-3		3 mg/kg OPC-41061 PO	5	3
11	NAR-O-10		10 mg/kg OPC-41061 PO	5	3
12	NAR-F-10		10 mg/kg Furosemide PO	5	3
13	NAR-F-30		30 mg/kg Furosemide PO	5	3
14	NAR-F-100		100 mg/kg Furosemide PO	5	3

Table 2. Experimental design for in vitro protein binding assay

Number	Group Name	Strain	Test Compound and Concentration	N or # replicates
1	SD-O-0.1	SD	0.1 µg/mL OPC-41061	3
2	SD-O-1		1 µg/mL OPC-41061	3
3	SD-F-1		1 µg/mL furosemide	3
4	SD-F-10		10 µg/mL furosemide	3
5	NAR-O-0.1	NAR	0.1 µg/mL OPC-41061	3
6	NAR-O-1		1 µg/mL OPC-41061	3
7	NAR-F-0.1		0.1 µg/mL furosemide	3
8	NAR-F-1		1 µg/mL furosemide	3

The following parameters were determined

- **PD Study**

- Change in body weight
 - Change in body weight was expressed as the ratio of before and 6h after administration.
- Change in urine volume and urine excretions of electrolytes (Na, K and Cl)
 - Change in urine volume and urine excretion of electrolytes was calculated by subtracting mean value after the administration of vehicle from mean value after the administration of OPC-41061 or furosemide.
- Urine osmolality
- Urine excretions of compounds

- **PK Study**

- Total and unbound plasma concentrations of compounds
 - Unbound plasma concentrations were calculated by multiplying total plasma concentrations by mean fraction unbound in two concentration groups.
- PK parameters (C_{max} , t_{max} , $t_{1/2}$, AUC_t , and AUC_{∞}) of compounds
- In vitro protein binding assay
- Protein binding ratio, fraction unbound and recovery of compounds

Results

Diuretic effects of OPC-41061 at doses of 1 to 10 mg/kg and furosemide at doses of 10 to 100 mg/kg were evaluated in SD rats and NAR. In SD rats, OPC-41061 dose-dependently increased urine volume relative to control with peak effect at 0 to 2 hr. Similar effect was noted with furosemide (Fig. 1). In NAR, OPC-41061 showed a similar diuretic effect with peak effect at 0 to 2 hr. In contrast, furosemide except at the high dose showed no significant change in urine volume at all 3 periods. A moderate increase in urine volume was observed with furosemide at 100 mg/kg. In summary, OPC-41061 showed similar increase ($P > 0.05$) in urine volume during 0 to 2 hr and 0 to 6 hr after the administration in SD rats and NAR. In contrast, furosemide showed significant decline in the increase in urine volume during 0 to 2 and 0 to 6 hours after the administration in NAR relative to SD rats. Additionally, each dose comparisons revealed significant reduction in urine volume during 0 to 2 hours after the administration at doses of 30 and 100 mg/kg and during 0 to 6 hours after the administration at all doses (Fig. 2).

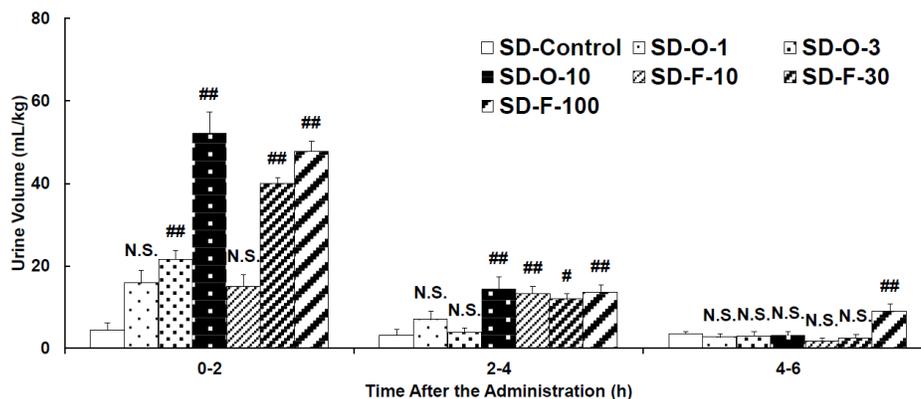
Urine osmolality: In SD rats, urine osmolality decreased significantly at all doses with both drugs. In NAR, OPC-41061 decreased urine osmolality in a dose-dependent manner. In contrast, furosemide did not decrease urine osmolality.

Plasma concentrations of OPC-41061 (Table 3) and furosemide (Table 4) were lower in NAR than those were in SD rats. Plasma unbound fraction of OPC-41061 in both SD rats and NAR were similar (Table 5). In contrast, the protein binding of furosemide in NAR serum was markedly lower than that was in SD rat serum (Table 6).

(A) SD rat

Dunnett's test using mixed effect model for repeated measures approach

Strain	vs Control	P values
SD rat	1 mg/kg OPC-41061	0.0505
	3 mg/kg OPC-41061	0.0126
	10 mg/kg OPC-41061	<.0001
	10 mg/kg Furosemide	0.0054
	30 mg/kg Furosemide	<.0001
	100 mg/kg Furosemide	<.0001



(B) NAR

Dunnett's test using mixed effect model for repeated measures approach

Strain	vs Control	P values
NAR	1 mg/kg OPC-41061	0.0754
	3 mg/kg OPC-41061	0.0005
	10 mg/kg OPC-41061	<.0001
	10 mg/kg Furosemide	0.906
	30 mg/kg Furosemide	0.9159
	100 mg/kg Furosemide	<.0001

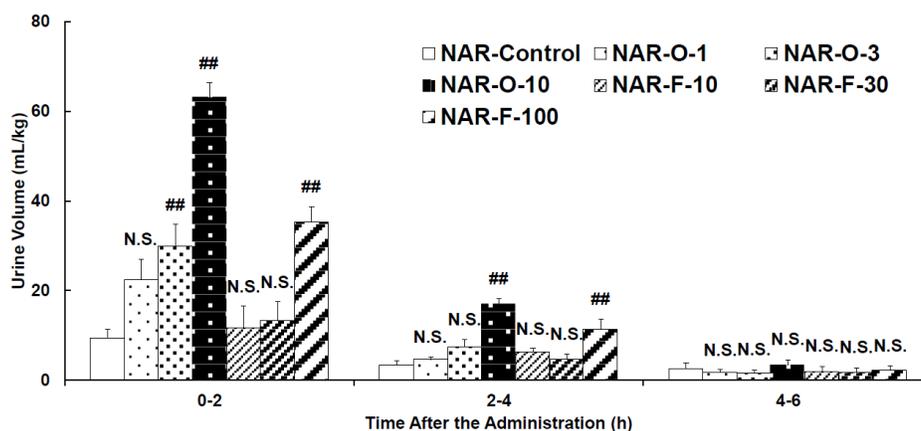


Figure 1. Time course of urine volume after the administration of OPC-41061 and furosemide in SD rats and NAR.

Urine volume during 0 to 2, 2 to 4 and 4 to 6 hours after the administration of vehicle, OPC-41061 or furosemide in SD rats (A) and NAR (B) was expressed as the mean ± SEM (n=5). Time courses of urine volume after the administration of vehicle, OPC-41061 or furosemide in SD rats and NAR were respectively analyzed by mixed effect model for repeated measures approach versus control group. P values are described in the upper table. Furthermore, differences of urine volume between OPC-41061 or furosemide-treated groups and control group at each time point were analyzed by Dunnett's test. #P<0.05, ##P<0.01. N.S.: not significant

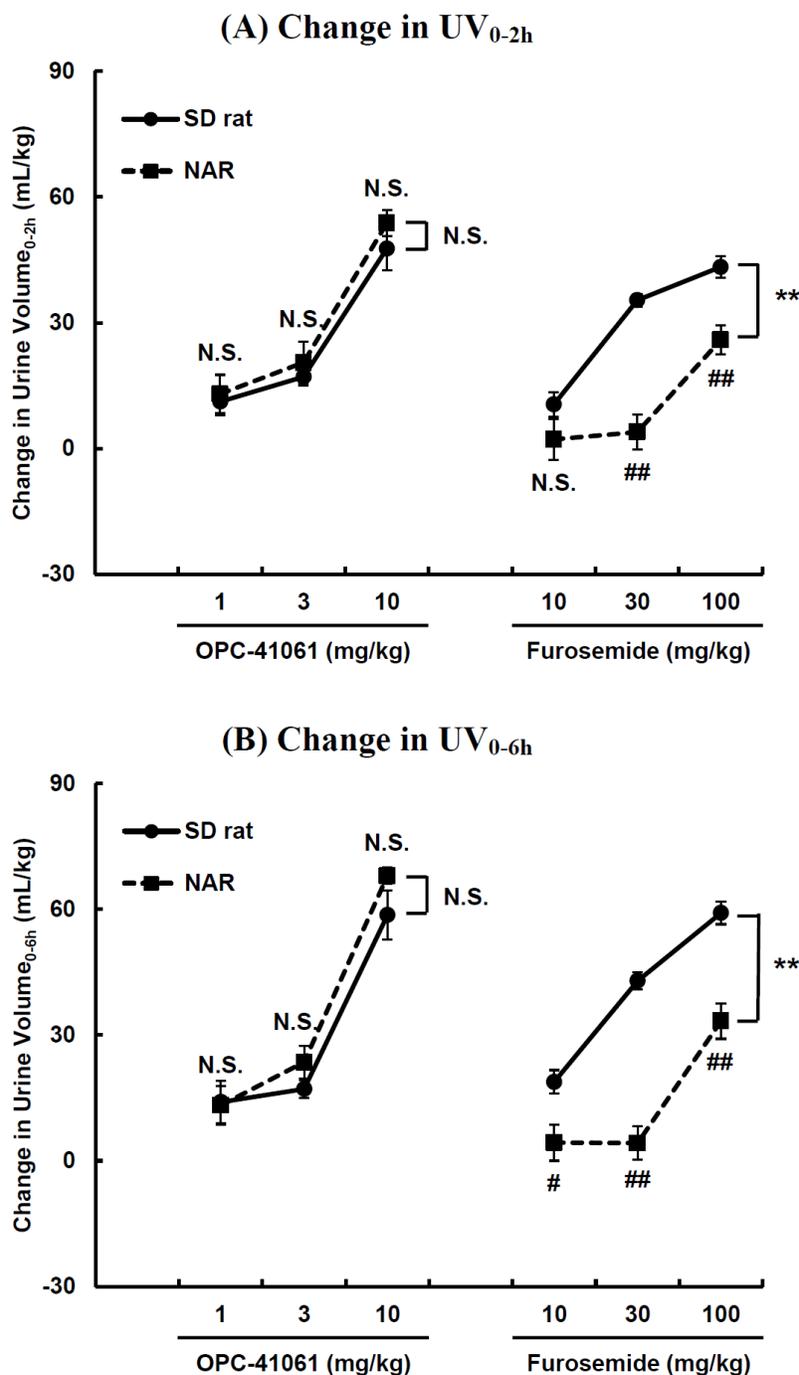


Figure 2. Effects on urine volume by the administration of OPC-41061 and furosemide in SD rats and NAR.

Change in urine volume during 0 to 2 (A) and 0 to 6 (B) hours after the administration of vehicle, OPC-41061 or furosemide in SD rats and NAR was expressed as the mean \pm SEM (n=5). Differences between in SD rats and NAR of change in urine volume in OPC-41061 or furosemide-treated groups were analyzed by 2-way ANOVA. Furthermore, differences between SD rats and NAR of change in urine volume in each OPC-41061 or furosemide-treated group were analyzed by unpaired t test.

**P<0.01, #P<0.05, ##P<0.01. N.S.: not significant.

Table 3. Pharmacokinetics parameters after the administration of OPC-41061 in SD rats and NAR

Strain	Dose (mg/kg)	C _{max} (ng/mL)	t _{max} (h)	AUC _t (ng·h/mL)	AUC _∞ (ng·h/mL)	t _{1/2} (h)
SD	1	8.202± 1.237	0.667± 0.289	21.47± 6.27	23.12± 6.22	1.58± 0.26
	3	26.25± 6.71	1.00± 0.87	80.19± 24.11	90.96± 32.05	1.71± 0.46
	10	169.3± 60.1	0.667± 0.289	416.8± 118.2	437.7± 133.7	1.24± 0.20
NAR	1	1.529± 0.325	0.833± 0.289	3.236± 1.274	6.258*	2.63*
	3	5.699± 1.928	0.500± 0.000	15.97± 3.20	19.29± 3.19	2.30± 0.26
	10	19.76± 3.60	0.833± 0.289	78.01± 10.34	110.5± 12.4	3.19± 0.86

Pharmacokinetics parameters after the administration of OPC-41061 are expressed as the mean ± SD (n=3) or the mean (*; n=2). C_{max}; maximum plasma concentration, t_{max}; time to reach maximum plasma concentration, AUC_t; area under the plasma concentration-time curve from zero to time t, AUC_∞; area under the plasma concentration-time curve from zero to time infinity, t_{1/2}; elimination half-life.

Table 4. Pharmacokinetics parameters after the administration of furosemide in SD rats and NAR

Strain	Dose (mg/kg)	C _{max} (µg/mL)	t _{max} (h)	AUC _t (µg·h/mL)	AUC _∞ (µg·h/mL)	t _{1/2} (h)
SD	10	1.567± 0.223	2.00± 0.00	4.561± 0.601	4.989± 0.747	1.44± 0.12
	30	8.272± 4.290	0.500± 0.000	17.13± 9.02	20.77± 9.88	2.55± 0.42
	100	54.94± 12.04	0.500± 0.000	114.8± 1.3	136.1± 24.8	2.15± 1.24
NAR	10	0.04281± 0.01742	1.00± 0.87	0.1287± 0.0574	0.1774± 0.0409	3.18± 1.48
	30	0.2921± 0.1305	0.500± 0.000	0.5983± 0.1137	0.7303± 0.1471	2.46± 0.33
	100	1.402± 0.303	0.500± 0.000	3.024± 0.067	4.035± 0.221	3.04± 0.27

Pharmacokinetics parameters after the administration of furosemide are expressed as the mean ± SD (n=3). C_{max}; maximum plasma concentration, t_{max}; time to reach maximum plasma concentration, AUC_t; area under the plasma concentration-time curve from zero to time t, AUC_∞; area under the plasma concentration-time curve from zero to time infinity, t_{1/2}; elimination half-life.

Table 5. Serum protein binding of OPC-41061 in SD rats and NAR

Strain	Concentration ($\mu\text{g/mL}$)	Protein Binding (%)
SD	0.1	96.2 \pm 0.6
	1	95.4 \pm 0.6
NAR	0.1	95.5 \pm 0.2
	1	95.1 \pm 0.5

The in vitro protein binding ratio of OPC-41061 in SD rats and NAR serum are expressed as the mean \pm SD (n=3).

Table 6. Serum protein binding of furosemide in SD rats and NAR

Strain	Concentration ($\mu\text{g/mL}$)	Protein Binding (%)
SD	1	98.5 \pm 0.6
	10	98.8 \pm 0.1
NAR	0.1	47.9 \pm 2.2
	1	47.8 \pm 1.3

The in vitro protein binding ratio of furosemide in SD rats and NAR serum are expressed as the mean \pm SD (n=3).

OPC-41061 demonstrated similar diuretic response in both SD rats and NAR as did similar protein binding. In contrast, furosemide showed the significant difference in its natriuretic effect between SD rats and NAR. The resistance to diuretic effect is probably related to lower value of protein binding in NAR serum, thereby decreasing in plasma concentration and urine excretion of furosemide. It is argued that the lack of albumin which is an important protein for binding to furosemide, unbound furosemide immediately disappears from the blood circulation to the extravascular organ. Thus, the binding of furosemide to circulating plasma albumin is essential for the delivery of furosemide to the site for its action. OPC-41061 binds not only albumin but also several other proteins such as human α 1-acidglycoprotein and immunoglobulinG. It is suggested that, OPC-41061 binds other unidentified proteins in NAR.

In another study (#037262, Report #030642), the plasma concentration-time profiles of OPC-41061 in the NAR and SD rats were determined. These results suggested that there was no difference in the intravenous plasma pharmacokinetics of OPC-41061 between the albumin deficient rats and SD rats. The data showed that the profiles were similar in normal and albumin deficient rats (Table 7). The changes in the C_0 and AUC_{∞} values were within 1.15-fold.

Table 7. Pharmacokinetic parameters of OPC-41061 in plasma after single intravenous administration at 1 mg/kg in male rats

Species/Strain	Rat/Sprague-Dawley (SD)	Rat/Nagase analbuminemic (NAR)
Gender (M/F)/Number of Animals	M/3 per group	M/3 per group
Feeding Condition	Non-fasted	Non-fasted
Vehicle/Formulation	Dimethylsulfoxide (2 mg/mL)	Dimethylsulfoxide (2 mg/mL)
Method of Administration	Intravenous	Intravenous
Dose (mg/kg)	1 mg/kg	1 mg/kg
Sample	plasma	plasma
Analyte	OPC-41061	OPC-41061
Assay	LC-ESI-MS/MS	LC-ESI-MS/MS
PK Parameters:		
C₀ (ng/mL)	565.6	647.8
AUC_t (ng·h/mL)	262.6	269.2
AUC_∞ (ng·h/mL)	263.0	269.4
t_{1/2} (h) (Time for calculation – h)	0.652 (0.25 - 6 h)	0.609 (0.5 - 6 h)
V_{ss} (mL/kg)	2850	2471
V_z (mL/kg)	3576	3261
CL (mL/h/kg)	3802	3711
Report No.	030642	030642

AUC_∞ = area under the concentration-time curve from time zero to infinity; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t; C₀ = concentration of the drug in plasma at time zero; CL = clearance; LC-ESI-MS/MS = liquid chromatography-electrospray ionization-tandem mass spectrometry; M = male; t_{1/2} = half-life; V_{ss} = volume of distribution at the steady state; V_z = volume of distribution at the terminal phase.

In summary, NAR showed no diuretic resistance to tolvaptan, differing from furosemide, indicating that tolvaptan may provide new therapeutic approach in hypoalbuminemia patients with various disorder of body fluid balance.

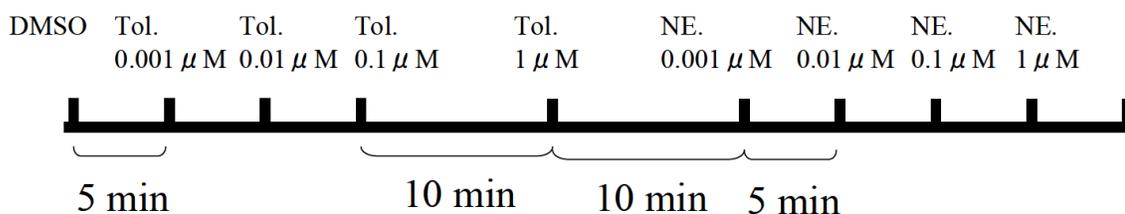
3.1.1.2 Effects of tolvaptan on the portal vein pressure in rats

This non-GLP study (#036768, Report No. 030056) was conducted at [REDACTED] (b) (4). The study was initiated on April 30, 2013 and completed on April 1, 2014. The rationale behind this study is that vasopressin stimulates V1 receptors to constrict visceral blood vessels resulting in reduction in portal blood flow and portal venous pressure (PVP). The study was designed to determine the effect of tolvaptan on PVP by using isolated perfused rat livers, anesthetized intact rats and anesthetized rats with liver fibrosis induced by bile duct ligation.

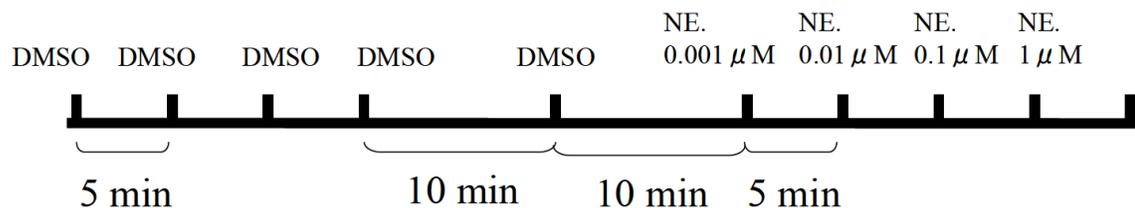
Methods

- a) Perfused rat liver: Male Sprague-Dawley rats (n=10; 354 ± 4 gm), were anesthetized and mechanically ventilated. The hepatic artery was ligated and the bile duct was cannulated. The inferior vena cava (IVC) above the renal veins was ligated, and both the IVC and portal vein were cannulated. Heparinized blood was withdrawn from the right carotid artery diluted with 5% bovine albumin in Krebs solution for portal perfusion. Once perfusion started, the livers were rapidly removed, suspended from the isometric transducer and weighed continuously throughout the experiment. The liver was perfused in a recirculating manner at a constant flow rate through the portal vein with blood that was pumped from the venous reservoir at 37°C, bubbled with 95% O₂ and 5% CO₂. After the baseline measurements, tolvaptan (or vehicle DMSO) and norepinephrine were consecutively administered as a bolus in a cumulative manner (final concentration 0.001, 0.01, 0.1, and 1 μM) into the reservoir. Tolvaptan and norepinephrine were dissolved in DMSO and saline, respectively.

1) Tolvaptan group



2) Control group



- b) Normal anesthetized rat experiment: Male Sprague-Dawley rats (n=5; 271 ± 3 g) were anesthetized followed by cannulation of the right carotid artery (for blood pressure measurement) and right external jugular vein (for measurement of the central venous pressure (CVP) and administration of drugs). Following an incision of the abdominal wall, the main portal vein was cannulated for measurement of PVP and portal venous blood flow (PBF). The abdomen was closed and the MAP, PVP, PBF and CVP were continuously measured with pressure transducers. Tolvaptan was intravenously injected as a bolus consecutively at the doses of 0.1, 0.3 and 1 mg/kg. Tolvaptan was dissolved in the solution containing 10% PEG400 and 3% cremophor EL.
- c) Bile duct ligation-induced liver cirrhotic rat experiment: Male Sprague-Dawley rats (n=4; 358 ± 5 g) were anesthetized followed by a midline incision of the abdominal wall to expose and ligate the common bile duct. The animals were allowed to recover and used 5 weeks after surgery in a manner similar to that of anesthetized rat experiment (see (b) above) except that PBF was not measured. At the end of the study, liver was removed and processed for histological examination to confirm the presence of liver fibrosis.

Results

In isolated blood-perfused rat livers, tolvaptan at dose ranging 0.001 to 1 µM did not affect any hemodynamic variables, liver weight, or bile flow rate. In contrast, norepinephrine (in presence or absence of tolvaptan) dose-dependently produced portal venoconstriction with increase in PVP and hepatic vascular resistance, and decrease in liver weight and bile flow. These findings suggest that tolvaptan does not affect not only the portal vein but also the reactivity of the portal vein to norepinephrine.

In anesthetized intact rats, bolus consecutive doses (0.1, 0.3 and 1 mg/kg) of tolvaptan did not significantly alter baseline values of mean arterial blood pressure, central venous pressure, PVP, mean portal venous blood flow and the calculated vascular resistance of the splanchnic vascular beds. Similar observations were made in anesthetized bile duct-ligated rats to tolvaptan. Histological examination of the liver showed marked architectural distortion of lobular structure and extensive bile duct proliferation. In some sections, there was a large area of parenchymal necrosis and inflammatory cell infiltration.

In conclusion, tolvaptan did not affect portal venous pressure in all three different set of experiments.

4 Reproductive and Developmental Toxicology

4.1 Nine-week repeat dose toxicity study in 4-day old juvenile rats

Key Study Findings: Tolvaptan was administered orally by gavage to neonates from PND 4 to PND 67 at doses of 10, 30 or 100 mg/kg/day. Two pups receiving 100 mg/kg/day died or euthanized on study day 9 or day 28. Histopathology revealed dilatation of the pelvis in the kidney, decreased hematopoiesis in bone marrow in the femur, decreased extramedullary hematopoiesis in the spleen and atrophy in the thymus. In animals sacrificed at the end of dosing period, dilatation of the renal pelvis was observed in males at ≥ 30 mg/kg/day and in females at all doses. Focal hemorrhage in lungs was observed in male rats at ≥ 30 mg/kg, and of female rats at 100 mg/kg. At the end of recovery, both lesions were still observed, but at reduced incidence and severity. According to the sponsor, the NOAEL for this study was 30 mg/kg/day. However, it is incorrect based on the observation of renal pelvic dilatation and elevated BUN at all doses. A NOAEL could not be determined for this study.

Purpose: The objective of the study was to evaluate the adverse effects and to determine the pharmacokinetic profile of tolvaptan when administered to pups from day 4 for 9 weeks followed by 4 week recovery.

Study No.: (b) (4) 7420, Sponsor report #029808

Location of Report: EDR

Conducting Laboratory and Location: (b) (4)

Dates of Study: The pups were initially dosed on June 25 and last necropsied on September 24, 2013.

GLP Compliance: Yes

QA'd Report: Yes

Drug, Lot #: OPC-156, 121217-1, 99.9% pure

Formulation: OPC-156 was dissolved in hypromellose (TC-5) 1% solution in water for injection. The dosing formulations were prepared weekly, refrigerated and protected from light until use. Concentration and homogeneity was analyzed 5 times. The doses were expressed as base.

Animals

Species/Strain: Sprague-Dawley Rats (CrI:CD[SD]) (from (b) (4))

#/Animals/Group: Pregnant females were received on day 18 of gestation and were allowed to deliver at the facility. Healthy neonates showing no external abnormalities were used in this study from 4 days after birth. There were 10/sex/group for the main study, 5/sex/group were assigned to the recovery subset (control and high dose groups only) and 26/sex/group (8/sex for control) to the toxicokinetics study (Table 8).

Age: Post-partum day 4 at initiation of dosing

Weight: Males: 13.1 to 22.3 gm; Females: 13.1 to 21.7 gm, at initiation of dosing

Husbandry: Dams with their neonates were housed in plastic cages until day 21 of lactation. After weaning (21 days after birth), animals were housed individually.

Food and water were available *ad libitum*. Food but not water was not withheld prior to blood sampling.

Dosing

Doses: OPC-156 was administered at three dose levels: 10, 30 or 100 mg/kg/day (Table 8). Control animals received the vehicle at the same dosage volume as treated animals. Doses were selected based on the results of 1-week and 4-week repeat dose range-finding juvenile toxicity studies in rats of the same strain (Sponsor report #028960 and 029331). In the first study, 4-day or 7-day old pups, all males and females treated at the high dose (1000 mg/kg/day), were found dead or moribund sacrificed on day 2. In the 2nd study, the high dose was reduced to 100 mg/kg/day and 4-day pups were treated for 4 weeks. There were no treatment related deaths or clinical signs during the conduct of the study. Mean body weights of the high dose groups were significantly lower than the control throughout the treatment period. There were no treatment-related gross findings observed at necropsy.

Table 8. Study design and group allocation of animals

Test group	Dose level (mg/kg/day)	Dose concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Recovery group	
					No. of animals	Animal number	No. of animals	Animal number
Control ^{a)}	0	0	10	Male	10	1001-1010	5	1011-1015
				Female	10	1101-1110	5	1111-1115
Low dose	10	1	10	Male	10	2001-2010	–	–
				Female	10	2101-2110	–	–
Middle dose	30	3	10	Male	10	3001-3010	–	–
				Female	10	3101-3110	–	–
High dose	100	10	10	Male	10	4001-4010	5	4011-4015
				Female	10	4101-4110	5	4111-4115

a): The control group was given the vehicle (1 w/v% TC-5 solution)

Test group	Dose level (mg/kg/day)	Dose concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Satellite group for TK analysis on Day 1 of administration		Satellite group for TK analysis on Days 32 and 63 of administration	
					No. of animals	Animal number	No. of animals	Animal number
Control ^{a)}	0	0	10	Male	3	1201-1203	5	1204-1208
				Female	3	1301-1303	5	1304-1308
Low dose	10	1	10	Male	15	2201-2215	11	2216-2226
				Female	15	2301-2315	11	2316-2326
Middle dose	30	3	10	Male	15	3201-3215	11	3216-3226
				Female	15	3301-3315	11	3316-3326
High dose	100	10	10	Male	15	4201-4215	11	4216-4226
				Female	15	4301-4315	11	4316-4326

a): The control group was given the vehicle (1 w/v% TC-5 solution)

Route, Mode and Duration of Administration: Main study animals were treated orally by gavage (10 ml/kg), once daily for 9 weeks, from day 4 post-partum to day 67 post-partum. Recovery subset animals were treated for the same duration but were killed 4 weeks after cessation of dosing.

Observations and Measurements

Clinical Signs: From the day following birth, neonates were observed once daily for clinical signs, such as external appearance, nutritional condition and behavior, and litter size. During the treatment period, all neonates were observed 3 times a day for general condition. During the recovery period, all neonates were observed once daily in the morning. Dams were observed once daily for lactation condition during the lactation period.

Body Weight: Pups were weighed on days 1, 4, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59 and 63 of administration before dosing of the day. Also weighed during the recovery period on days 1, 7, 14, 21 and 28 of recovery period.

Food Consumption: One-day food consumption per animal was determined based on 2-, 3- or 4-day cumulative food consumption measured before dosing on the days of measurement (days 19 to 21, 21 to 24, 24 to 28, 28 to 31, 31 to 35, 35 to 38, 38 to 42, 42 to 45, 45 to 49, 49 to 52, 52 to 56, 56 to 59 and 59 to 63 of administration). During the recovery period, 6- or 7-day cumulative food consumption was measured (days 1 to 7, 7 to 14, 14 to 21 and 21 to 28 of recovery) and one-day food consumption was calculated for each time period.

Water intake: One-day water intake was measured before dosing on days 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59 and 63 of administration. During the recovery period, one-day water intake was measured on days 2, 7, 10, 14, 17, 21, 24 and 28 of recovery.

Postnatal Evaluation: On all pups, opening of the vagina (females, days 32 and 39 of administration) and cleavage of the balanopreputial gland (males, days 39 and 46 of administration) were observed. For 2 high dose group males in the in which differentiation was not observed on day 46 of administration, observation was continued from day 47 of administration until differentiation was observed.

Ophthalmology: Conducted on all animals in week 9 (day 60) of administration and all animals in the recovery group in week 4 (day 25) of recovery.

Clinical Pathology: Blood samples were collected (from the abdominal aorta under isoflurane anesthesia) at termination from all animals (fasted overnight, 16 to 21 hr). Specimens were divided for hematology¹ and for clinical chemistry² evaluations. Urine samples³ were collected from 5 males and 5 females in each group after dosing on days 58 to 59 and all animals in the recovery groups on days 23 to 24 of recovery. Samples were collected under deprivation of food but providing water freely as 4-hour urine samples, and then 20-hour urine samples were collected with free access to food and water.

Toxicokinetics: Animals assigned exclusively to the toxicokinetics section of the study (satellite group, see Table 8) were used for blood sample collection at each

¹ Erythrocytes, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte ratio and count, white blood cell count, white blood cell differential, platelets, prothrombin time, activated partial thromboplastin time.

² ALT, AST, AP, LDH, CPK, γ -GTP, total bilirubin, total protein, glucose, BUN, creatinine, creatine kinase, sodium, potassium, chloride, calcium, inorganic phosphorus, triglycerides, cholesterol, phospholipids, A/G ratio, protein fraction ratio.

³ Volume, specific gravity, color, pH, blood, protein, bilirubin, glucose, ketones, urobilinogen, Na, K, Cl, creatinine.

time point on days 1 (Table 9), 32 and 63 (Table 10) of administration. Carcasses were discarded without further examination except for a male receiving 30 mg/kg/day. This animal died on study day 8 and necropsy revealed gavage error.

Table 9. Subject animals and time points of blood collection (day 1 of administration)

Blood collection time point	Control		10 mg/kg group		30 mg/kg group		100 mg/kg group	
	Male	Female	Male	Female	Male	Female	Male	Female
1 hour after dosing	–	–	2201	2301 a)	3201	3301	4201	4301
			2202	2302	3202	3302	4202	4302
			2203	2303	3203 a)	3303 a)	4203	4303
2 hours after dosing	1201	1301	2204	2304	3204	3304	4204	4304
	1202	1302	2205	2305	3205	3305	4205	4305
	1203	1303	2206	2306	3206	3306	4206	4306
4 hours after dosing	–	–	2207	2307	3207	3307	4207	4307
			2208	2308	3208	3308	4208	4308
			2209	2309	3209	3309	4209	4309
6 hours after dosing	–	–	2210	2310	3210	3310	4210	4310
			2211	2311	3211	3311	4211	4311
			2212	2312	3212	3312	4212	4312
24 hours after dosing	–	–	2213	2313	3213	3313	4213	4313
			2214	2314	3214	3314	4214	4314
			2215	2315	3215	3315	4215	4315

The numbers indicate the animal number of the subject animals.

a): Since blood could not be collected from these animals, blood was collected from the animals for adjustment of litter size in case of deaths.

Table 10. Subject animals and time points of blood collection (days 32 and 63 of administration)

Blood collection time point	Control		10 mg/kg group		30 mg/kg group		100 mg/kg group	
	Male	Female	Male	Female	Male	Female	Male	Female
1 hour after dosing	–	–	2216	2316	3216	3316	4216	4316
			2217	2317	3217	3317	4217	4317
			2218	2318	3218	3318	4218	4318
2 hours after dosing	1204	1304	2219	2319	3219	3319	4219	4319
	1205	1305	2220	2320	3220	3320	4220	4320
	1206	1306	2221	2321	3221	3321	4221	4321
4 hours after dosing	–	–	2222	2322	3222	3322	4222	4322
			2223	2323	3223	3323	4223	4323
			2224	2324	3225 a)	3324	4224	4324
6 hours after dosing	–	–	2216	2316	3216	3316	4216	4316
			2217	2317	3217	3317	4217	4317
			2218	2318	3218	3318	4218	4318
24 hours after dosing	–	–	2219	2319	3219	3319	4219	4319
			2220	2320	3220	3320	4220	4320
			2221	2321	3221	3321	4221	4321

The numbers indicate the animal number of the subject animals.

a): The animal numbered 3224 died on Day 8 of administration. Therefore, a spare animal (Animal No. 3225) was used instead.

Gross Pathology: All animals were euthanized following blood collection. Animals were observed for any abnormality in the external appearance and all the organs and tissues in the cephalic, thoracic and abdominal cavities were examined macroscopically. Animals that died or sacrificed moribund were necropsied immediately after they were discovered.

Organ Weight: The terminal body weights and weights of the organ collected (Table 11) were recorded for all surviving animals at scheduled necropsy. Absolute weight and organ weight ratio relative to body weight were calculated.

Histopathology: On completion of the necropsy, representative samples of the protocol tissues (Table 11) were collected from all animals (including those found dead or euthanized) and fixed. All organs or tissues in the control and high dose groups and all animals that died or were sacrificed in a moribund state were examined microscopically. The kidney in males and females, lung (including bronchus), bone marrow in the femoral, spleen and thymus in males, for which test article-related effects were suspected in the high dose group, were examined in the mid dose group, and the kidney in males was examined in the low dose group. At scheduled necropsy, small pieces of the left lateral lobe liver were removed from 2 animals of each sex in the control and high dose groups in the main and recovery groups. The liver samples from the main group were prefixed and examined by electron microscopy.

Table 11. Tissues/organs sampled for histopathological examination

Organ/tissue	Histopathology	Organ weight	Electron microscopy
	H&E		
Cerebrum	√	√ (as brain)	
Cerebellum	√		
Spinal cord, thoracic	√		
Sciatic nerve	√*		
Eye	√*		
Optic nerve	√*		
Harderian gland	√*		
Pituitary	√	√	
Thyroid	√*	√	
Parathyroid	√*	(as thyroid glands)	
Adrenal	√*	√	
Thymus	√	√	
Spleen	√	√	
Lymph node, submandibular	√		
Lymph node, mesenteric	√		
Heart	√	√	
Aorta, thoracic	√		
Trachea	√		
Lung (bronchus)	√	√	
Tongue	√		
Esophagus	√		
Stomach	√		
Intestine, duodenum	√		
Intestine, jejunum	√		
Intestine, ileum (Peyer's patch)	√		
Intestine, cecum	√		
Intestine, colon	√		
Intestine, rectum	√		
Salivary gland, submandibular	√*	√	
Salivary gland, sublingual	√*	(as salivary glands)	
Liver	√	√	√
Pancreas	√		
Kidney	√*	√	
Urinary bladder	√		
Testis/Ovary	√* / √*	√/√	
Epididymis/Uterus (including cervix)	√* / √*	√/√	
Prostate/Vagina	√/√	√/√	
Seminal vesicle	√*		
Mammary gland, inguinal, only female	√*		
Bone + Bone marrow, sternal	√		
Bone + Bone marrow, femoral + joint	√*		
Skeletal muscle, femoral	√*		
Skin, inguinal	√*		
Gross pathological abnormalities	√		
Nasal cavity		preservation only	

In addition, parts for identification (auricle with an ear tag) were removed and preserved.

Items marked with √ were examined.

* Removed bilaterally, but examined unilaterally.

Results

Analysis of Formulations: The dosing formulations were stable for at least 7 days and were refrigerated and protected from light. The concentration of OPC-156 in each suspension was in the range from 97.7 to 106.0% of the nominal concentration (acceptable range: percentage to the nominal concentration within $100\% \pm 10\%$) and the coefficient of variance (C.V.) in the range from 0.0 to 2.0% (acceptable range: C.V. less than 10%), both of which were within the acceptable range.

Mortality: A male (#4009) and a female (#4111) receiving 100 mg/kg/day died or euthanized on study day 9 or day 28. The male had no abnormal clinical signs before death and no abnormal findings at necropsy. Histopathology revealed dilatation of the pelvis in the kidney, decreased hematopoiesis in bone marrow in the femur, decreased extramedullary hematopoiesis in the spleen and atrophy in the thymus. The female sacrificed moribund demonstrated malocclusion on day 21, and decreased spontaneous movement and unkempt fur just before dosing on the day of necropsy. Furthermore, the animal lost body weight as a result of eating difficulty (food spillage). Additionally, as noted previously, a male in the 30 mg/kg/day group in satellite group died because of dosing error.

Clinical Signs: No test substance-related clinical signs were noted at all doses. Also, no abnormality was noted during the recovery.

Postnatal Development: Developmental landmark such as vaginal opening was complete in all females on day 39. However, cleavage of the balanopreputial gland was incomplete in males at all doses (3/10, 2/10 and 0/14 in low, mid and high dose groups, respectively). On day 46, differentiation was observed in all males except for 2 high dose group males. It was complete in these 2 males on day 51.

Body Weights and Food Consumption: Statistically significant reductions in mean body weight relative to control were observed in males and females at the 100 mg/kg/day through the dosing period and continued until day 14 of the recovery period (Table 12). The lower body weights correlated with lower food consumption values, up to day 45 in males and day 35 in females. A decrease in body weight was also observed in the 30 mg/kg/day group for the duration of the dosing period, but attained statistical significance on study day 7 (Table 12). Food consumption was comparable to the control group throughout the recovery period.

Water Intake: Water consumption was statistically significantly high in all dose groups relative to control group through the dosing period (Table 13).

Ophthalmology: There were no ophthalmology findings.

Hematology: A statistically significant prolongation of prothrombin time (+12% relative to control) was observed in males receiving 100 mg/kg/day. This trend continued until the end of recovery period. There were no changes in the hematology parameters related to the administration of tolvaptan.

Table 12. Body weight and food consumption

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Number of Animals								
Main Group	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Recovery Group	M: 5	F: 5	M: 0	F: 0	M: 0	F: 0	M: 5	F: 5

BODY WEIGHT								
Dosing period								
Day 7 (g)	24.8	23.7	24.1	22.5	23.2*	21.4*	19.4*	19.1*
Day 14 (g)	43.6	41.9	41.4	39.1*	41.5	39.3	32.4*	32.6*
Day 28 (g)	125.3	114.3	119.0	109.8	116.3	110.1	90.9*	86.3*
Day 42 (g)	257.0	184.4	251.1	178.8	244.4	173.6	199.0*	158.4*
Day 63 (g)	419.5	243.5	422.4	236.4	403.3	226.1	354.2*	210.4*
Recovery period								
Day 1 (g)	421.4	242.7	NA	NA	NA	NA	344.7*	194.3*
Day 14 (g)	483.7	262.7	NA	NA	NA	NA	419.6*	224.2*
Day 28 (g)	530.4	275.9	NA	NA	NA	NA	486.0	259.5

FOOD CONSUMPTION								
Dosing period								
Day 21 (g/rat/day)	11.5	10.7	11.4	10.5	10.6	10.5	7.5*	7.7*
Day 42 (g/rat/day)	30.0	22.0	29.9	21.1	29.7	21.1	26.3*	21.2
Day 63 (g/rat/day)	34.3	22.7	34.9	22.0	34.3	21.8	33.2	21.7
Recovery period:								
Day 14 (g/rat/day)	33.4	22.6	NA	NA	NA	NA	35.2	23.0
Day 28 (g/rat/day)	32.2	21.1	NA	NA	NA	NA	35.3	24.3

* Significantly different from the control value

Table 13. Water consumption

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Number of Animals								
Main Group	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Recovery Group	M: 5	F: 5	M: 0	F: 0	M: 0	F: 0	M: 5	F: 5
Dosing period								
Day 21 (mL/rat/day)	17.2	17.5	24.2*	21.7*	26.5*	27.6*	31.6*	32.2*
Day 42 (mL/rat/day)	36.1	30.9	54.0*	71.8*	85.2*	97.5*	94.2*	109.4*
Day 63 (mL/rat/day)	45.4	36.7	74.7*	65.4*	96.9*	103.5*	119.3*	143.5*
Recovery period								
Day 14 (mL/rat/day)	52.3	45.0	NA	NA	NA	NA	47.3	36.0
Day 28 (mL/rat/day)	48.3	38.8	NA	NA	NA	NA	40.0	33.9

* Significantly different from the control value

Urinalysis: A statistically significant increase in urine volume with a decrease in pH and osmolality, and increases ($p < 0.05$) in excretion of Na, K, and Cl were observed in all male and female tolvaptan treated groups (Table 14). This action is consistent with the pharmacological action of tolvaptan. There were no treatment related effects on urinalysis at the end of the 4-week recovery period.

Table 14. Summary of urinalysis (week 9 of administration)

Sex	Male				Female			
	0	10	30	100	0	10	30	100
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals	5	5	5	5	5	5	5	5
Urine volume (mL/24h)	13.8	39.9** (+189%)	53.3** (+286%)	72.5** (+425%)	5.8	37.7** (+550%)	63.8** (+1000%)	81.3** (+1302%)
Osmotic pressure (mOsm/kg)	1416	1280	685* (-52%)	370* (-74%)	1962	636* (-68%)	242* (-88%)	191* (-90%)
Na (mmol/24h)	1.6	3.8** (+138%)	3.1** (+94%)	2.6** (+63%)	0.7	2.0** (+186%)	1.4* (+100%)	1.3* (+86%)
K (mmol/24h)	3.8	10.3* (+171%)	7.6* (+100%)	5.6* (+47%)	2.0	4.3** (+115%)	2.9 (+45%)	2.9 (+45%)
Cl (mmol/24h)	2.3	6.2* (+170%)	4.9* (+113%)	3.6* (+57%)	1.2	2.8** (+133%)	2.0* (+67%)	2.0* (+67%)

Values in the table indicate group mean.

Values in the parentheses indicate percentage of change against the control mean (+: increase, -: decrease).

*: p≤0.05, **: p≤0.01 (statistically significant difference from the control group)

Clinical Chemistry: Statistically significant dose-dependent increase (+23% to 56%) in blood urea nitrogen was observed in males and females at all dose groups (Table 15). At the end of the recovery period, the value was still high (+35%) in females in the 100 mg/kg/day group. High dose group males showed a statistically significant increase in ALT and inorganic phosphorus, while high dose females showed an increase in total cholesterol. In addition, mid and high dose group animals showed a statistically significant decrease in sodium and chloride (Table 15) that were considered incidental.

Organ Weights: At the end of the dosing period, there were statistically significant decreases in the absolute and/or relative weights of the brain, salivary glands, thymus, pituitary, lung, spleen, kidney, adrenal, testis and epididymis in the high dose group animals. Also, statistically significant increases in the absolute and/or relative weights of the heart, liver and uterus was noted in the high dose group (Table 16). However, the sponsor rules out any relation to treatment and contends that these changes were related to the lower body weights at the end of treatment. No notable changes in organ weights were noted at the end of the recovery period.

Table 15. Summary of blood chemistry (end of administration period)

Sex	Male				Female				
	Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals	10	10	10	9	10	10	10	10	10
ALT (IU/L)	28	30	30	35** (+25%)	23	21	21	24	
T-CHO (mg/dL)	63	72	65	62	66	71	74	81* (+23%)	
BUN (mg/dL)	13	17** (+31%)	16** (+23%)	19** (+46%)	16	21** (+31%)	23** (+44%)	25** (+56%)	
Na (mmol/L)	145	141** (-3%)	142** (-2%)	143	143	140** (-2%)	141* (-1%)	143	
Cl (mmol/L)	106	103** (-3%)	103** (-3%)	106	107	104** (-3%)	106	107	
P (mg/dL)	7.1	7.1	7.4	8.1** (+14%)	5.8	5.9	6.3	6.1	
TP (g/dL)	6.1	5.9** (-3%)	5.8** (-5%)	5.9	6.1	6.0	5.9	6.0	

Values in the table indicate group mean.

Values in the parentheses indicate percentage of change against the control mean (+: increase, -: decrease).

*: p<0.05, **: p<0.01 (statistically significant difference from the control group)

Table 16. Summary of organ weight (end of administration period)

Sex	Male				Female			
	0	10	30	100	0	10	30	100
Dose (mg/kg/day)	10	10	10	9	10	10	10	10
No. of animals								
Brain								
Absolute weight (mg)	2120	2023*	1990**	1845**	1908	1872	1834	1711**
		(-5%)	(-6%)	(-13%)				(-10%)
Relative weight (mg/100 g)	550	529	549	564	854	867	893	879
Pituitary								
Absolute weight (mg)	12.5	12.0	12.4	10.7*	12.0	12.3	11.8	11.1
				(-14%)				
Relative weight (mg/100 g)	3.2	3.1	3.4	3.3	5.4	5.7	5.8	5.7
Salivary gland								
Absolute weight (mg)	638.2	605.4	588.3	529.2**	389.4	379.5	377.6	342.3*
				(-17%)				(-12%)
Relative weight (mg/100 g)	165.3	158.2	161.8	161.3	173.5	175.4	182.5	175.0
Thymus								
Absolute weight (mg)	588	525	526	479*	466	439	453	383*
				(-19%)				(-18%)
Relative weight (mg/100 g)	152	138	145	146	208	202	222	197
Heart								
Absolute weight (mg)	1281	1358	1339	1211	836	814	793	809
Relative weight (mg/100 g)	332	352	367*	369*	373	376	385	413**
			(+11%)	(+11%)				(+11%)
Lung								
Absolute weight (mg)	1337	1391	1298	1181*	955	939	909	891
				(-12%)				
Relative weight (mg/100 g)	346	361	356	361	426	434	440	456*
								(+7%)
Liver								
Absolute weight (g)	11.54	11.54	11.23	10.50	6.40	6.34	6.35	6.43
Relative weight (g/100 g)	2.98	3.00	3.08	3.19**	2.85	2.92	3.07**	3.27**
				(+7%)			(+8%)	(+15%)
Spleen								
Absolute weight (mg)	849	789	817	649**	479	470	477	423
				(-24%)				
Relative weight (mg/100 g)	220	206	225	197	214	216	230	217
Kidney								
Absolute weight (mg)	2874	2857	2894	2544*	1786	1786	1704	1674
				(-11%)				
Relative weight (mg/100 g)	745	744	794	773	796	825	828	856
Adrenal								
Absolute weight (mg)	59.3	58.3	52.7*	48.2**	62.2	59.8	59.0	59.8
			(-11%)	(-19%)				
Relative weight (mg/100 g)	15.4	15.2	14.5	14.7	27.8	27.7	28.7	30.5
Testis								
Absolute weight (mg)	3229	3196	3016	2697*	NA	NA	NA	NA
				(-16%)				
Relative weight (mg/100 g)	836	832	829	822	NA	NA	NA	NA
Epididymis								
Absolute weight (mg)	818	789	770	653**	NA	NA	NA	NA
				(-20%)				
Relative weight (mg/100 g)	211	206	212	199	NA	NA	NA	NA
Uterus								
Absolute weight (mg)	NA	NA	NA	NA	438	419	440	501
Relative weight (mg/100 g)	NA	NA	NA	NA	197	194	214	258*
								(+31%)

Values in the table indicate group mean.

Values in the parentheses indicate percentage of change against the control mean (+: increase, -: decrease).

*: $p \leq 0.05$, **: $p \leq 0.01$ (statistically significant difference from the control group)

NA: Not applicable

Gross Pathology: At unscheduled necropsy, the high dose male (#4009) had no abnormal findings. The unscheduled euthanized female (#4111) had unkempt fur, malocclusion and cyst in the kidney. Gross findings were limited to the kidneys and lungs. At scheduled necropsy, treatment-related renal pelvic dilatation was observed in females at all dose groups and in the males at mid and high dose groups. Dark red foci (hemorrhage) in lungs was noted in high incidence in males receiving 100 mg/kg/day followed by females (Table 17). At the end of recovery period, renal pelvis dilatation was present in all males (5/5) and reduced in incidence in females (2/4) at 100 mg/kg/day. Focal hemorrhage was observed in the male rats (1/5), but at reduced incidence and severity.

Table 17. Summary of necropsy findings (end of administration period)

Sex	Male				Female				
	Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals	10	10	10	9	10	10	10	10	10
Kidney									
Dilatation, pelvic	1	0	4	7	0	1	1	5	
Lung									
Focus, dark red	2	0	1	7	0	0	0	2	

Values in the table indicate the number of animals with findings.

Histopathology: Both unscheduled necropsied animals showed mild renal pelvic dilatation. The male (#4009) showed minimal decrease in hematopoiesis in bone marrow and spleen, and moderate atrophy in the thymus.

In animals that survived to scheduled euthanasia and at the end of the recovery period, microscopic findings were limited to the kidneys and lungs. At the end of the dosing period, pelvic dilatation was observed in males at 30 and 100 mg/kg and in females at all doses. The incidence and severity of the kidney dilatation was higher at 100 mg/kg/day than was at lower dose groups (Table 18). At the end of the recovery period, renal pelvis dilatation was reduced in severity in the males and in incidence females at 100 mg/kg (Table 19). At the end of the dosing period, mild focal hemorrhage was observed in the lungs of male rats at 30 and 100 mg/kg, and of female rats at 100 mg/kg (Table 18). At the end of recovery, focal hemorrhage was observed only in male rats, and at reduced incidence and severity (Table 19).

As noted in the 'Observations section', liver lobe samples from the high dose group animals were subjected to electron microscopy. No remarkable changes were observed in the liver from either sex in any high dose group animals at the end of administration period.

Table 18. Summary of histopathology findings (end of administration period)

Sex	Male				Female			
	0	10	30	100	0	10	30	100
Dose (mg/kg/day)								
No. of animals	10	10	10	9	10	10	10	10
Kidney								
No. examined	10	10	10	9	10	1	10	10
Dilatation, pelvic	1	0	5	7	0	1	1	5
minimal	0	0	1	0	0	1	1	1
mild	1	0	2	4	0	0	0	4
moderate	0	0	2	3	0	0	0	0
Lung (bronchus)								
No. examined	10	0	10	9	10	0	0	10
Hemorrhage, focal	1	0	2	6	0	0	0	2
minimal	1	0	2	4	0	0	0	2
mild	0	0	0	2	0	0	0	0

Values in the table indicate the number of animals with findings.

Table 19. Summary of histopathology findings (end of recovery period)

Sex	Male		Female	
	0	100	0	100
Dose (mg/kg/day)				
No. of animals	5	5	5	4
Kidney				
No. examined	5	5	5	4
Dilatation, pelvic	1	5	0	1
minimal	1	1	0	0
mild	0	4	0	1
Lung (bronchus)				
No. examined	5	5	5	4
Hemorrhage, focal	0	2	0	0
minimal	0	2	0	0

Values in the table indicate the number of animals with findings.

Toxicokinetics: The C_{max} and AUC_{24 hr} of tolvaptan and its two metabolites (DM-4103 and DM-4107) increased in a dose-related manner for both sexes on all days of measurement. For tolvaptan, there was no notable sex difference on day 1; however, on days 32 and 63, the C_{max} and AUC_{24 hr} in females were higher than those in males (Table 20).

Table 20. The toxicokinetic parameters of tolvaptan and its two metabolites in the treated groups

Sex	Analyte	C_{max} ($\mu\text{g/mL}$)								
		Day 1			Day 32			Day 63		
		Dose (mg/kg/day)			Dose (mg/kg/day)			Dose (mg/kg/day)		
		10	30	100	10	30	100	10	30	100
Male	OPC-156	0.05082	0.1937	1.585	0.08851	0.4109	1.004	0.2621	0.5401	1.271
	DM-4103	0.04830	0.2100	1.295	0.1634	0.5119	4.620	0.2085	1.194	8.786
	DM-4107	0.01085	0.1125	1.162	0.04723	0.1930	2.059	0.1168	0.5947	3.165
Female	OPC-156	0.1374	0.2165	1.712	0.5595	2.229	3.861	1.174	2.682	5.495
	DM-4103	0.08605	0.2333	1.342	0.09087	0.4948	4.478	0.08505	0.3592	2.746
	DM-4107	0.05509	0.1314	1.638	0.06434	0.3444	3.294	0.1059	0.5419	3.116
Sex	Analyte	AUC_{24h} ($\mu\text{g}\cdot\text{h/mL}$)								
		Day 1			Day 32			Day 63		
		Dose (mg/kg/day)			Dose (mg/kg/day)			Dose (mg/kg/day)		
		10	30	100	10	30	100	10	30	100
Male	OPC-156	0.2507	1.407	7.279	0.1617	1.558	4.223	1.013	2.522	7.518
	DM-4103	0.6146	3.524	21.92	0.9646	5.056	34.46	1.920	13.08	79.24
	DM-4107	0.1344	1.664	15.86	0.04723	1.077	12.45	0.6063	3.858	27.29
Female	OPC-156	0.3089	1.284	9.501	2.245	9.442	31.42	4.710	12.60	29.63
	DM-4103	1.103	2.875	21.42	0.8635	3.192	26.50	0.8753	3.011	32.41
	DM-4107	0.1711	1.175	21.22	0.1259	1.616	19.46	0.5316	3.147	27.17

C_{max} : maximum serum concentration after administration

AUC_{24h} : area under the concentration-time curve from time zero to 24 hours after administration calculated using the trapezoidal method

The lower limit of quantification was set at 0.02 $\mu\text{g/mL}$ (Days 1 and 32) or 0.005 $\mu\text{g/mL}$ (Day 63).

5 Other Toxicity Studies

5.1 Toxicogenetic analysis of tolavaptan using collaborative cross mice

This non-GLP study (lab study #MWMOTS141, sponsor study # 037757, report #031040) was conducted at [REDACTED] (b) (4) [REDACTED] between February 19 and April 10, 2014. The objective of the study was to evaluate the liver response to tolavaptan in the genetically diverse recombinant inbred mouse strains of the Collaborative Cross (CC). The study might identify potential risk factors associated with susceptibility to tolavaptan-induced liver injury.

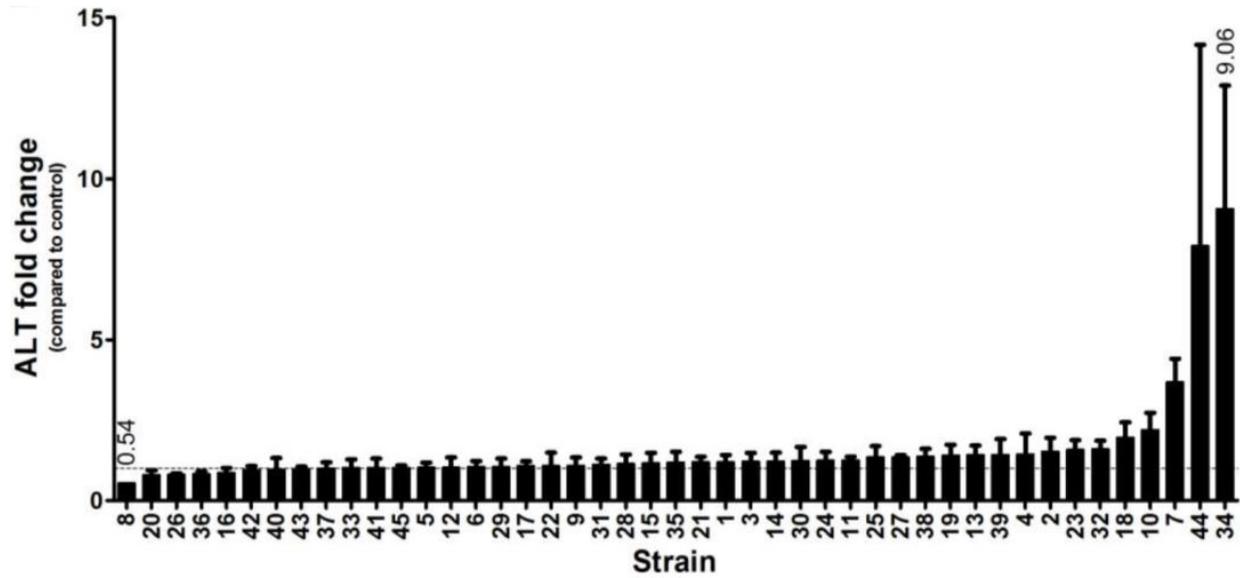
Methods

For the study, 45 genetically different inbred strains of the Collaborative Cross mice (8 to 10 weeks of age, body weight 10-30 gm), allocating randomly 8 male mice from each strain with 4 each to treatment and vehicle groups (total 360 animals) were used. A single dose of tolavaptan (100 mg/kg in 1% HPMC) was administered orally by gavage (10 ml/kg). Control animals received vehicle. Based on the previous toxicity studies, a dose of 100 mg/kg was chosen that did not result in overt liver toxicity and expected to be the maximally tolerated dose in CC strains. Furthermore, this dose is approximately 4 times (based on BSA) the maximally recommended human 120 mg dose. Whole blood was collected from all mice at 2 hr post-dosing for analysis of plasma drug and human metabolite DM-4103 concentration. Animals were killed 24 hr post dose to collect blood (for determination of drug concentration and clinical chemistry) and liver (weight, histopathological examination and gene expression microarray analysis). Phenotypic findings in combination with toxicogenomics were used to identify variation in specific genes and pathways associated with susceptibility to the tolavaptan hepatic response in sensitive CC strains.

Results

Six accidental deaths occurred shortly after the interim blood draw. No abnormal clinical findings were reported for animals in the following strains: 1, 2, 3, 4, 9, 11, 12, 13, 18, 19, 21, 28, 33, 34, 35, 38, 41, 43, and 44. Clinical findings in other strains included dehydration, hunched posture, lethargy, and excessive bleeding at the interim blood draw. Based on the pharmacology of tolavaptan, dehydration was frequently observed in all drug-treated animals.

Clinical chemistry: ALT, AST, and total bilirubin (TBIL) levels were higher ($p < 0.001$ for treatment, two-way ANOVA with variables treatment and strain) than the vehicle control. Plasma ALT levels were significantly ($p < 0.05$) elevated in strains 7, 34, and 44 in comparison to control (Fig. 3). However, miR-122 (specific marker for liver injury) levels were highly variable (Fig. 4) and did not reach statistical significance for a treatment effect ($p > 0.05$ for treatment, two-way ANOVA with variables treatment and strain).



However, miR-122-fold change values by pair were significantly correlated with ALT (Pearson $r = 0.3304$, $p < 0.0001$). Furthermore, AST, TBIL, and miR-122 were all significantly correlated with ALT fold change particularly for strain #44.

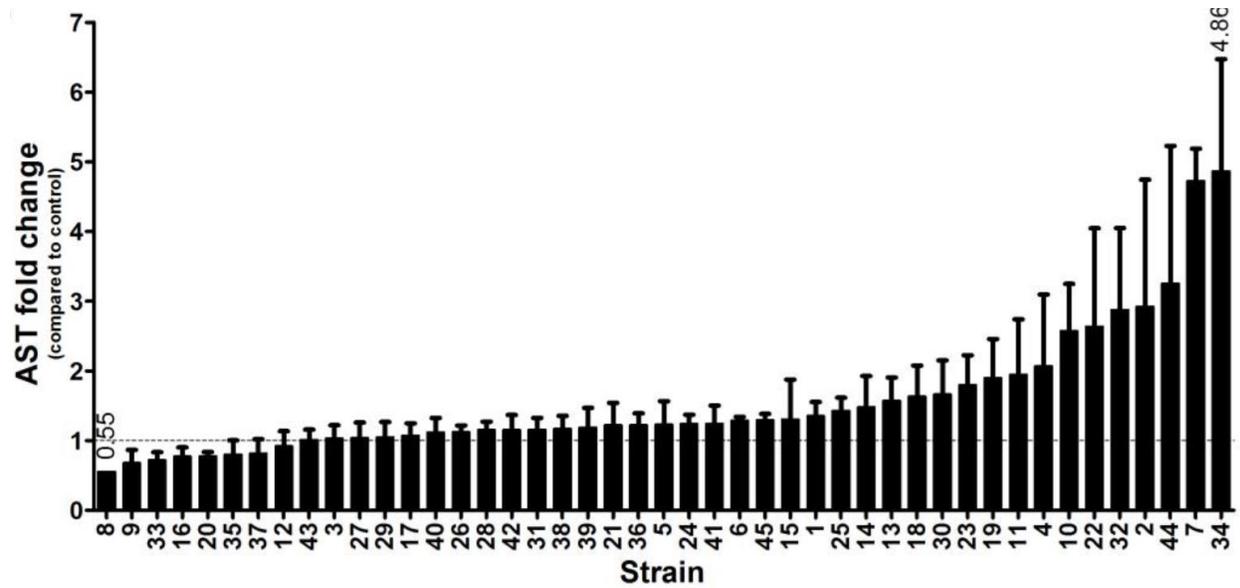


Figure 3. Average ALT(top) and AST (bottom) fold change (tolvaptan-treated animal over vehicle-treated paired control) for each strain in order by increasing average fold change from left to right. Data are represented as mean ± standard error of N=4 pairs per strain.

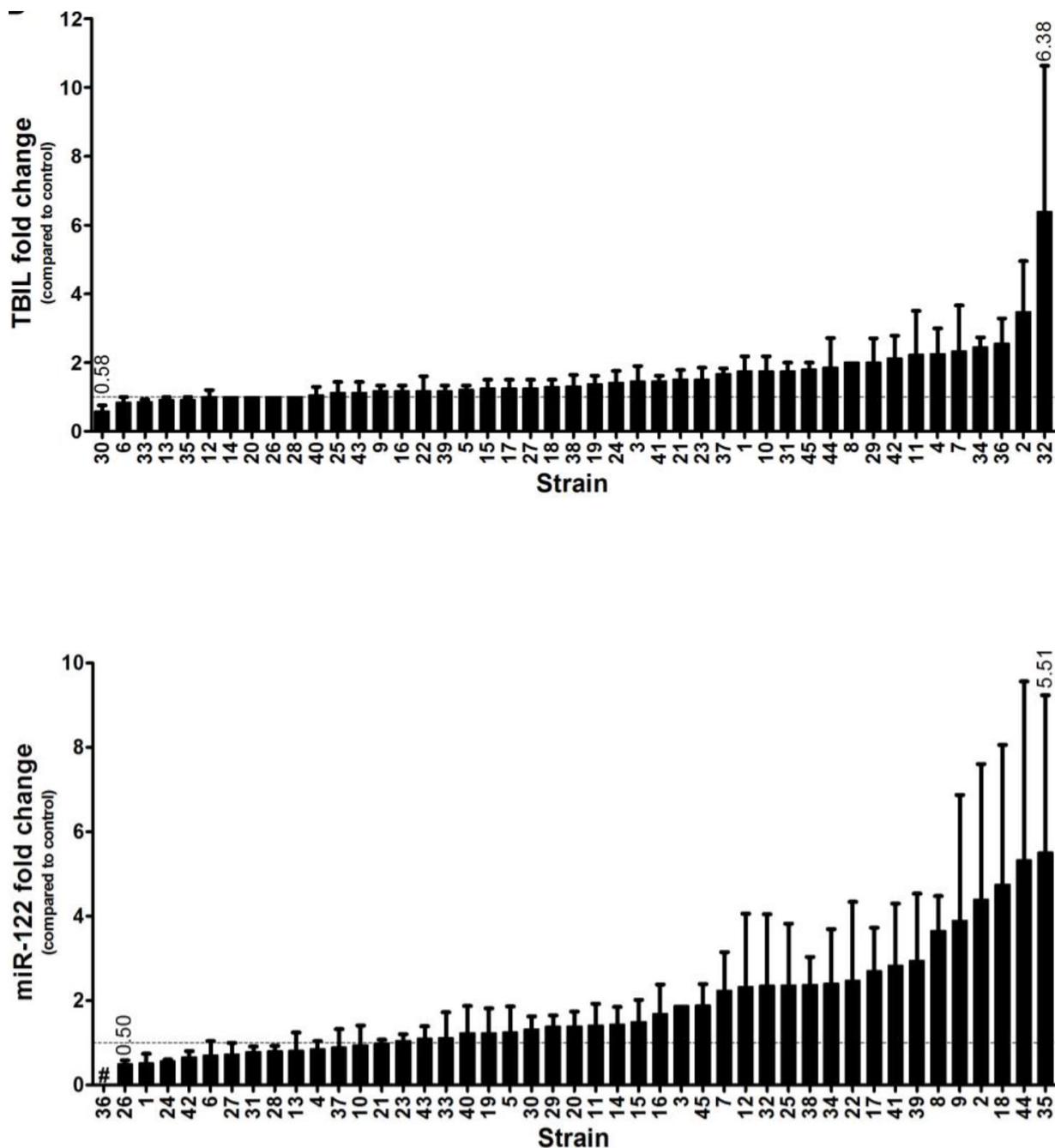


Figure 4. Average total bilirubin (top) and miR-122 (bottom) fold change (tolvaptan-treated animal over vehicle-treated paired control) for each strain in order by increasing average fold change from left to right.

Data are represented as mean ± standard error of N=4 pairs per strain.

Plasma concentration of test substance and its metabolite: Plasma tolvaptan concentrations varied significantly by strain and within individual strains at 2 h post dose. Tolvaptan plasma concentrations at 2 hr were significantly correlated (Pearson $r = 0.1571$, $p=0.0466$) with ALT fold change with the highest observed in the 3 sensitive

mouse strains (Fig. 5A). Plasma tolvaptan concentrations decreased in all strains by 24 hr, and many strains had values below the limit of quantitation (Fig. 5B). Plasma concentrations of metabolite DM-4103 also varied significantly by strain at 2 hr post dose. DM-4103 plasma concentrations at 2 hr were not significantly correlated with plasma tolvaptan concentrations at 2 hr. Mean plasma DM-4103 concentrations at 24 hr were significantly correlated with plasma tolvaptan concentrations at 24 hr. However, plasma levels of DM-4103 were not correlated with ALT fold change in the corresponding pairs.

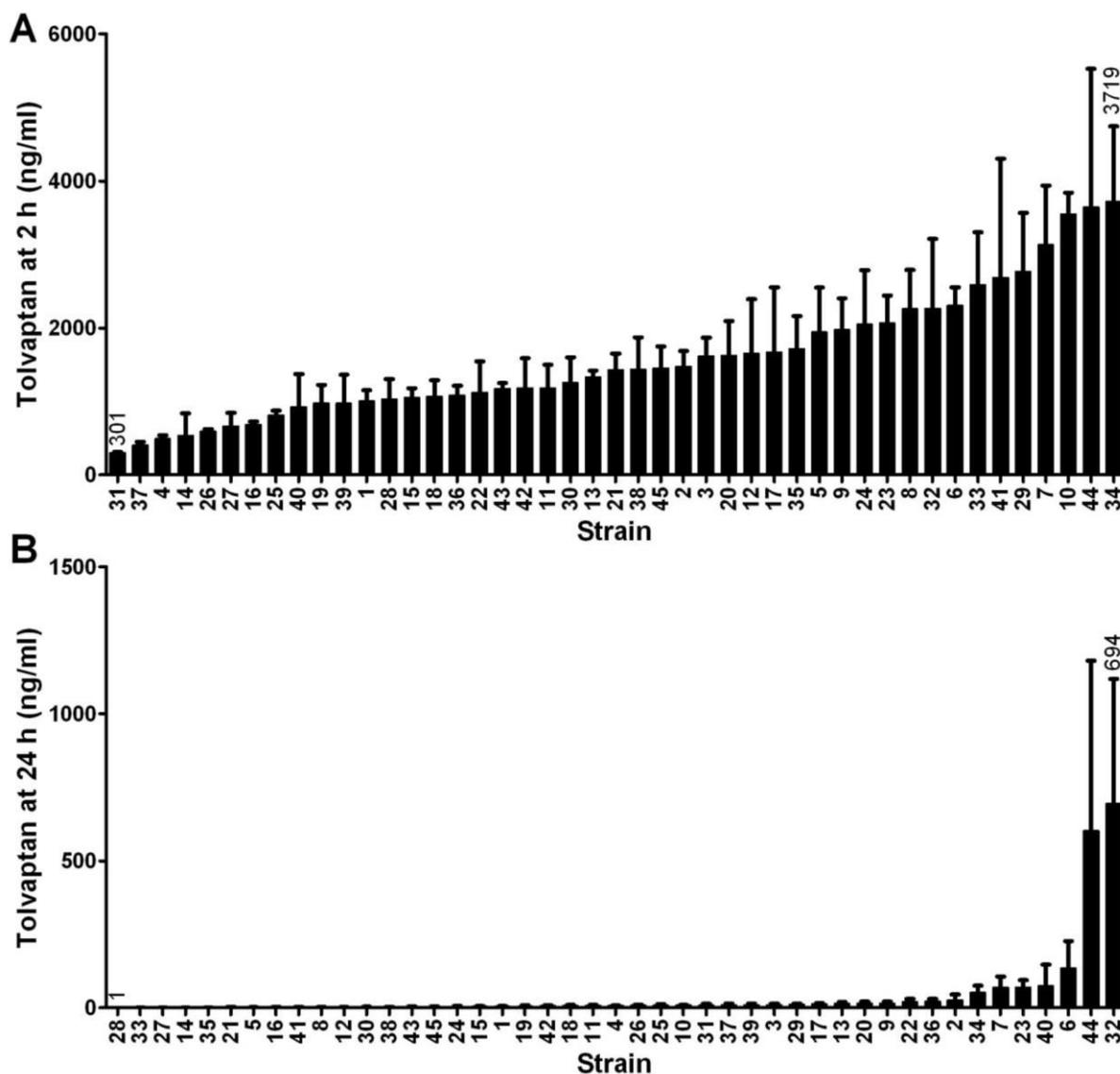


Figure 5. Average plasma tolvaptan concentrations at (A) 2 h and (B) 24 h by strain. Data are represented as mean \pm standard error of N=4 mice per strain.

Histopathology: No statistically significant difference in liver weight was seen between tolvaptan- and vehicle-treated animals. There were no differences in liver microscopic findings observed between tolvaptan-treated mice of any strain and vehicle control.

Quantitative trait loci mapping:

Genetic mapping using ALT fold change values for each strain pair across all 45 strains identified a locus on chromosome 14 (Fig. 6). At this locus, C57BL/6 founder strain (#45) DNA is highly represented among the pairs of mice with the greatest ALT elevations in response to tolvaptan.

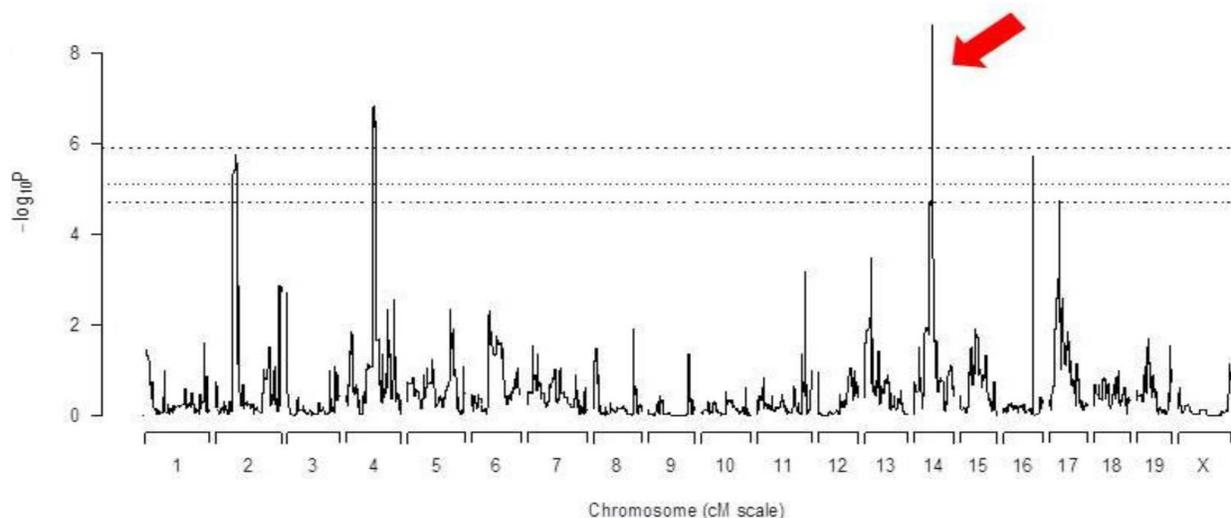


Figure 6. QTL mapping using ALT fold change

Genome scan plot illustrating QTL associated with tolvaptan-induced ALT fold change. The x-axis plots the location in the genome. The y-axis gives statistical significance of association with the phenotype. Dashed horizontal lines indicate genome wide significance at the 0.01, 0.05, and 0.1 levels. The genomic interval with the highest LOD score (Marker: JAX00053588, Chr14: 61.3 Mb) is indicated with a red arrow.

Microarray analysis: Microarray analysis identified gene expression changes correlated with the liver response and yielded a list of several hundred genes associated with significantly elevated ALT. Several nuclear receptor pathways enriched with these genes were differentially expressed after treatment with tolvaptan, independent of the liver (plasma ALT) response. The top pathways affected with treatment are oxidative stress response, superoxide radicals degradation, mitochondrial dysfunction, altered bile acid homeostasis, and fatty acid β -oxidation (Fig. 7). A different analysis was performed to identify gene expression changes that were not only associated with tolvaptan treatment, but also the liver (plasma ALT) response. One of the top 10 pathways enriched among these genes is 'bile acid biosynthesis', which was not identified with the drug treatment (Fig 8).

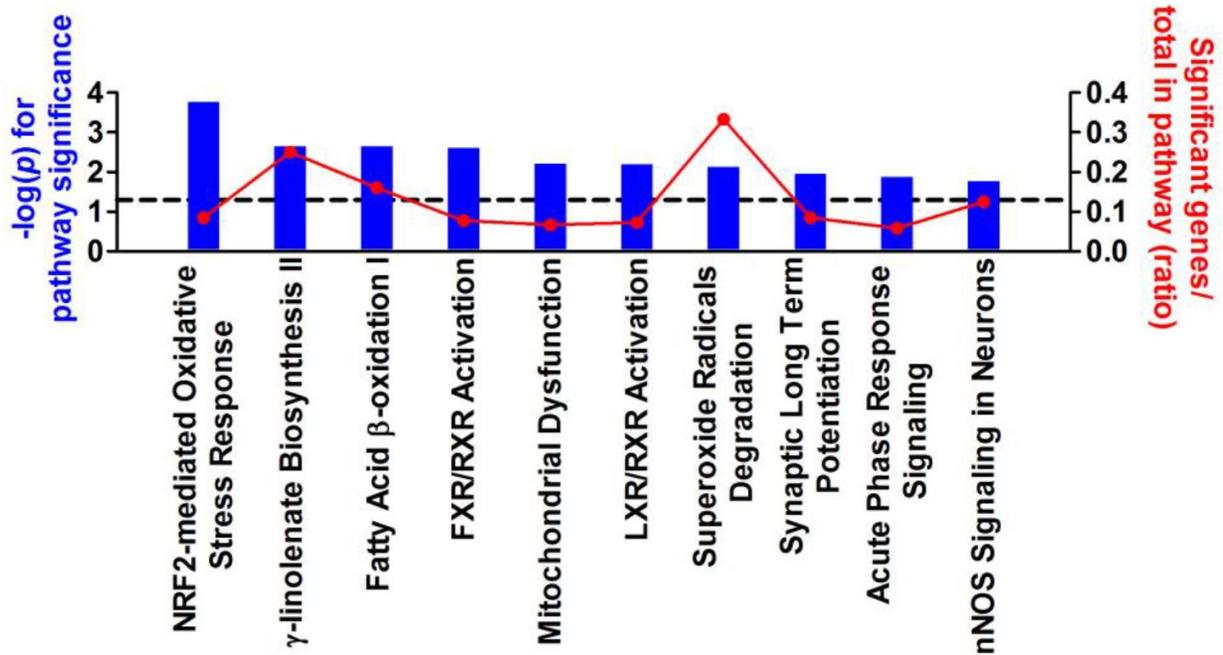


Figure 7. Pathway enrichment analysis of genes associated with tolavaptan treatment
 Top 10 pathways enriched among genes differentially expressed with tolavaptan treatment, independent of a liver response.

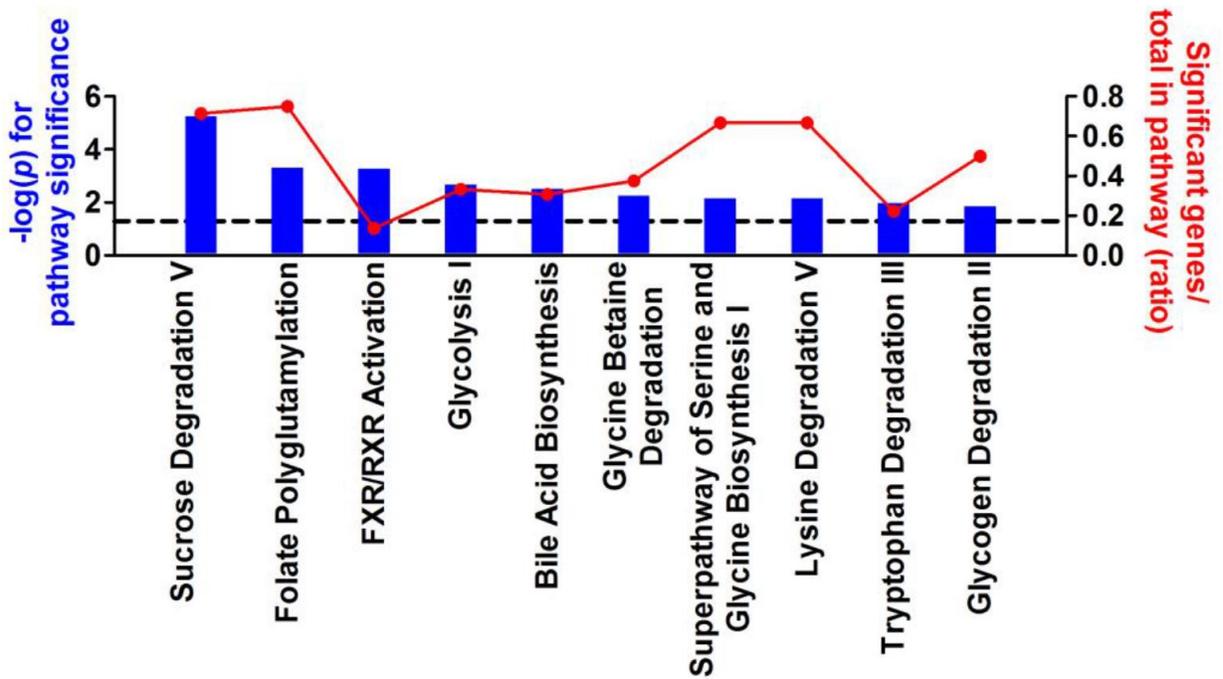


Figure 8. Pathway enrichment analysis of genes correlated with ALT fold change
 Top 10 pathways enriched among genes significantly correlated with ALT elevations.

Discussion: In recombinant inbred mouse strains of the Collaborative Cross, tolvaptan elevated ALT levels in 3 of 45 CC strains (#7, 34, and 44), which are genetically sensitive to tolvaptan-induced liver response than other CC strains or traditional rodents. AST, total bilirubin, and miR-122 (a specific marker for liver injury) were all statistically significantly correlated with the fold changes in ALT. Genetic mapping identified a locus on chromosome 14 associated with tolvaptan-induced liver response in CC mice. Microarray analysis identified gene expression changes that were associated with liver injury and ALT fold change. Gene expression changes related to treatment with tolvaptan were associated with the activation of several nuclear receptor pathways. The top pathways enriched among genes affected with treatment that could result in liver injury are mitochondrial dysfunction, bile acid transport inhibition and ROS generation (oxidative stress). Tolvaptan and its metabolites are known to inhibit several human hepatic transporters involved in bile acid transport, thus affecting bile acid homeostasis.

Thus, evidence suggests liver gene expression analysis after a single, maximally tolerated dose may in fact be a more sensitive and translational method to predict an adverse drug response in humans.^{1,2} Furthermore, evaluation of molecular signaling pathways can provide insight into mechanisms of drug toxicity as well as phenotypes for quantitative trait loci (QTL) mapping, even in the absence of overt injury.

According to the sponsor, ALT elevations may reflect early liver toxicity although no liver injury was observed in any previous repeat dose toxicity studies in any animals. The current study suggests that the 3 CC strains (#7, 34, and 44) may be genetically sensitive to tolvaptan-induced liver response than other CC strains or traditional rodents. The sponsor cautions that the liver response observed in the sensitive CC strains does not directly correspond to the pattern of liver injury observed in ADPKD patients (e.g., elevations in serum aminotransferases exceeding 3 times the upper limits of normal and corresponding elevations in serum bilirubin, delayed onset)³ in the tolvaptan clinical trials. Furthermore, the sponsor hypothesizes that it is possible that the liver response observed in the sensitive mice strain in this study could have mechanistic similarities to the early hepatocellular events that may have occurred in the ADPKD patients. The results suggest that genetic sensitivity potentiates the toxic response to tolvaptan.

¹ Laifenfeld, D., et al. (2014). Utilization of causal reasoning of hepatic gene expression in rats to identify molecular pathways of idiosyncratic drug-induced liver injury. *Toxicol Sci*, 137, 234-248.

² Leone, A., et al. (2014). Oxidative stress/reactive metabolite gene expression signature in rat liver detects idiosyncratic hepatotoxicants. *Tox Applied Pharmacol* 275, 189-197.

³ Torres, VE., et al. (2012). Tolvaptan in patients with autosomal dominant polycystic kidney disease. *New Eng J Med* 367, 2407-2418.

5.2 Stress response pathways initiated in human hepatocytes exposed to tolvaptan

This non-GLP study (lab study #MWMOTS142, sponsor study # 038008, report #031329) was conducted at [REDACTED] (b) (4) [REDACTED] between October 22, 2014 and April 24, 2015. The objective of the study was to evaluate tolvaptan-induced stress response pathways in primary human hepatocytes using a combined experimental approach of toxicogenomics, high content imaging (HCI), and exosome profiling. The study might provide insight into the cellular mechanisms contributing to the idiosyncratic drug-induced liver injury (IDILI) observed with tolvaptan.

Methods

Primary human hepatocytes from 3 adult donors (a male and two females) were seeded on micropatterned plates to create cocultures with 3T3-J2 murine embryonic fibroblasts. The cultures were exposed to serial concentrations of tolvaptan (0.01 to 50 μ M) or 0.1% DMSO (control) continuously for 4, 24, or 72 hr. Valinomycin and acetaminophen were used as reference compounds. This was followed by high content imaging to measure cell count/loss, membrane permeability, mitochondrial function, and apoptosis. In another experiment, cells were evaluated for changes in morphology, viability, gene expression, and exosome release. Media aliquot was assayed for ALT, urea, and miR-122.

Results and Discussion

Minimal changes in the number of hepatocytes were observed in response to tolvaptan treatment at all time points. The percentage of hepatocytes with both small and textured nuclei was significantly ($p < 0.001$) increased in serum-free medium at 50 μ M tolvaptan concentration relative to the vehicle-treated control (Fig. 9). The combination of both small and textured nuclei is indicative of cells undergoing programmed cell death (PCD). Additional observation showed that PCD that was not apoptosis was induced with the translocation of cytochrome c from the mitochondria to the cytoplasm resulting in increased cytoplasmic cytochrome c intensity (high cytochrome c) reflecting mitochondrial-induced apoptosis. The percentage of hepatocytes with high cytoplasmic cytochrome c spot was statistically significantly increased at the 50 μ M tolvaptan concentration relative to the vehicle-treated control (Fig. 10). Tolvaptan had a significant effect on mitochondrial function. At 24 hr, the percentage of hepatocytes with low mitochondrial function was significantly ($p < 0.05$) increased at the 50 μ M tolvaptan concentration relative to the control suggesting tolvaptan induced mitochondrial dysfunction. Under basal conditions, urea is released by hepatocytes and accumulates in the culture medium. Any decline in urea release indicates a decrease in cellular function. At 24 hr and 72 hr, tolvaptan at 20 and 50 μ M had a significant ($p < 0.05$) effect on urea release relative to the vehicle-treated control.

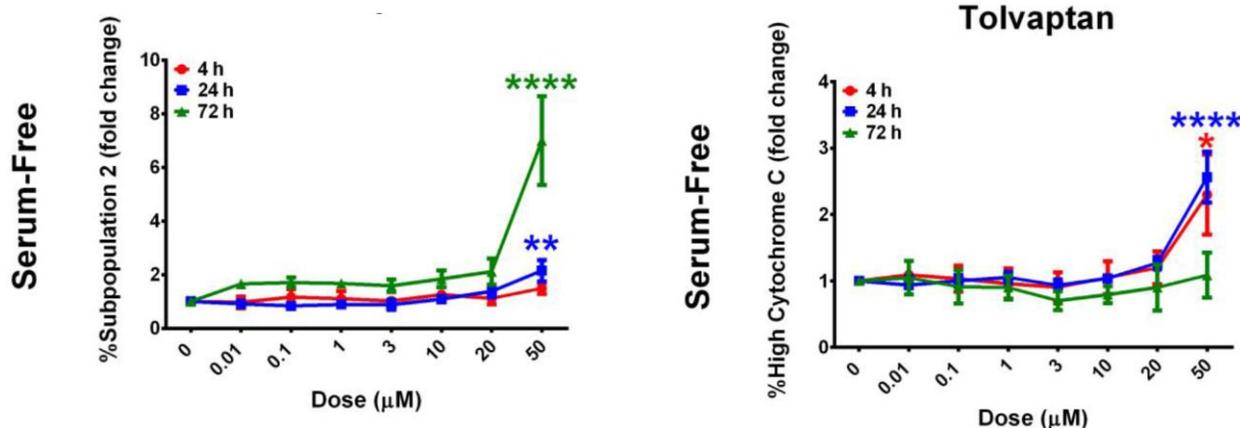


Figure 9. Left: Average fold change (relative to control) in % hepatocytes with both small and textured nuclei.

Figure 10. Right: Average fold change (relative to control) in % of hepatocytes with high cytoplasmic cytochrome c.

Cytotoxicity and exosome release were evaluated using hepatocyte-specific endpoints including hepatocyte morphology, ALT, miR-122 and urea. Statistically significant treatment-induced changes in these endpoints were typically observed at the highest concentration and varied by duration of exposure. Overt cytotoxicity was observed with acetaminophen, the reference control, as assayed by morphology, media-based endpoints and exosome release. ALT is a marker of hepatocyte death as cellular membrane permeability is required for ALT to leak into the culture medium. Tolvaptan did not ($p > 0.05$) affect ALT release (Fig. 11, left panel). On the other hand, miR-122 released into the culture medium by two mechanisms: 1) protein bound which occurs predominantly during overt cell death, 2) inside of membrane-bound vesicles called exosomes which are released from cells under basal conditions but are known to increase during cellular stress. Exosomal miR-122 was significantly ($p < 0.05$) increased at both 20 and 50 μM concentrations of tolvaptan at 24 hr, indicative of cellular stress. However, tolvaptan did not significantly affect total miR-122 release into the culture medium but the data are marred by high standard error (Fig. 11, right panel).

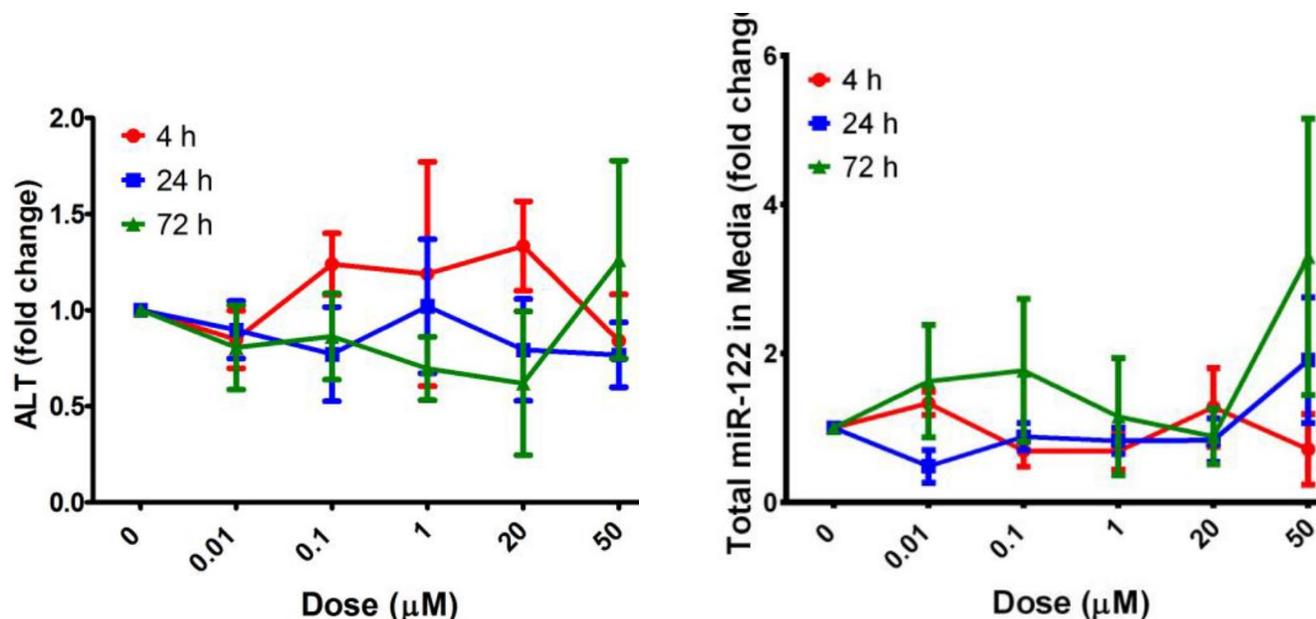


Figure 11. Average fold change (relative to control) in ALT (left) and miR-122 (right) release into culture medium.

Genome expression profiling: Gene expression changes were associated with the activation of several nuclear receptor pathways and toxicity with 20 (Fig. 12) and 50 (Fig. 13) μM at 24 and 72 hr. The top pathways enriched among genes affected with treatment were oxidative stress, superoxide radicals degradation, mitochondrial dysfunction, altered bile acid homeostasis, apoptosis and fatty acid β -oxidation. Furthermore, findings from this study suggest that tolvaptan-induced apoptosis may be the result of oxidative stress. Mitochondrial dysfunction and alterations in bile acid homeostasis may also contribute to the stress response. The pathway level changes associated with tolvaptan exposure in primary human hepatocytes identified in this study are listed in Table 21. Activation of these pathways as a result of tolvaptan exposure may precipitate liver injury in susceptible patients. Additionally, the sponsor suggests noninvasive biomarkers associated with the activation of these pathways (Table 21) may provide a way to test hepatocellular stress in vivo and could potentially identify patients susceptible to liver injury, particularly after initiating treatment with tolvaptan. Furthermore, according to the sponsor, the identification of activated stress response pathways and demonstration of key signaling mechanisms may provide biological plausibility for genetic or non-genetic biomarkers that emerge from the other research activities conducted to identify risk factors for drug-induced liver injury in tolvaptan treated patients.

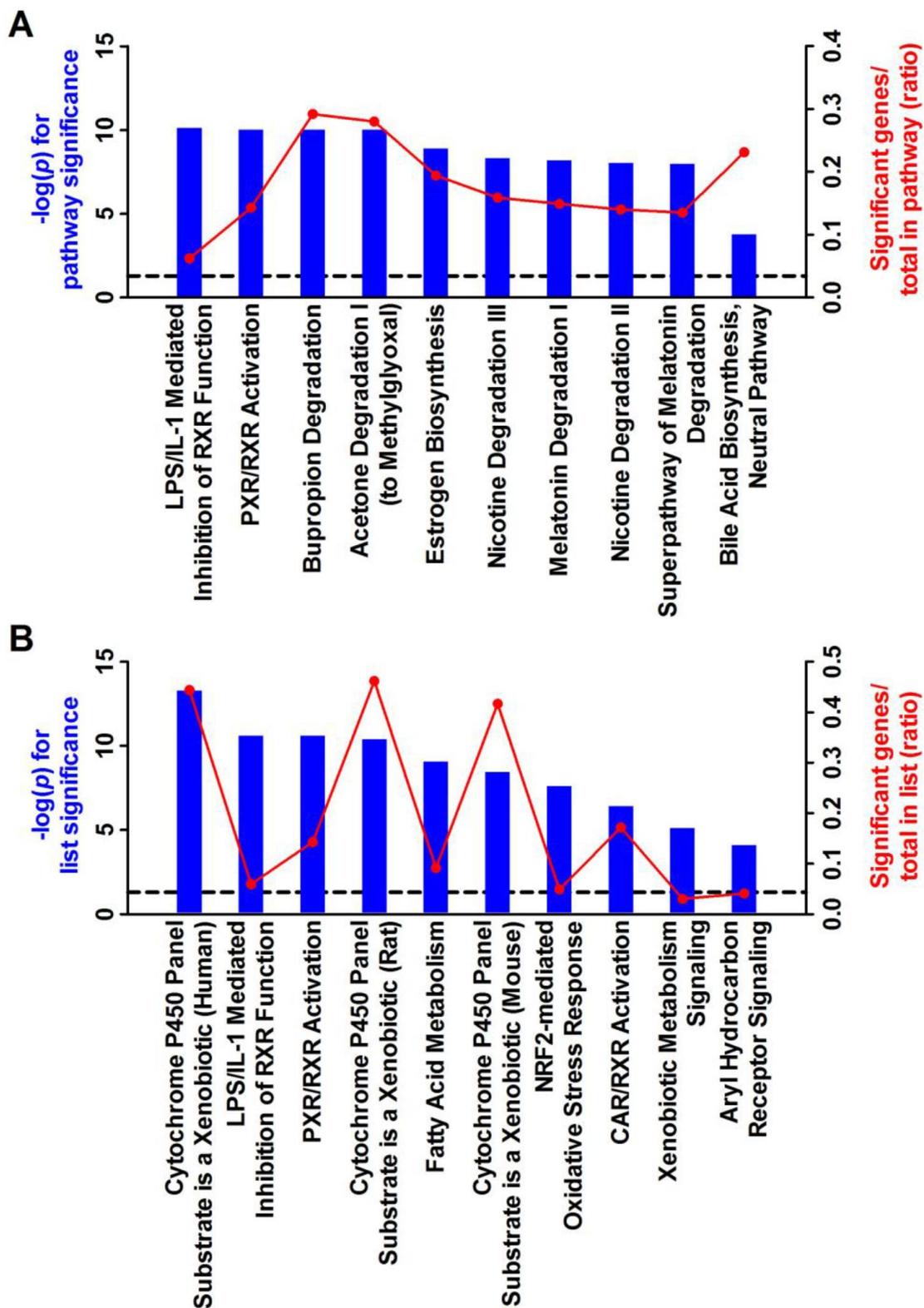


Figure 12. Top 10 (A) pathways and (B) toxicity lists enriched among genes significantly differentially expressed with 20 μ M tolvaptan at 72 h.

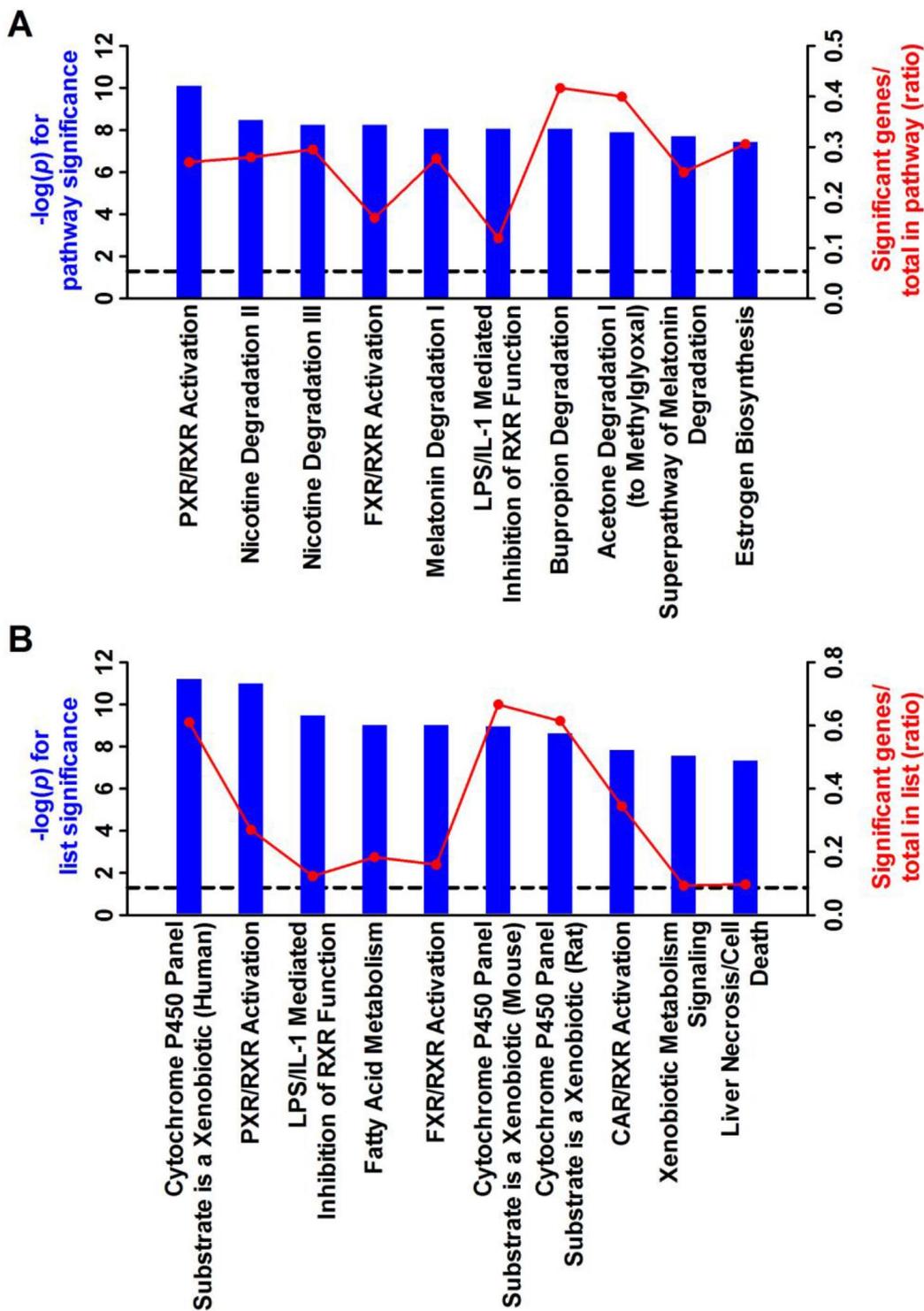


Figure 13. Top 10 (A) pathways and (B) toxicity lists enriched among genes significantly differentially expressed with 50 μ M tolvaptan at 72 hr. The dashed line indicates a $-\log(p) > 1.3$ threshold for significance.

Table 21. Pathway-level changes associated with tolvaptan exposure in primary human hepatocytes, and proposed biomarkers to measure pathway activation in vivo

Stress Response	Biomarker
Altered Bile Acid Homeostasis	Bile Acids
Mitochondrial Dysfunction	Acylcarnitines
Oxidative Stress	Exposure
Apoptosis	Cleaved Keratin-18, Oxidized High Mobility Group Box-1 Protein
Danger Signal	miR-122, Full-Length Keratin-18, Reduced High Mobility Group Box-1 Protein
Immune Response	Acetylated High Mobility Group Box-1 Protein

The enrichment of biological processes among the lists of significantly differentially expressed genes at 24 and 72 hr with 20 and 50 μM were analyzed with GoFigure Maps software. It showed many more processes were enriched at the 50 μM tolvaptan concentration relative to the 20 μM concentration. At the 20 μM concentration (at both 24 and 72 hr), most enriched processes are associated with drug metabolism. Although these processes were still enriched at the 50 μM concentration, additional processes such as apoptotic signaling and inflammatory response were enriched at 24 and 72 hr, respectively. For simplicity, a GoFigure Map of gene ontology biological processes enriched among genes differentially expressed with 50 μM at 72 hr is shown in Fig. 14.

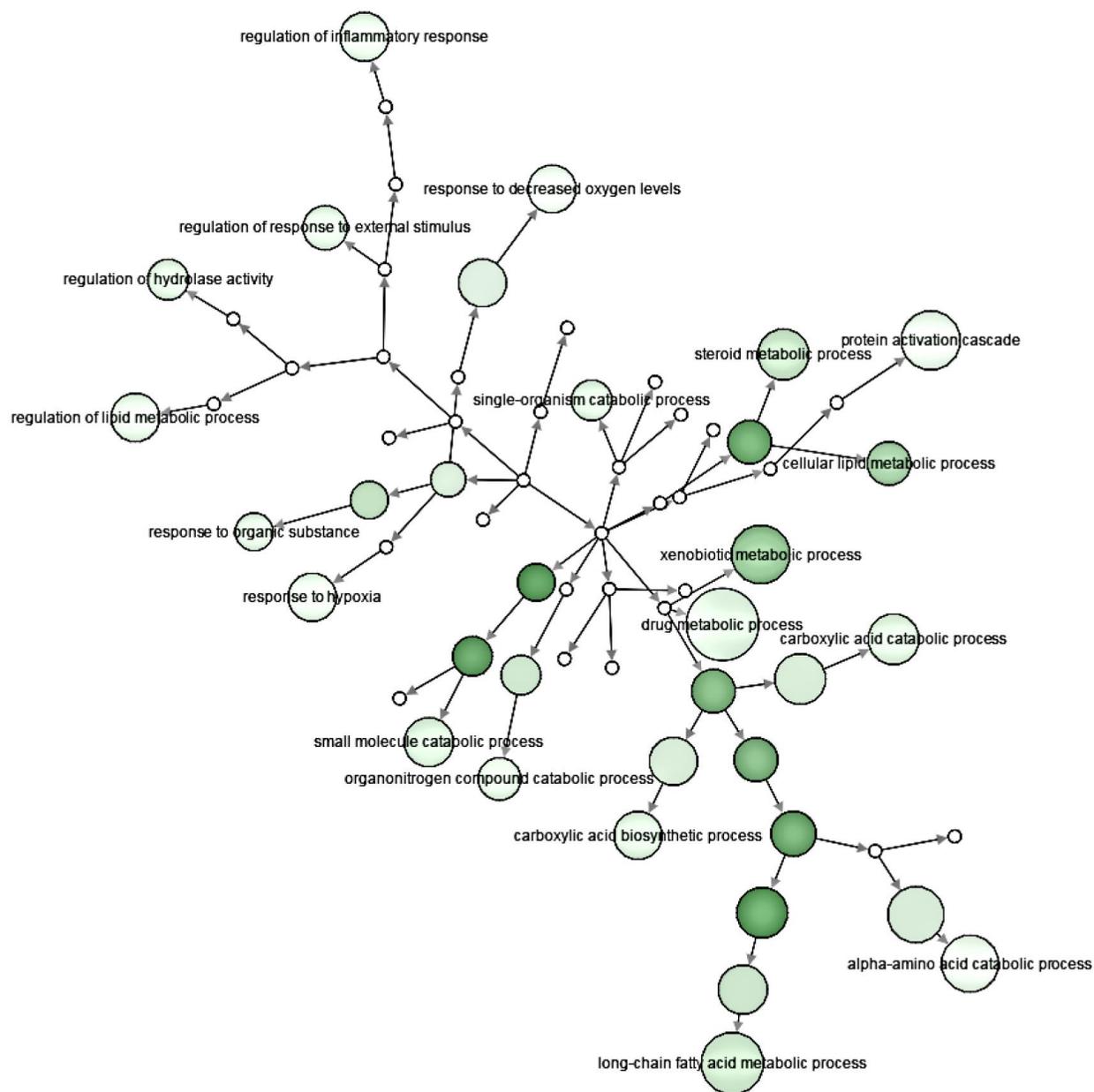


Figure 14. GoFigure Map of GO biological processes enriched among the genes differentially expressed with 50 µM tolvaptan at 72 h.

The number of genes annotated with each term is reflected by node size and the significance of the enrichment by node color. The names of biological processes represented by strongly enriched and/or terminal nodes are listed.

In conclusion, tolvaptan-induced apoptosis may be the result of **mitochondrial dysfunction** and **oxidative stress**. Mitochondrial dysfunction and alterations in bile acid homeostasis may contribute to the stress response. Additionally, hepatocytes released exosomes containing miR-122 in response to tolvaptan exposure provides a potential link between changes elicited at the hepatocyte level and activation of an innate immune response. According to the sponsor, in tolvaptan clinical trials, the profile of DILI observed suggest the involvement of the adaptive immune system in the liver response. Based on this observation, the sponsor hypothesizes that events that are necessary for the liver injury begins at the hepatocyte level. It is possible that in susceptible patients, the cellular stress, apoptosis, and release of extracellular vesicles observed provokes an innate immune response that in combination with adaptive immune attack ultimately results in liver injury. The hypothesized pathogenesis of tolvaptan-induced liver injury is schematically illustrated in Figure 15. Biomarkers associated with the activation of nuclear receptor pathways, **altered bile acid homeostasis, mitochondrial dysfunction**, as well as **oxidative stress** and **apoptosis** (Fig. 15) may provide a noninvasive way to test hepatocellular stress in vivo and could potentially identify patients susceptible to liver injury, particularly after initiating treatment with tolvaptan.

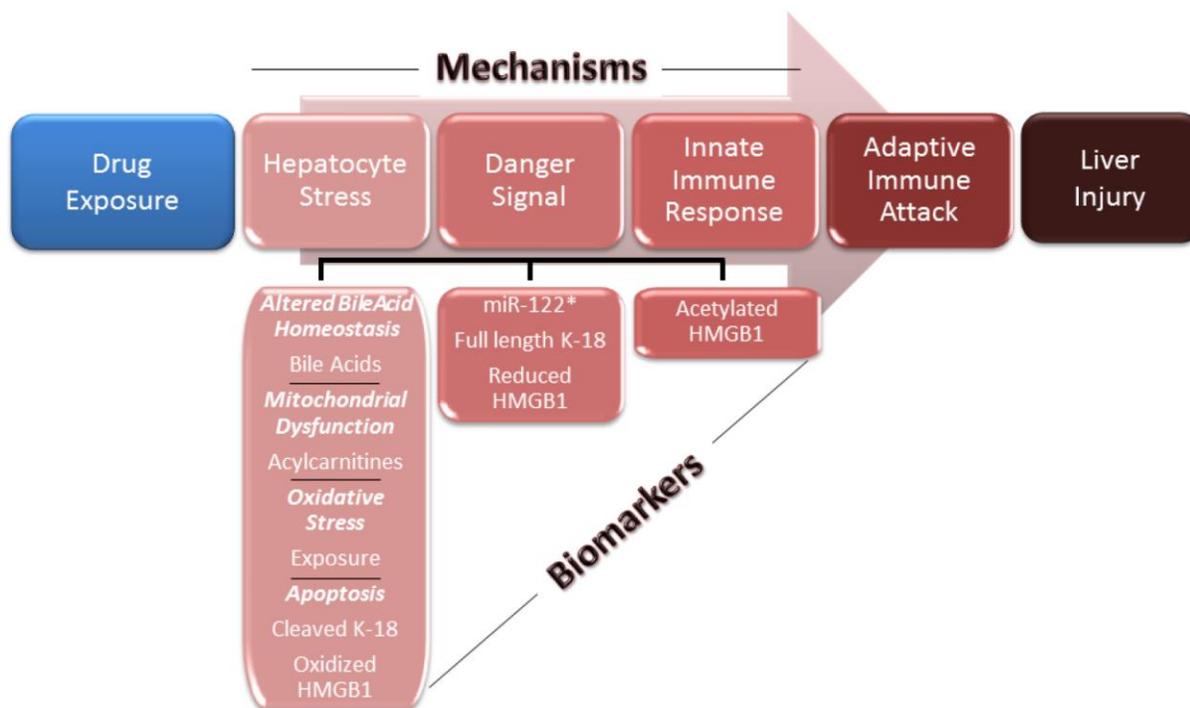


Figure 15. Hypothesis for the pathogenesis of tolvaptan-induced liver injury.

Associated biomarkers are listed for each toxicity mechanism. The * next to miR-122 highlights it as a biomarker that was identified in this study.

5.3 Tolvaptan in the DILIsym® software platform

This non-GLP study (# 038007, report #031328) was conducted at (b) (4) and the report was issued on July 2, 2015.

As part of a collaboration between the sponsor and (b) (4) to identify risk mitigation strategies for tolvaptan liver liability in ADPKD, this project applied the DILIsym® software for the mechanistic representation of tolvaptan. It is a computational model that will predict whether new drug candidates will cause DILI in patients and provides an enhanced understanding of the mechanisms underlying compounds that generate liver signals in the clinic. The animal studies did not provide evidence for any overt liver toxicity. However, clinical trials suggested that a small percentage of ADPKD patients treated with tolvaptan develop ALT elevations, with a small subset of those individuals developing significant bilirubin elevations as well and progressing to Hy's Law cases. It was hypothesized that ADPKD pathophysiology may impact liver function, thereby sensitizing an individual to tolvaptan-mediated liver injury. The identification of such functional differences in liver physiology could potentially be used to create a customized SimPops™ (simulated population samples) within DILIsym® reflecting those differences.

In this regard, the current study was designed to meet the following specific objectives using DILIsym®:

- Determine if bile acid, mitochondrial, and/or reactive oxygen species (ROS)-mediated toxicity (the three major mechanisms involved in DILI) are sufficient to drive tolvaptan hepatotoxicity
- Discern the contribution of each mechanism to hepatotoxicity
- Discern the contribution of each tested molecular species (tolvaptan, or its metabolites DM-4103 and DM-4107) to hepatotoxicity
- Identify candidate patient susceptibility factors that generate testable hypotheses for hepatotoxic susceptibility in ADPKD patients and guide blood and urine genetic and non-genetic biomarker identification/qualification

DILIsym® represents a) bile acid enterohepatic circulation via bile acid transporters, b) mitochondrial dynamics leading to adenosine triphosphate (ATP) production in hepatocytes, and c) the generation of ROS in response to test substance exposure.

The following data were collected to represent tolvaptan within DILIsym®.

- a) The inhibition constant IC₅₀ for tolvaptan and its metabolites DM-4103 and DM-4107 for human Na⁺-Taurocholate Cotransporting Polypeptide (NTCP).
- b) The inhibition constant (K_i) for tolvaptan and its metabolites DM-4103 and DM-4107 for human Bile Salt Export Pump (BSEP).

- c) The potential for tolvaptan and its metabolites DM-4103 and DM-4107 to cause direct mitochondrial dysfunction
- d) The potential for tolvaptan and its metabolites DM-4103 and DM-4107 to cause hepatocyte oxidative stress
- e) Use DILIsym® software and pharmacokinetic, bile acid transport, and mitochondrial dysfunction data to assess the likelihood of liver toxicity for tolvaptan and metabolites in the following types of simulated individuals:
 - Healthy subject (i.e., baseline human)
 - Human simulated population samples (SimPops™) from DILIsym® that include variability in bile acid transport and mitochondrial dysfunction
 - Customized SimPops™ for Otsuka with traits similar to those of ADPKD patients, in addition to variability in tolvaptan pharmacokinetic and drug metabolism parameters
- f) Analyze simulation results to provide a mechanistic rationale for the liver injury signals observed with tolvaptan in ADPKD patients
- g) Identify the most significant susceptibility factors in ADPKD simulations that will generate testable hypotheses regarding enhanced hepatotoxic susceptibility of ADPKD patients and that could guide additional genetic investigation.

Results

Using in vitro toxicity data and clinical PK data, DILIsym® correctly simulated tolvaptan as relatively safe for healthy volunteers using dosing regimens representative of those used for non-ADPKD indications. On the other hand, simulation of the higher dose, longer duration protocol used in the pivotal ADPKD trial did result in substantial hepatotoxicity, which was qualitatively but not quantitatively consistent with findings in the pivotal ADPKD trial. The occurrence of many simulated individuals with hepatotoxicity was critical in positioning the project for analysis of factors that could differentiate tolvaptan responders from non-responders. Although simulated toxicity was found to be more commonly driven by tolvaptan, it is also influenced by DM-4103-mediated toxicity.

DILIsym® simulations indicated that while the increased exposure to tolvaptan was a risk factor, tolvaptan hepatotoxicity was multifactorial in nature. Increased exposure revealed patient susceptibility factors related to mitochondrial electron transport chain inhibition and bile acid transport accumulation. The DILIsym® results provided biological plausibility to emerging biomarkers related to bile acid accumulation/metabolism and mitochondrial dysfunction and supported a decision to de-emphasize exposure-related biomarkers. Tolvaptan and its metabolites inhibited several human hepatic transporters involved in bile acid transport, thus affecting bile acid homeostasis. DILIsym® analysis also suggested potential areas to investigate for new biomarkers that could be predictive of delayed (3 and 14 months of treatment) tolvaptan hepatotoxicity.

6. Integrated Summary and Safety Evaluation

Tolvaptan selectively inhibits arginine vasopressin (AVP) induced water reabsorption at the renal collecting ducts by blocking V2 receptors located therein,¹ which results in increased water excretion without any change in electrolyte excretion. Based on this pharmacologic action, tolvaptan (Samsca®) was approved by the agency in May 2009 (NDA 22275) for the treatment of clinically significant hypervolemic and euvolemic hyponatremia. Subsequently, the sponsor developed tolvaptan under IND 72,975 for the treatment autosomal dominant polycystic kidney disease (ADPKD). Although it was approved for ADPKD in other countries, the agency did not approve the NDA 204441 in 2013. Based on the agency's Complete Response Letter, the sponsor has resubmitted the NDA for tolvaptan (Jynarque®). All the pharmacology and toxicology studies submitted for the two NDAs have been reviewed by Dr. Xavier Joseph. In the current NDA re-submission, additional information has been included from a few studies that were completed after the submission of previous NDA 22275 and NDA 204441.

The studies reviewed here examine the therapeutic benefit of tolvaptan in certain rat models followed by juvenile rat toxicity studies. The observation that tolvaptan can cause serious and fatal liver injury in clinical trials, has prompted the sponsor to conduct mechanistic studies, which included genomic analyses and in silico predictions of tolvaptan-induced hepatotoxicity. The results of these studies are reviewed and discussed in the current NDA.

Pharmacology

The diuretic effects of tolvaptan (oral, 1 to 10 mg/kg) and furosemide (oral, 10 to 100 mg/kg) were studied in both Nagase analbuminemic rats (NAR) and SD rats. Tolvaptan exhibited a sufficient diuretic effect in an analbuminemia model, which showed the diuretic resistance to furosemide suggesting that tolvaptan may provide therapeutic benefit for the patients with hypoalbuminemia. The lack of albumin, which is an important protein for binding to furosemide, caused unbound furosemide to immediately disappear from the blood circulation to the extravascular organ. In contrast, tolvaptan binds not only albumin but also several other proteins that can deliver it to the site for its action. In another study, tolvaptan was studied on hemodynamic variables in intact anesthetized rats, in anesthetized rats with portal hypertension induced by bile duct ligation and in isolated perfused rat livers. In all these experiments, tolvaptan did not affect portal venous pressure and portal venous blood flow.

¹ Robben JH, et al. Regulation of the vasopressin V2 receptor by vasopressin in polarized renal collecting duct cells. *Mol Biol Cell*. 2004;15: 5693–9.

Toxicology

Juvenile toxicity study in rats

According to the sponsor, these studies were conducted to support pediatric clinical trials for hyponatremia indication, but was not necessarily conducted in support of PKD. The effect of tolvaptan was evaluated in 4-, 7- and 25-day old rats for 1 week to 9 weeks at oral doses up to 1000 mg/kg/day. A study in neonatal rats from postnatal day 25 to postnatal day 67 was reviewed previously by Dr. Xavier Joseph (see Appendix). In this study, the MTD was 1000 mg/kg/day.

In a dose range-finding study, 4- or 7-day old male and female pups were treated up to a maximum dose of 1000 mg/kg/day for a week. All pups were found dead or were euthanized on day 2 suggesting the MTD in 4- or 7-day old pups was 100 mg/kg/day. There were no gross findings. A 9-week study in 4-day old pups showed increased BUN (at all doses), focal hemorrhage in lungs, renal pelvis dilatation, atrophy in the thymus and decreased hematopoiesis in bone marrow in the femur and in the spleen at ≥ 30 mg/kg/day. At the end of 4 week recovery, pelvis dilatation and focal hemorrhage in lungs were still present but at reduced incidence and severity. In neonates, the principal organs of toxicity were kidneys and lungs. A NOAEL could not be documented in the study.

Mechanistic studies in delineating tolvaptan-induced hepatotoxicity

Three nonclinical studies investigated possible mechanisms of tolvaptan-induced hepatotoxicity observed during studies in ADPKD patients.

- a) Previous repeat dose toxicity studies in rats and dogs (including PKD model animals) showed no significant evidence of liver injury. In recombinant inbred mouse strains of the Collaborative Cross (CC), tolvaptan at a single 100 mg/kg dose (4X MRHD) and 24 hr sacrifice, produced elevated ALT levels in 3 of 45 CC strains (#7, 34, and 44), which are genetically sensitive to tolvaptan-induced liver response than other CC strains or traditional rodents. AST, total bilirubin, and miR-122 (a specific marker for liver injury) were all significantly correlated with the fold changes in ALT particularly for strain #44 (C57BL/6). Tolvaptan plasma concentrations at 2 hr were significantly correlated with ALT fold change with the highest observed in the 3 sensitive mouse strains. Genetic mapping identified a locus on chromosome 14 associated with tolvaptan-induced liver response in CC mice. Microarray analysis identified gene expression changes that associated with liver injury and ALT fold change. **Gene expression changes related to treatment with tolvaptan were associated with the activation of several nuclear receptor pathways.** The top pathways enriched among genes affected with treatment were oxidative stress, superoxide radicals degradation,

- mitochondrial dysfunction and altered bile acid homeostasis. It may be noted that tolvaptan and its metabolites inhibit several human hepatic transporters involved in bile acid transport, thus affecting bile acid homeostasis. This mouse study was not designed to allow for the evaluation of an adaptive immune response. In this study, tolvaptan did not produce microscopic changes in the liver. The sponsor hypothesizes that it is possible that the liver response observed in the sensitive mouse strain could have mechanistic similarities to the early hepatocellular events that may have occurred in the ADPKD patients. The results further suggest that genetic sensitivity, that may be unique to ADPKD patients, potentiates the toxic response to tolvaptan.
- b) In an invitro study of cultured human hepatocytes treated with tolvaptan (20 and 50 μ M), programmed cell death was observed with a statistically significantly increase in the percentage of hepatocytes with both small and textured nuclei. Presence of mitochondrial-induced apoptosis was noted with the translocation of cytochrome c from the mitochondria to the cytoplasm. This suggests tolvaptan had a significant effect on mitochondria to cause mitochondrial dysfunction. Furthermore, a decrease in cellular function was noted with the decline in the release of urea into the culture medium. Tolvaptan did not significantly affect ALT and total miR-122 release into the culture medium. Gene expression changes were associated with the activation of several nuclear receptor pathways and toxicity. The top pathways enriched among genes affected with treatment were oxidative stress, superoxide radicals degradation, mitochondrial dysfunction, altered bile acid homeostasis, apoptosis and fatty acid β -oxidation. Furthermore, findings from this study suggest that tolvaptan-induced apoptosis may be the result of oxidative stress, mitochondrial dysfunction and alterations in bile acid homeostasis. The sponsor suggests six non-invasive biomarkers associated with the activation of nuclear receptor pathways as ways to test hepatocellular stress and liver injury.
- c) The DILIsym® software is a computational model that will predict whether new drug candidates will cause DILI in patients and provide an enhanced understanding of the mechanisms underlying compounds that generate liver signals in the clinic. Using in vitro toxicity data and clinical PK data, DILIsym® correctly simulated tolvaptan as relatively safe for healthy volunteers using dosing regimens representative of those used for non-ADPKD indications. On the other hand, simulation of the higher dose, longer duration protocol used in the pivotal ADPKD trial did result in substantial hepatotoxicity, which was qualitatively, but not quantitatively, consistent with findings in the pivotal

ADPKD trial. Simulated toxicity was found to be more commonly driven by tolvaptan, but also influenced by active metabolite DM-4103- mediated toxicity. Tolvaptan hepatotoxicity is multifactorial in nature with contributions from both bile acid accumulation and inhibition of mitochondrial electron transport chain. Qualification of novel genetic and non-genetic biomarkers might be required that could be predictive of delayed tolvaptan hepatotoxicity.

Conclusions

The pharmacodynamic study with tolvaptan showed that it reduced the rate of growth of the total kidney volume in PKD mice and rats. Consistent with its pharmacological action as a selective inhibitor of V2 receptors in the basolateral membrane of renal collecting duct, mechanistically, tolvaptan reduced the mitogenic source cAMP levels. This action inhibited collecting duct cysts growth and enlargement. Finally, there are no non-clinical findings that preclude from the approvability of tolvaptan to patients with PKD.

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APPENDIX

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 204441
Supporting document/s: # 0 (original submission)
Applicant's letter date: 11/15/2012
CDER stamp date: 11/15/2012
Product: Tolvaptan (OPC-41061) tablets, 15-, 30-, 45-, 60-, 90 mg
Indication: Indicated to slow progressive kidney disease in adults with autosomal dominant polycystic kidney disease (ADPKD)
Applicant: Otsuka Pharmaceutical Company, Ltd., 1 University Square Drive, Princeton, NJ08540
Review Division: Division of Cardiovascular and Renal Products
Reviewer: Xavier Joseph, D.V.M.
Supervisor/Team Leader: Thomas Papoian, Ph.D.
Division Director: Norman Stockbridge, M.D., Ph.D.
Project Manager: Anna Park

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TABLE OF CONTENTS

	Page #
1. EXECUTIVE SUMMARY	3
2. DRUG INFORMATION	7
3. PHARMACOLOGY	8
4. GENERAL TOXICOLOGY	16
5. INTEGRATED SUMMARY	24

EXECUTIVE SUMMARY

1.1 Introduction

Tolvaptan (Samsca[®]), a vasopressin V₂-receptor antagonist, was approved in 2009 for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (NDA 22-275). Tolvaptan blocks the binding of arginine vasopressin (AVP) at the V₂ receptors of the distal portions of the nephron, thereby preventing water reabsorption, and inducing water diuresis (aquaresis) without the depletion of electrolytes. AVP, a neuropeptide hormone synthesized in the hypothalamus and released into the blood from posterior pituitary, causes fluid retention and hyponatremia. The maximum approved human dose for the treatment of hyponatremia is 60 mg/day.

For the current NDA (# 204441), Otsuka is seeking approval for tolvaptan for the treatment of autosomal dominant polycystic kidney disease (ADPKD). ADPKD is an inherited renal disease characterized by progressive development of cysts that destroy normal kidney architecture. cAMP has been shown to play a major role in cyst formation by promoting transepithelial fluid secretion and stimulating cyst-derived cell proliferation. The maximum recommended human dose for ADPKD is 120 mg/day.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies conducted with tolvaptan were previously reviewed under NDA 22-275.

In receptor binding studies using human AVP receptors expressed in HeLa cells, tolvaptan blocked the binding of labeled AVP to human V₂ 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). For tolvaptan, these binding studies demonstrated 29, 61 and 259 times higher affinity for V₂ receptors than V_{1a} in human, canine and rat species, respectively. It was shown that tolvaptan also inhibited AVP-induced cAMP production. The metabolites of tolvaptan showed no activity at human V₂ and V_{1a} receptors or weak antagonistic activity compared to tolvaptan.

Tolvaptan increased urine excretion and decreased urine osmolality in the dose range of 0.3 to 10 mg/kg, when given orally (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. Serum sodium concentration was elevated at 4 hours postdose in rats, rabbits and dogs.

From *in vitro* studies, CYP3A4 was identified as responsible for catalyzing the primary metabolic reactions. CYP1A1 also plays a role in the formation of the metabolite DM-4128.

Following oral administration of 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 and lactating women.

Chronic oral toxicity studies conducted in both rats (30, 100 and 1000 mg/kg/day for 26 weeks) and dogs (30, 100 and 1000 mg/kg/day for 52 weeks) did not reveal any notable toxicity in either species except for reduced adrenocorticyte vacuolation and/or increased cortical width in the adrenal glands of dogs from all groups. These adrenocortical findings were attributed to a physiological adaptive response to stress (chronic marked diuresis).

No increased incidence of tumors were seen after two years of oral administration of tolvaptan to male and female rats at doses up to 1000 mg/kg/day (81 times the 120 mg maximum recommended human dose [MRHD] on a body surface area basis), to male mice at doses up to 60 mg/kg/day (2 times the MRHD) or to female mice at doses up to 100 mg/kg/day (4 times the MRHD).

Tolvaptan tested negative for genotoxicity in *in vitro* and *in vivo* test systems.

In a fertility study in which male and female rats were orally administered tolvaptan at doses of 100, 300 or 1000 mg/kg/day, the highest dose level was associated with significantly fewer corpora lutea and implants than control. Oral administration of tolvaptan in pregnant rats at 10, 100 and 1000 mg/kg/day during organogenesis was associated with reduced fetal weight and delayed ossification of fetuses at 1000 mg/kg/day (81 times the MRHD on a body surface area basis). In pregnant rabbits, oral administration of tolvaptan at 100, 300 and 1000 mg/kg/day during organogenesis was associated with abortions (mid and high doses) and increased incidences of embryo-fetal death, fetal microphthalmia, brachymelia and skeletal malformations (high dose).

In animal models of polycystic kidney disease (PKD), administration of tolvaptan lowered renal cAMP and exerted a protective effect on the development of PKD as reflected by lower kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices.

The sponsor recently studied tolvaptan vs placebo for delay in progression of renal disease in about 1400 patients with autosomal dominant polycystic kidney disease (ADPKD). In this study, more patients with tolvaptan group experienced elevations of serum alanine aminotransferase (ALT) compared to placebo. At least 3 Hy's law cases were reported in the tolvaptan group indicating the potential for the drug to cause liver injury capable of progression to liver failure. It is noted that the ADPKD studies used a dose (120 mg/day) higher than that currently approved for the treatment of hyponatremia (60 mg/day).

On April 30, 2013, the US FDA issued a Drug Safety Communication (DSC) for Samsca (tolvaptan). The DSC recommends limiting the duration of treatment to 30 days, and that tolvaptan not be used in patients with underlying liver disease.

In summary, chronic oral toxicity studies conducted in rats (26 weeks) and dogs (52 weeks), at doses up to 1000 mg/kg/day [81 times the maximum recommended human dose (MRHD) in rats and 270 times the MRHD in dogs on a body surface area basis], did not reveal any liver toxicity

potential. 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 embryofetal development in rats and rabbits occurred at relatively high doses, and may have been secondary to maternal toxicity at these doses. We do not consider the reprotoxicity findings observed at the very high multiples of MRHD to constitute an approvability issue. However, since the 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 nonclinical toxicity-testing program.

1.3 Recommendations

1.3.1 Approvability

There are no approvability issues for tolvaptan based on nonclinical toxicity testing program.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following sponsor's version of the labeling is considered to be acceptable. (b) (4)

[Redacted]

8.1 Pregnancy

[Redacted] (b) (4)

(b) (4)

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13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

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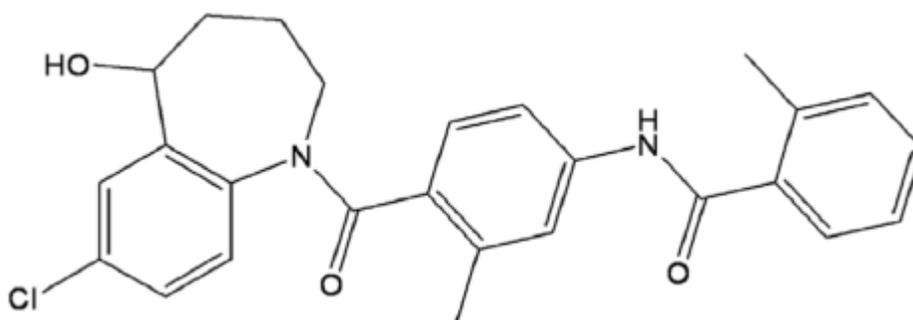
2 Drug Information

2.1 Drug: (b) (4) immediate release tablets.

Generic Name: Tolvaptan

Code Names: OPC-41061 and OPC-156

Chemical Structure



MW 448.94

Pharmacologic Class: Selective vasopressin V₂ receptor antagonist

2.2 Relevant NDAs: NDA 22-275 - Samsca[®] tablets (for the treatment of hypervolemic and euvolemic hyponatremia)

2.3 **Drug Formulation:** (b) (4) immediate release tablets for oral administration are available in 15, 30, 45, 60 and 90 mg strengths of tolvaptan. Inactive ingredients include corn starch, hydroxypropyl cellulose, lactose monohydrate, low – substituted hydroxypropyl cellulose, magnesium stearate and microcrystalline cellulose and FD & C Blue No.2 Aluminum Lake as colorant.

Pharmacology

In Vivo Animal Models

1. Pkd2^{WS/-} Mouse –An Animal Model of ADPKD

[Sponsor's Tables and Figures are used for this review.]

The effects of tolvaptan for the treatment of polycystic kidney disease were investigated in Pkd2^{WS25/-} mouse, an animal model of human autosomal dominant polycystic kidney disease (ADPKD). The goal of the present study was to determine whether tolvaptan, a vasopressin V2 receptor antagonist used in clinical trials for the treatment of water retaining states, also inhibits the development of polycystic kidney disease in a mouse model of human ADPKD.

The study was conducted at [REDACTED] ^{(b) (4)} (Otsuka Study No. 021276 and Report No. 017968, and study period from April 1, 2004 to November 4, 2005.)

Tolvaptan was administered for 12 weeks to groups of mice (9-12/group/sex; between 4 and 16 weeks of age) via diet at concentrations of 0 (control), 0.01, 0.03 and 0.1%. The mice were given water ad libitum. The animals were sacrificed at sixteen weeks of age and the following parameters were evaluated: 1) body weight at sacrifice; 2) kidney and liver weights (% BW); 3) histomorphometric analysis of kidneys and livers (cysts and fibrosis volumes, % BW) ; 4) renal proliferative (immunostaining for proliferating cell nuclear antigen) and apoptotic (using the TUNEL assay) indices (%); 5) renal cAMP concentrations (pmol/mg protein); 6) plasma BUN, creatinine and sodium concentrations; and urine volumes (ml/24 hours), urine osmolality, and blood pressure.

Results are presented in the following Figures.

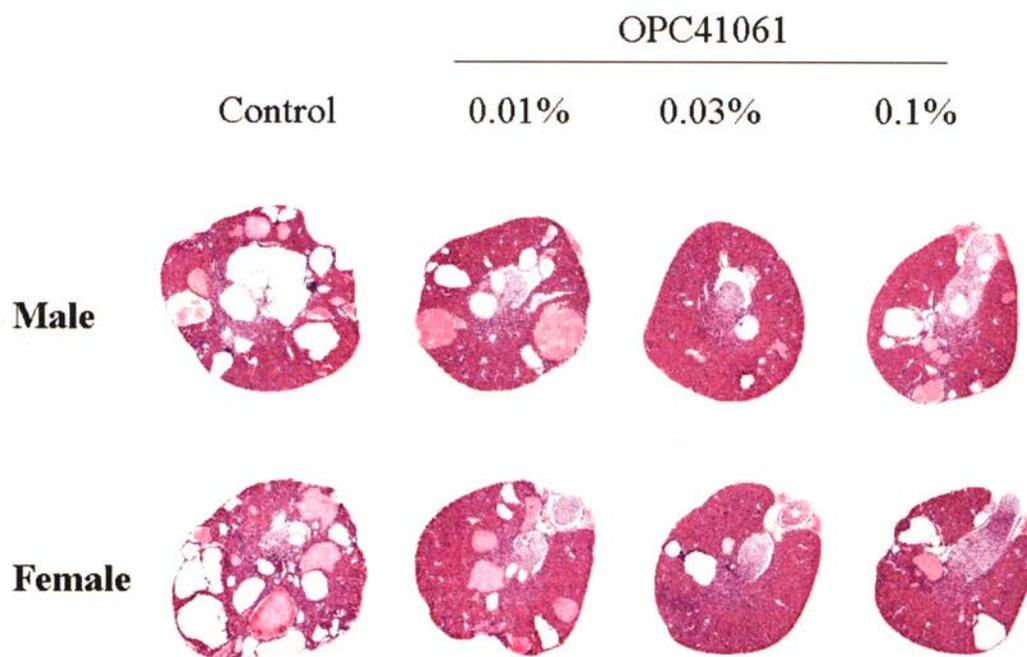


Figure 1 **Representative kidney sections from male and female $Pkd2^{WS25/-}$ mice treated with OPC-41061 between 4-16 weeks of age.**

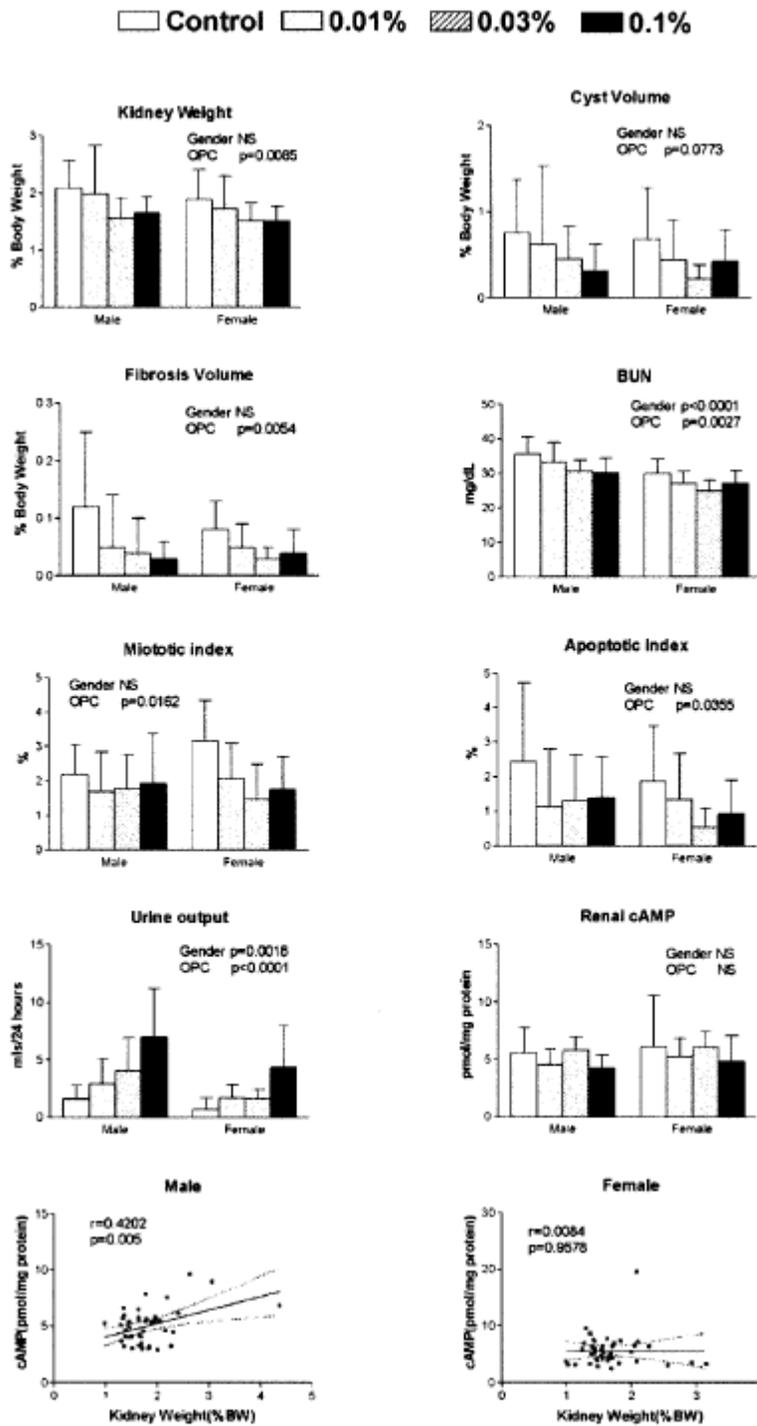


Figure 2

Effects of treatment with OPC-41061 at the specified concentrations on the development of polycystic kidney disease in male and female *Pkd2*^{W525/-} mice between 4–16 weeks of age.

Administration of tolvaptan between 4 and 16 weeks of age exerted a protective effect on the development of polycystic kidney disease in Pkd2^{WS25/-} mice, as evidenced by significantly lower kidney weights, fibrosis volumes, mitotic and apoptotic indices, and plasma BUN concentrations (Figures 1 and 2). Cyst volumes were also lower in drug treated groups, without reaching statistical significance (p=0.077). Renal cAMP levels tended to be lower in the treated animals without reaching statistical significance. A statistically significant positive correlation was noted between the tissue levels of cAMP and the severity of polycystic kidney disease in male (r=0.42, p=0.005) but not in female mice. Most of the protective effect of tolvaptan was reached at a concentration of 0.03%, a dose that caused modest aquaresis. No additional protection was achieved by increasing the dose to 0.1%. The administration of tolvaptan resulted in a dose dependent increase in urine output and reduction in urine osmolality. These were accompanied by a significant elevation in plasma sodium.

Administration of tolvaptan did not inhibit the development of polycystic liver disease, consistent with the absence of vasopressin V₂ receptors in the liver.

2. PCK Rat – An Animal Model of ARPKD

The goals of the present study were to determine whether tolvaptan, a vasopressin V₂ receptor antagonist, currently approved for the treatment of hyponatremia, inhibits the development of polycystic kidney disease in PCK rats.

The PCK rat is a model of human autosomal recessive polycystic kidney disease (ARPKD) caused by a splicing mutation that leads to a frameshift in the ortholog Pkhd1 gene due to skipping of exon 36. Progressive cystogenesis and impairment of renal function characterize this model.

The effects of tolvaptan for the treatment of PKD in PCK rats were investigated at the (b) (4) (Otsuka Study No. 021276 and Report No. 016916; Study period April 1, 2004 to May 12, 2005).

Tolvaptan was administered for 7 weeks to groups of PCK rats on a Sprague-Dawley strain (10/group/sex) via the diet at concentrations of 0 (control), 0.01, 0.03, and 0.1% beginning at 3 weeks of age. The rats were given water ad libitum. Animals were sacrificed at 10 weeks of age and the following parameters were evaluated: 1) body weight at sacrifice; 2) kidney and liver weights (% BW); 3) histomorphometric analysis of the kidneys and livers (cyst and fibrosis volumes, % BW); 4) renal proliferative (for proliferating cell nuclear antigen) and apoptotic (using TUNEL assay) indices (%); 5) renal cAMP concentrations (pmol/mg protein); 6) plasma BUN, creatinine and sodium concentrations; and 7) urine volumes (ml/24 hours) and blood pressure.

Results are presented in the following Figures.

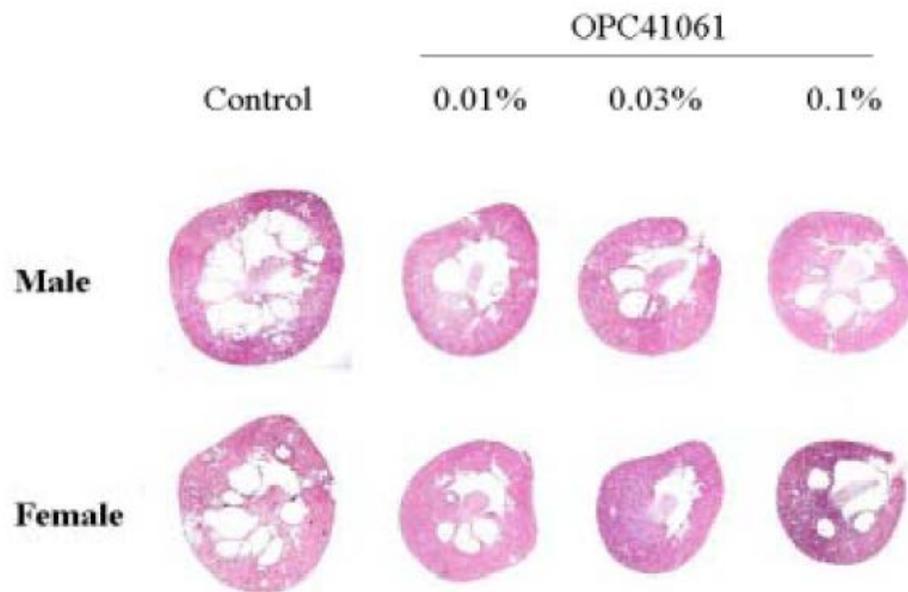
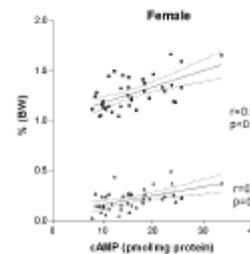
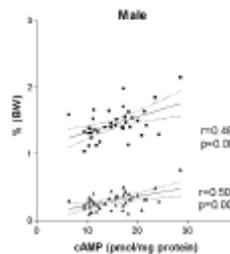
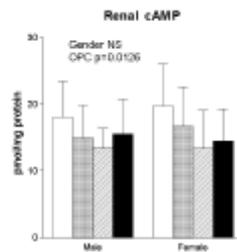
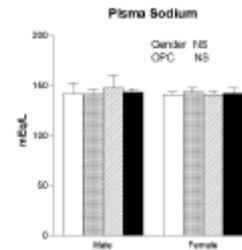
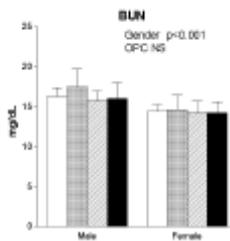
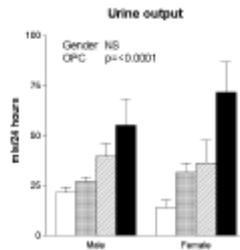
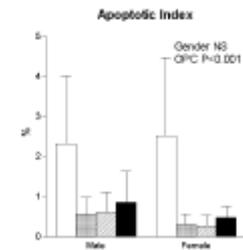
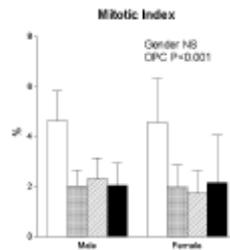
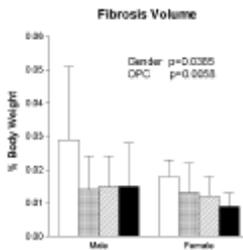
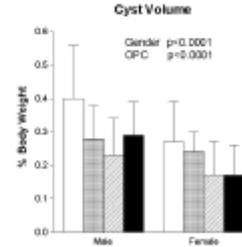
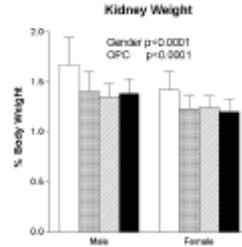
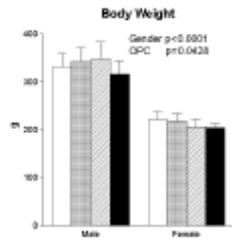


Figure 1A

Representative kidney sections from male and female PCK rats treated with OPC-41061 between 3-10 weeks of age.

control 0.01% 0.03% 0.1%



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Figure 1B

Effects of OPC-41061 treatment at the specified concentrations on the development of polycystic kidney disease, urine output, and renal cAMP concentration in PCK rats. Data are expressed as means±SD of 10 animals. Significant positive correlation was observed between renal cAMP concentrations and kidney weights or cyst volumes at sacrifice in male and female animals

Mitotic and apoptotic indices are shown as percent of cells that are positive for proliferating cell nuclear antigen staining or ApopTag peroxidase positive in the TUNEL assay.

Results indicated that the administration of tolvaptan between 3 and 10 weeks of age lowered renal cAMP levels and exerted a protective effect on the development of polycystic kidney disease in PCK rats, as reflected by significantly lower kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices (Figure 1A and 1B). Plasma sodium, BUN and creatinine levels were normal and not significantly different between groups. A statistically significant positive correlation was detected between the tissue levels of cAMP and the severity of polycystic kidney disease. Most of the protective effect of tolvaptan was detectable with the lowest dose used in the study, a dose that caused only modest aquaresis. Limited additional protection was achieved by a ten-fold increase in dose.

In a separate study conducted at ^{(b) (4)} the effects of tolvaptan on the development of polycystic disease were studied in male PCK rats. Tolvaptan was administered by oral gavage at a dose of 10 mg/kg once or twice a day for 8 weeks from 5 to 13 weeks of age. PCK control rats were treated with the vehicle (1% hydroxypropylmethyl cellulose). A nontreated control group of Sprague-Dawley rats was included in the study. The following parameters were evaluated: clinical signs, body weight, food and water consumption, urine volume, urinalysis including urinary Na levels, osmolality, urinary cAMP excretion, blood chemistry, renal cAMP levels, organ weights, histopathologic evaluations (cyst and fibrosis volumes of kidney), and PCNA and TUNEL labeling indices.

Water intake and urine volume were increased and urinary osmolality decreased with both dose regimens (once or twice daily dose administrations). After 8 weeks of treatment, no changes were observed on kidney weight, kidney cAMP content, or on kidney histopathology (cyst and fibrosis volumes) findings, indicating that tolvaptan did not affect the progression of PKD based on the lack of kidney changes. Gavage administration of tolvaptan at 10 mg/kg exerted a short duration of aquaretic action that was not sustained for 24 hours in male Sprague-Dawley rats even in the case of twice daily administration. These findings suggested that the duration of V2R inhibition by this administration method (oral gavage) may not be sufficient to protect the development of PKD in PCK rats.

Studies in Other Animal Models of PKD

Studies were also conducted in male **pcy mouse** (orthologous to human adolescent nephronophthisis). Tolvaptan was administered at dietary concentrations of 0, 0.01, 0.03, 0.1 or 0.3% for 10 weeks, beginning at 5 weeks of age. A dose-related decrease in kidney weights was observed in tolvaptan-treated mice, compared to controls, with a statistically significant reduction observed at tolvaptan concentration of 0.03% and higher. A decreasing trend was observed for kidney cyst volume, kidney fibrosis volume and mitotic index with a significant reduction observed at 0.1 or 0.3%. A dose-related aquaretic effect was observed on urine volume (increased) and urine osmolality (decreased) with a maximum effect seen at 0.1%. Consistent with the reduced kidney volume, a dose-related reduction in kidney cAMP content and ERK activity were observed in pcy mice compared to controls.

Serum levels of tolvaptan were determined in the pcy mouse treated via the diet (0.01 or 0.1%) for 14 days. Blood samples were collected at Day 7 (morning) and on 3 occasions (morning, evening and midnight) on Day 14. Serum levels of tolvaptan increased with dietary concentration, and the levels tended to be higher at midnight. The data demonstrate exposure to tolvaptan in the pcy mouse at pharmacologically active doses, and the levels observed are considered to be generally similar to those found with clinical administration of tolvaptan.

In Vitro Studies examined cell proliferation in normal human kidney cells (NHK) and cells obtained from the cysts of the ADPKD patients. Addition of AVP to NHK cultures resulted in an increase of intracellular cAMP, however, the rate of cell proliferation did not change. By contrast, addition of AVP to ADPKD cultures resulted in increased intracellular cAMP and increased cell proliferation. Tolvaptan significantly reduced cAMP and cell proliferation of the AVP stimulated ADPKD cells.

General Toxicology

Six-Week Oral Dose Toxicity Study of Tolvaptan With a 4-Week Recovery Period in Juvenile Rats

(Note: This juvenile rat study was undertaken to support pediatric clinical trials for hyponatremia, and not PKD.)

Study numbers: (b) (4) 5805 (CRO #)
030396 (Sponsor's study #)
025612 (Sponsor's Report #)

Study report location: Otsuka Pharmaceutical Co., Ltd, Tokushima, Japan

Conducting laboratory and location: (b) (4)

Study dates: April 1, 2010 – October 29, 2010

GLP compliance: The study was conducted in accordance with the GLP Ordinances issued by the Ministry of Health and Welfare, Japan.

QA statement: Inspections were conducted periodically and the final Quality Assurance Statement has been issued by the Lead QA manager on October 29, 2010.

Drug, lot # and % purity: code number – OPC-156, lot # - 090821-1, drug content – 66.5%

Key study findings: Six-week oral administration of tolvaptan to male and female juvenile rats (25 days of age at the study initiation), at 0, 30, 100 and 1000 mg/kg/day, was associated with significantly increased urine volume and water intake in all drug-treated groups. Other treatment related effects, noted only at the high dose level, included congestion and pigmentation of the spleen red pulp, hypertrophy of adrenal cortical cells and thyroid follicular cells and centrolobular hypertrophy of hepatocytes. These changes were found to be reversible.

Methods

Doses: 0, 30, 100 and 1000 mg/kg/day

Frequency of dosing: once daily for six weeks

Route of administration: oral (gavage)

Dose volume: 5 ml/kg

Formulation/Vehicle: formulated as a suspension in 1% hypromellose. Dosing formulations were prepared weekly and were found to be stable for 8 days when stored at temperature not exceeding 10°C

Species/Strain: Rats/Sprague-Dawley [CrI:CD(SD)] (b) (4)

Number/Sex/Group: 10 – An additional 5 rats/sex were included in the control and high dose groups for the 4-week recovery period. Satellite animals (6 rats/sex in the control and 26 rats/sex in drug treated groups) were used for toxicokinetic evaluations.

Age: 25 days of age at the initiation of the study [the treatment period of six weeks which extended from about 3.5 to 9.5 weeks of age included the age of rats (7 to 8 weeks) at the initiation of 6-month rat toxicity study that was conducted earlier].

Weight: main study group – males – 76 to 108 g; females – 73 to 91 g; satellite group – males – 74 to 101 g; females -71 to 95 g

Rationale for dose selection: The doses were selected based on the results of a 3-week oral range-finding study of tolvaptan (0, 100, 300 and 1000 mg/kg) in juvenile rats. No deaths were seen in the study; significantly increased water intake was noted in all groups. Other treatment-related findings included reduced body weight gain, decreased RBC count, hemoglobin and hematocrit values and enlargement of liver at the high dose. No histopathology was conducted in the study. Based on the above results, the 1000 mg/kg/day was selected as the high dose for the 6-week toxicity study in juvenile rats. (It is also noted that the results from the previous repeated dose oral toxicity studies showed that the 1000 mg/kg/day is considered to be the maximum feasible dose based on the viscosity and volume of the dosing suspension.)

Unique study design: none

Deviation from study protocol: Minor deviations noted from protocol (such as, at the initiation of the study, a body weight deviation (+3g) for 1 animal from the upper limit specified by the protocol; a 1°C temperature increase on one day for about 30 minutes in one of the animal rooms, and missing of parathyroid tissue for one control female) were judged to have no significant effect on study results.

Observations

Clinical signs and mortality: All animals in the main study and recovery groups were examined twice daily during the drug treatment period and once daily during the recovery period. Satellite group animals were examined only for mortality.

Detailed clinical examinations were performed on 5 rats/sex/group from the main study group on Day 40 of drug treatment and on all recovery phase animals on Day 26 of the recovery period. These examinations included home cage observations (posture and abnormal behavior), in hand observations [skin conditions, secretions from eyes/nose, visible mucous membrane, autonomic

nervous system function (lacrimation, salivation, piloerection, pupil size and abnormal respiration) and reactivity to handling] and open field observations (arousal, convulsion, abnormal behavior, gait, posture, defecation and urination).

Sensory/reflex function examinations were performed on 5 rats/sex/group from the main study group (1 to 4 hr post-dose) on Day 40 of drug treatment and on all recovery phase animals on Day 26 of recovery. These examinations included manipulative tests (auditory response, touch response, tail pinch response, pupillary reflex, aerial righting reflex and landing foot splay) and measurement of grip strength using CPU gauge.

Motor activity was measured in 5 rats/sex/group from the main study animals on Day 40 of treatment and in all recovery phase animals on Day 26 of recovery. The motor activity, using the spontaneous movement sensor for the experimental animals NS-AS01, was measured for 1 hour and the counts were recorded at 10 minute intervals and also as total counts.

Body weight: All animals from the main study and recovery groups were weighed every 3 to 4 day intervals during the drug treatment period. Animals were weighed weekly during the recovery period. On the day of necropsy, animals were weighed after fasting for calculating the relative organ weight. Satellite group animals were weighed for calculating the dose volume, but the data were not subjected to toxicological evaluation.

Food consumption: During the drug administration period, for all main study and recovery phase animals, a 3 or 4-day cumulative food consumption was measured and one day food consumption was calculated. For recovery phase animals, a 6 or 7-day cumulative food consumption was measured for determining the one day consumption.

Water intake: A 3 to 4 day cumulative water intake was determined to calculate the one day water intake for the main study and recovery group animals during the drug treatment and recovery periods.

External differentiation : Females were observed for vaginal opening (Days 11 and 18 of drug treatment) and males for balanopreputial separation (Days 18 and 25 of drug treatment) and the parameter was expressed as the external differentiation percentage.

Urinalysis was conducted on main study animals (5 rats/sex/group) on Day 36 of drug treatment and on all recovery phase animals on Day 22 of recovery. Four-and twenty- hour urine samples were collected. Urine volume, color, pH, sediment, protein, ketones, glucose, occult blood, bilirubin and urobilinogen were determined on 4-hour sample, and urine volume, osmolality, sodium, potassium, chloride and creatinine determinations were made on 24- hour sample.

Hematology: Blood samples were collected from the main study animals at the end of the treatment period, and from the recovery animals at the end of the recovery period. The following parameters were evaluated: RBC, WBC (total and differential), reticulocyte and platelet counts, hemoglobin, hematocrit, MCV, MCH, MCHC, prothrombin time and activated partial thromboplastin time.

Blood chemistry: The following parameters were evaluated: alkaline phosphatase, total cholesterol, triglyceride, phospholipids, total bilirubin, glucose, BUN, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, A/G ratio, protein fraction ratio and concentrations, aminotransferases, LDH, CPK and γ -GTP.

Gross pathology: At the end of the treatment and treatment-free periods, a complete necropsy was performed on all animals. All macroscopic abnormalities were recorded.

Organ weight: Brain, pituitary, thyroid, adrenal, thymus, spleen, heart, lung, salivary glands, liver, kidney, testis, ovary, epididymus, uterus and prostate were weighed.

Histopathology: All organs and tissues from all animals were fixed, preserved, processed and stained with H&E. The tissues shown in the following Table from the control and high dose groups were examined microscopically. The adrenals, bone marrow (femur), liver, spleen (including Berlin staining for hemosiderin), thyroid and urinary bladder from the middle dose group were also examined. In addition, spleen (males) and urinary bladder (both sexes) from the low dose group were examined.

Toxicokinetic evaluation: Blood was collected from 3 rats/sex at one time point (2 hour post-dose) from the control group and 5 time points (1, 2, 4, 6 and 24 hour post-dose) from drug treated groups on Days 1 and 42. The TK parameters for OPC-156 and its metabolites (DM-4103 and DM-4107) were determined.

List of organs/tissues for histopathological examination

Organ/tissue	Histopathology		Organ weight
	H&E	Special staining ^{Note 1)}	
cerebrum	√		√ (as brain)
cerebellum	√		
spinal cord, thoracic	√		
sciatic nerve	√*		
eye	√*		
optic nerve	√*		
harderian gland	√*		
pituitary	√		√
thyroid	√*		√ (as thyroid glands)
parathyroid	√*		
adrenal	√*		√
thymus	√		√

Organ/tissue	Histopathology		Organ weight
	H&E	Special staining ^{Note 1)}	
spleen	√	√	√
lymph node, submandibular	√		
lymph node, mesenteric	√		
heart	√		√
aorta, thoracic	√		
trachea	√		
lung (bronchus)	√		√
tongue	√		
esophagus	√		
stomach	√		
intestine, duodenum	√		
intestine, jejunum	√		
intestine, ileum (Peyer's patch)	√		
intestine, cecum	√		
intestine, colon	√		
intestine, rectum	√		
salivary gland, submandibular	√*		√
salivary gland, sublingual	√*		(as salivary glands)
liver	√		√
pancreas	√		
kidney	√*		√
urinary bladder	√		
testis/ovary	√* / √*		√/ √
epididymis/uterus(cervix)	√* / √*		√/ √
prostate/vagina	√/ √		√/ /
seminal vesicle	√*		
mammary gland, inguinal, only female	√*		
bone + bone marrow, sternal	√		
bone + bone marrow, femoral + joint	√*		
skeletal muscle, femoral	√*		
skin, inguinal	√*		
gross pathological abnormalities	√		
nasal cavity		preservation only	

In addition, parts for identification (auricle with an ear-tag) were removed and preserved.

Items marked with √ were examined.

* Removed bilaterally, but examined unilaterally.

Note 1): For all animals, Berlin blue staining was performed.

Results

Mortality: There was no death in the study.

Clinical signs: No treatment-related clinical signs were noted in the routine clinical observation or in the detailed clinical examination.

Body weight: The body weights in the low and mid dose groups were comparable to that in the control group. In the high dose group, reduced body weights were noted which became significantly different from control on two occasions (Days 4 and 18 of drug treatment) in males

and on Days 4 to 14 of treatment in females. There were no differences in body weight between control and the high dose group for the rest of the treatment period or during the recovery period.

Food consumption: The food consumption in the high dose group (both sexes) was lower than control during the first week of treatment. No significant differences between control and the high dose group were seen during the rest of the treatment period. At lower doses, the food consumption was comparable to control. During the recovery period, the food consumption in previously treated females was higher than control during the first half of the recovery period.

Water consumption: The water consumption was significantly increased in all drug treated groups during the treatment period, but during the recovery period, it was comparable in control and treated groups.

Manipulative tests and grip strength: No treatment-related effects were seen in manipulative tests (auditory response, approach response, touch response, tail pinch response, pupillary reflex and aerial righting reflex) and in grip strength during treatment and recovery periods.

Motor activity: A decrease in motor activity count was observed in all treated groups, the values achieving statistical significance at some time intervals and in total counts. During the recovery period, no significant difference was noted between control and the treated group.

External differentiation : On Day 18 of treatment, the balanopreputial differentiation percentage in the high dose male group was significantly lower compared to control (6.7% vs 66.7% in control), but on Day 25, no significant difference in differentiation was noted. No treatment-related effects were seen at lower dose levels. For vaginal opening, though not statistically significant, the differentiation percentage for the high dose female group on Day 11 of treatment was lower than control. However, the value in the high dose female group was similar to control on Day 18.

Urinalysis: Urine volume and urine sodium, potassium, chloride and creatinine levels (except for creatinine in high dose males) were increased in treated groups (both sexes), compared to control, while osmolality was decreased in treated groups. During the recovery period, no significant differences in these parameters were noted between control and treated groups.

Hematology: Increased reticulocyte and platelet counts, and prolongation of prothrombin and activated partial thromboplastin times in high dose male group, and increased reticulocyte count in high dose female group were noted. Also, increased basophil ratio and count, and decreased eosinophil ratio were seen in high dose males. No significant differences between control and the treated group were noted at the end of the recovery period.

Blood chemistry: Significantly increased total bilirubin and inorganic phosphorus levels were seen in high dose males and females.

Organ weights: Significantly increased absolute and relative liver, thyroid, kidney and adrenal weights were seen in high dose females, while only relative weights for these organs were

increased in high dose males. At the end of the recovery period, increased absolute and relative kidney weights were noted in high dose males.

Gross pathology: There were no treatment-related macroscopic findings.

Histopathology: Treatment-related microscopic findings were seen in adrenals, liver, spleen and thyroid of high dose males and females. The incidence and severity of these lesions are given below.

Sex	Male				Female			
	0	30	100	1000	0	30	100	1000
No. of animals	10	10	10	10	10	10	10	10
Adrenal								
Hypertrophy, cortical cell, diffuse								
minimal	0	–	0	3	0	–	0	1
Liver								
Hypertrophy, hepatocytic, central								
minimal	0	–	0	6	0	–	0	6
mild	0	–	0	3	0	–	0	1
Spleen								
Congestion								
minimal	0	0	0	1	0	–	0	1
Pigmentation, red pulp								
minimal	0	0	0	0	0	–	0	2
Spleen (Berlin blue stain)								
Positive granule, red pulp								
minimal	0	–	0	0	4	–	1	2
mild	0	–	0	0	6	–	6	4
moderate	0	–	0	0	0	–	0	4
Thyroid								
Hypertrophy, follicular cell								
minimal	0	–	0	1	0	–	0	5

–: Not examined

The microscopic lesions observed included minimal diffuse hypertrophy of adrenal cortical cells, centrilobular hypertrophy of hepatocytes, congestion and pigmentation of the red pulp of the spleen and hypertrophy of the follicular cells of the thyroid. Reversibility of lesions was noted in recovery animals.

Toxicokinetics: The toxicokinetic parameters of OPC-156 and its metabolites are given below.

Sex	Analyte	C _{max} (µg/mL)					
		Day 1			Day 42		
		Dose (mg/kg/day)			Dose (mg/kg/day)		
		30	100	1000	30	100	1000
Male	OPC-156	0.4639	2.236	10.55	0.4471	1.137	1.380
	DM-4103	1.891	9.294	28.52	1.268	11.08	40.96
	DM-4107	0.8136	4.427	19.07	0.4957	3.335	26.26
Female	OPC-156	1.117	4.638	11.61	2.013	5.511	5.356
	DM-4103	1.978	6.444	27.58	0.3048	1.823	26.31
	DM-4107	0.8045	3.902	15.16	0.4868	2.314	20.88
Sex	Analyte	AUC _{24h} (µg·h/mL)					
		Day 1			Day 42		
		Dose (mg/kg/day)			Dose (mg/kg/day)		
		30	100	1000	30	100	1000
Male	OPC-156	1.752	10.03	66.62	2.437	5.918	13.14
	DM-4103	12.01	71.50	341.2	9.753	64.78	558.1
	DM-4107	2.678	20.96	217.8	3.864	20.20	258.2
Female	OPC-156	2.826	16.94	111.1	10.82	39.13	60.65
	DM-4103	13.64	60.49	262.7	3.248	23.51	325.3
	DM-4107	2.951	22.63	196.0	4.002	24.26	274.1

C_{max}: maximum serum concentration after administration

AUC_{24h}: area under the concentration-time curve from 0 to 24 hours after administrations calculated using the trapezoidal method

The lower limit of quantification: 0.005 µg/mL

Dose-related increases in C_{max} and AUC values were seen for the parent compound and its metabolites in both sexes. These values for the parent compound were generally lower at the high dose on Day 42 than on Day 1 in both sexes. Also, the parent drug C_{max} and AUC values were higher in females than in males on both days. The C_{max} and AUC values for the metabolites at the high dose were higher on Day 42 than on Day 1 in both sexes. The TK parameters for the metabolite DM-4103 were lower in females than in males. No notable sex difference in TK parameters was noted for the metabolite DM-4107.

In summary, a 6-week oral toxicity study with tolvaptan (0, 30, 100 and 1000 mg/ kg/day) was conducted in juvenile rats (25 days of age at the study initiation) to support pediatric studies for hyponatremia. Animals were exposed to tolvaptan beginning PND 25 to 67 (drug exposure from weaning to puberty) which is comparable to the timing of exposure in the intended pediatric population [childhood to puberty (6 to 17 years of age)].

In the above toxicity study, significantly increased urine output and water intake, compared to control, were noted in all drug treated groups, indicating that the desired pharmacological effect of tolvaptan (blocking the binding of arginine vasopressin at the V₂ receptors, thereby inducing water diuresis) was observed in juvenile rats. The other treatment-related effects, observed only at the high dose level (1000 mg/ kg/day) included increased reticulocyte and platelet counts, prolongation of prothrombin and activated partial thromboplastin times, increased total bilirubin levels, congestion and pigmentation of the red pulp of the spleen, and hypertrophy of adrenal cortical cells, thyroid follicular cells and hepatocytes. These changes were found to be reversible within 4 weeks. The middle dose of 100 mg/kg/day was considered to be the NOAEL for the study.

Based on AUC comparison, systemic exposures for the parent compound, after 42 days of treatment at the NOAEL dose in male and female rats, were 0.7 and 5 times, respectively, greater

than the human exposures (at the second week of treatment) at the maximum recommended daily dose of 120 mg.

Integrated Summary

Tolvaptan (Samsca[®]), a vasopressin V₂-receptor antagonist, was approved for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (NDA 22-275). Tolvaptan blocks the binding of arginine vasopressin (AVP) at the V₂ receptors of the distal portions of the nephron, thereby preventing water reabsorption, and inducing water diuresis (aquaresis) without the depletion of electrolytes. For the current NDA (# 204441), Otsuka is seeking approval for tolvaptan for the treatment of autosomal dominant polycystic kidney disease (ADPKD). ADPKD is an inherited renal disease characterized by progressive development of cysts that destroy normal kidney architecture. The maximum recommended human dose for ADPKD is 120 mg/day.

Approximately, 85% of ADPKD is caused by a mutation in PKD1 gene and 15% by mutation in the PKD2 gene. Although less common but still clinically significant is autosomal recessive PKD (ARPKD). This condition is associated with a mutation in the polycystic kidney and hepatic disease-1 (PKHD1) gene.

It is well known that the renal cysts in PKD originate from collecting ducts where V₂ receptors are predominantly expressed. The combination of increased V₂ receptor expression and increased circulating levels of AVP may give rise to persistent cAMP production leading to cellular proliferation and cyst growth.

Inhibition of V₂ receptors reduces renal cAMP levels that lead to an inhibition of both cyst formation and kidney enlargement. In several studies, vasopressin V₂ receptor antagonists have demonstrated efficacy in PKD by decreasing intracellular levels of cAMP which plays a major role in cyst formation by promoting transepithelial fluid secretion and stimulating cyst-derived cell proliferation.

In animal models of ADPKD (Pkd2^{WS/-} mouse) and ARPKD (PCK rat), it has been shown that dietary administration of tolvaptan lowered renal cAMP levels and exerted a protective effect on the development of PKD as evidenced by lower kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices. A statistically positive correlation was observed between the tissue levels of cAMP and the severity of polycystic kidney disease.

A juvenile rat study was conducted to support pediatric clinical trials for hyponatremia indication, but was not necessarily conducted in support of PKD. In this study, six-week oral administration of tolvaptan to male and female juvenile rats (25 days of age at the study initiation), at 0, 30, 100 and 1000 mg/kg/day, was associated with significantly increased urine volume and water intake in all drug-treated groups. Other treatment related effects, noted only at the high dose level, included congestion and pigmentation of the spleen red pulp, hypertrophy of adrenal cortical cells and thyroid follicular cells and centrolobular hypertrophy of hepatocytes. These changes were found to be reversible after 4 weeks of drug discontinuation. The middle

dose (100 mg/kg/day) was considered to be the NOAEL for the study. Based on AUC comparison, systemic exposures for the parent compound, after 42 days of treatment at the NOAEL dose in male and female juvenile rats, were 0.7 and 5 times, respectively, greater than the human exposures (at the second week of treatment) at the maximum recommended daily dose of 120 mg.

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

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/s/

XAVIER JOSEPH
07/12/2013

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 204441
Supporting document/s: # 0 (original submission)
Applicant's letter date: 11/15/2012
CDER stamp date: 11/15/2012
Product: Tolvaptan (OPC-41061) tablets, 15-, 30-, 45-, 60-, 90 mg
Indication: Indicated to slow progressive kidney disease in adults with autosomal dominant polycystic kidney disease (ADPKD)
Applicant: Otsuka Pharmaceutical Company, Ltd., 1 University Square Drive, Princeton, NJ08540
Review Division: Division of Cardiovascular and Renal Products
Reviewer: Xavier Joseph, D.V.M.
Supervisor/Team Leader: Thomas Papoian, Ph.D.
Division Director: Norman Stockbridge, M.D., Ph.D.
Project Manager: Anna Park

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TABLE OF CONTENTS

	Page #
1. EXECUTIVE SUMMARY	3
2. DRUG INFORMATION	7
3. PHARMACOLOGY	8
4. GENERAL TOXICOLOGY	16
5. INTEGRATED SUMMARY	24

EXECUTIVE SUMMARY

1.1 Introduction

Tolvaptan (Samsca[®]), a vasopressin V₂-receptor antagonist, was approved in 2009 for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (NDA 22-275). Tolvaptan blocks the binding of arginine vasopressin (AVP) at the V₂ receptors of the distal portions of the nephron, thereby preventing water reabsorption, and inducing water diuresis (aquaresis) without the depletion of electrolytes. AVP, a neuropeptide hormone synthesized in the hypothalamus and released into the blood from posterior pituitary, causes fluid retention and hyponatremia. The maximum approved human dose for the treatment of hyponatremia is 60 mg/day.

For the current NDA (# 204441), Otsuka is seeking approval for tolvaptan for the treatment of autosomal dominant polycystic kidney disease (ADPKD). ADPKD is an inherited renal disease characterized by progressive development of cysts that destroy normal kidney architecture. cAMP has been shown to play a major role in cyst formation by promoting transepithelial fluid secretion and stimulating cyst-derived cell proliferation. The maximum recommended human dose for ADPKD is 120 mg/day.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies conducted with tolvaptan were previously reviewed under NDA 22-275.

In receptor binding studies using human AVP receptors expressed in HeLa cells, tolvaptan blocked the binding of labeled AVP to human V₂ 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). For tolvaptan, these binding studies demonstrated 29, 61 and 259 times higher affinity for V₂ receptors than V_{1a} in human, canine and rat species, respectively. It was shown that tolvaptan also inhibited AVP-induced cAMP production. The metabolites of tolvaptan showed no activity at human V₂ and V_{1a} receptors or weak antagonistic activity compared to tolvaptan.

Tolvaptan increased urine excretion and decreased urine osmolality in the dose range of 0.3 to 10 mg/kg, when given orally (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. Serum sodium concentration was elevated at 4 hours postdose in rats, rabbits and dogs.

From *in vitro* studies, CYP3A4 was identified as responsible for catalyzing the primary metabolic reactions. CYP1A1 also plays a role in the formation of the metabolite DM-4128.

Following oral administration of 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 and lactating women.

Chronic oral toxicity studies conducted in both rats (30, 100 and 1000 mg/kg/day for 26 weeks) and dogs (30, 100 and 1000 mg/kg/day for 52 weeks) did not reveal any notable toxicity in either species except for reduced adrenocorticyte vacuolation and/or increased cortical width in the adrenal glands of dogs from all groups. These adrenocortical findings were attributed to a physiological adaptive response to stress (chronic marked diuresis).

No increased incidence of tumors were seen after two years of oral administration of tolvaptan to male and female rats at doses up to 1000 mg/kg/day (81 times the 120 mg maximum recommended human dose [MRHD] on a body surface area basis), to male mice at doses up to 60 mg/kg/day (2 times the MRHD) or to female mice at doses up to 100 mg/kg/day (4 times the MRHD).

Tolvaptan tested negative for genotoxicity in *in vitro* and *in vivo* test systems.

In a fertility study in which male and female rats were orally administered tolvaptan at doses of 100, 300 or 1000 mg/kg/day, the highest dose level was associated with significantly fewer corpora lutea and implants than control. Oral administration of tolvaptan in pregnant rats at 10, 100 and 1000 mg/kg/day during organogenesis was associated with reduced fetal weight and delayed ossification of fetuses at 1000 mg/kg/day (81 times the MRHD on a body surface area basis). In pregnant rabbits, oral administration of tolvaptan at 100, 300 and 1000 mg/kg/day during organogenesis was associated with abortions (mid and high doses) and increased incidences of embryo-fetal death, fetal microphthalmia, brachymelia and skeletal malformations (high dose).

In animal models of polycystic kidney disease (PKD), administration of tolvaptan lowered renal cAMP and exerted a protective effect on the development of PKD as reflected by lower kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices.

The sponsor recently studied tolvaptan vs placebo for delay in progression of renal disease in about 1400 patients with autosomal dominant polycystic kidney disease (ADPKD). In this study, more patients with tolvaptan group experienced elevations of serum alanine aminotransferase (ALT) compared to placebo. At least 3 Hy's law cases were reported in the tolvaptan group indicating the potential for the drug to cause liver injury capable of progression to liver failure. It is noted that the ADPKD studies used a dose (120 mg/day) higher than that currently approved for the treatment of hyponatremia (60 mg/day).

On April 30, 2013, the US FDA issued a Drug Safety Communication (DSC) for Samsca (tolvaptan). The DSC recommends limiting the duration of treatment to 30 days, and that tolvaptan not be used in patients with underlying liver disease.

In summary, chronic oral toxicity studies conducted in rats (26 weeks) and dogs (52 weeks), at doses up to 1000 mg/kg/day [81 times the maximum recommended human dose (MRHD) in rats and 270 times the MRHD in dogs on a body surface area basis], did not reveal any liver toxicity

potential. 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 embryofetal development in rats and rabbits occurred at relatively high doses, and may have been secondary to maternal toxicity at these doses. We do not consider the reprotoxicity findings observed at the very high multiples of MRHD to constitute an approvability issue. However, since the 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 nonclinical toxicity-testing program.

1.3 Recommendations

1.3.1 Approvability

There are no approvability issues for tolvaptan based on nonclinical toxicity testing program.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following sponsor's version of the labeling is considered to be acceptable. (b) (4)

[Redacted]

8.1 Pregnancy

[Redacted] (b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

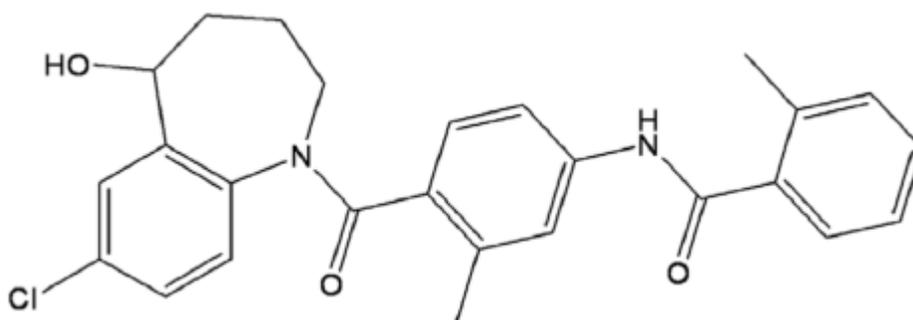
2 Drug Information

2.1 Drug: (b) (4) immediate release tablets.

Generic Name: Tolvaptan

Code Names: OPC-41061 and OPC-156

Chemical Structure



MW 448.94

Pharmacologic Class: Selective vasopressin V₂ receptor antagonist

2.2 Relevant NDAs: NDA 22-275 - Samsca[®] tablets (for the treatment of hypervolemic and euvolemic hyponatremia)

2.3 **Drug Formulation:** (b) (4) immediate release tablets for oral administration are available in 15, 30, 45, 60 and 90 mg strengths of tolvaptan. Inactive ingredients include corn starch, hydroxypropyl cellulose, lactose monohydrate, low – substituted hydroxypropyl cellulose, magnesium stearate and microcrystalline cellulose and FD & C Blue No.2 Aluminum Lake as colorant.

Pharmacology

In Vivo Animal Models

1. Pkd2^{WS/-} Mouse –An Animal Model of ADPKD

[Sponsor's Tables and Figures are used for this review.]

The effects of tolvaptan for the treatment of polycystic kidney disease were investigated in Pkd2^{WS25/-} mouse, an animal model of human autosomal dominant polycystic kidney disease (ADPKD). The goal of the present study was to determine whether tolvaptan, a vasopressin V2 receptor antagonist used in clinical trials for the treatment of water retaining states, also inhibits the development of polycystic kidney disease in a mouse model of human ADPKD.

The study was conducted at (b) (4) (Otsuka Study No. 021276 and Report No. 017968, and study period from April 1, 2004 to November 4, 2005.)

Tolvaptan was administered for 12 weeks to groups of mice (9-12/group/sex; between 4 and 16 weeks of age) via diet at concentrations of 0 (control), 0.01, 0.03 and 0.1%. The mice were given water ad libitum. The animals were sacrificed at sixteen weeks of age and the following parameters were evaluated: 1) body weight at sacrifice; 2) kidney and liver weights (% BW); 3) histomorphometric analysis of kidneys and livers (cysts and fibrosis volumes, % BW) ; 4) renal proliferative (immunostaining for proliferating cell nuclear antigen) and apoptotic (using the TUNEL assay) indices (%); 5) renal cAMP concentrations (pmol/mg protein); 6) plasma BUN, creatinine and sodium concentrations; and urine volumes (ml/24 hours), urine osmolality, and blood pressure.

Results are presented in the following Figures.

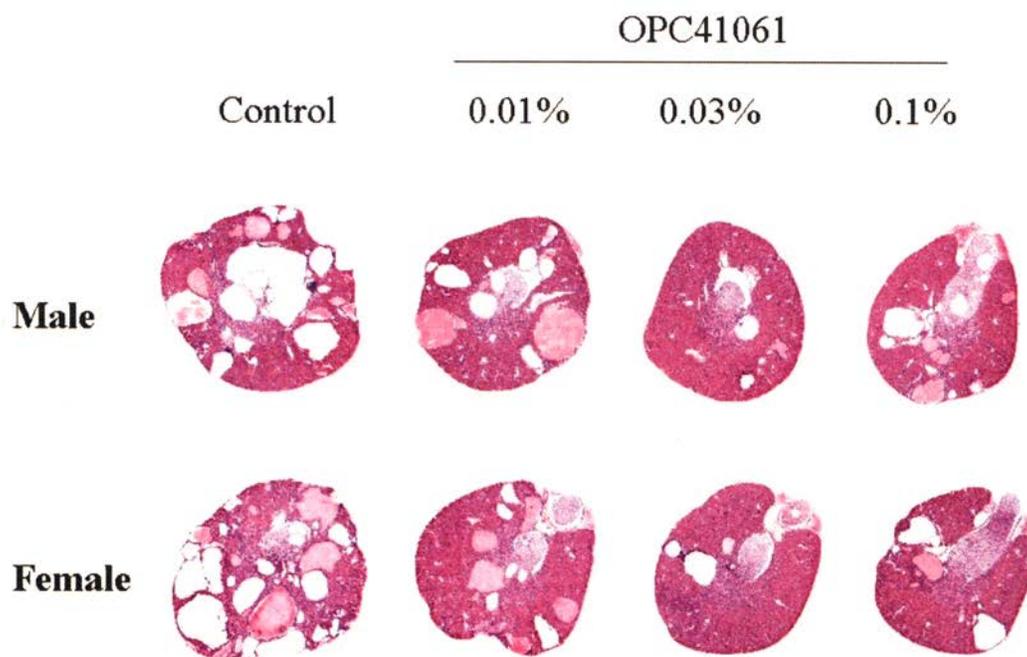


Figure 1 **Representative kidney sections from male and female $Pkd2^{WS25/-}$ mice treated with OPC-41061 between 4-16 weeks of age.**

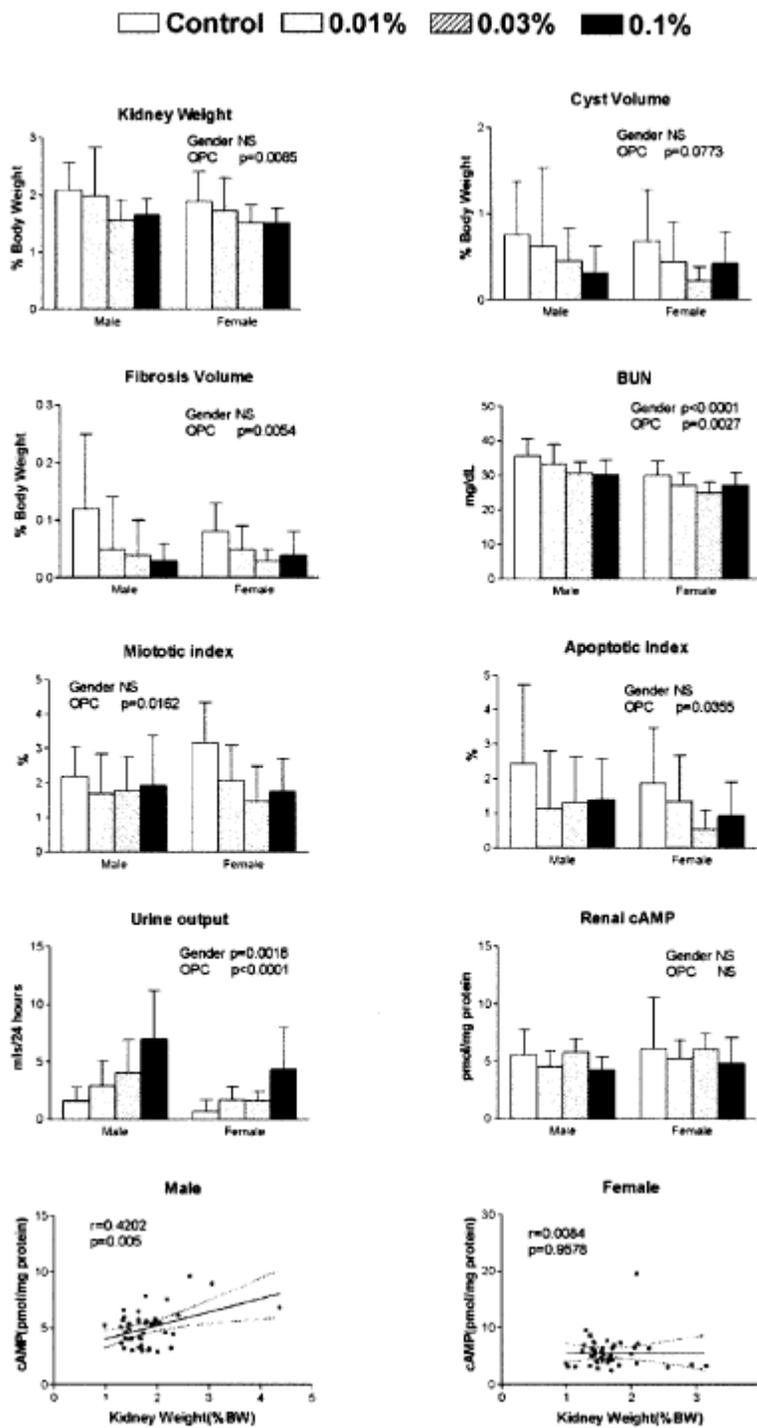


Figure 2

Effects of treatment with OPC-41061 at the specified concentrations on the development of polycystic kidney disease in male and female *Pkd2*^{W525/-} mice between 4–16 weeks of age.

Administration of tolvaptan between 4 and 16 weeks of age exerted a protective effect on the development of polycystic kidney disease in Pkd2^{WS25/-} mice, as evidenced by significantly lower kidney weights, fibrosis volumes, mitotic and apoptotic indices, and plasma BUN concentrations (Figures 1 and 2). Cyst volumes were also lower in drug treated groups, without reaching statistical significance (p=0.077). Renal cAMP levels tended to be lower in the treated animals without reaching statistical significance. A statistically significant positive correlation was noted between the tissue levels of cAMP and the severity of polycystic kidney disease in male (r=0.42, p=0.005) but not in female mice. Most of the protective effect of tolvaptan was reached at a concentration of 0.03%, a dose that caused modest aquaresis. No additional protection was achieved by increasing the dose to 0.1%. The administration of tolvaptan resulted in a dose dependent increase in urine output and reduction in urine osmolality. These were accompanied by a significant elevation in plasma sodium.

Administration of tolvaptan did not inhibit the development of polycystic liver disease, consistent with the absence of vasopressin V₂ receptors in the liver.

2. PCK Rat – An Animal Model of ARPKD

The goals of the present study were to determine whether tolvaptan, a vasopressin V₂ receptor antagonist, currently approved for the treatment of hyponatremia, inhibits the development of polycystic kidney disease in PCK rats.

The PCK rat is a model of human autosomal recessive polycystic kidney disease (ARPKD) caused by a splicing mutation that leads to a frameshift in the ortholog Pkhd1 gene due to skipping of exon 36. Progressive cystogenesis and impairment of renal function characterize this model.

The effects of tolvaptan for the treatment of PKD in PCK rats were investigated at (b) (4)
 (Otsuka Study No. 021276 and Report No. 016916; Study period April 1, 2004 to May 12, 2005).

Tolvaptan was administered for 7 weeks to groups of PCK rats on a Sprague-Dawley strain (10/group/sex) via the diet at concentrations of 0 (control), 0.01, 0.03, and 0.1% beginning at 3 weeks of age. The rats were given water ad libitum. Animals were sacrificed at 10 weeks of age and the following parameters were evaluated: 1) body weight at sacrifice; 2) kidney and liver weights (% BW); 3) histomorphometric analysis of the kidneys and livers (cyst and fibrosis volumes, % BW); 4) renal proliferative (for proliferating cell nuclear antigen) and apoptotic (using TUNEL assay) indices (%); 5) renal cAMP concentrations (pmol/mg protein); 6) plasma BUN, creatinine and sodium concentrations; and 7) urine volumes (ml/24 hours) and blood pressure.

Results are presented in the following Figures.

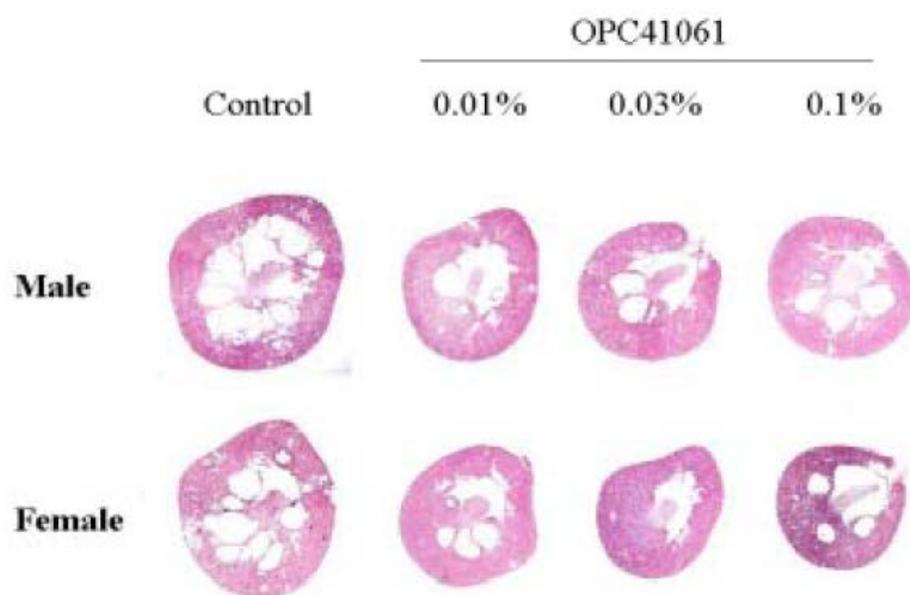
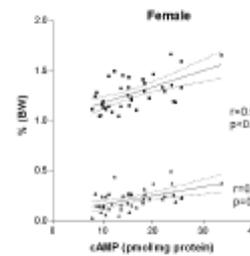
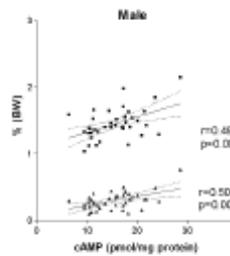
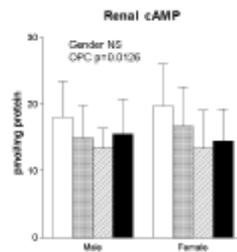
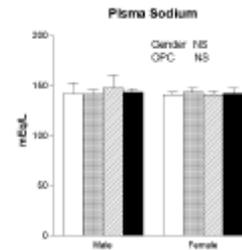
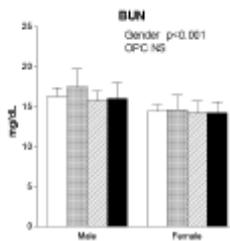
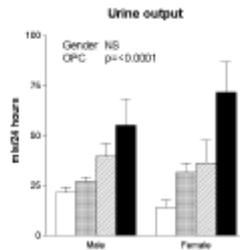
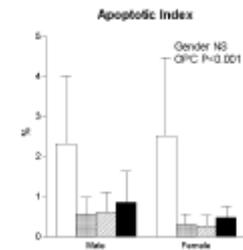
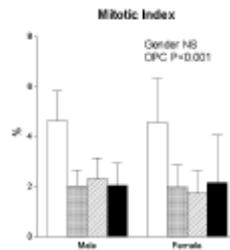
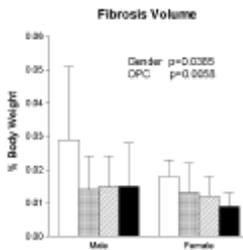
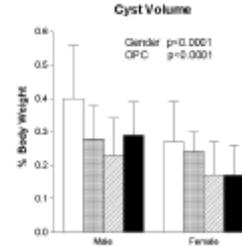
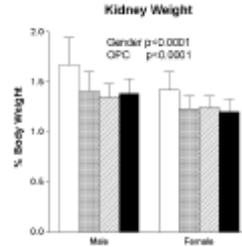
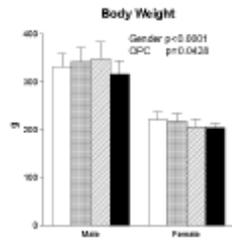


Figure 1A

Representative kidney sections from male and female PCK rats treated with OPC-41061 between 3-10 weeks of age.

control 0.01% 0.03% 0.1%



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Figure 1B

Effects of OPC-41061 treatment at the specified concentrations on the development of polycystic kidney disease, urine output, and renal cAMP concentration in PCK rats. Data are expressed as means±SD of 10 animals. Significant positive correlation was observed between renal cAMP concentrations and kidney weights or cyst volumes at sacrifice in male and female animals

Mitotic and apoptotic indices are shown as percent of cells that are positive for proliferating cell nuclear antigen staining or ApopTag peroxidase positive in the TUNEL assay.

Results indicated that the administration of tolvaptan between 3 and 10 weeks of age lowered renal cAMP levels and exerted a protective effect on the development of polycystic kidney disease in PCK rats, as reflected by significantly lower kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices (Figure 1A and 1B). Plasma sodium, BUN and creatinine levels were normal and not significantly different between groups. A statistically significant positive correlation was detected between the tissue levels of cAMP and the severity of polycystic kidney disease. Most of the protective effect of tolvaptan was detectable with the lowest dose used in the study, a dose that caused only modest aquaresis. Limited additional protection was achieved by a ten-fold increase in dose.

In a separate study conducted at ^{(b) (4)} the effects of tolvaptan on the development of polycystic disease were studied in male PCK rats. Tolvaptan was administered by oral gavage at a dose of 10 mg/kg once or twice a day for 8 weeks from 5 to 13 weeks of age. PCK control rats were treated with the vehicle (1% hydroxypropylmethyl cellulose). A nontreated control group of Sprague-Dawley rats was included in the study. The following parameters were evaluated: clinical signs, body weight, food and water consumption, urine volume, urinalysis including urinary Na levels, osmolality, urinary cAMP excretion, blood chemistry, renal cAMP levels, organ weights, histopathologic evaluations (cyst and fibrosis volumes of kidney), and PCNA and TUNEL labeling indices.

Water intake and urine volume were increased and urinary osmolality decreased with both dose regimens (once or twice daily dose administrations). After 8 weeks of treatment, no changes were observed on kidney weight, kidney cAMP content, or on kidney histopathology (cyst and fibrosis volumes) findings, indicating that tolvaptan did not affect the progression of PKD based on the lack of kidney changes. Gavage administration of tolvaptan at 10 mg/kg exerted a short duration of aquaretic action that was not sustained for 24 hours in male Sprague-Dawley rats even in the case of twice daily administration. These findings suggested that the duration of V2R inhibition by this administration method (oral gavage) may not be sufficient to protect the development of PKD in PCK rats.

Studies in Other Animal Models of PKD

Studies were also conducted in male **pcy mouse** (orthologous to human adolescent nephronophthisis). Tolvaptan was administered at dietary concentrations of 0, 0.01, 0.03, 0.1 or 0.3% for 10 weeks, beginning at 5 weeks of age. A dose-related decrease in kidney weights was observed in tolvaptan-treated mice, compared to controls, with a statistically significant reduction observed at tolvaptan concentration of 0.03% and higher. A decreasing trend was observed for kidney cyst volume, kidney fibrosis volume and mitotic index with a significant reduction observed at 0.1 or 0.3%. A dose-related aquaretic effect was observed on urine volume (increased) and urine osmolality (decreased) with a maximum effect seen at 0.1%. Consistent with the reduced kidney volume, a dose-related reduction in kidney cAMP content and ERK activity were observed in pcy mice compared to controls.

Serum levels of tolvaptan were determined in the pcy mouse treated via the diet (0.01 or 0.1%) for 14 days. Blood samples were collected at Day 7 (morning) and on 3 occasions (morning, evening and midnight) on Day 14. Serum levels of tolvaptan increased with dietary concentration, and the levels tended to be higher at midnight. The data demonstrate exposure to tolvaptan in the pcy mouse at pharmacologically active doses, and the levels observed are considered to be generally similar to those found with clinical administration of tolvaptan.

In Vitro Studies examined cell proliferation in normal human kidney cells (NHK) and cells obtained from the cysts of the ADPKD patients. Addition of AVP to NHK cultures resulted in an increase of intracellular cAMP, however, the rate of cell proliferation did not change. By contrast, addition of AVP to ADPKD cultures resulted in increased intracellular cAMP and increased cell proliferation. Tolvaptan significantly reduced cAMP and cell proliferation of the AVP stimulated ADPKD cells.

General Toxicology

Six-Week Oral Dose Toxicity Study of Tolvaptan With a 4-Week Recovery Period in Juvenile Rats

(Note: This juvenile rat study was undertaken to support pediatric clinical trials for hyponatremia, and not PKD.)

Study numbers: (b) (4) 6805 (CRO #)
030396 (Sponsor's study #)
025612 (Sponsor's Report #)

Study report location: Otsuka Pharmaceutical Co., Ltd, Tokushima, Japan

Conducting laboratory and location (b) (4)

Study dates: April 1, 2010 – October 29, 2010

GLP compliance: The study was conducted in accordance with the GLP Ordinances issued by the Ministry of Health and Welfare, Japan.

QA statement: Inspections were conducted periodically and the final Quality Assurance Statement has been issued by the Lead QA manager on October 29, 2010.

Drug, lot # and % purity: code number – OPC-156, lot # - 090821-1, drug content – 66.5%

Key study findings: Six-week oral administration of tolvaptan to male and female juvenile rats (25 days of age at the study initiation), at 0, 30, 100 and 1000 mg/kg/day, was associated with significantly increased urine volume and water intake in all drug-treated groups. Other treatment related effects, noted only at the high dose level, included congestion and pigmentation of the spleen red pulp, hypertrophy of adrenal cortical cells and thyroid follicular cells and centrolobular hypertrophy of hepatocytes. These changes were found to be reversible.

Methods

Doses: 0, 30, 100 and 1000 mg/kg/day

Frequency of dosing: once daily for six weeks

Route of administration: oral (gavage)

Dose volume: 5 ml/kg

Formulation/Vehicle: formulated as a suspension in 1% hypromellose. Dosing formulations were prepared weekly and were found to be stable for 8 days when stored at temperature not exceeding 10°C

Species/Strain: Rats/Sprague-Dawley [CrI:CD(SD)] (b) (4)

Number/Sex/Group: 10 – An additional 5 rats/sex were included in the control and high dose groups for the 4-week recovery period. Satellite animals (6 rats/sex in the control and 26 rats/sex in drug treated groups) were used for toxicokinetic evaluations.

Age: 25 days of age at the initiation of the study [the treatment period of six weeks which extended from about 3.5 to 9.5 weeks of age included the age of rats (7 to 8 weeks) at the initiation of 6-month rat toxicity study that was conducted earlier].

Weight: main study group – males – 76 to 108 g; females – 73 to 91 g; satellite group – males – 74 to 101 g; females -71 to 95 g

Rationale for dose selection: The doses were selected based on the results of a 3-week oral range-finding study of tolvaptan (0, 100, 300 and 1000 mg/kg) in juvenile rats. No deaths were seen in the study; significantly increased water intake was noted in all groups. Other treatment-related findings included reduced body weight gain, decreased RBC count, hemoglobin and hematocrit values and enlargement of liver at the high dose. No histopathology was conducted in the study. Based on the above results, the 1000 mg/kg/day was selected as the high dose for the 6-week toxicity study in juvenile rats. (It is also noted that the results from the previous repeated dose oral toxicity studies showed that the 1000 mg/kg/day is considered to be the maximum feasible dose based on the viscosity and volume of the dosing suspension.)

Unique study design: none

Deviation from study protocol: Minor deviations noted from protocol (such as, at the initiation of the study, a body weight deviation (+3g) for 1 animal from the upper limit specified by the protocol; a 1°C temperature increase on one day for about 30 minutes in one of the animal rooms, and missing of parathyroid tissue for one control female) were judged to have no significant effect on study results.

Observations

Clinical signs and mortality: All animals in the main study and recovery groups were examined twice daily during the drug treatment period and once daily during the recovery period. Satellite group animals were examined only for mortality.

Detailed clinical examinations were performed on 5 rats/sex/group from the main study group on Day 40 of drug treatment and on all recovery phase animals on Day 26 of the recovery period. These examinations included home cage observations (posture and abnormal behavior), in hand observations [skin conditions, secretions from eyes/nose, visible mucous membrane, autonomic

nervous system function (lacrimation, salivation, piloerection, pupil size and abnormal respiration) and reactivity to handling] and open field observations (arousal, convulsion, abnormal behavior, gait, posture, defecation and urination).

Sensory/reflex function examinations were performed on 5 rats/sex/group from the main study group (1 to 4 hr post-dose) on Day 40 of drug treatment and on all recovery phase animals on Day 26 of recovery. These examinations included manipulative tests (auditory response, touch response, tail pinch response, pupillary reflex, aerial righting reflex and landing foot splay) and measurement of grip strength using CPU gauge.

Motor activity was measured in 5 rats/sex/group from the main study animals on Day 40 of treatment and in all recovery phase animals on Day 26 of recovery. The motor activity, using the spontaneous movement sensor for the experimental animals NS-AS01, was measured for 1 hour and the counts were recorded at 10 minute intervals and also as total counts.

Body weight: All animals from the main study and recovery groups were weighed every 3 to 4 day intervals during the drug treatment period. Animals were weighed weekly during the recovery period. On the day of necropsy, animals were weighed after fasting for calculating the relative organ weight. Satellite group animals were weighed for calculating the dose volume, but the data were not subjected to toxicological evaluation.

Food consumption: During the drug administration period, for all main study and recovery phase animals, a 3 or 4-day cumulative food consumption was measured and one day food consumption was calculated. For recovery phase animals, a 6 or 7-day cumulative food consumption was measured for determining the one day consumption.

Water intake: A 3 to 4 day cumulative water intake was determined to calculate the one day water intake for the main study and recovery group animals during the drug treatment and recovery periods.

External differentiation : Females were observed for vaginal opening (Days 11 and 18 of drug treatment) and males for balanopreputial separation (Days 18 and 25 of drug treatment) and the parameter was expressed as the external differentiation percentage.

Urinalysis was conducted on main study animals (5 rats/sex/group) on Day 36 of drug treatment and on all recovery phase animals on Day 22 of recovery. Four-and twenty- hour urine samples were collected. Urine volume, color, pH, sediment, protein, ketones, glucose, occult blood, bilirubin and urobilinogen were determined on 4-hour sample, and urine volume, osmolality, sodium, potassium, chloride and creatinine determinations were made on 24- hour sample.

Hematology: Blood samples were collected from the main study animals at the end of the treatment period, and from the recovery animals at the end of the recovery period. The following parameters were evaluated: RBC, WBC (total and differential), reticulocyte and platelet counts, hemoglobin, hematocrit, MCV, MCH, MCHC, prothrombin time and activated partial thromboplastin time.

Blood chemistry: The following parameters were evaluated: alkaline phosphatase, total cholesterol, triglyceride, phospholipids, total bilirubin, glucose, BUN, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, A/G ratio, protein fraction ratio and concentrations, aminotransferases, LDH, CPK and γ -GTP.

Gross pathology: At the end of the treatment and treatment-free periods, a complete necropsy was performed on all animals. All macroscopic abnormalities were recorded.

Organ weight: Brain, pituitary, thyroid, adrenal, thymus, spleen, heart, lung, salivary glands, liver, kidney, testis, ovary, epididymus, uterus and prostate were weighed.

Histopathology: All organs and tissues from all animals were fixed, preserved, processed and stained with H&E. The tissues shown in the following Table from the control and high dose groups were examined microscopically. The adrenals, bone marrow (femur), liver, spleen (including Berlin staining for hemosiderin), thyroid and urinary bladder from the middle dose group were also examined. In addition, spleen (males) and urinary bladder (both sexes) from the low dose group were examined.

Toxicokinetic evaluation: Blood was collected from 3 rats/sex at one time point (2 hour post-dose) from the control group and 5 time points (1, 2, 4, 6 and 24 hour post-dose) from drug treated groups on Days 1 and 42. The TK parameters for OPC-156 and its metabolites (DM-4103 and DM-4107) were determined.

List of organs/tissues for histopathological examination

Organ/tissue	Histopathology		Organ weight
	H&E	Special staining ^{Note 1)}	
cerebrum	√		√ (as brain)
cerebellum	√		
spinal cord, thoracic	√		
sciatic nerve	√*		
eye	√*		
optic nerve	√*		
harderian gland	√*		
pituitary	√		√
thyroid	√*		√ (as thyroid glands)
parathyroid	√*		
adrenal	√*		√
thymus	√		√

Organ/tissue	Histopathology		Organ weight
	H&E	Special staining ^{Note 1)}	
spleen	√	√	√
lymph node, submandibular	√		
lymph node, mesenteric	√		
heart	√		√
aorta, thoracic	√		
trachea	√		
lung (bronchus)	√		√
tongue	√		
esophagus	√		
stomach	√		
intestine, duodenum	√		
intestine, jejunum	√		
intestine, ileum (Peyer's patch)	√		
intestine, cecum	√		
intestine, colon	√		
intestine, rectum	√		
salivary gland, submandibular	√*		√
salivary gland, sublingual	√*		(as salivary glands)
liver	√		√
pancreas	√		
kidney	√*		√
urinary bladder	√		
testis/ovary	√* / √*		√ / √
epididymis/uterus(cervix)	√* / √*		√ / √
prostate/vagina	√ / √		√ /
seminal vesicle	√*		
mammary gland, inguinal, only female	√*		
bone + bone marrow, sternal	√		
bone + bone marrow, femoral + joint	√*		
skeletal muscle, femoral	√*		
skin, inguinal	√*		
gross pathological abnormalities	√		
nasal cavity		preservation only	

In addition, parts for identification (auricle with an ear-tag) were removed and preserved.

Items marked with √ were examined.

* Removed bilaterally, but examined unilaterally.

Note 1): For all animals, Berlin blue staining was performed.

Results

Mortality: There was no death in the study.

Clinical signs: No treatment-related clinical signs were noted in the routine clinical observation or in the detailed clinical examination.

Body weight: The body weights in the low and mid dose groups were comparable to that in the control group. In the high dose group, reduced body weights were noted which became significantly different from control on two occasions (Days 4 and 18 of drug treatment) in males

and on Days 4 to 14 of treatment in females. There were no differences in body weight between control and the high dose group for the rest of the treatment period or during the recovery period.

Food consumption: The food consumption in the high dose group (both sexes) was lower than control during the first week of treatment. No significant differences between control and the high dose group were seen during the rest of the treatment period. At lower doses, the food consumption was comparable to control. During the recovery period, the food consumption in previously treated females was higher than control during the first half of the recovery period.

Water consumption: The water consumption was significantly increased in all drug treated groups during the treatment period, but during the recovery period, it was comparable in control and treated groups.

Manipulative tests and grip strength: No treatment-related effects were seen in manipulative tests (auditory response, approach response, touch response, tail pinch response, pupillary reflex and aerial righting reflex) and in grip strength during treatment and recovery periods.

Motor activity: A decrease in motor activity count was observed in all treated groups, the values achieving statistical significance at some time intervals and in total counts. During the recovery period, no significant difference was noted between control and the treated group.

External differentiation : On Day 18 of treatment, the balanopreputial differentiation percentage in the high dose male group was significantly lower compared to control (6.7% vs 66.7% in control), but on Day 25, no significant difference in differentiation was noted. No treatment-related effects were seen at lower dose levels. For vaginal opening, though not statistically significant, the differentiation percentage for the high dose female group on Day 11 of treatment was lower than control. However, the value in the high dose female group was similar to control on Day 18.

Urinalysis: Urine volume and urine sodium, potassium, chloride and creatinine levels (except for creatinine in high dose males) were increased in treated groups (both sexes), compared to control, while osmolality was decreased in treated groups. During the recovery period, no significant differences in these parameters were noted between control and treated groups.

Hematology: Increased reticulocyte and platelet counts, and prolongation of prothrombin and activated partial thromboplastin times in high dose male group, and increased reticulocyte count in high dose female group were noted. Also, increased basophil ratio and count, and decreased eosinophil ratio were seen in high dose males. No significant differences between control and the treated group were noted at the end of the recovery period.

Blood chemistry: Significantly increased total bilirubin and inorganic phosphorus levels were seen in high dose males and females.

Organ weights: Significantly increased absolute and relative liver, thyroid, kidney and adrenal weights were seen in high dose females, while only relative weights for these organs were

increased in high dose males. At the end of the recovery period, increased absolute and relative kidney weights were noted in high dose males.

Gross pathology: There were no treatment-related macroscopic findings.

Histopathology: Treatment-related microscopic findings were seen in adrenals, liver, spleen and thyroid of high dose males and females. The incidence and severity of these lesions are given below.

Sex	Male				Female			
	0	30	100	1000	0	30	100	1000
No. of animals	10	10	10	10	10	10	10	10
Adrenal								
Hypertrophy, cortical cell, diffuse								
minimal	0	–	0	3	0	–	0	1
Liver								
Hypertrophy, hepatocytic, central								
minimal	0	–	0	6	0	–	0	6
mild	0	–	0	3	0	–	0	1
Spleen								
Congestion								
minimal	0	0	0	1	0	–	0	1
Pigmentation, red pulp								
minimal	0	0	0	0	0	–	0	2
Spleen (Berlin blue stain)								
Positive granule, red pulp								
minimal	0	–	0	0	4	–	1	2
mild	0	–	0	0	6	–	6	4
moderate	0	–	0	0	0	–	0	4
Thyroid								
Hypertrophy, follicular cell								
minimal	0	–	0	1	0	–	0	5

–: Not examined

The microscopic lesions observed included minimal diffuse hypertrophy of adrenal cortical cells, centrilobular hypertrophy of hepatocytes, congestion and pigmentation of the red pulp of the spleen and hypertrophy of the follicular cells of the thyroid. Reversibility of lesions was noted in recovery animals.

Toxicokinetics: The toxicokinetic parameters of OPC-156 and its metabolites are given below.

Sex	Analyte	C _{max} (µg/mL)					
		Day 1			Day 42		
		Dose (mg/kg/day)			Dose (mg/kg/day)		
		30	100	1000	30	100	1000
Male	OPC-156	0.4639	2.236	10.55	0.4471	1.137	1.380
	DM-4103	1.891	9.294	28.52	1.268	11.08	40.96
	DM-4107	0.8136	4.427	19.07	0.4957	3.335	26.26
Female	OPC-156	1.117	4.638	11.61	2.013	5.511	5.356
	DM-4103	1.978	6.444	27.58	0.3048	1.823	26.31
	DM-4107	0.8045	3.902	15.16	0.4868	2.314	20.88
Sex	Analyte	AUC _{24h} (µg·h/mL)					
		Day 1			Day 42		
		Dose (mg/kg/day)			Dose (mg/kg/day)		
		30	100	1000	30	100	1000
Male	OPC-156	1.752	10.03	66.62	2.437	5.918	13.14
	DM-4103	12.01	71.50	341.2	9.753	64.78	558.1
	DM-4107	2.678	20.96	217.8	3.864	20.20	258.2
Female	OPC-156	2.826	16.94	111.1	10.82	39.13	60.65
	DM-4103	13.64	60.49	262.7	3.248	23.51	325.3
	DM-4107	2.951	22.63	196.0	4.002	24.26	274.1

C_{max}: maximum serum concentration after administration

AUC_{24h}: area under the concentration-time curve from 0 to 24 hours after administrations calculated using the trapezoidal method

The lower limit of quantification: 0.005 µg/mL

Dose-related increases in C_{max} and AUC values were seen for the parent compound and its metabolites in both sexes. These values for the parent compound were generally lower at the high dose on Day 42 than on Day 1 in both sexes. Also, the parent drug C_{max} and AUC values were higher in females than in males on both days. The C_{max} and AUC values for the metabolites at the high dose were higher on Day 42 than on Day 1 in both sexes. The TK parameters for the metabolite DM-4103 were lower in females than in males. No notable sex difference in TK parameters was noted for the metabolite DM-4107.

In summary, a 6-week oral toxicity study with tolvaptan (0, 30, 100 and 1000 mg/ kg/day) was conducted in juvenile rats (25 days of age at the study initiation) to support pediatric studies for hyponatremia. Animals were exposed to tolvaptan beginning PND 25 to 67 (drug exposure from weaning to puberty) which is comparable to the timing of exposure in the intended pediatric population [childhood to puberty (6 to 17 years of age)].

In the above toxicity study, significantly increased urine output and water intake, compared to control, were noted in all drug treated groups, indicating that the desired pharmacological effect of tolvaptan (blocking the binding of arginine vasopressin at the V₂ receptors, thereby inducing water diuresis) was observed in juvenile rats. The other treatment-related effects, observed only at the high dose level (1000 mg/ kg/day) included increased reticulocyte and platelet counts, prolongation of prothrombin and activated partial thromboplastin times, increased total bilirubin levels, congestion and pigmentation of the red pulp of the spleen, and hypertrophy of adrenal cortical cells, thyroid follicular cells and hepatocytes. These changes were found to be reversible within 4 weeks. The middle dose of 100 mg/kg/day was considered to be the NOAEL for the study.

Based on AUC comparison, systemic exposures for the parent compound, after 42 days of treatment at the NOAEL dose in male and female rats, were 0.7 and 5 times, respectively, greater

than the human exposures (at the second week of treatment) at the maximum recommended daily dose of 120 mg.

Integrated Summary

Tolvaptan (Samsca[®]), a vasopressin V₂-receptor antagonist, was approved for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (NDA 22-275). Tolvaptan blocks the binding of arginine vasopressin (AVP) at the V₂ receptors of the distal portions of the nephron, thereby preventing water reabsorption, and inducing water diuresis (aquaresis) without the depletion of electrolytes. For the current NDA (# 204441), Otsuka is seeking approval for tolvaptan for the treatment of autosomal dominant polycystic kidney disease (ADPKD). ADPKD is an inherited renal disease characterized by progressive development of cysts that destroy normal kidney architecture. The maximum recommended human dose for ADPKD is 120 mg/day.

Approximately, 85% of ADPKD is caused by a mutation in PKD1 gene and 15% by mutation in the PKD2 gene. Although less common but still clinically significant is autosomal recessive PKD (ARPKD). This condition is associated with a mutation in the polycystic kidney and hepatic disease-1 (PKHD1) gene.

It is well known that the renal cysts in PKD originate from collecting ducts where V₂ receptors are predominantly expressed. The combination of increased V₂ receptor expression and increased circulating levels of AVP may give rise to persistent cAMP production leading to cellular proliferation and cyst growth.

Inhibition of V₂ receptors reduces renal cAMP levels that lead to an inhibition of both cyst formation and kidney enlargement. In several studies, vasopressin V₂ receptor antagonists have demonstrated efficacy in PKD by decreasing intracellular levels of cAMP which plays a major role in cyst formation by promoting transepithelial fluid secretion and stimulating cyst-derived cell proliferation.

In animal models of ADPKD (Pkd2^{WS/-} mouse) and ARPKD (PCK rat), it has been shown that dietary administration of tolvaptan lowered renal cAMP levels and exerted a protective effect on the development of PKD as evidenced by lower kidney weights, cyst and fibrosis volumes, and mitotic and apoptotic indices. A statistically positive correlation was observed between the tissue levels of cAMP and the severity of polycystic kidney disease.

A juvenile rat study was conducted to support pediatric clinical trials for hyponatremia indication, but was not necessarily conducted in support of PKD. In this study, six-week oral administration of tolvaptan to male and female juvenile rats (25 days of age at the study initiation), at 0, 30, 100 and 1000 mg/kg/day, was associated with significantly increased urine volume and water intake in all drug-treated groups. Other treatment related effects, noted only at the high dose level, included congestion and pigmentation of the spleen red pulp, hypertrophy of adrenal cortical cells and thyroid follicular cells and centrolobular hypertrophy of hepatocytes. These changes were found to be reversible after 4 weeks of drug discontinuation. The middle

dose (100 mg/kg/day) was considered to be the NOAEL for the study. Based on AUC comparison, systemic exposures for the parent compound, after 42 days of treatment at the NOAEL dose in male and female juvenile rats, were 0.7 and 5 times, respectively, greater than the human exposures (at the second week of treatment) at the maximum recommended daily dose of 120 mg.

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

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/s/

XAVIER JOSEPH
07/12/2013

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07/15/2013
Concur.