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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA-204-485
Supporting document/s:	0000
Applicant's letter date:	September 25, 2012
CDER stamp date:	September 26, 2012
Product:	Pitressin (Vasopressin) Injection, USP. (20 pressure unit/mL)
Indication:	Treatment of vasodilatory shock, including post-cardiotomy shock and septic shock
Applicant:	JHP Pharmaceuticals, LLC
Review Division:	Cardiovascular and Renal Products
Reviewer:	Rama Dwivedi, Ph.D.
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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

Arginine Vasopressin (AVP, Pitressin®), as vasoconstrictor and antidiuretic drug has been in the market as an unapproved product for the treatment of vasodilatory shock for the last several decades (prior to 1938 by Parke-Davis). Low arterial blood pressure and slow tissue perfusion as a result of decreased systemic vascular resistance are the major causes of vasodilatory shock. Severe shock may also occur as a result of either sepsis or surgery requiring cardiopulmonary bypass. The major challenge for the treatment of vasodilatory shock is a decreased plasma vasopressin (Landry 2001) concentration and poor response to catecholamine (the usual vasopressor drug) leading to an increased mortality rate (30 to 60%).

The proposed treatment for vasodilatory shock (0.01 to 0.067 units/minute) including post-cardiotomy (0.005 to 0.10 unit/minute) and septic shock (0.01 to 0.03 unit/minute) is an intravenous administration of AVP for continuous infusion to maintain a MAP of ≥ 65−70mm Hg. Due to an enormous non-clinical data (PubMed, TOXNET (including TOXLINE, HSDB, DART, etc., EMBASE, Registry of Toxic Effects of Chemical Substances (RTECS), CAplus, and Beilstein) available on vasopressin (8-AVP), a full battery of non-clinical studies were not conducted by the Sponsor to support the present NDA.

1.2 Brief Discussion of Nonclinical Findings

AVP is mainly synthesized in the hypothalamus, and its cellular effects are mediated via V1a, V1b and V2 receptors. Binding to the V1a receptor results in vasoconstriction, glycogenolysis, platelet aggregation and adrenocorticotropic hormone release. AVP binding to the V1a receptor, that is present on vascular smooth muscle cells, causes vasoconstriction mediated by an increase in intracellular calcium via the phosphatidyl-inositol-bisphosphonate pathway (see Fig. 3 pg. # 16).

Thus, the cardiac effects of AVP are largely due to an indirect mechanism resulting from coronary vasoconstriction, decreased coronary blood flow, and altered vagal and sympathetic tones. Plasma half-life of AVP in humans ranges from 17 to 35 minutes (Jackson 2006). Due to inactivation by trypsin in the gastrointestinal tract, AVP treatment for vasodilatory shock is given by continuous IV administration.

Peripheral vasoconstriction as a result of an increased blood pressure and a compensatory decrease in heart rate are the adverse effects of AVP treatment in rats and dogs given IV bolus and IV infusion, however, no mortalities were reported in these studies (Miyazaki et al 2000, Rysa et al 2006, Tsukada et al 2002, Groszmann et al 1982, Barr et al 1975). A 14-Day dose range finding (DRF) study and 28-Day repeated dose toxicity study in Sprague-Dawley rats , bacterial mutagenesis (Ames) and CHO chromosome aberration assays were conducted by the Sponsor for safety assessment of AVP and AVP degradation products ((b) (4) to evaluate the toxic and mutagenic potential of AVP.

No adverse effects were reported in 28-Day repeated dose toxicity study in rats. AVP did not show any genotoxic potential in mutagenicity and clastogenicity studies.

The no observed adverse effect level (NOAEL) was considered to be 0.76 µg/kg for AVP and 1.14 µg/kg for impurities in 4-week repeated dose study in rats.

The Sponsor did not conduct any formal carcinogenic or fertility impairment studies in animals with Pitressin in evaluating the effects of AVP, however, studies from published literature have shown that high doses of AVP might have adverse effects on reproductive function, fetal growth and development, and therefore, there may be a potential risk to the developing embryo and fetus. No safety concerns from a pharmacology or toxicology perspective have been identified toward the approvability this submission.

1.3 Recommendations

1.3.1 Approvability

Yes

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Under the 'Pregnancy' section (8.1), the phrase "(b) (4)" should be deleted from the title. The Sponsor's statement that, "*It is not known if vasopressin can cause fetal harm when administered to pregnant women or if it can affect reproductive capacity*" does not seem to be substantiated in light of available information in published literature, and should be changed as follows:

(b) (4)

Sponsor's labeling statement as in Paragraph # 2 of this section does not seem to be supported by the data provided by Davison et al (1989), "*There is a decrease in endogenous plasma concentration of vasopressin during the first and second trimesters due to increased levels of vasopressinase which could interfere with the effectiveness of Pitressin. However, low doses of AVP sufficient for a pressor effect are not likely to produce tonic uterine contractions that could be deleterious to the fetus or threaten the continuation of the pregnancy*".

The article by Davison et al. (1989) did not show decreased AVP levels during pregnancy, but rather showed that a basal level of endogenous AVP plasma concentration is maintained even in the presence of increased vasopressinase levels, indicating that synthesis of vasopressin was

likely increased in response to increased degradation by vasopressinase. Because of this reviewer's different view of the Sponsor's interpretation of the data, a MHT consult has been requested for this part of labeling.

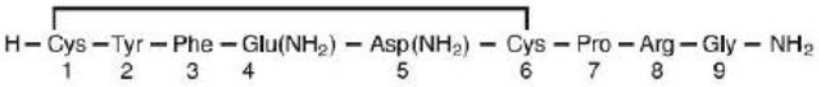
In addition to this, (b) (4) as suggested by the Sponsor needs supporting data to validate the claim.

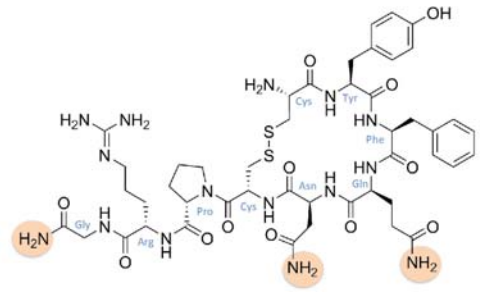
In the 'Nonclinical Toxicology Section' the title, "*Carcinogenicity, mutagenicity and fertility*" was replaced by "Carcinogenesis, Mutagenesis, Impairment of Fertility" and the section has been changed as follows in view of information available in the published literature:

"No formal carcinogenicity or fertility studies with Pitressin have been conducted in animals. Pitressin was found to be negative in the *in vitro* bacterial mutagenicity (Ames) test and the *in vitro* Chinese hamster ovary cell chromosome aberration test. In mice, vasopressin has been reported to have a detrimental effect on sperm function and on the fertilizing ability of spermatozoa."

2 Drug Information

2.1 Drug	Pitressin® (Vasopressin Injection, USP)
CAS Registry Number (Optional)	113-79-1
Generic Name	Vasopressin
Code Name	Pitressin®
Chemical Name	Cyclo (1-6) L-Cysteinyl-L, Tyrosil-L, Phenylalanyl-L-Glutaminyl-L-Asparaginyl-LCysteinyl- L-Propyl-L-Arginyl-L-Glycineamide
Molecular Formula	$C_{46}H_{65}N_{15}O_{12}S_2$
Molecular Weight	1084.23Structure or Biochemical Description





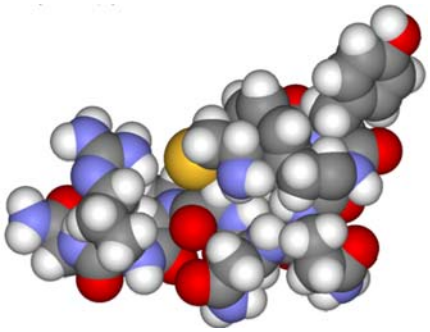


Figure 1: Space filling model of Vasopressin (Wikipedia)

Pharmacologic Class	Vasopressin

2.2 Relevant INDs, NDAs, BLAs and DMFs

PINDs: 112,944, (b) (4)
NDA: 22-275 (Tolvaptan), 021795 (Desmopressin), 018261 (Pitocin), 003402 (withdrawn), 19286 (withdrawn).

Drug Formulation

As listed below.

Table 1: JHP Pitressin® (Vasopressin Injection, USP) Drug Formulation (Sponsor's table)

Ingredient	Function	Amount/mL
Vasopressin, USP	Active Ingredient	(b) (4)
Chlorobutanol, NF	Preservative	
Acetic acid, NF	pH Adjustment	
Water for Injection, USP	Diluent	

*Based on USP Reference Standard conversion of (b) (4)

2.4 Comments on Novel Excipients

The list of excipients in drug formulation is given below (Table 2):

Table 2: Novel Excipients (Sponsor's table)

Ingredient	Function	Standard
Vasopressin	Active	USP
Chlorobutanol	Preservative	NF
Acetic Acid	pH Adjustor	NF
Water for Injection	Solvent	USP

Chlorobutanol is used as preservative while acetic acid is used to adjust the pH of the drug product in a range of 3.4 to 3.6. All excipients comply with USP/NF monographs.

The (b) (4) being used for manufacturing of (b) (4) are in accordance with and FDA's approved limits (b) (4) Table 3.

Table 3: Quantitative Composition to FDA's Inactive Ingredients (IIG) Database (Sponsor's table)

Ingredient	Composition (mg/mL)	Inactive Ingredients Guide Acceptable Levels* (As listed for Injection, IM)
Pressor Activity	20 units (b) (4)	Not Applicable
Chlorobutanol	(b) (4)	0.5%
Acetic Acid	(b) (4)	
Water for Injection	(b) (4)	Not Applicable

*FDA Inactive Ingredient Database at http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.

(b) (4) a degradation product of (b) (4) contains a structural alert for potential genotoxicity; and Sponsor should tighten control of this impurity commensurate with its potential toxicity observed on the eyes, skin, kidneys, liver, and lungs in exposed animals (b) (4)

(b) (4)

(b) (4)

A consult request for QSAR analysis data has confirmed the non-mutagenic potential of (b) (4) as data obtained in Salmonella mutagenicity test (Ames assay), and detailed study conducted by (b) (4) is available at:

(b) (4)

Comments on Impurities/Degradants of Concern

Impurities identified in the currently marketed unapproved AVP product are the same as referenced in DMF and summarized below (Table 4).

Table 4: Summary of 8-AVP Related Impurities (Sponsors table)

[redacted] are identified degradants, and RRT denotes their relative retention time on the gradient HPLC method.

Test	25°C/60%RH		40°C/75%RH	
	Slope (month^{-1})	R^2	Slope (month^{-1})	R^2
Vasopressin Assay	-0.11 U (-0.56%)	0.98	-0.85 U (-4.25%)	0.99
Total Imps	[redacted]			
[redacted]				

* p = 0.10 for pooled slopes. For all other models, p>0.25.

The 14-Day dose range finding study (# 249985) and 28-Day rat IV bolus repeat-dose toxicity (# 253051) studies conducted by the Sponsor qualified the limit of NMT [redacted]% for two major [redacted] products of AVP [redacted] by comparison to the reference listed drug (RLD) as addressed in *Q3B®* for degradation products (*Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, Dec 2008).*

These impurities do not have any dose related toxicity in rats. The NOAEL for these impurities was determined as1.14 µg/kg in rats when administered intravenously.

Based on the structural similarity of [redacted] with [redacted] and [redacted], [redacted] ([redacted]% NMT) does not seem to possess any inherent toxicity different from [redacted] and [redacted] ([redacted]%) already qualified by the Sponsor and no additional safety studies are warranted.

2.6 Proposed Clinical Population and Dosing Regimen

Vasodilatory Shock

In adult ICU patients AVP (Pitressin®) will be given as a continuous IV infusion, with no loading dose and hemodynamics will be continuously monitored. A dose range from 0.01 to 0.067 U/min has been proposed by the Sponsor (Dunser et al 2003, Holmes et al 1001) to maintain a MAP of ≥ 65−70mm Hg.

Post-cardiotomy Shock

A starting dose of 0.03 to 0.1 U/min has been proposed by the Sponsor (Hasija et al 2010, Argenziano et al 1999) in adult patients to maintain a MAP of ≥ 65−70mm Hg..

Pediatric Dose
In pediatric patients the dose is calculated based on weight, derived from an average weight-based on adult dose. In several clinical studies conducted in pediatric population, AVP doses of 0.0001 to 0.002 U/kg/min (b) (4) were safely administered (Lechner et al 2007, Alten et al 2012, Rosenzweig et al 1999) on post-cardiotomy hemodynamics in children, including neonates.

Septic Shock
Sponsor has proposed an AVP dose of 0.01 U/min and increased to 0.03 U/min to maintain a MAP of 60–70 mm Hg (Holmes at al 2001) in septic shock patients. The AVP dose will be tapered by 0.005 U/min every hour and discontinued when the patient has been stable for at least 8 hrs.

Pediatric Dose
No evidence for benefit in the use of vasopressin in pediatric patients has been demonstrated.

2.7 Regulatory Background

Pitressin (vasopressin injection, USP) was submitted by JHP Pharmaceuticals (September 26, 2012) for treatment of vasodilatory shock including post-cardiotomy shock and septic shock as a 505(b) (2) application (NDA 204485) based on published literature.

The Pitressin product was previously manufactured and distributed by Parke-Davis for the treatment of diabetes insipidus. JHP Pharmaceuticals acquired all the intellectual property and trademark from Parke-Davis associated with Pitressin drug product.

The Pitressin formulation in oil with a trade name of Pitressin Tennate (5 unit/mL) was approved by FDA (NDA 003402), however; withdrawn by the Sponsor later for unknown reason(s).

3 Studies Submitted

3.1 Studies Reviewed

3.1.1 Repeat-dose Toxicity

Maximum Tolerated Dose and Dose-Range Finding Study for Arginine Vasopressin and its Degradation Products in Sprague- Dawley Rats (Study No. 249985)

A 28-Day Repeated Dose Intravenous Toxicity Study in Sprague- Dawley Rats Given Arginine Vasopressin Alone and in Combination with Arginine Vasopressin Impurities (Study No. 253051)

3.1.2 Genotoxicity

Bacterial Reverse Mutation Assay of Pitressin® Drug Substance/API (Study No. 249866)

In vitro Chromosome Aberration Test of Pitressin® Drug Substance/API in Chinese Hamster Ovary Cells (Study No. 249865)

3.2 Studies Not Reviewed

Development and validation of an LC/MS/MS method for the measurement of vasopressin in dosing solutions and its use in support of toxicology studies (Calibration range: 20.00 ng/mL to 300.00 ng/mL) (Study No. 253921-T)

Development and validation of an LC/MS/MS method for the measurement of vasopressin in dosing solutions and its use in support of toxicology studies (Calibration range: 200.0 µg/mL to 3000.00 µg/mL) (Study No. 253921-GT)

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

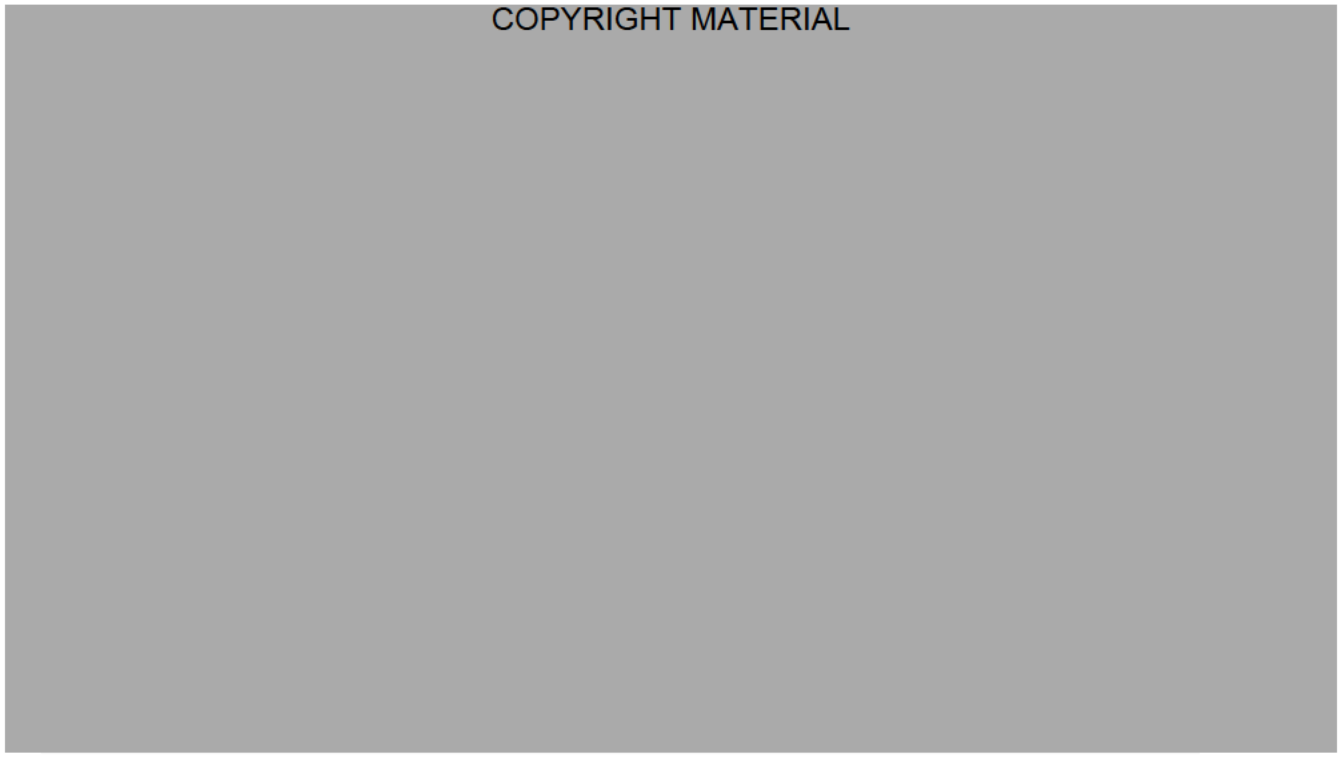
No new pharmacological studies were conducted by the Sponsor and the pharmacological studies submitted were taken from the review chapter by Jackson (2006) that was updated by Reilly and Jackson (2010).

Human AVP is synthesized in hypothalamus as a 168-amino acid pro-hormone with a ~23 amino acid signal peptide (Fig. 2). The pro-hormone contains three domains: vasopressin (residues 1-9), vasopressin (VP)-neurophysin (residues 13-105), and VP-glycopeptide (residues 107-145).

Monooxygenase, and lyase act sequentially on the pro-hormone to produce AVP, VP-neurophysin (sometimes referred to as neurophysin II or MSEL-neurophysin), and VP-glycopeptide (sometimes called copeptin) in secretory granules. AVP is also synthesized in heart and adrenal glands.

An increase in plasma osmolality is one of the main physiological factors for vasopressin secretion and release. A 2% increase in plasma osmolality causes ~ 2- to 3-fold increase in plasma vasopressin levels, which in turn causes increased solute-free water reabsorption, with an increase in urine osmolality.

AVP PREPROHORMONE (HUMAN)



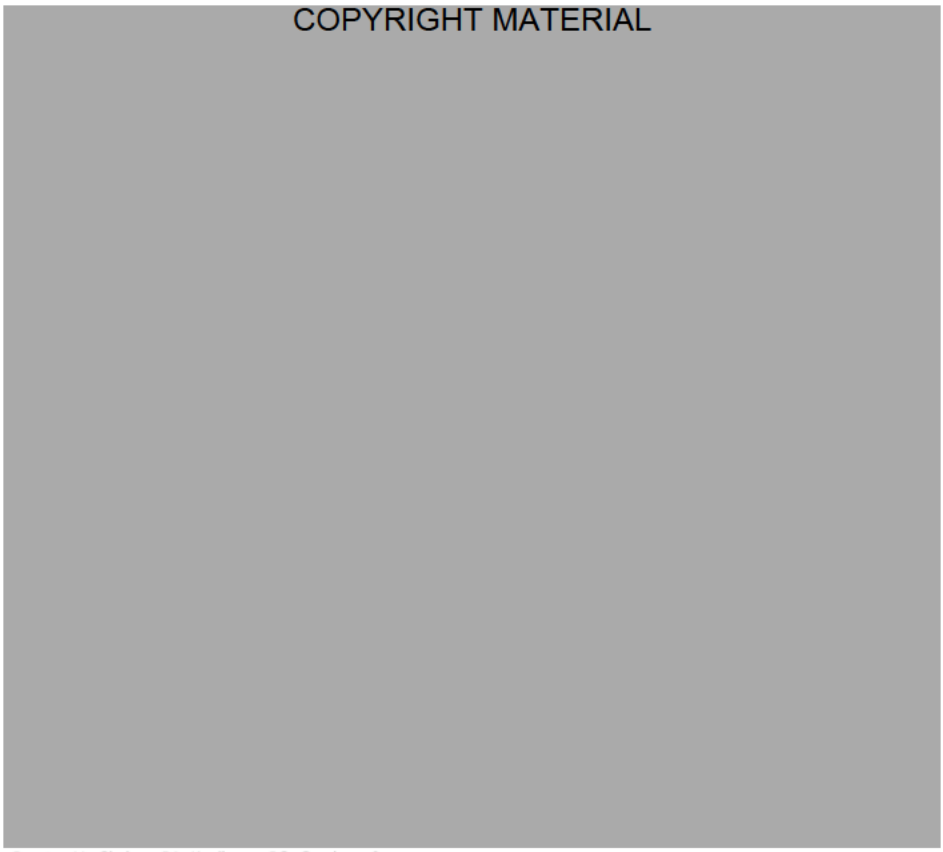
Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

Figure 2: Vasopressin Synthesis

Vasopressins act as an antidiuretic hormone in human and are involved in regulation of body fluid osmolality. 8-Arginine vasopressin (AVP) in human and other related peptides have been reported as follows:

AVP	Cys-Tyr-Phe-Gln-Asp-Cys-Pro-Arg-Gly-(NH3)
Lysine Vasopressin	Cys-Tyr-Phe-Gln-Asp-Cys-Pro-**Lys**-Gly-(NH3)
Oxytocin	Cys-Tyr-**Ile**-Gln-Asp-Cys-Pro-**Leu**-Gly-(NH3)

There are mainly three types of receptors; V1a, V1b and V2 that are involved toward the cellular effects of vasopressin on physiological system. The V1a receptor found in vascular smooth muscle, the adrenal gland, myometrium, the bladder, adipocytes, hepatocytes, platelets, renal medullary interstitial cells, vasa recta in the renal microcirculations, epithelial cells in the renal cortical collecting ducts, spleen, testis and many central nervous system (CNS) structures, is the most predominant one. AVP binding to V1 receptors activates the G_q-PLC-IP_3 pathway, mobilizing intracellular Ca^{2+}, activating PKC, and causing vasoconstriction, glycogenolysis, platelet aggregation, and ACTH release in smooth muscle cells (Fig. 3).



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

Figure 3: AVP and V1 receptor binding

Mechanism of V_1 receptor coupling. Binding of AVP to V_1 receptor (V_1) stimulates several membrane-bound phospholipases. Stimulation of the G_q-PLCβ pathway results in IP_3 formation, mobilization of intracellular Ca^{2+}, and activation of PKC. Activation of V_1 receptors also causes influx of extracellular Ca^{2+} by an unknown mechanism. PKC and Ca^{2+}/calmodulin-activated protein kinases phosphorylate cell-type-specific proteins leading to cellular responses.

A further component of the AVP response derives from the production of eicosanoids secondary to the activation of PLA_2; the resulting mobilization of arachidonic acid (AA) provides substrate for eicosanoid synthesis by the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to local production of prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT), which may activate a variety of signaling pathways, including those linked to G_S and G_q.

Biological effects mediated by the V_1 receptor include vasoconstriction, glycogenolysis, platelet aggregation, ACTH release, and growth of vascular smooth muscle cells. The effects of vasopressin on cell growth involve transcriptional regulation by the FOS/JUN AP-1 transcription complex. The V1b receptor is located in the anterior pituitary, several brain regions, the pancreas and the adrenal medulla. V2 receptors (Fig. 4) are found in the renal collecting-duct system, epithelial cells in the thick ascending limb and on vascular endothelial cells.

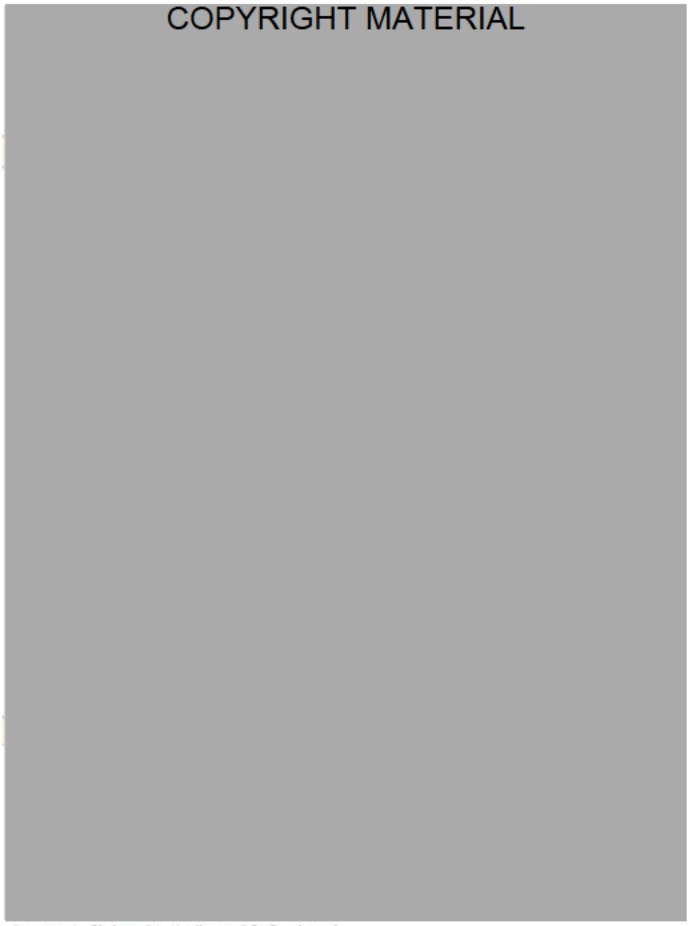


Figure 4: V2 receptor coupling.

Mechanism of V_2 receptor coupling. Binding of AVP to the V_2 receptor activates the G_S-adenylyl cyclase-cAMP-PKA pathway and shifts the balance of aquaporin-2 trafficking toward the apical membrane of the principal cell of the collecting duct, thus enhancing water permeability. Although phosphorylation of serine 256 of aquaporin 2 is involved in V_2 receptor signaling, other proteins located both in the water channel-containing vesicles and the apical membrane of the cytoplasm also may be involved.

AVP binding to the V1 receptors causes vasoconstriction, glycogenolysis, platelet aggregation and adrenocorticotropic hormone release, while binding to V2 receptors increases the water permeability of the apical membrane of the cells of the renal collecting duct, increases permeability to urea in the medullary collecting duct, and increases sodium transport in the thick ascending limb and collecting duct. The cardiovascular effects of vasopressin, such as decreased cardiac outflow and heart rate (HR), are due to indirect effects of AVP as a result of V_1 receptor mediated coronary vasoconstriction.

4.2 Safety Pharmacology

No formal safety pharmacological studies were conducted by the Sponsor. The effects reported in published literature are given below:

Cardiovascular Effects

Effects of AVP on hemodynamic system, cardiac resuscitation, and angina/ischemia and ECG endpoints are reported in literature and provided by the Sponsor. No mortalities were observed following intravenous administration of AVP in rats (0.053 U/kg) and dogs (0.014 U/kg/min) as reported in hemodynamic studies (Table 5).

AVP administration (IV) resulted in a decreased ventricular fibrillation in rats (0.8 U/kg, Studer et al, 2002) and increased diastolic blood pressure, mean arterial blood pressure, coronary perfusion in pig (0.4 U/kg, Prengel et al, 2005), models of cardiac arrest and cardiac arrhythmia, and aid in restoring blood pressure (Table 6).

In models of cardiac angina and ischemia (rat/rabbit), a depression of the ST segment of the ECG, decreased heart rate, myocardial hypoxia, and fibrosis were recorded (Table7/8).

Table 5: Effects of AVP on Hemodynamics (Sponsor's table)

Species, Strain	Treatment and Treatment Regimen	Summary of Results Source of VP	Ref.
Rat Wistar	IV bolus 0.053 U/kg - 0.1 ug/kg	Increased mean arterial pressure Source = AVP (Sigma)	Miyazaki 2000 [87-91]
Rat Spraque-Dawley	IV infusion 0.026 U/kg/min - 0.05 ug/kg/min for 30 minutes and 4 hours	29% maximum increase in mean arterial pressure 32% maximum decrease in HR Source = AVP (Peninsular Laboratories Europe)	Rysa 2006 [375 - 377]
Rat Wistar	IV repeat bolus 8 times every 0.5 hr 0.03 U/kg - 0.057 ug/kg	Increased diastolic blood pressure Source = AVP (Peptide Institute)	Tsukada 2002 [129-135]
Dog	IV infusion 0.0055 U/kg/min - 0.010 ug/kg/min	Increased arterial pressure Decreased heart rate, cardiac output, portal pressure Source = AVP (unk)	Groszmann 1982 [757-759]
Dog	IV infusion 0.014U/kg/min - 0.026 ug/kg/min for 20 minutes	Increased systemic blood pressure Decreased portal vein blood flow, decreased cardiac output Source = AVP (unk)	Barr 1975 [13-18]

AVP = 8-arginine vasopressin
HR = heart rate
U = international units
IV = intravenous
unk = unknown
VP = vasopressin

Table 6: Effects of AVP on Cardiac Resuscitation (Sponsor's table)

Species, Strain	Treatment and Treatment Regimen	Summary of Results Source of VP	Ref.
Rat Sprague-Dawley	IV bolus 0.8 U/kg - 1.5 ug/kg	Decreased ventricular fibrillation Source = AVP (Sigma)	Studer 2002 [201-204]
Pig domestic	IV bolus 0.4 U/kg - 0.76 ug/kg	Increased diastolic blood pressure, mean arterial blood pressure, coronary perfusion Source = AVP (unk)	Prengel 2005 [2587-2589]
Pig	IV bolus 0.8 U/kg - 1.5 ug/kg	Recovery from ventricular fibrillation Source = VP (unk)	Wenzel 2001 [529-533]
Pig	IV bolus 0.8 U/kg - 1.5 ug/kg	Increased blood pressure and recovery from ventricular arrhythmias Source = VP (unk)	Little 2006 [810-814]

AVP = 8-arginine vasopressin
U = international units
IV = intravenous
unk = unknown
VP = vasopressin

Table 7: Effects of AVP on Angina/Ischemia and ECG Endpoints (Sponsor's table)

Species, Strain	Treatment and Treatment Regimen, Sample Size	Summary of Results Source of VP	Ref.
Rat Donryu	IV bolus 0.1 U/kg - 0.19 ug/kg n=5	S-wave suppression Source = AVP (unk)	Hirata 1998 [322-323, 325]
Rat Donryu	IV bolus 0.2 U/kg - 0.38 ug/kg n=6	Coronary vasospasm ST-segment of the ECG transiently increased, followed by a continuous depression Source = AVP (Sigma)	Sasaki 2005 [812-813]
Rat Sprague-Dawley (burn shock)	IV bolus 0.21 ug/kg - 0.4 ug/kg	Decrease heart rate, elevation of S-T segment, ventricular fibrillation, increased survival rate Source = AVP (Peninsula Lab)	Sun 1990 [17-19]
Rat Donryu	IV bolus 0.25 U/kg - 0.47 ug/kg	Depression of the ST segment Source = AVP (unk)	Mori 1995 [1668-1669]
Rat Donryu	IV bolus 0.5 U/kg - 0.95 ug/kg n=unk	Myocardial hypoxia and altered ECG Depression of the ST-segment of the ECG Source = ox pituitary extract, less than 1/13 oxytocin	Hiramatsu 1970 [313-315]
Rat Donryu	IV bolus 0.5 U/kg - 0.95 ug/kg	ST segment depression, myocardial fibrosis Source = AVP (unk)	Satoh 2002 [103-106]

Table 8: Effects of AVP on Angina/Ischemia and ECG Endpoints (Sponsor's table).

Species, Strain	Treatment and Treatment Regimen, Sample Size	Summary of Results Source of VP	Ref.
Rat Wistar, and spontaneous hypertensive rats (SHR)	IV bolus Wistar 3 U/kg SHR 1 U/kg - 1.9 ug/kg	Wistar rats - no effect on ECG SHR - ST wave depression, arrhythmias and QRS widening Source = AVP (Sigma)	Karasawa 1988 [1702-1704]
Rat Donryu	IV bolus 1 U/kg - 1.9 ug/kg n=9	Transient ischemic changes, capillary density increased Source = VP (unk)	Xie 1997 [261-265]
Rat Sprague-Dawley	IV bolus 1 U/kg - 1.9 ug/kg n=8	T-wave elevation Source = AVP (unk)	Benedini 1995 [130-133]
Rabbit unk	IV bolus 2 U/kg - 3.8 ug/kg n=unk	Myocardial hypoxia and altered ECG Depression of the ST-segment and elevated T wave Source = ox pituitary extract, less than 1/13 oxytocin	Hiramatsu 1970 [318]

AVP = 8-arginine vasopressin
ECG = electrocardiogram
IV = intravenous
SHR = spontaneous hypertensive rat
U = international units
unk = unknown
VP = vasopressin

5 Pharmacokinetics/ADME/Toxicokinetics

No formal pharmacokinetic or absorption/distribution/metabolism/excretion (ADME) studies were conducted by the Sponsor. Information obtained from published literature is as below.

AVP is metabolized in liver and kidney, and cleaved at NH_2 terminal by enzyme aminopeptidases and at the COOH terminal by reductases. AVP is also inactivated by trypsin in GI tract, and orally not bioavailable. The physiological plasma level of vasopressin in human varies from 0.20-0.50 pg/mL (Oosterbaan 1989) to 0.76-1.19 pg/mL (Risberg 2009).

The variation across reports in values is likely due to different methodology used in measuring the vasopressin levels in different studies. The plasma half-life for vasopressin is reported to vary from 17 to 35 min in human (Jackson, 2006). The C_{max} values in rats following intravenous administration of AVP are given as below (Miyazaki et al 2000 and Ginsberg et al 1953):

dose (ng/kg)	C_{max} plasma levels (pg/mL)
25	47
50	160
250	450
1900	242

6 General Toxicology

6.1 Single-Dose Toxicity

No single dose toxicity studies were conducted by the Sponsor and others for this application.

6.2 Repeat-Dose Toxicity

6.2.1. A 28-Day Repeated Dose Intravenous Toxicity Study in Sprague-Dawley Rats Given Arginine Vasopressin Alone and in Combination with Arginine Vasopressin Impurities.

Conducting laboratory and location:	(b) (4)
Study number(s):	253051 (JHP-2011-012-D)
Date of study initiation:	April 4, 2012
Drug lot/batch number:	Pitressin® (8-arginine vasopressin (310571-1)
Molecular Weight:	1084.24 daltons
Molecular Formula:	C46H65N15O12S2
Lot No.:	310571-1
Impurity 1:	(b) (4)
Molecular Weight:	(b) (4)
Molecular Formula:	
Lot No.:	
Impurity 2:	(b) (4)
Molecular Weight:	(b) (4)
Molecular Formula:	
Lot No.:	
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

8-arginine vasopressin (Pitressin® AVP) and its degradation products (b) (4) and (b) (4) did not reveal any related toxicity in rats when administered intravenously daily for 28 days at a dose of 0.76 µg/kg AVP alone, or with the addition of impurities at 0.1, 0.32 and 1.14 µg/kg. The no observed adverse effect level (NOAEL) was considered to be 0.76µg/kg for AVP and 1.14 µg/kg for impurities.

Purpose

The 28 Day repeated dose study with 8-arginine vasopressin (Pitressin® AVP) and its degradation products (b) (4) and (b) (4) was conducted to evaluate their toxic effects in rats following intravenous administration.

Methods

The intravenous dose level of AVP (0.76 µg/kg) and its impurities ((b) (4) and (b) (4)) at 0.1, 0.32 and 1.14 µg/kg (or 0.05, 0.16 and 0.57 µg/kg each) for 28 days was determined from the Dose Range Finding (DRF) study where AVP at 0.76 µg/kg/day, plus (b) (4) and (b) (4) , at doses each up to 0.45 µg/kg/day for 14 days was well tolerated. 8-arginine vasopressin (Pitressin® AVP) and its degradation products (b) (4) and (b) (4) were administered to rats as per following treatment groups (Table 9).

Table 9: Animal Assignment and Treatment Groups

Treatment Group	% Total Peptide Content as each Impurity	% Total Peptide Content as Impurities	AVP Dose (µg/kg/ day)	Impurities[1] Dose (µg/kg/day, of each)	Dose Conc. AVP/ each Impurity (µg/mL)	Dose Volume (mL/kg)	Number of Animals (Main Study)	Number of Animals (TK)
1. Vehicle	0	0	0	0	0 / 0	5	10 M	3 M
2. AVP	0	0	0.76	0	0.152 / 0	5	10 M	6 M
3. AVP + 12% Impurities[2]	6	12	0.76	0.05	0.152 / 0.010	5	10 M	6 M
4. AVP + 30% Impurities[2]	15	30	0.76	0.16	0.152 / 0.032	5	10 M	6 M
5. AVP + 60% Impurities[2]	30	60	0.76	0.57	0.152 / 0.114	5	10 M	6 M

M = Male
AVP = Arginine Vasopressin
[1]Impurity 1 = (b) (4)
Impurity 2 =
[2]Percent is percent of total peptides

Mortality and clinical signs were observed twice a day while body weights and food consumption was monitored on weekly basis during the period of study. Ophthalmic evaluations (funduscopic and biomicroscopic) were performed once prior to pretreatment and prior to necropsy. Blood samples for TK analyses were collected at Days 1 and 28. Gross pathological examination was conducted at Day 29 following 12 to 18 hrs. of fasting. Following organ weights were taken:

Adrenals	Pituitary Gland
Brain	Prostate
Heart	Spleen
Kidneys	Testes
Liver	Thymus
Lungs	

Following tissues were preserved in 10% neutral buffered formalin for histopathological evaluation. Smears from three femoral bones were prepared from each sacrificed animal for any hematological findings.

Abnormal Tissues	Kidneys	Seminal Vesicles
Adrenals	Lacrimal Glands	Spinal Cord (Cervical)
Animal Identification	Liver (sample of central & left lobes)	Spleen
Aorta (Thoracic)	Lungs (left & right diaphragmatic lobes)***	Sternum & Marrow
Brain	Lymph Node (Mandibular)	Stomach
Cecum	Lymph Node (Mesenteric)	Testes*
Colon	Optic Nerves*	Thymus
Duodenum	Pancreas	Thyroid/Parathyroids
Epididymides	Pituitary	Tongue
Esophagus	Prostate	Trachea
Eyes*	Salivary Glands (Mandibular)	Urinary Bladder
Heart**	Sciatic Nerve	Injection site
Ileum	Skeletal Muscle (quadriceps)	
Jejunum	Skin (Inguinal) & Subcutis	

* Fixed in alcoholic formalin (euthanized animals only)
** Sections of left and right ventricles and atria, septum with papillary muscle
*** Lungs of euthanized animals infused with formalin

Results

Mortality

No mortality was observed except one animal (#047) that died on Day 21 in AVP+30% impurities group. Analysis of necropsy and histpathological data implicated the urinary obstruction (urolithiasis) leading to cystis, hydronephrosis and pyelonephrosis for the cause of death, and was not related to the treatment.

Body weights and Food Consumption

A slight to moderate gain was observed in body weights; however, no difference in food consumption was seen in treated animals throughout the study period (Table 10)

Table 10: Changes in Body Weights and Food Consumption

Group	Mean Body Weights ± S.D. (g)[2] n=10					Mean[3] Body Weight Changes	Mean Total Food Consumption (g)
	Day 1	Day 8	Day 15	Day 22	Day 28		
1. Vehicle	305.8 ± 16.1	347.2 ± 24.1	383.9 ± 25.4	422.4 ± 27.6	440.3 ± 29.6	134.5 g (44%)	802.3
2. AVP	309.5 ± 22.3	348.0 ± 28.9	383.1 ± 34.2	408.2 ± 38.4	415.1 ± 35.5	105.6 g (34%)	806.9
3. AVP + 12% Impurities	313.3 ± 17.3	352.4 ± 24.5	391.9 ± 28.7	423.6 ± 31.8	436.1 ± 31.2	122.8 g (39%)	809.8
4. AVP + 30% Impurities	305.9 ± 18.6	343.4 ± 22.5	376.2 ± 23.7	407.7[1] ± 23.4	422.6[1] ± 27.6	116.7 g (38%)	822.8
5. AVP + 60% Impurities	307.3 ± 15.8	348.1 ± 24.0	381.8 ± 33.5	414.4 ± 35.0	425.8 ± 40.2	118.5 g (39%)	815.4

[1] n = 9 (one rat died on Day 21)
[2] Body Weights during acclimation period were recorded, but were not reported.
[3] Body Weight Changes over the 28-day period.

Ophthalmology
Crystalline corneal deposits were seen in one animal in each group of treated animals, and characterized as spontaneous occurring lesions in Sprague Dawley rats (11-46%) with a background incidence of cataracts (9.8%). These findings were not considered to treatment related.

Hematology
There were no statistically significant differences observed in hematology parameters (Table 11), coagulation data (Table 12), and biochemical indices (Table 13). The RBC counts, reticulocytes, hemoglobin, hematocrit and RBC indices (MCV, MCH and MCHC) were within the normal physiological range when compared with treated group of animals.

Table 11: Hematological Parameters (Sponsor's table)

		1-M Vehicle Control	2-M AVP Alone	3-M AVP + 12% Impurities	4-M AVP + 30% Impurities	5-M AVP + 60% Impurities	Normal Range
RBC	Mean	7.68 a	7.55	7.39	7.56	7.44	6.06 - 9.46
[x10e12/L]	S.D.	0.28	0.26	0.35	0.39	0.25	
Day 29	N	10	10	10	9	10	
HEMO	Mean	147 a	143	143	146	145	120 - 181
[g/L]	S.D.	6	4	7	6	5	
Day 29	N	10	10	10	9	10	
HEMA	Mean	44.4 a	43.3	43.4	44.4	43.7	37.3 - 50.2
[%]	S.D.	1.8	1.3	2.1	1.7	1.2	
Day 29	N	10	10	10	9	10	
MCV	Mean	57.8 a	57.3	58.8	58.8	58.8	47.5 - 66.1
[fL]	S.D.	1.3	1.7	2.0	1.7	1.2	
Day 29	N	10	10	10	9	10	
MCH	Mean	19.1 a	18.9	19.3	19.3	19.4	15.8 - 23.1
[pg]	S.D.	0.6	0.5	0.7	0.5	0.5	
Day 29	N	10	10	10	9	10	
MCHC	Mean	331 a	331	329	329	331	287 - 401
[g/L]	S.D.	5	3	5	4	6	
Day 29	N	10	10	10	9	10	
PLAT	Mean	1,087 a	1,206	1,155	1,161	1,198	579 - 1641
[x10e9/L]	S.D.	138	179	133	155	152	
Day 29	N	10	10	10	9	10	
WBC	Mean	8.85 a	8.83	7.66	8.60	9.17	5 - 15.28
[x10e9/L]	S.D.	2.29	2.49	1.87	2.81	2.57	
Day 29	N	10	10	10	9	10	
NEU	Mean	0.73 a	1.40	0.76	0.73	0.67	0.05 - 2.37
[x10e9/L]	S.D.	0.28	1.48	0.20	0.28	0.18	
Day 29	N	10	10	10	9	10	
LYM	Mean	7.74 a	6.99	6.55	7.47	8.10	1.67 - 14
[x10e9/L]	S.D.	2.03	1.82	1.71	2.50	2.35	
Day 29	N	10	10	10	9	10	
MO	Mean	0.17 a	0.22	0.17	0.18	0.17	0 - 0.46
[x10e9/L]	S.D.	0.11	0.12	0.05	0.07	0.04	
Day 29	N	10	10	10	9	10	
EOS	Mean	0.08 a	0.07	0.09	0.08	0.07	0 - 0.21
[x10e9/L]	S.D.	0.04	0.02	0.04	0.03	0.02	
Day 29	N	10	10	10	9	10	
BAS	Mean	0.02 a	0.02	0.02	0.02	0.02	0 - 0.06
[x10e9/L]	S.D.	0.01	0.01	0.01	0.01	0.01	
Day 29	N	10	10	10	9	10	
LUC	Mean	0.11 a	0.13	0.09	0.12	0.13	0 - 0.14
[x10e9/L]	S.D.	0.04	0.08	0.04	0.08	0.04	
Day 29	N	10	10	10	9	10	
RET	Mean	244.6 a	250.7	305.9	311.3	291.0	100 - 400
[x10e9/L]	S.D.	39.3	99.5	71.0	68.3	57.7	
Day 29	N	10	10	10	9	10	

Table 12: Coagulation Data (Sponsor's table)

		1-M Vehicle Control	2-M AVP Alone	3-M AVP + 12% Impurities	4-M AVP + 30% Impurities	5-M AVP + 60% Impurities	Normal Range
PT	Mean	16.3 d	17.6 **	17.2	16.8	16.7	11.6 - 23.3
[seconds]	S.D.	1.0	1.2	0.7	0.9	0.7	
Day 29	N	10	10	10	9	10	
APTT	Mean	17.6 a	18.8	19.7	17.9	18.1	4.7 - 37.4
[seconds]	S.D.	2.5	2.7	1.6	2.4	1.8	
Day 29	N	10	10	10	9	10	

d=ANOVA-DUNNETT; ** = p < 0.01; a=ANOVA

Table 13: Biochemical Parameters (Sponsor's table)

		1-M Vehicle Control	2-M AVP Alone	3-M AVP + 12% Impurities	4-M AVP + 30% Impurities	5-M AVP + 60% Impurities	Normal Range
A/G	Mean	1.0 a	1.0	1.1	1.1	1.1	0.7 - 1.6
	S.D.	0.1	0.1	0.1	0.0	0.1	
Day 29	N	10	10	10	9	10	
ALB	Mean	28 a	28	30	30	30	23 - 43
[g/L]	S.D.	2	3	2	2	2	
Day 29	N	10	10	10	9	10	
GLOB	Mean	26 a	27	26	27	27	22 - 36
[g/L]	S.D.	1	3	1	1	1	
Day 29	N	10	10	10	9	10	
ALKP	Mean	171 a	149	161	168	153	47 - 426
[U/L]	S.D.	38	29	35	37	21	
Day 29	N	10	10	10	9	10	
TBIL	Mean	5.8 a	5.4	5.8	6.3	6.1	1.7 - 5.7
[µmol/L]	S.D.	1.1	0.7	0.7	1.4	1.1	
Day 29	N	10	10	10	9	10	
BUN	Mean	6.2 a	6.6	6.4	7.0	6.5	3 - 8.4
[mmol/L]	S.D.	1.1	1.5	0.8	1.7	1.1	
Day 29	N	10	10	10	9	10	
Ca	Mean	2.49 d	2.51	2.57	2.60 *	2.58	2.24 - 3
[mmol/L]	S.D.	0.07	0.10	0.12	0.08	0.07	
Day 29	N	10	10	10	9	10	
Cl	Mean	102 a	102	102	102	103	90 - 116
[mmol/L]	S.D.	2	2	1	2	1	
Day 29	N	10	10	10	9	10	
CREA	Mean	37 a	39	36	41	35	24 - 66
[µmol/L]	S.D.	5	8	4	9	3	
Day 29	N	10	10	10	9	10	
GLU	Mean	9.7 a	10.4	9.2	9.3	9.0	0.8 - 11.2
[mmol/L]	S.D.	2.1	3.1	1.6	2.8	1.3	
Day 29	N	10	10	10	9	10	
LDH	Mean	2,470 a	1,748	1,860	2,319	3,260	1050 - 6401
[U/L]	S.D.	1,784	1,217	1,314	1,801	1,776	
Day 29	N	10	10	10	9	10	
Phos	Mean	2.66 a	2.61	2.69	2.82	2.71	1.83 - 3.94
[mmol/L]	S.D.	0.24	0.19	0.15	0.19	0.16	
Day 29	N	10	10	10	9	10	
K	Mean	4.9 a	5.0	4.8	4.9	5.0	3.7 - 7
[mmol/L]	S.D.	0.4	0.5	0.3	0.2	0.3	
Day 29	N	10	10	10	9	10	
TP	Mean	54 a	56	56	57	56	47 - 75
[g/L]	S.D.	2	4	3	2	2	
Day 29	N	10	10	10	9	10	
AST	Mean	85 a	78	77	82	90	42 - 149
[U/L]	S.D.	16	14	15	18	18	
Day 29	N	10	10	10	9	10	
ALT	Mean	45 a	43	44	46	44	26 - 71
[U/L]	S.D.	8	5	5	5	8	
Day 29	N	10	10	10	9	10	
Na	Mean	142 d	142	144 *	143	144	136 - 152
[mmol/L]	S.D.	1	1	1	2	1	
Day 29	N	10	10	10	9	10	
TRIG	Mean	0.57 a	0.46	0.43	0.42	0.49	0.1 - 1.55
[mmol/L]	S.D.	0.21	0.16	0.14	0.11	0.17	
Day 29	N	10	10	10	9	10	
CK	Mean	300 a	215	214	259	382	228 - 529
[U/L]	S.D.	175	116	120	187	195	
Day 29	N	10	10	10	9	10	
CHOL	Mean	1.49 a	1.68	1.57	1.68	1.49	1 - 3
[mmol/L]	S.D.	0.23	0.50	0.46	0.35	0.35	
Day 29	N	10	10	10	9	10	
GGT	Mean	5 a	5	5	5	5	4 - 6
[U/L]	S.D.	0	0	0	0	0	
Day 29	N	10	10	10	9	8	

a=ANOVA; d=ANOVA-DUNNETT; * = p < 0.05

Histopathology
The histopathological data did not show any adverse outcome or toxicity of daily administration of AVP (0.76 µg/kg/day) or AVP impurities (1.14 µg/kg/day) in Sprague-Dawley rats when compared with respective control treated animals.

Mild focal inflammatory lesions found in lung, liver and kidney of control and treated animals had no histopathological concern related to the treatment.

A small increase in weight of adrenal, lung and heart in treated group of animals compared to controls did not indicate any histopathological correlation of toxicological significance.

Conclusion
There were no toxicities of concern when AVP was administered intravenously daily for 28 days at a dose of 0.76 µg/kg alone, or with impurities at 0.1, 0.32 and 1.14 µg/kg.

The no observed adverse effect level (NOAEL) was considered to be 0.76 µg/kg for AVP and 1.14 µg/kg for impurities.

7 Genetic Toxicology

7.1 Bacterial Reverse Mutation Assay of Pitressin Drug Substance/API

Conducting laboratory and location: (b) (4)

Study number(s):	249866
Date of study initiation:	March 20, 2012
Drug lot/batch number:	VP1002-1
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

Pitressin did not show any mutagenic potential in *S. typhimurium* strains, TA98, TA100, TA1535, TA1537, and *E. coli* strain, WP2 *uvrA*.

Purpose

To assess the mutagenic potential of Pitressin® in inducing the point mutations in strains of *S. typhimurium* and *E. coli*.

Methods

S. typhimurium strains, TA98, TA100, TA1535, TA1537 and *E. coli* strain, WP2 *uvrA* were procured from (b) (4) and used in this study. The 0.6% BactoTM agar, containing 0.5% NaCl, supplemented with histidine and biotin (50 µM) was used as top agar for the selection of *S. typhimurium* revertants while 0.7% BactoTM agar was used for *E. coli* revertants. The 0.9% NaCl (saline) was used as negative control. The positive controls for experiments with or without S9 metabolic activation system (9,000 x *g* fraction of liver homogenate from male Sprague-Dawley rats treated with Aroclor 1254) are as below (Table. 14):

Table 14: Positive Controls with or without S9 Mix.

Tester Strains	- S9 mix Positive control	per plate	+ S9 mix Positive control	per plate
TA98	2-Nitrofluorene	5.0 µg	Benzo[α]pyrene	5.0 µg
TA100	Sodium Azide	5.0 µg	Benzo[α]pyrene	5.0 µg
TA1535	Sodium Azide	5.0 µg	Cyclophosphamide	100 µg
TA1537	9-Aminoacridine	100 µg	Benzo[α]pyrene	5.0 µg
WP2 *uvrA*	Methyl methanesulfonate	1.0 µl	2-Aminoanthracene	100 µg

S. typhimurium and E. coli cultures were grown overnight to obtain a density of 0.11 to 1.38 x 10^9 colony forming units per mL. Pitressin® (0, 0.31, 0.62, 1.3, 2.5 and 5.0 mg per plate), negative or positive controls were mixed with 0.1 mL of overnight bacterial culture and incubated at 37^0C for 48 to 72 hours in the presence and absence of S9 mix. The number of revertant colonies was counted. A positive result was defined as a significant increase ($p<0.01$, at least ≥2 fold) in number of revertant colonies in a dose dependent and reproducible manner in the presence and absence of S9 mix when compared with controls.

Results

The positive controls exhibited a significant increase (≥2 fold) in revertant colonies, however, Pitressin® did not show a ≥2 fold increase in revertant colonies at any dose level in any of the strains of *S. typhimurium* or *E. coli* (Table 15/16/17).

Table 15: Plate Incorporation Assay (Sponsor's table)

Pitressin mg per plate	Colony Counts			Mean ± SD	Lawn
TA98, -S9					
0	20	26	19	22 ± 4	NL
0.31	29	25	28	27 ± 2	NL
0.62	21	28	17	22 ± 6	NL
1.3	31	21	20	24 ± 6	NL
2.5	24	27	20	24 ± 4	NL
5.0	19	31	25	25 ± 6	NL
2-NF, 5 µg	3237	2850	3323	3137 ± 252	NL
TA98, +S9					
0	27	32	37	32 ± 5	NL
0.31	22	32	30	28 ± 5	NL
0.62	27	31	26	28 ± 3	NL
1.3	39	34	31	35 ± 4	NL
2.5	20	25	28	24 ± 4	NL
5.0	33	27	32	31 ± 3	NL
B[α]P, 5 µg	407	413	395	405 ± 9	NL
TA100, -S9					
0	150	102	106	119 ± 27	NL
0.31	111	130	104	115 ± 13	NL
0.62	137	100	121	119 ± 19	NL
1.3	110	105	116	110 ± 6	NL
2.5	125	127	142	131 ± 9	NL
5.0	121	124	116	120 ± 4	NL
NaAz, 5 µg	1926	1875	1875	1892 ± 29	NL
TA100, +S9					
0	132	135	129	132 ± 3	NL
0.31	155	112	134	134 ± 22	NL
0.62	109	113	120	114 ± 6	NL
1.3	112	127	128	122 ± 9	NL
2.5	133	138	121	131 ± 9	NL
5.0	118	100	127	115 ± 14	NL
B[α]P, 5 µg	1063	1091	1062	1072 ± 16	NL

Table 16: Plate Incorporation Assay (Sponsor's table)

Pitressin mg per plate	Colony Counts			Mean ± SD	Lawn
TA1535, -S9					
0	19	19	13	17 ± 3	NL
0.31	14	15	15	15 ± 1	NL
0.62	12	20	12	15 ± 5	NL
1.3	21	13	20	18 ± 4	NL
2.5	16	14	12	14 ± 2	NL
5.0	17	14	13	15 ± 2	NL
NaAz, 5 µg	1885	1728	1647	1753 ± 121	NL
TA1535, +S9					
0	16	11	15	14 ± 3	NL
0.31	14	12	10	12 ± 2	NL
0.62	15	10	11	12 ± 3	NL
1.3	11	12	11	11 ± 1	NL
2.5	9	16	11	12 ± 4	NL
5.0	10	7	11	9 ± 2	NL
CP, 100 µg	146	128	142	139 ± 9	NL
TA1537, -S9					
0	12	9	5	9 ± 4	NL
0.31	13	12	12	12 ± 1	NL
0.62	8	11	10	10 ± 2	NL
1.3	14	10	13	12 ± 2	NL
2.5	16	8	12	12 ± 4	NL
5.0	8	6	11	8 ± 3	NL
9-AA, 100 µg	1547	1877	1945	1790 ± 213	NL
TA1537, +S9					
0	16	18	14	16 ± 2	NL
0.31	19	21	11	17 ± 5	NL
0.62	14	12	11	12 ± 2	NL
1.3	11	16	15	14 ± 3	NL
2.5	18	24	14	19 ± 5	NL
5.0	13	19	18	17 ± 3	NL
B[α]P, 5 µg	176	218	212	202 ± 23	NL

Table 17: Plate Incorporation Assay (Sponsor's table)

Pitressin mg per plate	Colony Counts			Mean ± SD	Lawn
WP2 *uvrA*, -S9					
0	27	33	23	28 ± 5	NL
0.31	30	26	27	28 ± 2	NL
0.62	20	22	23	22 ± 2	NL
1.3	24	27	28	26 ± 2	NL
2.5	32	21	28	27 ± 6	NL
5.0	17	28	27	24 ± 6	NL
MMS, 1 µl	441	451	447	446 ± 5	NL
WP2 *uvrA*, +S9					
0	46	34	34	38 ± 7	NL
0.31	40	36	44	40 ± 4	NL
0.62	40	47	35	41 ± 6	NL
1.3	38	39	37	38 ± 1	NL
2.5	37	35	34	35 ± 2	NL
5.0	35	41	35	37 ± 3	NL
2-AMA, 100 µg	173	160	182	172 ± 11	NL

Note:
9-AA, 9-aminoacridine
2-AMA, 2-aminoanthracene
2-NF, 2-nitrofluorene
NaAz, sodium azide
B[α]P, benzo[α]pyrene
CP, cyclophosphamide
MMS, methyl methanesulfonate

Note:
NL, Normal Background Lawn
SR, Slight Reduction of Lawn
MR, Moderate Reduction of Lawn
ER, Extreme Reduction of Lawn

7.2 *In Vitro* Chromosome Aberration Test of Pitressin Drug Substance/API in Chinese Hamster Ovary Cells

Conducting laboratory and location: (b) (4)

Study number(s):	249865
Date of study initiation:	March 20, 2012
Drug lot/batch number:	VP1002-1
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

Pitressin did not show any clastogenic potential in chromosomal aberration assay using the Chinese hamster ovary (CHO) cell line.

Purpose

To evaluate the clastogenic potential of Pitressin in inducing chromosomal aberrations (clastogenicity) in cultured Chinese hamster ovary (CHO) cells.

Methods

Cell line:	Chinese hamster ovary (CHO) cells (0.5 x 10^6 cells/T-25 cm^2 culture flask, passage # 7)
Concentrations in definitive study:	0 to 100 mg/mL
Concentrations in main study:	0, 0.47, 1.0, 2.3 and 5.0 mg/mL
Basis of concentration selection:	Cytotoxicity
Negative control:	0.9% Sodium chloride (saline)
Positive control:	Mitomycin C (MMC) in the absence and Cyclophosphamide (CP) in the presence of S9-metabolic activation system. The S9-homogenate (mitochondrial 9,000xg fraction) was prepared from livers of male Sprague Dawley rats pretreated with Phenobarbital-5,6-benzoflavone.
Formulation/Vehicle:	0.9% Sodium chloride (saline)
Incubation & sampling time:	Duplicate cultures for 3 or 17 hrs treatment in presence or absence of S9 activation system.

The Chinese hamster ovary (CHO-WBL) cells obtained from (b) (4), (b) (4) were treated in duplicate with Pitressin for 3 hrs. (0, 0.47, 1.0, 2.3 and 5.0 mg/mL) or 21 hrs. (0, 0.097, 0.21, 0.47, 1.0, 2.3 and 5.0 mg/mL) in absence or presence of S9 metabolic activation system (Galloway et al., 1987) at 37 ± 2°C and 5 ± 2% CO2.

For short term treatment (3 hrs.), cells were incubated for an additional 18 to 19 hrs in fresh medium without S9 system. All cultures were harvested at the end of 21 ± 1 hrs. after the initiation of treatment. Following the incubation period and 3-4 hrs. prior to harvest, colcemid (0.1 µg/mL) was added to arrest cells in metaphase stage. The total number of harvested cells

was counted using trypan blue exclusion procedure and relative cell growth (RCG) was calculated using the following equation:

$$RCG\ (\%) = \frac{\text{Viable cell count in test article culture}}{\text{Viable cell count in solvent control culture}} \times 100$$

Chromosomal slides were prepared from cell suspension using fresh fixative and 10% Giemsa stain (Evans, 1976) and relative mitotic index (RMI) was calculated to evaluate the toxic effect of Pitressin as follows:

$$RMI\ (\%) = \frac{\text{Test Article concentration MI}}{\text{Solvent control MI}} \times 100$$

Cells in metaphase with 19-23 chromosomes were analyzed for chromosome aberrations as defined in Protocol JHP/249865 (Appendix I; Scott, *et al*., 1990; OECD, 1997).

Study Validity

The positive result of a test article is defined as a significant increase ($p<0.001$) in chromosomal aberration (including gaps, chromatid/chromosomal breaks, chromatid/chromosomal exchange) when tested in presence or absence of S9 metabolic activation system and compared with historical negative/positive controls.

Results

The short term treatment (3 hrs) of CHO cells with Pitressin at 1.0, 2.3 and 5.0 mg/mL showed a relative cell growth (RCG) of 103, 107 and 103% in the absence and 92, 105 and 109%, in the presence of S9, respectively. In the absence of S9, the 21 hrs. treatment showed a RCG of 94, 92 and 51% at 1.0, 2.3 and 5.0 mg/mL concentration of Pitressin, respectively (Table 18).

Table 18: Relative Cell Growth (Sponsor's table)

Pitressin (mg/mL)	3 hrs -S9			Pitressin (mg/mL)	3 hrs +S9			Pitressin (mg/mL)	21 hrs -S9		
	Cells ($x\ 10^6$)	Mean ($x\ 10^6$)	RCG (%)		Cells ($x\ 10^6$)	Mean ($x\ 10^6$)	RCG (%)		Cells ($x\ 10^6$)	Mean ($x\ 10^6$)	RCG (%)
0	2.955 2.865	2.91	100	0	2.700 2.580	2.64	100	0	3.945 4.260	4.10	100
								0.097	4.035 4.170	4.10	100
								0.21	3.090 3.705	3.40	83
0.47	2.850 3.015	2.93	101	0.47	3.120 2.850	2.99	113	0.47	2.880 4.050	3.47	84
1.0	3.105 2.865	2.99	103	1.0	2.430 2.430	2.43	92	1.0	3.975 3.720	3.85	94
2.3	3.090 3.120	3.11	107	2.3	2.910 2.610	2.76	105	2.3	3.735 3.810	3.77	92
5.0	3.045 2.925	2.99	103	5.0	2.805 2.940	2.87	109	5.0	1.935 2.220	2.08	51

RCG = Relative Cell Growth

A relative mitotic index (RMI) of 101, 89 and 85% and of 101, 96 and 72% was seen in absence and presence of S9 in Pitressin treated cells at 1.0, 2.3 and 5.0 mg/mL concentrations, respectively. In the absence of S9, the 21 hr. treatment of Pitressin at 1.0, 2.3 and 5.0 mg/mL showed (Table 19) a RMI of 101, 96 and 72%, respectively.

Table 19: Relative Mitotic Index (Sponsor's Table)

Pitressin (mg/mL)	3 hrs -S9			Pitressin (mg/mL)	3 hrs +S9			Pitressin (mg/mL)	21 hrs -S9		
	M/500 cells	MI (%)	RMI (%)		M/500 cells	MI (%)	RMI (%)		M/500 cells	MI (%)	RMI (%)
0	79 76	15.5	100	0	119 111	23.0	100	0	77 84	16.1	100
1.0	80 76	15.6	101	1.0	113 117	23.0	100	1.0	78 85	16.3	101
2.3	75 63	13.8	89	2.3	109 98	20.7	90	2.3	79 76	15.5	96
5.0	60 71	13.1	85	5.0	108 105	21.3	93	5.0	51 65	11.6	72

M = Metaphases
MI = Mitotic Index
RMI = Relative Mitotic Index
Note: Slides not analyzed for concentrations 0.47 mg/mL and below

As recommended for a non-toxic compound, Pitressin was tested at the maximum concentration (5 mg/mL) in chromosomal aberration assay. A low level of chromosome aberrations was observed at all concentrations including the solvent control (Table 20).

Table 20: Chromosomal Aberrations* due to Pitressin Treatment (Sponsor's Table)

Pitressin mg/mL	3 hours -S9	3 hours +S9	21 hours -S9
0	2.0	1.0	2.5
1.0	1.0	2.0	1.5
2.3	1.5	2.0	3.0
5.0	1.5	2.0	3.5

**Chromosomal aberrations per 100 cells in absence or presence of metabolic activation system (S9) are shown in this table. The positive control (MMC 0.2µg/Ml) has shown a* significantly ($p<0.001$) high level of aberrations (37/100 cells).

Conclusions

The results from chromosomal aberration assay using the CHO cells have demonstrated that Pitressin treatment did not produce any structural or numerical chromosomal aberration beyond those observed in vehicle treated solvent controls, and, therefore, was considered to be non-clastogenic.

7.2 Other Genetic Toxicity Studies

No additional genetic studies were conducted by the Sponsor for this indication.

8 Carcinogenicity

There are no formal carcinogenic studies available in published literature and none were conducted by the Sponsor for this application.

9 Reproductive and Developmental Toxicology

There were no formal reproductive toxicological studies conducted by the Sponsor to assess the effects of AVP on reproductive competence in sexually mature males and females. Available reports from published literature are given below.

Reproductive Toxicity

A recent study has shown that VP has a detrimental effect on sperm function (the percentage of motility, capacitation status, and protein tyrosine phosphorylation), fertilization, and embryonic development, suggesting its critical role in the acquisition of fertilizing ability of mouse spermatozoa (Kwon et al 2013). The data available in published literature have shown that AVP affects the renal function of rats during the reproductive phase when it was administered intravenously on each of the four days of estrus cycle (10, 20 and 40 fmol/min of AVP for one hour followed by a recovery period of 90 min). A dose dependent antidiuresis response was seen at pro-estrous and disastrous day -1 (Hartley 2002).

An intravenous injection of AVP has been reported to increase basal level of pre-surge leutinizing hormone (LH) in ovariectomized female rats (Salisbury et al, 1980). Another study (Wube 2008) has shown a suppressed reproduction and a significant decrease in spermatogenic index (as measured by distribution of seminiferous tubules) in *Acomys russatus* spiny mice when AVP was administered intraperitoneally (50 ug/kg AVP at 3-day interval for 4 weeks) and compared to controls (Table 21). There were no effects on female reproductive function (as determined by uterine weight and number of estrous cycles). Testis mass was also decreased to almost 50% in experimental group compared to controls in *Acomys russatus* mice, however, effects were not noticed in *Acomys* cahirinus.

Table 21: Testis mass, body mass and spermatogenic index values in *Acomys russatus* and Acomys cahirinus (mean ± SD, $p<0.01$).

	Absolute testis mass (g)	Relative testis mass (%)	Spermatogenic index	Body mass (g)
A. russatus				
Control	0.23±0.11	0.38±0.15	329.83±25.64[A]	60.82±4.21
Experimental	0.11±0.02	0.17±0.04	243.5±39.58[B]	65.35±5.47
Recovery	0.27±0.04	0.48±0.11	407.25±1.77[C]	56.45±4.7
A. cahirinus				
Control	0.18±0.06	0.33±0.11	419.17±19.64	52.63±2.86
Experimental	0.25±0.03	0.39±0.01	411.83±17.76	63.18±8.61
Recovery	0.26±0.04	0.44±0.06	430.00±22.63	59.66±0.76

Placental Transfer
Following an intravenous administration (25 to 50 mU AVP), placental transfer of AVP was detected in 43% (3/7) guinea pigs (Forsling 1977) having the similar type of hemochorial placenta as in humans, however, no placental transfer of endogenous AVP was detected in humans (Harding and Bocking 2001). Radiolabeled study with AVP has shown that 50% of radiolabeled AVP is degraded during *in vitro* perfusion of maternal intervillous space (Landon 1988) in humans.

Results from ovine placental study have shown an absence of AVP gene expression in the placenta and fetal membranes eliminating the possibility of AVP synthesis therein, however, V1a receptor gene expression was evidently present and increased during the gestation period from Day 45 to 66 (Koukoulas et al, 2003) and correlated with the maximum placental growth in the sheep.

Data from recent study (Ray 2004) has shown that the desmopressin (DDAVP), a synthetic analogue of AVP does not cross the placenta within detectable limits at therapeutic doses (30 pg/mL). At higher concentration (60,000 pg/mL), it may cross the placenta in small amounts. Likewise oxytocin that is closely related with vasopressin and binds to vasopressin receptors crosses the placental barrier by simple diffusion (Malek et al 1996).

Developmental toxicity
Results of *in vitro* exposure of AVP (of 65 and 130 mU/kg/hr.) to fetal lambs (epitheliochorial placenta in contrast to humans with haemochorial placenta are less permeable to hydrophilic molecules) have shown increased level of Na^+/K^+ in amniotic fluid followed by a significant decrease in fetal heart rate, increase in systolic and diastolic blood pressure (Ross 1985).

Evaluation of cardiovascular response following an infusion of AVP (1.06 to 2.33 mU/kg/minute) in fetal lambs (120 to 140 days of gestation), has shown a persistent decrease in heart rate (HR) and an increase in mean arterial pressure (MAP) that returned to normal after dosing was stopped (Miyake 1991).

Dose response effects of AVP on fetal heart rate, and arterial pressure were also observed (Tomita 1985) when fetal sheep (122 to 136 days of gestation) were infused with AVP (0.5, 3.3, 10, 33 and 700 ng/minute). Plasma concentration of AVP was significantly increased.

Heart rate and arterial blood pressure
A 3-day IV infusion of AVP (45 mU/kg/hour) to fetal sheep (121-136 day old) has shown acute effects of decreased HR, rise in arterial blood pressure, acidemia and failure of glomerulotubular balance and were reversed by Day 3 (Gibson 1997). Role of AVP has also been suggested in brain development in particular to learning and memory processes (Ermisch 1987 and Swenson 1990).

Subcutaneous treatment of AVP to pregnant rats (1 U/day) and their offspring (0.5 U/day up until Day 30 post-parturition) led to 50% fetal mortality and 15% decrease in body weight with an increased learning indices in surviving pups (Ermisch 1987).

An intravenous administration of AVP (1 µg/24 hour) to pregnant rats (gestations days 13-19) led to visual discrimination and passive avoidance responses at 65 days postpartum. Impaired memory retrievals were observed in males while females were not affected (Tinius 1987). The subcutaneous administration of AVP (1 µg /day) to female rats (gestation days 13 – 19) demonstrated an enhanced retention of the responses while males were not affected when pups were evaluated at 65 days postpartum (Swenson 1990).

Behavioral Effects
Subcutaneous treatment of AVP (1 µg every 24 hours) to homozygous Brattleboro rats (deficient in brain AVP) did not cause an expected low body and brain growth, eye opening or any adverse effects (Snijdewint 1985). Intracisternal administration of AVP (10 or 100 ng) directly into the CNS of fetal rats (gestation day 20) led to a significant increase in fetal movement and effects were ceased within few minutes after the treatment (Varlinskaya 1994). A significant increase in forelimb activity, increase in head and mouth activity was evident at higher dose levels (10.0 to 100.0 ng)

Effects of AVP on Lactation
Intravenous administration of AVP (0.1, 0.3 and 1.0 ng/kg/minute over 90 minutes) to goats (2-3 months postpartum) has shown a ~8 fold increase in milk flow over the base line that returned to normal within 60 minutes (Olsson 2003). No effects of AVP (10 mU followed by 6 mU/minute for 60 minutes) were observed on plasma osmolality or volume of lactation in lactating goats (Dahlborn 1990).

10 Special Toxicology Studies

No special toxicological studies were conducted by the Sponsor for this indication.

11 Integrated Summary and Safety Evaluation

Vasopressin (AVP) has been in the market for a long time (prior to 1938) for a number of indications. The present submission for the treatment of vasodilatory, postcardiotomy and septic shock deals with an intravenous infusion of vasopressin ranging from 0.01 to 0.10 units/minute as continued infusion for up to several days.

Due to vast amount of information available in published data base (PubMed, TOXNET, TOXLINE, HSDB, DART, EMBASE, Registry of Toxic Effects of Chemical Substances (RTECS), CAplus, and Beilstein) on vasopressin and its extensive use in experience in humans. Except for a 28 day repeated dose study no formal single dose studies in animals were conducted by the Sponsor for pharmacological, toxicological and safety evaluation of vasopressin in present submission.

Primary Pharmacology
Vasopressin (VP) synthesis (Fig. 2) occurs in the two specific hypothalamic nuclei, the supraoptic nucleus (SON) and paraventricular nucleus (PVN) and is regulated at transcriptional level in CNS as a result of an increase in plasma osmolality. It is synthesized as 168- amino acid

prohormone and 23 amino acid signal peptide. The VP domain (1-9 residues) in the prohormone is linked to VP neurophysin domain (13-105 residues) that is linked to VP glycopeptide domain (107 to 145 residues).

In addition to this, VP is also synthesized in heart and adrenal gland.
Physiological stimulus such as hypervolemia and hypotension play an important in VP synthesis and release. Severe decreases (20-30%) in blood volume could increase VP concentration to 20-30 times to normal physiological level.

Three types of VP receptors: V_{1a}, V_{1b}, and V_2 are responsible toward the pharmacological action of VP. The V_{1a} receptor is the main subtype of VP receptor found in vascular smooth muscle, adrenal gland, myometrium, and bladder that activates the G_q-PLC-IP_3 pathway causing vasoconstriction, glycogenolysis, and platelet aggregation (Fig. 3).

The V_{1b} receptors are found in the anterior pituitary, several brain regions, pancreas, and adrenal medulla. The V_2 receptors are located on renal collecting-duct system and on vascular endothelial cells. The most prominent response to V_2 receptors is an increased water permeability of the renal collecting duct at concentrations as low as 50 fM.

Safety Pharmacology
There were no safety pharmacological studies conducted by the Sponsor, and cardiovascular effects of VP are reported below.

Cardiovascular Effects
Intravenous administration of AVP in rats (0.026 U/kg/min) and dogs (0.0055 U/kg/min- 0.026 U/kg/min) as reported in hemodynamics studies submitted by the Sponsor did not cause any mortalities. Decreased heart rate (HR), decreased cardiac output, increased mean arterial pressure (MAP) were the primary outcomes from these studies.

AVP administration (IV) resulted into an increased diastolic blood pressure, mean arterial blood pressure, coronary perfusion in pig (0.4 U/kg) models of cardiac arrest and cardiac arrhythmia.

Central Nervous System (CNS)
As a neurotransmitter and/or neuromodulator VP can modulate CNS autonomic systems controlling heart rate, arterial blood pressure, respiration rate, and sleep patterns, however, physiological significance is not understood. VP may also have an effect on certain learned behaviors, in the development of some complex social problems, and in the pathogenesis of complex psychiatric diseases such as depression.

Pharmacokinetics
AVP is metabolized in liver and kidney by aminopeptidases and inactivated by trypsin in GI tract. The physiological plasma level of vasopressin in human varies from 0.20-0.50 pg/mL (Oosterbaan 1989) to 0.76-1.19 pg/mL (Risberg 2009).

The variation across reports in values is likely due to different methodology used in measuring the vasopressin levels in different studies.

The plasma half-life for vasopressin is reported to vary from 17 to 35 min in human (Jackson, 2006). Antidiuretic effects of vasopressin last 2-8 hours following intramuscular or subcutaneous injection.

General Toxicology
No single dose toxicity studies were conducted by the Sponsor in animals and little information is available in published literature. The Sponsor conducted a dose range finding (DRF) study, and a 28-Day repeat dose study to evaluate the toxic effects of AVP and impurities in rats.

Data from 28-Day repeat dose study did not reveal any AVP related toxicity in rats when administered intravenously daily for 28 days at a dose of 0.76 μg/kg AVP alone, or with the addition of impurities at 0.1, 0.32 and 1.14 μg/kg. No adverse effects have been reported in animal studies other than expected pharmacological effects.

The NOAEL dose of AVP administered intravenously to rats over a 28-day period was considered to be 0.76 μg/kg and for impurities 1.14 μg/kg. AVP impurities did not have any additive or synergistic toxic effects in repeated dose rat study.

Genetic Toxicology
Data from genetic toxicity studies did not show any mutagenic or clastogenic potential of AVP in mammalian cell chromosomal aberration assay .

Reproductive and Developmental Toxicology
Published studies assessing the effects on *in utero* exposure to the fetus have not resulted in any morphological teratogenic effects. A significant decrease in spermatogenic index was noticed in spiny mice when AVP was administered intraperitoneally (50 μg/kg AVP at 3-day interval for 4 weeks), while no effects were seen on female reproductive function.

VP has a detrimental effect on sperm function (the percentage of motility, capacitation status, and protein tyrosine phosphorylation), fertilization, and embryonic development, suggesting its critical role in the acquisition of fertilizing ability of mouse spermatozoa.

Results of *in vitro* exposure of AVP (of 65 and 130 mU/kg/hr) to fetal lambs (epitheliochorial placenta in contrast to humans with haemochorial placenta are less permeable to hydrophilic molecules) have shown to be associated with an increased level of Na^+/K^+ in amniotic fluid followed by a significant decrease in fetal heart rate, increase in systolic and diastolic blood pressure.

Intracisternal administration of AVP (10 or 100 ng) directly into the CNS of fetal rats (gestation day 20) led to a significant increase in fetal movement and effects were ceased within few minutes after the treatment. A significant increase in forelimb activity, increase in head and mouth activity was evident at higher dose levels (10.0 to 100.0 ng) of VP.

Safety Evaluation

Based upon the NOAEL (0.76 µg/kg) obtained in repeated dose toxicity study in rats, the calculated human equivalent dose of AVP (0.76x.16=0.12 µg/kg) is at least 57X than the proposed dose of intravenous administration of AVP (0.01 to 0.067 U/min = 0.019 to 0.13 µg/min equivalent to 0.00032 to 0.0021 µg/kg for a 60 kg adult).

The combination of the extensive historical use of Pitressin (AVP) in humans, and with an adequate safety margin, this reviewer has no nonclinical safety concern from the pharmacological and toxicological perspectives for current NDA 204485.

12 References

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13 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RAMA S DWIVEDI
04/10/2013

THOMAS PAPOIAN
04/10/2013
Concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/Number:	Applicant:	Stamp Date:
204485	JHP PHARMACEUTICALS LLC	09/26/2012

Drug Name: PITRESSIN (VASOPRESSIN)

NDA/BLA Type:

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		Although some studies were conducted by the Sponsor, submission is almost entirely based on published literature.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		See comment to # 4
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		See comment to # 4

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		No impurities were identified.
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

None

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Rama Dwivedi — October 31, 2012
Reviewing Pharmacologist — Date

Thomas Papoian — October 31, 2012
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RAMA S DWIVEDI
10/31/2012

THOMAS PAPOIAN
10/31/2012
Concur.