CENTER FOR DRUG EVALUATION AND RESEARCH

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NON-CLINICAL REVIEW(S)

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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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Product:	Vasopressin
Indication:	Increase of blood pressure in adults with vasodilatory shock (e.g., post-cardiotomy or sepsis) who remain hypotensive despite fluids and catecholamines.
Applicant:	American Regent, Inc., 800 Adams Avenue, Norristown, CA
Review Division:	Cardiovascular and Renal Products
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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

Hypoperfusion and inadequate cellular oxygenation with consequential cellular hypoxia, organ failure, and death are the hall marks of shock. Distributive shock is one of the 4 shocks that occurs in nearly 200,000 US adults yearly. The cause of distributive shock is systemic inflammation that renders blood vessels unresponsive to endogenous vasoconstrictors (1). It includes sepsis, anaphylaxis and vasodilatory shocks. Septic shock mostly arises from Gram negative bacteria that results in circulatory insufficiency characterized by systemic vasodilation, relative and absolute hypovolemia, myocardial dysfunction, severe hypotension and decreased tissue perfusion. Even short durations of hypotension are associated with increased severe adverse events such as myocardial infarction, stroke, and acute kidney injury (2). Vasodilatory shock results from hypotension stemming from a failure of vascular smooth muscle to constrict as a result of marked decrease in systemic vascular resistance (3).

Therapy includes specific treatment of the underlying disease, volume replacement, and use of vasopressor medications to restore arterial blood pressure (4). Currently, catecholamines (dopamine, epinephrine, and/or NE) are the vasopressor agents of choice for supporting arterial blood pressure and ensuring adequate organ perfusion. Norepinephrine is considered by many investigators to be the standard vasopressor to treat septic shock (5, 6). However, high doses of catecholamines may contribute to organ dysfunction and mortality in sepsis (7, 8). Based on the pharmacodynamic properties, AVP gained importance in the treatment of patients in the critical care environment and has been explored as a vasoconstrictor in the treatment of hypotension associated with shock (9, 10)**.** Additionally, AVP is part of the Advanced Cardiac Life Support protocol for the resuscitation of patients in cardiac arrest (11). It has also been suggested as an adjunct to norepinephrine either to raise mean arterial blood pressure to target or to decrease NE dose (6). However, in a large multicenter trial (VASST), vasopressin failed to reduce mortality in septic shock (12). In this context, it should be noted that in December 2017, Angiotensin II was approved for the treatment of hypotension in patients with distributive shock receiving a variety of other vasopressors (1).

The current drug substance, vasopressin (AVP), is an endogenous neuroendocrine peptide formed in the hypothalamus and released from the posterior pituitary in response to hypotension, decreased blood volume, decreased intravascular volume and increased plasma osmolality. Plasma osmolality is a primary stimulus for AVP's release (13). Under normal physiological conditions, AVP has a limited role in the regulation of blood pressure, and a drop of >10% is needed to stimulate AVP release (13). The reason is, its normal physiological concentration is < 4 pg/ml, a concentration insufficient to cause vasoconstriction. During hypotension and hypovolemia, AVP acts as a potent vasoconstrictor with little or no antidiuretic effect as its level surges (14, 15). AVP vasoconstriction effect is mediated by V1 (or V1a) vascular receptors (pressor)

coupled to Gq protein and the PLC/IP3 signal transduction pathway. Physiologically, AVP increases water and sodium reabsorption in the collecting duct by producing renal glomerular efferent arteriolar vasoconstriction. The effect is mediated by V2 renal receptors (antidiuresis) coupled to Gs and adenylyl cyclase pathway. This eventually enhances tissue oxygenation, increases hepatic arterial blood flow, decreases portal venous blood flow and normalizes cellular metabolism. The V1b (or V3) receptor in the anterior pituitary is also coupled to the Gq and PLC pathway. AVP through the V3 receptor stimulates ACTH and catecholamine release from the anterior pituitary gland and adrenal gland, respectively, contributing to rise in blood pressure (16). In addition, AVP acts on oxytocin (OT) receptors, and the P2 class of purinoreceptors (17).

Based on several published studies (15, 18, 19), the sponsor summarizes that AVP is safe and effective in increasing MAP in patients with several forms of shock. It decreases the requirement for catecholamines to maintain blood pressure in patients with vasodilatory shock. Through this NDA submission, American Regent company is seeking regulatory approval for AVP for the treatment of hypotension in adults with vasodilatory shock who remain hypotensive despite fluid and vasopressor therapy.

1.2 Brief Discussion of Nonclinical Findings

The primary effect of AVP is to maintain blood pressure and cardiac output by activating V1 receptor. In addition, AVP has been demonstrated to cause vasodilation in numerous vascular beds mediated by extra renal V2, V3, OT and purinergic P2 receptors distinguishing it from other vasoconstrictor agents (20) especially the recently approved Angiotensin II for shock (1). Vasoconstriction or vasodilation by AVP depends on the vascular bed studied, its receptor density, type of G protein and the dose and duration of exposure of AVP. It is not clear from the literature whether AVP can cause simultaneous vasoconstriction of some vascular beds and vasodilation of others (see discussion in reference number 20). The actions of AVP on the heart are mediated by V1 receptor and/or OT receptor (endothelial/NO/ANP effect). Thus, the actions are complex, and studies are contradictory. Its effect on the coronary vascular tone (vasoconstriction or vasodilation), force of contraction, cardiac output depends on dose and duration of infusion and condition of the patient. In neonatal rat, AVP has been shown to promote cardiac hypertrophy via direct effects on cardiomyocyte protein synthesis secondary to V1 receptor-mediated intracellular calcium release. In adult rat, AVP directly increases the rate of protein synthesis through the V1 receptor stimulation.

AVP exposure provokes thrombosis. At physiological concentrations (1 pM), AVP stimulates V1 receptors to activate platelets leading to an increased expression of the activation-dependent platelet antigen P-selectin (CD62, a sensitive marker of platelet activation) (25). At concentrations 1000-fold high (1 nM), a dose-dependent aggregation of platelets with a concomitant stimulation of thromboxane B2 release has been reported (26). A combination of AVP acting through V1 receptors and heparin interacting with platelet factor 4 might lead to aggregation of platelets and to further organ dysfunction (22). In addition, extrarenal V2 receptor stimulation is implicated in

the release of clotting factors - factor VIII, von Willebrand factor, von Willebrand factor multimers and tissue plasminogen activator when given intravenously in a manner similar to its synthetic analogue desmopressin (dDAVP), an antidiuretic V2 receptor specific agonist in both normal subjects and in patients with central diabetes insipidus (17, 27, 28). These studies suggest that AVP promotes thrombosis and may be a physiologic platelet agonist.

There is not much information on the effect of AVP on reproductive process. However, a few studies have shown that AVP has adverse effects on male reproductive organ. In vitro study with the mouse sperm, vasopressin dose-dependently impairs sperm function, including motility, fertilization and embryonic development. The responses are mediated by V2 receptors. It is unclear just how much vasopressin may be bioavailable to harm sperm in the human testis and reproductive tract (29). In the pregnant sheep, AVP decreases maternal plasma tonicity resulting in parallel fetal plasma hyponatremia, which in turn increases fetal urinary flow. The hypotonicity expands maternal blood volume and increases amniotic fluid volume at levels of plasma hyponatremia that does not incur significant fetal or maternal risks (30).

1.3 Recommendations

1.3.1 Approvability

Yes

1.3.2 Additional Non-Clinical Recommendations

Actions of AVP extend beyond blood pressure increases. AVP activates multiple receptors in the heart to cause positive inotropy and hypertrophy. As noted in the previous section, continuous administration of AVP for a long period provokes thrombosis.

1.3.3 Labeling

Recommendations and edits are made on the labeling document on SharePoint and presented at the Division labeling meeting held on October 30, 2019.

2 Drug Information

2.1 Drug

CAS Registry Number: 133-79-1

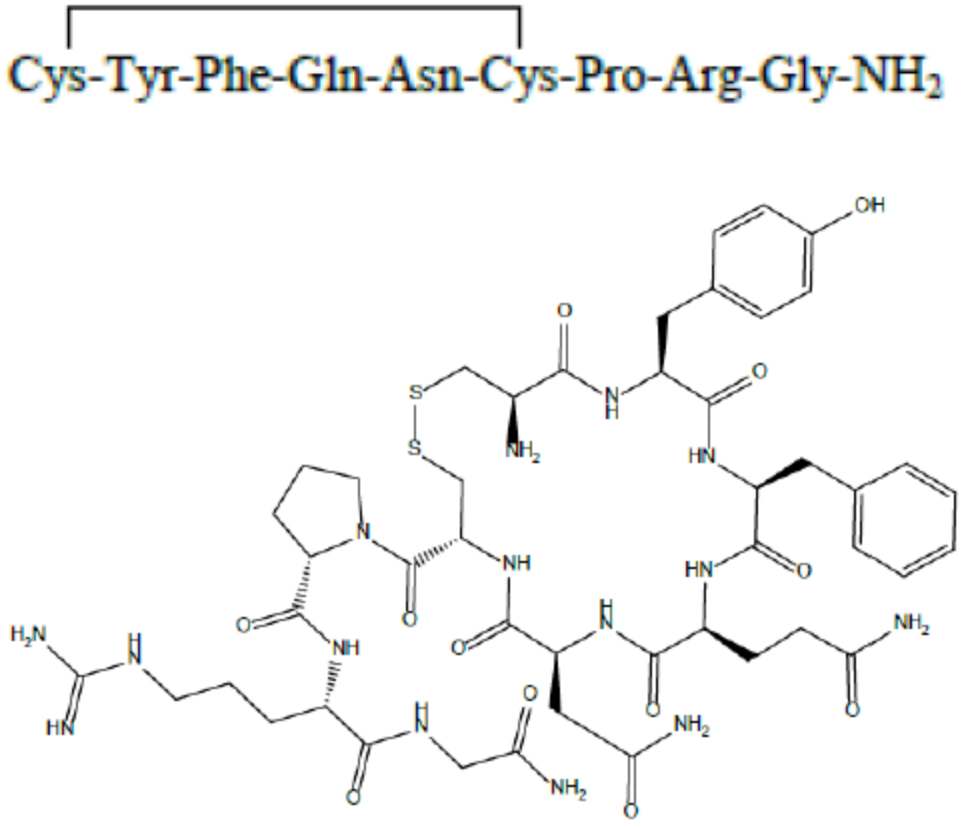
Generic Name: Vasopressin

Trade Name: None

Chemical Name: Vasopressin, 8-L-arginine
L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-arginyl-glycinamide (1→6)-disulfide, acetate salt.

Molecular Formula/Molecular Weight: $C_{46}H_{65}N_{15}O_{12}S_2$ (as free base)
Molecular Weight: 1084.23

Structure and Biochemical Description: Vasopressin is a cyclic peptide consists of nine amino acids. A disulfide bond is present between 1st and 6th amino acid. Vasopressin is a synthetic peptide hormone and is freely soluble in water. It is white to off-white powder/flakes. All 9 amino acids are present in their natural L-form.



Pharmacologic Class: Vasopressor

Proposed indication: Increase of blood pressure in adults with vasodilatory shock (e.g., post-cardiotomy or sepsis) who remain hypotensive despite fluids and catecholamines.

Route of Administration: Intravenous

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 204485

2.3 Drug Formulation

Vasopressin injection, USP is a sterile, aqueous solution containing 20 units/ml vasopressin. It is diluted in normal saline (0.9% sodium chloride) or 5% dextrose in water prior to use for intravenous administration.

2.4 Comments on Novel Excipients

There are no novel excipients. All of the excipients meet the Inactive Ingredient Database limits (Table 1).

Table 1. Vasopressin injection, USP 20 units/ml. Inactive ingredients

Name of Excipient	Per mL	Total Daily Intake	Maximum Potency per FDA Inactive Ingredient Database (IID) (per unit)
Sodium Chloride, USP/ Multi-Compendial	9 mg	9 mg	18 mg/mL
Chlorobutanol, NF	5 mg	5 mg	5 mg/mL
(b) (4) Acetic Acid, USP/Multi-Compendial	pH Adjust	Not Applicable	0.546 mg/mL
Water for Injection, USP	Q.S.	Not Applicable	Not Applicable
			(b) (4)

2.5 Comments on Impurities/Degradants of Concern

CMC Drug substance reviewer, Dr. Monica Cooper, in an email (dated August 6, 2019) sent a request to qualify the limits of the following impurities in the drug substance.

1. A limit of (b) (4)% (b) (4).
2. A limit of (b) (4)% for (b) (4)
3. A limit of (b) (4)% for (b) (4)
4. A limit of (b) (4)% for (b) (4)

(b) (4)

(b) (4): There is a lack of information not only on the limit but also on the toxicity of (b) (4). (b) (4) is listed in ICH Q3C as (b) (4) On extensive literature search, this reviewer could get the toxicity of (b) (4) in rats. In a reproductive and developmental toxicity study in rats, (b) (4)[1] observed a significant (b) (4) Another study[2] reported (b) (4) in rats. According to this study, (b) (4) is not a mutagenic. The study also noted that no carcinogenicity or reproductive toxicity tests on (b) (4) have been published. Human Equivalent Dose for NOAEL is (b) (4) mg/kg/day (or (b) (4) mg/day) (estimated rat NOAEL of (b) (4)).

According to the sponsor 470 (average) Units of AVP is equal to 1 mg AVP. Maximum daily dosage of AVP is 144 Units/day (that is, 0.1 unit/min infusion rate x 60 min x 24 hr = 144 Units/day). Thus, 144 U of AVP is equal to 0.3 mg/day x (b) (4) . With a maximum daily dose

[1] (b) (4)

[2] (b) (4)

of 144 Units of AVP, the patient will consume (b) (4)/day, which is far lower than HED ((b) (4) mg/day) of the NOAEL of (b) (4) mg/kg/day in rats.

Peptide impurities: Available information in the literature on vasopressin analogues is insufficient to evaluate the pharmacology and toxicology effects of peptide impurities present in the drug substance. All three AVP analogues have minimal endocrinological activity. In the work of (b) (4). According to the authors, these molecules are (b) (4). Based on the impurity levels ranging from (b) (4)%, the patient is expected to consume a total of (b) (4) µg/day with a maximum daily dose of 144 Units (equals to 300 µg)/day of AVP.

The above information was conveyed to Dr. Cooper via email on August 8, 2019.

2.6 Proposed Clinical Population and Dosing Regimen

Adults with distributive or vasodilatory shock who remain hypotensive despite fluid and vasopressor therapy. The maximum dose for post-cardiotomy shock is 0.1 units/minute and for septic shock 0.07 units/minute.

2.7 Regulatory Background

Vasostrict (NDA 204485) as the listed drug for this 505(b)(2).

3 Studies Not Reviewed

None

[3] (b) (4).

4 Pharmacology

No stand-alone Pharmacology studies were conducted by the sponsor on vasopressin. The Agency agreed that the information in the literature is enough and no nonclinical studies with vasopressin are required. Results from the literature summarized in this section (article summary edited for consistency) give an understanding of the pharmacologic effects of vasopressin for the intended indication.

4.1 Primary Pharmacology

4.1.1 Vasopressin in a hemorrhagic shock model

Reference: Bini, R., Chiara, O., Cimbanassi, S., Olivero, G., Trombetta, A., Cotogni, P. Evaluation of capillary leakage after vasopressin resuscitation in a hemorrhagic shock model. World Journal of Emergency Surgery. 2018; 13:11;1-9.

Background: Hemorrhagic shock (HS) is a major threat to patients with trauma and spontaneous bleeding. The aim of the study was to investigate early effects of vasopressin on metabolic and hemodynamic parameters and endothelium permeability by measuring capillary leakage compared to those of other resuscitation strategies in a HS model.

Methods: Forty-five Sprague-Dawley rats were randomized into five groups: S group *(n* = 5), sham-operated rats without shock or resuscitation; HS group *(n* = 10), HS and no resuscitation; RL group *(n* = 1 0), HS and resuscitation with Ringer's lactate (RL); RLB (Ringer lactate plus blood) group *(n* = 1 0), HS and resuscitation with two-third shed blood plus RL; and vasopressin group *(n* = 10), HS and resuscitation with RL, followed by continuous infusion of 0.04 U/kg/min vasopressin. The effects of resuscitation on hemodynamic parameters [mean arterial pressure (MAP), superior mesenteric artery blood flow (MBF), and mesenteric vascular resistances (MVR)], arterial blood gases, bicarbonate, base deficit, and lactate levels as well as on capillary leakage in the lung, ileum, and kidney were investigated. Capillary leakage was evaluated with Evans blue dye extravasation.

Results: In the vasopressin group, the MAP was higher than in the RL and RLB groups *(p* < 0.001), while MBF was decreased *(p* < 0.001). MVR were increased only in the vasopressin group *(p* < 0.001). Capillary leakage was increased in the lungs of the animals in the vasopressin group compared to that in the lungs of animals in the RLB group *(p* < 0.05); this increase was associated with the lowest partial pressure of

oxygen ($p < 0.05$). Conversely, decreased capillary leakage was observed with vasopressin in the ileum ($p < 0.05$). Increased capillary leakage was observed in the kidney in the RLB and vasopressin groups ($p < 0.05$). Lastly, vasopressin use was associated with higher base deficit and lactate levels when compared to the RL and RLB groups ($p < 0.001$).

Conclusion: Although vasopressin was proposed as a vasoactive drug for provisional hemodynamic optimization in the early phase of HS resuscitation, the overall findings of this experimental study focus on the possible critical side effects of vasopressin on metabolic parameters and endothelium permeability.

4.1.2 Vasopressin in the treatment of septic shock in sheep

Reference: Sun Q, Dimopoulos G, Nguyen D N, Tu Z, Nagy N, Hoang A D, Rogiers P, De Backer D, and Vincent J-L. Low-dose vasopressin in the treatment of septic shock in sheep. Am J Respir Crit Care Med. 2003;168: 481–486.

Septic shock is the circulatory insufficiency that develops in response to overwhelming systemic infection. The central characteristics of septic shock are systemic vasodilation and myocardial depression resulting in hypotension requiring vasopressor agents such as norepinephrine to maintain mean arterial blood pressure (MAP). Arginine vasopressin is a vasomodulatory hormone with unique properties including pulmonary vasodilation and systemic and renal efferent arteriolar vasoconstriction. It is also an intriguing vasopressor because it has little pressor effect in normal subjects but markedly increases blood pressure when sympathetic nerve function is impaired. The present study was designed to study the effects of low-dose vasopressin alone, or in combination with norepinephrine, on hemodynamics, histologic changes, and survival time in a sheep model of septic shock due to peritonitis.

After induction of cecal perforation, 20 anesthetized sheep were randomized to be treated, when arterial blood pressure fell below 75 mm Hg, with vasopressin (fixed dose of 0.02 U/minute), norepinephrine (0.5–5 µg/kg/minute titrated to maintain mean arterial pressure between 75 and 85 mm Hg), vasopressin plus norepinephrine (vasopressin at fixed dose 0.01 U/minute plus norepinephrine titrated as for norepinephrine only group), or no vasopressor (Ringer's lactate [control]). Mean arterial pressure was well maintained in all treatment groups. Superior mesenteric arterial blood flow was significantly lower in the vasopressin plus norepinephrine group than in the vasopressin group. Vasopressin alone or combined with norepinephrine limited the increase in blood lactate concentration and ileal P_{CO2}-gap compared with control and norepinephrine

groups. Urine output was higher in the vasopressin group than in control and norepinephrine groups. Survival time was longer in the vasopressin (30 ± 6 hours) and vasopressin plus norepinephrine (30 ± 3 hours) groups than in the norepinephrine group (20 ± 1 hours, $p < 0.05$) and in all treatment groups than in the control group (17 ± 2 hours, $p < 0.05$). Tissue injury was less severe in the vasopressin and vasopressin plus norepinephrine groups than in the others. In this clinically relevant model of septic shock due to peritonitis, vasopressin administration (alone or with norepinephrine) can prolong survival (Fig. 1).

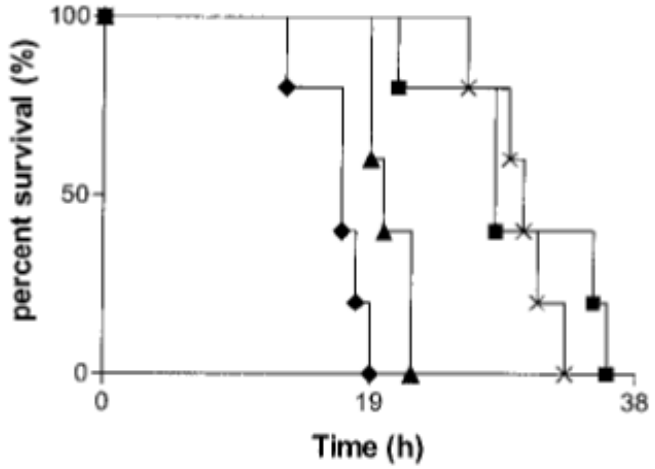


Figure 1. Kaplan–Meier survival curves for the four groups.
Control (closed diamonds), vasopressin (closed squares), norepinephrine (closed triangles), and vasopressin plus norepinephrine (X). $p < 0.05$, control versus vasopressin, or norepinephrine or vasopressin plus norepinephrine.

According to the authors, although the model shows many of the features of human septic shock, it may not replicate the human situation exactly, as the sheep is a ruminant. Second, peritonitis induced by fecal spillage is only one of many possible causes of human septic shock. Third, for experimental reasons, fecal spillage was allowed during the experiment, whereas eradication of the infectious focus is an important part of the management of the patient with septic shock. In addition, no antibiotic therapy was administered, as this was a lethal model. Fourth, anesthesia and ventilation were maintained in all animals throughout the study to minimize suffering and experimental bias, which may not reflect the clinical situation in all patients. Finally, for technical reasons it was not possible to measure plasma vasopressin concentrations in the animals. Despite these limitations, authors conclude that low doses vasopressin, alone or in combination with norepinephrine, maintain arterial blood pressure, mesenteric blood flow, and cardiac output, limit the increase in blood lactate concentrations and Pco2-gap, and prolong survival time in this clinically relevant model of sepsis. Prospective, randomized clinical studies are needed to test whether vasopressin administration can alter the outcome of septic shock.

4.1.3 Vasopressin plays a role in maintaining vasomotor tone during acute blood loss

Reference: Sims, C.A., Yuxia, G., Singh, K., Werlin, E.C., Reilly, P.M., Baur, J.A. 2017. Supplemental arginine vasopressin during the resuscitation of severe hemorrhagic shock preserves renal mitochondrial function. PLoS ONE:12(10):1-16.

With prolonged hemorrhagic shock, intense vasoconstriction will progress to vasodilation and catecholamine-resistant cardiovascular collapse. Identifying strategies to prevent or treat this state of “decompensated shock” could be lifesaving. AVP has been investigated as an adjunct during the resuscitation of severe trauma. Clinically, severely injured trauma patients demonstrate a high incidence of AVP deficiency with an increased need for vasopressor support, blood product transfusions and prolonged ICU care.

In this study, the authors have hypothesized that decompensated hemorrhagic shock is associated with decreased AVP stores and supplementation during resuscitation would improve both blood pressure and renal function. Using a decompensated hemorrhagic shock model, male Long-Evans rats were bled to mean arterial blood pressure (MAP) of 40 mm Hg and maintained until the MAP could not be sustained without fluid. Once 40% of the shed volume was returned in lactated Ringer's (Severe Shock), animals were resuscitated over 60 minutes with 4x the shed volume in lactated Ringer's (LR) or the same fluids with AVP (0.5 units/kg+ 0.03 units/kg/min). Animals (n = 6-9/group) were sacrificed before hemorrhage (Sham), at Severe Shock, following resuscitation (60R, 60R with AVP) or 18 hours post-resuscitation (18 hr, 18 hr with AVP). Blood samples were taken to measure AVP levels and renal function. Pituitaries were harvested and assayed for AVP. Kidney samples were taken to assess mitochondrial function, histology, and oxidative damage.

Baseline pituitary AVP stores (30,364 ± 5311 pg/mg) decreased with severe shock and were significantly depressed post-resuscitation (13,910 ± 3016 pg/ml, $p<0.05$) and at 18 hr (15,592 ±1169 pg/ml, $p<0.05$). Resuscitation with LR + AVP led to higher serum AVP levels at 60R (31 ± 8 vs 79 ± 12, $p<0.01$) with an improved MAP both at 60R (125 ± 3 vs 77 ± 7 mm Hg, $p<0.01$) and 18 hr (82 ± 6 vs 69 ± 5 mm Hg, $p<0.05$). AVP supplementation preserved mitochondrial complex I respiratory capacity at 60R and both complex I and II function at 18 hr ($p<0.05$). AVP was also associated with decreased reactive oxygen species at 60R (856 ± 67 vs 622 ± 48F RFU) and significantly decreased oxidative damage as measured by mitochondrial lipid peroxidation (0.9 ± 0.1 vs 1.7 ± 0.1-fold change, $p<0.01$) and nitrosylation (0.9 ± 0.1 vs 1.4 ± 0.2-fold change, $p<0.05$). With AVP, renal damage was mitigated at 60R and histologic architecture was conserved at 18 hr.

In conclusion, pituitary and serum AVP levels decreased during severe hemorrhage and may contribute to the development of decompensated hemorrhagic shock. Supplementing exogenous AVP during resuscitation improves blood pressure, preserves renal mitochondrial function, and mitigates acute kidney injury. AVP appears to be a beneficial adjunct during the resuscitation of severe shock.

4.1.4 Vasopressin in uncontrolled hemorrhagic shock rats

Reference: Yang, G., Hu, Y., Peng, X., Zhu, Y., Zang, J., Li, T., Liu, L. Hypotensive resuscitation in combination with arginine vasopressin may prolong the hypotensive resuscitation time in uncontrolled hemorrhagic shock rats. J. Trauma Acute Care Surg. 78(4):760-766, 2015.

Hemorrhage and subsequently hemorrhagic shock remain the major cause of mortality after trauma. Fluid resuscitation is the ‘‘cornerstone’’ of the therapy of hemorrhagic shock during the last decades. However, uncontrolled bleeding is often seen in trauma patients (known as uncontrolled hemorrhagic shock), and the ‘‘classic’’ fluid resuscitation (aggressive resuscitation) is deleterious for this condition because large volume of fluid infusion before bleeding is controlled can induce further blood loss and severe hemodilution. So, hypotensive resuscitation or limited fluid resuscitation emerged and was advocated. Animal studies and clinic trials showed that hypotensive resuscitation resulted in better outcomes than did large-volume fluid resuscitation.

Arginine vasopressin is an endogenous hormone, which has only a minor effect on blood pressure under physiologic condition. However, under shock state, AVP shows a potent vasoconstriction and vasopressor effect, both in vasodilatory septic shock and hemorrhagic shock patients. A recent study with a rat model of uncontrolled hemorrhagic shock by an 80% tail amputation showed improved hemodynamics and outcome after treatment with AVP or terlipressin. This study observed only a shorter ‘‘hypotension’’ time (40 minutes) before the active bleeding was controlled, and a bolus injection of AVP was given, which rapidly increased the mean arterial pressure (MAP) up to 120 mm Hg. However, the rapid elevation of blood pressure during uncontrolled hemorrhage is contradictory to the concept of the hypotensive resuscitation according to European trauma guidelines.

The objective of the present study was to investigate whether hypotensive resuscitation in combination with AVP can prolong the hypotensive resuscitation time by minimizing blood loss and stabilizing hemodynamics for uncontrolled hemorrhagic shock.

Methods: With an established rat model of uncontrolled hemorrhagic shock, investigators compared the beneficial effects of hypotensive resuscitation in combination with AVP to maintain blood pressure at 50 mm Hg for 3 hours to hypotensive resuscitation alone on animal survival, blood loss, and vital organ functions.

Results: Hypotensive resuscitation in combination with AVP maintenance for 3 hours significantly reduced total blood loss and fluid requirement during hypotensive resuscitation period and significantly improved the survival of shock rats as compared

with hypotensive resuscitation alone. Among the four concentrations of AVP, 5 X 10^{-4} U/ml had the best effect: it significantly improved hemodynamics and increased cardiac function, oxygen delivery, as well as hepatic blood flow and hepatic function in the shock rats. However, renal blood flow in the hypotensive resuscitation + AVP group was lower than that in the hypotensive resuscitation alone group.

Conclusion: Hypotensive resuscitation in combination with early application of AVP could prolong the tolerance time of hypotensive resuscitation and ‘‘buy’’ longer safe prehospital transport time by reducing blood loss and stabilizing hemodynamics. This strategy may be a promising strategy for the early management of trauma patients with active bleeding

4.1.5 Ischemia and reperfusion modulate arterial contraction triggered by AVP.

Reference: Szadujkis-Szadurska, K., Malinowski, B., Piotrowska, M., Grześk, G., Wiciński, M., Gajdus, M. 2016. The modulatory effect of ischemia and reperfusion on Arginine Vasopressin-induced arterial reactions. Biomed Res Int. http://dx.doi.org/10.1155/2016/3679048.

Blood vessel occlusion leads to ischemia. Cells have energy reserves and an ability to increase the amount of available oxygen, to survive a reduced blood supply. If ischemia lasts for longer period, changes in cellular structure occur with a possible necrosis. Studies have indicated an inhibitory effect of ischemia on arterial contractility and shows clear association between NO synthesis and activation of cGMP

The purpose of this study was to investigate the impact of ischemia and reperfusion on the resistance of arteries to AVP, with a particular emphasis on the role of smooth muscle cells in the action of vasopressin receptors and the role of the cGMP-associated signaling pathway.

Materials and Methods. Experiment was performed on isolated and perfused tail arteries collected from male Wistar rats under anesthesia. The constriction triggered by AVP after 30 min of ischemia and 30 and 90 min of reperfusion was analyzed. Analogous experiments were also carried out in the presence of 8Br-cGMP.

Results: Ischemia reduced, and reperfusion increased in a time-dependent manner the arterial reaction to AVP. The presence of 8Br-cGMP caused a significant decrease of arterial reactivity under study conditions. In the presence of 8Br-cGMP, the concentration response curves of AVP was shifted to the right, with a reduction of the maximum effect (Table 2).

Table 2. Values % Ea/Em, EC50, and pEC50 for AVP determined after ischemia and reperfusion in the presence of 8Br-cGMP.

	% Ea/Em	EC_{50}	pEC_{50}
Control	100,00	$9{,}696 \times 10^{-7}$	7,987
AVP + 8Br-cGMP	79,29	$3{,}865 \times 10^{-7*}$	7,587[a]
Ischemia + 8Br-cGMP	21,03	$3{,}492 \times 10^{-6**}$	6,543[b]
Reperfusion 30′ + 8Br-cGMP	117,70	$7{,}583 \times 10^{-8\dagger}$	8,880[c]
Reperfusion 90′ + 8Br-cGMP	147,70	$2{,}559 \times 10^{-9\dagger\dagger}$	9,408[d]

EC_{50} control versus $EC_{50}^{*/**/\dagger/\dagger\dagger}$, $p < 0.05$; pEC_{50} control versus $pEC_{50}^{b/c/d}$, $p < 0.05$; pEC_{50} control versus pEC_{50}^{a}, p = ns.
% Ea/Em: % of maximum reaction.
EC_{50}: concentration triggering 50% of the maximum reaction.
pEC_{50}: −log 10 of EC_{50}.

Conclusions. Ischemia and reperfusion modulate arterial contraction triggered by AVP. The effect of 8Br-cGMP on reactions, induced by AVP after ischemia and reperfusion, indicates that signaling pathway associated with nitric oxide and cGMP regulates the tension of the vascular smooth muscle cells triggered by the vasoconstrictor.

4.1.6 Apoptosis pathways in the hypothalamus during sepsis.

Reference: da Costa, L. H. A., dos Santos Júnior, N.N., Catalão, C. H. R., Sharshar, T., Chrétien, F., da Rocha, M. J. A. Vasopressin impairment during sepsis is associated with hypothalamic intrinsic apoptotic pathway and microglial activation. Mol Neurobiol: 54(7):5526-5533, 2017.

The inflammatory agents produced by immune cells at the infection site or in the blood can directly or indirectly reach the central nervous system. There they can lead to a cerebral dysfunction denominated sepsis-associated encephalopathy (SAE), resulting in cognitive, autonomic, and endocrine impairment. Regarding neuroendocrine changes during sepsis, the study group has focused its investigation on AVP secretion, an important vasopressor peptide synthetized in the supraoptic (SON) and paraventricular

(PVN) nuclei of the hypothalamus. By using cecal ligation and puncture as an experimental model of sepsis induction, a drop-in blood pressure and an increase in plasma vasopressin following 4–6 hr of surgery were observed. However, 24 h after sepsis induction, the circulating levels of the hormone remained low, despite of persistent hypotension. Moreover, at this time, an impairment in magnocellular activation and an increase in some markers of oxidative stress that could result in apoptosis of AVP-producing neurons were observed. Apoptosis, a form of programmed cell death, is an ATP-dependent highly complex process that comprises a sophisticated cascade of molecular events. The key elements in apoptosis are caspases, a family of cystenil proteases that, once cleaved, become activated and initiate a proteolytic cascade by activating other procaspases and amplifying the death signal.

Previous studies have shown that in the early phase of sepsis, the plasma concentration of AVP is increased, but in the late phase, its levels remain inadequately low, despite of persistent hypotension. One hypothesis suggested for this relative deficiency is apoptosis of vasopressinergic neurons. Previous studies from the authors laboratory have shown that during sepsis, there is an augmented expression of cleaved caspase-3 and annexin-V affinity accompanied by a decrease in copeptin (a component of the AVP precursor) in SON and PVN, strongly suggesting that AVP-producing magnocellular neurons undergo apoptosis. Here, the authors have investigated apoptosis pathways in the hypothalamus during sepsis, as well as mechanisms underlying the process.

Male Wistar rats were submitted to sepsis by cecal ligation and puncture (CLP) or nonmanipulated (naive) as control. After 6 and 24 hr, the animals were decapitated, and brain and blood were collected to assess hypothalamic apoptotic markers, IFN-γ (interferon-gamma) plasma levels, and evidence for breakdown of the blood-brain barrier (BBB).

Sepsis caused a decrease in mitochondrial antiapoptotic proteins (Bcl-2, Bcl-xL) in the hypothalamus, but had no effect on markers of cell death mediated by death receptors or immune cells. In the supraoptic nuclei of these animals, microglia morphology was consistent with activation, associated with an increase in plasma IFN-γ. A transitory breakdown of BBB in the hypothalamus was seen at 6 hr following CLP. The results indicate that the intrinsic but not extrinsic apoptosis pathway is involved in the cell death observed in vasopressinergic neurons, and that this condition is temporally associated with microglial activation and BBB leaking.

4.2 Secondary Pharmacology

The sponsor did not identify any published literature for the secondary pharmacology of vasopressin.

4.3 Safety Pharmacology

4.3.1. Coagulation and vasodilation responses by dDAVP

Reference: Bichet DG, et al.: Hemodynamic and coagulation responses to 1-desamino[8-D-arginine] vasopressin in patients with congenital nephrogenic diabetes insipidus. New Engl J Med 318:881-887,1988.

The antidiuretic hormone arginine vasopressin interacts with two types of receptors: V1, which mediates the effects of vasopressin on vascular smooth muscle, and V2, which mediates the antidiuretic effects on renal tubules. Resistance of the renal tubules to arginine vasopressin and to the antidiuretic V2-specific agonist 1-desamino[8-o-arginine] vasopressin (dDAVP) occurs in congenital nephrogenic diabetes insipidus, a rare X-linked disease, although the V1-receptor responses remain intact. The extrarenal actions of dDAVP in normal persons are a decrease in blood pressure, an increase in plasma renin activity, and stimulation of the release of factor VIIIc and von Willebrand factor.

The authors measured the response of mean arterial pressure, pulse rate, plasma renin activity, factor VIIIc, and von Willebrand factor to an infusion of dDAVP (0.3 μg/kg) in seven male patients with congenital nephrogenic diabetes insipidus, six obligatory carriers of the gene for nephrogenic diabetes insipidus, five patients with central diabetes insipidus and four normal subjects.

In the normal subjects and the patients with central diabetes insipidus, dDAVP decreased mean arterial pressure (by 10 to 15 percent) and increased pulse rate (by 20 to 25 percent), renin activity (by 65 percent), and the release of coagulation factors (two-fold to three-fold) (all changes were significant, $P<0.01$) (Fig. 2). None of these changes were observed in the patients with congenital nephrogenic diabetes insipidus, and minimal responses were observed in the obligatory carriers.

These results confirm the existence of extrarenal vasopressin V2-like receptors, which may be defective in patients with congenital nephrogenic diabetes insipidus. The extrarenal actions of dDAVP were to lower blood pressure, to increase heart rate, and to stimulate the release of factor VIIIc and von Willebrand factor in normal subjects and

in patients with central diabetes insipid us. The vasodilator action of dDAVP was not mediated through a competitive antagonism with endogenous arginine vasopressin on vascular V1 receptors. The rapid changes in mean arterial pressure, pulse rate, and plasma renin activity observed after dDAVP infusion would exclude the possible involvement of inhibition of water excretion. Extensive experimental data obtained in laboratory animals indicate that the "antidiuretic" activity of arginine vasopressin causes cardiovascular effects opposite to the classical vasoconstrictive actions of arginine vasopressin suggesting the existence of extrarenal vasodilator V2-like receptors.

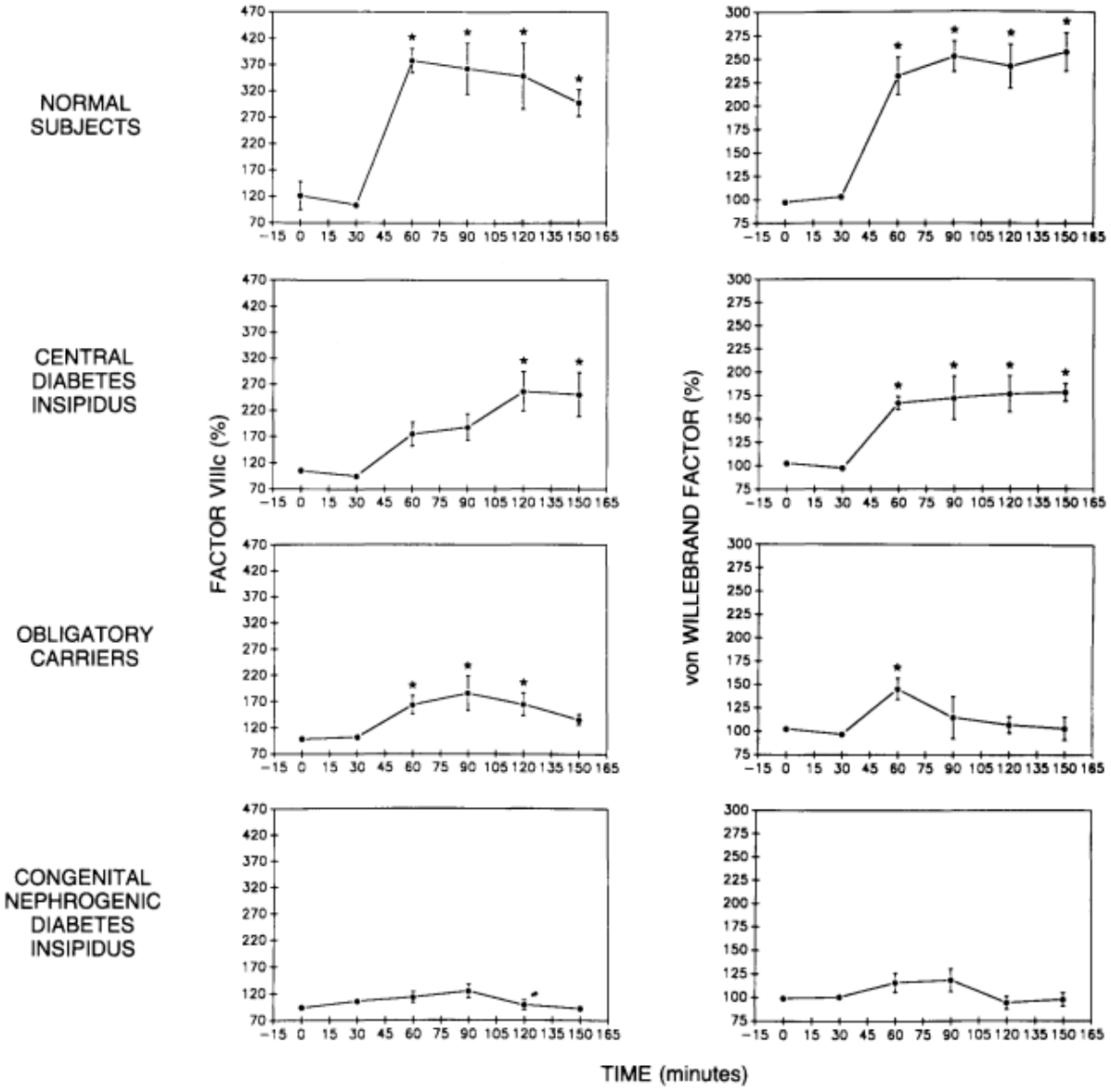


Figure 2. Factor VIIIc and von Willebrand Factor Responses to dDAVP Infusion.
All subjects received the dDAVP infusion from 30 to 50 min. Asterisks indicate significant differences from baseline (values at 0 and 30 min).

4.3.2. Platelet aggregation by AVP

Reference: Filep J, Rosenkranz B: Mechanism of vasopressin-induced platelet aggregation. Thromb Res 1987; 45:7-15.

As AVP has been shown to stimulate the formation of thromboxane in the isolated toad urinary bladder and in isolated hepatocytes, AVP might stimulate thromboxane synthesis in platelets and thus induce aggregation. The study was designed to determine the effect of AVP on thromboxane formation and to investigate the participation of calcium and lipoxygenase products in AVP-induced aggregation of platelets in healthy human platelet rich plasma.

AVP over the range of 1.8-113.6 mU/ml caused a dose-dependent aggregation of platelets with a concomitant stimulation of thromboxane B2 (TXB2) formation, with a half maximal stimulation occurring at about 20 mU/ml (Fig. 3). The aggregation was reversible within 3 min at lower concentrations, while it was irreversible at higher concentrations. d(CH2)gTyr (Me)AVP, an AVP antagonist, did not affect platelet aggregation or TXB2 release, but completely inhibited the action of AVP. dDAVP (V2 receptor selective agonist) up to the concentration of 280 pM/ml had no effect on aggregation. Pretreatment of platelets with verapamil, trifluoroperazine (a calmodulin antagonist) or methylimidazole (a TX synthetase blocker), prevented AVP-induced aggregation and TXB2 release. Neither phenidone (a dual inhibitor of cyclooxygenase / lipoxygenase pathways) in lower concentration nor nordihydroguaiaretic acid (a lipoxygenase inhibitor and antioxidant) inhibited the ability of AVP to induce aggregation and TXB2 release. These results are consistent with the hypothesis that human platelets possess AVP receptor of the calcium-dependent V1 subtype and suggest that AVP-induced platelet aggregation is mediated via TX release.

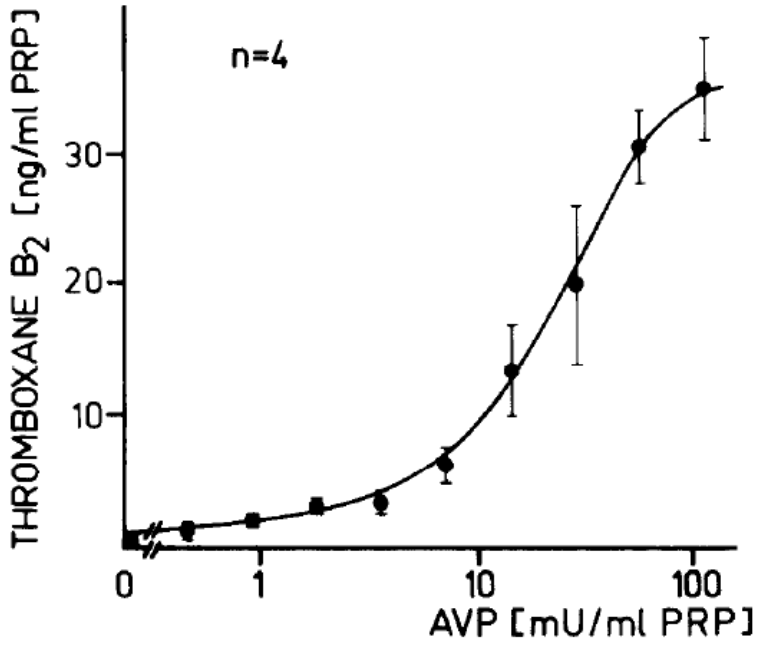


Figure 3. Effect of AVP on thromboxane formation.

Platelet rich plasma was prepared and TXB2 was determined as described in Methods.
n, number of volunteers. Values are means ± S.E.M.

4.3.4. Platelet activation by AVP at physiologic concentrations

Reference: Wun T, et al.: Physiologic concentrations of arginine vasopressin activate human platelets in vitro. Br J. Haematol 1996; 92:968–972.

Arginine vasopressin (AVP) is a neurohypophyseal peptide hormone with protean effects. Previous reports had shown that AVP stimulates platelets, but only at concentrations 3–6 logs higher than the normal plasma concentrations in humans. In this study, we tested the hypothesis that AVP, at physiologic concentrations, stimulated the expression of an activation-dependent platelet antigen.

Platelets obtained from normal volunteers were incubated with increasing concentrations of AVP and the expression of the activation dependent platelet antigen P-selectin (CD62) was determined by monoclonal antibodies and flow cytometry.

There was a concentration-dependent increase in CD62 expression with increasing AVP concentration (0.1 pM to 100 nM); at 1 pM AVP, 24.5% (1.3–88.5%) [median (range)] of platelets expressed CD62 (Fig. 4). The selective vasopressin V1 receptor antagonist d(CH2)5-Tyr(-me)AVP (TM-AVP) completely abolished AVP-stimulated CD62 expression. The concentration of unbound AVP in the plasma is 0.3–2 pM. However, the concentration of platelet membrane bound AVP is approximately 3–15 pM. The results of the present study show that physiologic (picomolar) concentrations of AVP can stimulate human platelets to express the activation-dependent platelet antigen P-selectin (CD62) in vitro. This response occurs at a concentration 3–6 logs lower than the concentration at which AVP can stimulate platelet aggregation, and transmembrane calcium influx. The authors conclude that this response is mediated by the platelet V1 receptor. AVP may be a physiologic platelet agonist.

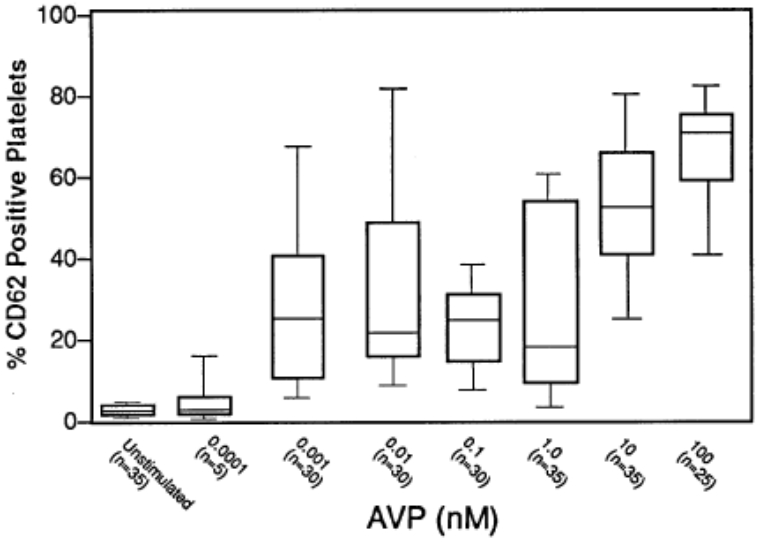


Figure 4. AVP-stimulated CD62 expression on normal human platelets

Platelet-rich plasmas were incubated with the indicated concentrations of AVP and CD62 expression quantitated by two-color flow cytometry. Data are shown as box plots with horizontal lines representing the 10th, 25th, 50th, 75th and 90th percentiles. There is a concentration-dependent increase in CD62 expression with an apparent intermediate plateau between 0.001 and 1.0 nM AVP.

5 Pharmacokinetics/ADME

No stand-alone PK studies were conducted by the sponsor on vasopressin. The Agency agreed that the information in the literature is sufficient and no nonclinical PK studies with vasopressin are required. A short summary based on the published literature is given below.

5.1 Biological half-life and organ distribution of arginine-vasopressin

Reference: Janáky, T., Lászió, F.A., Sirokmán, F., Morgat, J.L. 1982. Biological half-life and organ distribution of [^{3}H]8-arginine-vasopressin in the rat. J. Endocrinol: 93(3):295-303.

The biological half-life of synthetic, radiochemically pure, biologically active [^{3}H]8-arginine-vasopressin, the distribution of radioactivity among the organs and the in-vivo metabolism of the hormone were studied in the rat. The half-life calculated from the [^{3}H]AVP radioactivities isolated from the blood was found to be 1·74 ± 0·22 (S.D.) min in the fast phase, and 16·98 ± 1.01 min in the slow phase. The half-lives of total radioactivity were longer in both phases; fast phase was 2·49 ± 0·51 (S.D.) min and for the slow phase 27·9 ± 5.08 min.

Organ distribution of [3H]AVP and the accumulation of the radioactivity in the tissues, high activity was observed in those organs (kidney and liver) in which the vasopressin is quickly metabolized. However, considerable accumulation was noted in the small intestine, an amount exceeding that in the kidney and the liver (Table 3). The metabolic activity is more marked in the kidney than in the liver and thus, the results agree with the observations of other authors. The majority of the hormone is removed from the circulation by the kidney. The rate of inactivation, however, is more pronounced in the liver as supported by radio-chromatograms taken after 2 and 5 min; after 2 min the [^{3}H]AVP accounted for 5·4% of the radioactivity in the kidney but only 2.7% in the liver. The phenomenon may be explained by the different enzymatic activities, or by the fact that there are specific vasopressin receptors in the kidney, whereas only non-specific membrane binding is to be found in the liver. It is noteworthy that the highest radioactivity accumulation was observed in the adenohypophysis and neurohypophysis. It is reported that the neurohypophysis also contains vasopressin-inactivating aminopeptidase and trypsin-like enzymes.

Table 3. Mean (± S.E.M.) distribution of radioactivity (% total activity administered)/100 mg organ weight) in various organs of rats

Organ	[^{3}H]Arginine-vasopressin (10 rats)
Kidney	0·129 ± 0.034
Liver	0·090 ± 0. 013
Muscle	0·031± 0·007
Small intestine	0·195± 0.049
Cerebral cortex	0.042 ± 0·009
Neurohypophysis	0.140 ± 0.026
Adenohypophysis	0.227± 0.051
Hypothalamus	0.043 ±0.008

5.2 Biological half-life and organ distribution of of [3H]dDAVP

Reference: Laszlo, F.A., Janaky, T., Balaspiri, L., Morgat, J.L. 1981. Biological half-life and organ distribution of [3H]1-deamino-8-D-arginine-vasopressin in the rat. J. Endocrinol. 88(2):181-186.

The vasopressin derivative, 1-deamino-8-D-arginine-vasopressin (dDAVP), a selective V2 receptor agonist, is widely and effectively used in clinical practice for the treatment of diabetes insipidus. It is known that dDAVP has a slight pressor effect, whereas its antidiuretic activity is several times that of the natural hormone and the duration of its action is considerably longer. The difference between the biological effects raises the question of whether the variation in the vasopressin molecule is accompanied by a change in the metabolism of the hormone and this has been studied using biologically active, tritiated dDAVP. The biological half-life and organ distribution of the labelled dDAVP were examined in male rats.

The biological activity was parallel with the radioactivity. The biological half-life of synthetic, radiochemically pure, biologically active [^{3}H]1-deamino-8-D-arginine-vasopressin (dDAVP) in rats was found to be 5·33 ± 0·28 (S.E.M) min in the initial, transitional, fast phase and 56·28 ± 3·27 min in the second, slow phase. The extended biological half-life may play a role in the more marked and longer antidiuretic effect of dDAVP. The substance accumulated to the greatest extent in the kidney and small

intestine and only slightly in the adenohypophysis (Table 4). The poor accumulation in the adenohypophysis suggests that dDAVP does not possess an effect similar to that of corticotrophin releasing factor.

Table 4. Mean (±S.E.M) distribution of [^{3}H]1-deamino-8-D-arginine-vasopressin radioactivity (total activity %/ 100 mg organ weight) in various organs of ten rats

Organ	Radioactivity	Organ	Radioactivity
Kidney	0·83 ± 0·05	Neurohypophysis	0·20 ± 0·03
Liver	0·20 ± 0·01	Adenohypophysis	0·14 ± 0·01
Muscle	0·07 ± 0·01	Hypothalamus	0·06 ± 0·01
Small intestine	0·57 ± 0·05	Cerebral cortex	0·08 ± 0·01

6 Toxicology

No stand-alone toxicology studies were conducted by the sponsor on vasopressin. According to the sponsor, no studies with vasopressin were found in the literature which would permit an evaluation on the carcinogenic, mutagenic or teratology effects of vasopressin. Results and the follow up discussion are based on the published articles included in the submission.

6.1 Reproductive and Developmental Toxicology

6.1.1 Effect of vasopressin on ovine fetal renal response

Reference: Roberts, T.J., Nijland, M.J.M., Curran, M., Ross M.G. 1999. Maternal 1-deamino-8-D-arginine-vasopressin-induced sequential decreases in plasma sodium concentration: Ovine fetal renal responses. Am. J. Obstet. Gynecol. 180:82-90.

Background: The mammalian fetus acquires water from the maternal circulation through the placenta. Although there is extensive bidirectional water diffusion across the placenta, net placental water flux toward the fetus averages only 20 to 30 mL/day throughout gestation. This fluid contributes to the fetal intravascular, intracellular, and interstitial fluid compartments, as well as to amniotic fluid, which may be considered a fetal extracellular fluid compartment. Maternal hyperosmolality may decrease or reverse transplacental water flow and eventually lead to decreases in amniotic fluid volume. Maternal hyponatremia induces fetal plasma hyponatremia, increases ovine fetal urinary flow, and increases amniotic fluid volume in both sheep and human beings.

Objective: Acute maternal plasma hypotonicity induces a reduced placental osmotic gradient that contributes to augmented maternal-to-fetal water flow. Subsequently, maternal plasma hyponatremia results in fetal plasma hyponatremia, increased fetal urinary flow, and ultimately increased amniotic fluid volume. The authors have hypothesized that both the degree of reduction in the placental osmotic gradient and the degree of fetal plasma hyponatremia influence fetal urinary diuretic responses. To differentiate the roles of these factors, authors have determined fetal urinary responses to graded levels of plasma hyponatremia during a constant placental osmotic gradient. Furthermore, authors have sought to establish the minimum level of plasma hyponatremia necessary to facilitate an increase in fetal urine production.

Study Design: Seven pregnant ewes (130 ± 2 days) were prepared with maternal and fetal vascular catheters and a fetal bladder catheter. After 6 days of recovery, fetal urinary flow and urine and plasma compositions were measured during a 2-hour control period. At 2 hours, tap water (2 L, 38°C) with a 20 gm bolus of 1-deamino-8-D-arginine-vasopressin immediately followed by 4 gm/hr DDAVP infusion together with a maintenance i.v. infusion of 5% dextrose in water (5 to 25 mg/kg/hr) was administered to the ewe. Maternal plasma sodium concentration was decreased from control by 5 to 7, 10 to 12, and 15 to 17 mEq/L, and held at each level (levels 1, 2, and 3) for 60 min.

Results: DDAVP administration induced sequential decreases in maternal and fetal plasma sodium concentrations (control 146.9 ± 0.5 mEq/L and 141.0 ± 0.5 mEq/L, respectively) at level 1 (140.1 ± 0.6 mEq/L and 136.7 ± 0.7 mEq/L, respectively), level 2 (132.5 ± 0.7 mEq/L and 130.6 ± 1.1 mEq/L, respectively), and level 3 (125.4 ± 1.2 mEq/L and 123.0 ± 1.5 mEq/L, respectively). The maternal fetal placental osmolality and sodium gradients were constant at each hypotonicity level. Fetal urinary flow significantly increased in association with the degree of hyponatremia (from 0.17 ± 0.03 mL/kg/min to 0. 26 ± 0.04 mL/kg/min, 0.33 ± 0.05 mL/kg/min, and 0.38 ± 00.5 mL/kg/min at levels 1, 2, and 3, respectively).

Conclusions: These results indicate the following: (1) Sequential decreases in maternal plasma tonicity result in parallel decreases in fetal plasma tonicity. (2) The fetal urinary diuretic response is highly correlated with the degree of fetal plasma hypotonicity, despite a constant placental osmotic gradient. A fetal therapeutic response (53% increase in fetal urine production) may be induced by a maternal plasma sodium concentration decrease of only 5 to 7 mEq/L. (3) dAVP-induced hypotonicity contributes to increased amniotic fluid volume.

The results of this study demonstrate the potential role of maternal dDAVP-induced hypotonicity in expanding maternal blood volume and increasing amniotic fluid volume at levels of plasma hyponatremia that do not incur significant fetal or maternal risks.

6.1.2 Effect of vasopressin on male fertility, fertilization and embryonic development

Reference: Kwon, W-S., Park, Y-J., Kim, Y-H., You, Y-A., Kim, I.C., Pang, M-G. 2013. Vasopressin effectively suppresses male fertility. PLoS ONE. 8: e54192, pages 1-8.

Introduction: Arginine vasopressin (VP), a neurohypophysial hormone, has been implicated in stimulating contractile activity of the male reproductive tract in the testis. High concentrations of immunoreactive VP and specific vasopressin receptor have been found in the testis. VP increases the volume of seminal fluid and the concentration

of spermatozoa in the ejaculate of the rabbit. VP increases the accumulation of cytosolic cAMP in vas deferens epithelial cells and can modulate ion transport across vas deferens epithelia by independent mechanisms. Higher levels of VP decrease sperm count and motility in healthy human but infertile men. However, very little is known about the involvement of VP in controlling mammalian reproductive process. The present study was designed to explore the expression of VP receptor (AVPR2) in spermatozoa and the roles of VP in sperm function in capacitation condition (the change undergone by spermatozoa in the female genital tract that enables them to penetrate and fertilize an egg), fertilization, and embryonic development. For the study, deamino [Cys 1, D-ArgS] vasopressin (dDAVP), a selective AVPR2 agonist, was used.

Methods: Mouse sperm suspension was prepared using 8 to 12-week-old male ICR mice. The caput and cauda epididymides from mice were separated, the fat was removed. Caput or cauda epididymides were cut with a surgical blade. The excised cauda epididymides minced a few pieces to let spermatozoa flow out from the ducts. The spermatozoa were incubated with various concentrations of dDAVP (10 pM to 10 µM) and sperm motility, capacitation status, Protein Kinase A activity (PKA), tyrosine phosphorylation, fertilization, and embryo development were assessed using computer-assisted sperm analysis, Combined Hoechst 33258/chlortetracycline fluorescence, Western blotting, and in vitro fertilization, respectively. AVPR2 was placed on the acrosome region and mid-piece in cauda epididymal spermatozoa, but the caput epididymal spermatozoa was mid-piece only. For in vitro fertilization study, 8 to 12-week-old female mice super ovulated with mare serum gonadotrophin and human chorionic gonadotrophin were used. Cumulus-oocyte were collected from oviduct and inseminated with dDAVP-treated spermatozoa for 6 hr at 37ºC. Fertilization rate was assessed following 18 hr of insemination. All embryos that developed up to the blastocyst stage were counted.

Results: Treatment with dDAVP dose-dependently decreased sperm motility reaching statistical significance at high concentrations (10 nM and 10 µM). At these concentrations, intracellular pH and PKA substrates (approximately 55 and 22 kDa) were significantly decreased and Ca^{2+} concentration was increased. At the highest concentration (10 µM) tested, dDAVP significantly decreased PKA substrate (approximately 23 kDa) and tyrosine phosphorylation (approximately 30 kDa). Treatment detrimentally affected capacitation and acrosome reaction. At high concentrations (10 nM and 10 µM), dDAVP significantly decreased capacitated spermatozoa and increased non-capacitated spermatozoa relative to control. Treatment dose-dependently decreased fertilization and the embryonic development. A statistically significant decrease was noted at concentrations, 10 nM and 10 µM (Fig. 5). There was no effect of dDAVP on any of the parameters at the lowest concentration, 10 pM.

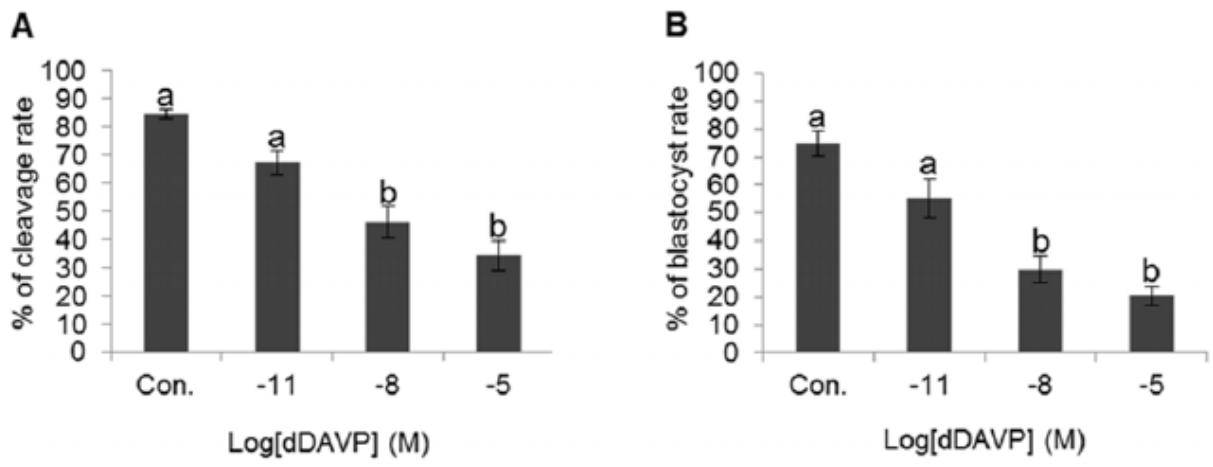


Figure 5. Effect of dDVAP on fertilization and embryo development.
(A) Change of cleavage rate in various treatment conditions. (B) Change of blastocyst rate in various treatment conditions. Data represent mean 6 SEM, n = 6. Values with different superscripts ($^{a, b}$) were significantly different between control and treatment groups by One-way ANOVA ($p < 0.05$).

Discussion: Sperm motility is an important feature and is the most reliable actual predictor of male factor infertility. VP through AVPR2 dose-dependently decreased sperm motility. The result implies that VP is detrimental to sperm motility when the spermatozoa undergo capacitation. The observation strongly suggests that over- or under-expression of AVPR2 and/or AVPR2 mutation, and that aberrant concentration of VP in semen or the male reproductive tract may contribute to male infertility. Previous studies have similarly reported that oxytocin and VP significantly decreased the percentage of motility in spermatozoa from human and mouse. To fertilize oocytes in the female reproductive tract and in vitro, the mammalian spermatozoa must undergo the capacitation process that is a prerequisite for the acrosome reaction. VP significantly decreased in capacitation and the acrosome reaction. Protein tyrosine phosphorylation has an important role in the regulation of processes such as sperm maturation, motility, hyperactivation, cell recognition, and the acrosome reaction all of which are essential for fertilization to occur. VP potently inhibited PKA activity that directly phosphorylates tyrosine in a dose-dependent manner. In addition, in vitro fertilization system demonstrated that VP decreased fertilization and embryonic development in a dose-dependent fashion achieving significant effect at the high concentration.

The study concludes that VP is a specific agonist for AVPR2 and its strong activation is detrimental to normal sperm function, especially related with fertility. It is worth noting that the VP concentrations tested in the current and previous studies were much higher than the VP concentration actually present in seminal plasma and blood (1.84 ± 1.23 pg/ml).

7 Integrated Summary and Safety Evaluation

Vasopressin (AVP) is an endogenous hormone synthesized by the magnocellular neurons of the hypothalamus. Subsequently, it migrates along the supraoptic-hypophyseal tract to the posterior pituitary, where it is released into the circulation (17). Vasopressin is involved in cardiovascular and osmotic homeostasis. A deficiency (early release in shock followed by depletion of neurohypophyseal stores and consequently reduction in circulating concentration) of vasopressin exists in some shock states and replacement of physiologic levels of vasopressin can restore vascular tone (20). During hemorrhagic shock, V1 receptors, in particular, play a pivotal role in maintaining cardiovascular homeostasis and mitigate acute kidney injury. Several published studies reviewed here have examined the therapeutic benefit of AVP in vasodilatory shock states. Additional published literature summarizes some of the adverse effects and male reproductive toxicity of AVP.

Pharmacology

Pharmacodynamics

The actions of AVP are mediated by 5 different receptors (V1, V2, V3, oxytocin (OT), and purinergic P2) with different tissue specificity and intracellular pathways (16, 17). V1 receptors are found primarily on vascular smooth muscle, platelets, liver, CNS but are also expressed in high density in the renal medullary interstitial cells, vasa recta and epithelial cells of the collecting duct (17). AVP causes vasoconstriction by increasing intracellular calcium via the Gq/11-PLC-coupled phosphatidyl-inositol-triphosphate and calcium signaling pathway. Additional mechanisms that contribute to V1R led vasoconstriction are (a) closure or inhibition of K_{ATP} channels, (b) blunting the increase in cGMP induced by nitric oxide and atrial natriuretic peptide, and (c) by decreasing the synthesis of inducible nitric oxide synthase that is stimulated by lipopolysaccharide (20). On a molar basis, VP is more potent vasoconstrictor than is angiotensin II or NE (22). In the renal medulla, however, AVP selectively constricts the efferent but not the afferent arterioles, thus leading to an increase in glomerular filtration [23] and diuresis, which limits the antidiuretic effect of V2 receptors.

The V2 receptors differs functionally from the V1 receptors. The antidiuretic effect of AVP occurs via activation of V2 receptors in the renal medulla. V2 receptors are coupled to Gs signaling pathway that activates cAMP. Additionally, V2 receptors are expressed in endothelium as its stimulation causes vasodilation both centrally and peripherally and releases procoagulant glycoprotein von Willebrand factor

The V3 pituitary receptor has a profile that distinguishes from V1 and V2 receptors as it activates several signaling pathways via different G proteins (Gq/11, Gi and Gs) depending on the level of receptor expression and the concentration of AVP (21). Its activation stimulates the release of ACTH from the anterior pituitary and catecholamines from the adrenal gland.

AVP has a 30-fold higher affinity for V1 receptors than is for OT receptors (endothelial/NO effect). OT receptors coupled to Gq/11 stimulates PLC to generate 2 second messengers, IP3 and DAG. The secondary transduction mechanism is Gi/Go family coupled adenylyl cyclase inhibition. These receptors are present in both reproductive and non-reproductive tissues. Their presence on vascular endothelium mediates nitric oxide-dependent vasodilation. In the heart, OT receptors stimulation releases atrial natriuretic peptide. In smooth muscle cells, e.g., myometrium, OT receptors stimulation initiates contraction. Additionally, AVP activates intravascular purinergic P2 receptors in the heart to cause positive inotropy (17, 24). The discovery that AVP activates purinergic P2 receptors (ligand-gated ion channel P2X and G protein-coupled P2Y) is intriguing.

Pharmacology studies of AVP relevant to the proposed indication, to increase blood pressure in adults with vasodilatory shock who remain hypotensive despite fluids and catecholamines, and the intended intravenous route of administration have been reported in the literature. In animal (rats, sheep) models of hemorrhagic shock or septic shock, intravenous AVP infusion improved blood pressure, restored effective tissue perfusion, normalized cellular metabolism, preserved renal mitochondrial function and mitigated acute kidney injury. The studies showed that AVP appears to be a beneficial adjunct during the resuscitation of severe shock.

Safety pharmacology

AVP was shown to exert cardiac effects through the activation of intravascular purinergic P2, V1 and OT receptors. In neonatal rat heart, AVP promotes cardiac hypertrophy by accelerating cardiomyocyte protein synthesis secondary to V1 receptors -mediated IP3-calcium release. Stimulation of cardiac OT receptors releases ANP (20).

Several published both animal and clinical studies suggest that AVP induces thrombosis. AVP activates platelets via V1 receptors that parallels its pressor action. In healthy human platelet rich plasma, AVP thru stimulation of V1 receptors has been shown to cause a dose-dependent aggregation of platelets with a concomitant stimulation of thromboxane B2 formation. The aggregation was reversible within 3 min at lower concentrations, while it was irreversible at higher concentrations. In another similar study, AVP at physiological concentrations, was shown to stimulate platelets to express the activation -dependent platelet antigen P-selectin (CD62). In addition to V1 receptors, extrarenal V2 receptors stimulation releases clotting factors - factor VIIIc, von Willebrand factor and tissue plasminogen activator when given intravenously in a manner similar to its synthetic analogue desmopressin (dDAVP), an antidiuretic V2-specific agonist in both normal subjects and in patients with central diabetes insipidus. These studies suggest AVP may be a physiologic platelet agonist.

Pharmacokinetics

The intravenous biological half-life of synthetic AVP in the rat was 1·74 min in the fast phase, and 17 min in the slow phase. The peak effect occurs within 15 minutes after infusion. After stopping the infusion, the pressor effect declines within 20 minutes. The half-lives of total radioactivity were longer in both phases. The radioactivity accumulated to the greatest extents in the adenohypophysis and small intestine. The radioactive substance was accumulated more in the kidney than in the liver, but the hormone underwent inactivation more quickly in the liver than in the kidney.

Toxicology

The published literature on the toxicities (including carcinogenic, mutagenic and teratology) of exogenously administered AVP is scanty except for two research papers discussed below. The lack of these studies is reflected in the label.

In pregnancy, the fetus acquires water from the maternal circulation through the placenta and that this fluid contributes to the fetal intravascular, intracellular, interstitial fluid fetal compartments and the amniotic fluid. Intravenous administration of dDAVP, a selective V2 receptor agonist, to pregnant ewes showed that sequential maternal plasma hyponatremia results in parallel decreases in fetal plasma sodium concentrations and increases fetal urinary flow. The decreased plasma tonicity contributes to increased amniotic fluid volume. Thus, the study demonstrated the potential role of maternal dDAVP-induced hypotonicity in expanding maternal blood volume and increasing amniotic fluid volume at levels of plasma hyponatremia that do not incur significant fetal or maternal risks.

Little is known on the effect of AVP on mammalian reproductive process. Higher levels of AVP decreases sperm count and motility in healthy humans. A mechanistic in vitro study has shown that dDAVP dose-dependently ($P<0.05$ at ≥10 nM) decreased the mouse sperm motility and function in capacitation condition, thus contributing to male infertility. In addition, in vitro fertilization system demonstrated that V2 receptor stimulation decreases fertilization and embryonic development in the mouse.

Evaluation

AVP is a natural vasopressor and antidiuretic hormone of the posterior pituitary gland that regulates cardiovascular and renal physiology. It stimulates a family of receptors (V1, V2, V3, OT and purinergic P2 receptors). Although vasoconstriction is a predominant effect, more diversified responses are noted in heart, kidney, blood and different vascular beds. These effects depend on the dose and duration. It mediates many of its effects by binding to three G protein-coupled vasopressin receptors, V1, V2 and V3. Additionally, it binds to oxytocin (OT) and purinergic P2 receptors. Although the predominant effect is vasoconstriction mediated by V1 receptors, vasodilation is mediated by V2 and OT receptors. The cardiac effects (primarily positive inotropy) is mediated at least in part through P2X and P2Y receptors (24). The rationale for the use

of AVP is the putative relative deficiency and hypersensitivity to its vasopressor effects during shock wherein all three vasopressin receptors are downregulated. Hypotension is the potent stimulus for release of stored AVP. The current drug product is the same form of vasopressin that was used in many studies and manufactured by several companies. Primary pharmacodynamic studies involving animal models of shock demonstrated therapeutic benefit of AVP in humans with vasodilatory, the proposed indication of the product.

Although AVP is a natural vasopressor hormone of the body, it is not devoid of serious adverse and toxic effects when its circulating concentration exceeds physiological levels (~4 pg/ml). The toxicities of exogenously administered AVP is manifested as excessive activation of V1 and V2 receptors. Thus, the adverse effects of AVP are dose- and duration-dependent. At physiological concentrations (1 pM), AVP activates platelets via V1 receptors leading to an increased expression of the activation-dependent platelet antigen P-selectin (CD62, a sensitive marker of platelet activation) (25, 22). At concentrations 1000-fold high (1 nM), a dose-dependent aggregation of platelets with a concomitant stimulation of thromboxane B2 formation has been reported (26). Extrarenal V2 receptors stimulation is implicated in the release of clotting factors - factor VIIIc, von Willebrand factor, von Willebrand factor multimers and tissue plasminogen activator (17, 27, 28) when given intravenously in a manner similar to its synthetic analogue desmopressin (dDAVP), an antidiuretic V2 receptor specific agonist in both normal subjects and in patients with central diabetes insipidus. These studies suggest that AVP promotes thrombosis and may be a physiologic platelet agonist.

The published literature on the reproductive effect of AVP is scanty. A few publications report that activation of V2 receptors causes a dose-dependent inhibition of sperm motility. AVP in a dose-dependent manner detrimentally affected capacitation and acrosome reaction causing decreased fertilization and embryonic development. It should be noted that these studies used very high concentrations of AVP than is actually present in seminal plasma and blood. There is not much information on how much vasopressin may be bioavailable and harm sperm in the human testes and reproductive tract.

There are not many deleterious effects of AVP that occur with excess stimulation of the multiple vasopressin and OT receptor if therapeutic doses of the current product are not exceeded. The main objective in the clinical use of AVP, is to restore systemic blood pressure from the hypotensive to normotensive state. Any excessive pharmacologic and toxicologic effects of exogenously administered AVP on the vasculature and heart observed in normotensive subjects is unlikely to occur in patients with distributive or vasodilatory shock if titrated to lowest dose compatible with a clinically acceptable blood pressure response.

Conclusions

AVP is intended to raise blood pressure from the hypotensive to normotensive state, and not to induce a hypertensive state. The duration of infusion of AVP is for a limited

period. Additionally, AVP is short acting with the pressor effect declines within 20 min. Identified risks are related to the mechanism of action of AVP, wherein it stimulates multiple receptors in vascular smooth muscle, heart, platelets and kidney. The detrimental effects, if any, on the target organs are dose- and duration-dependent.

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