Blockage of Arginine Vasopressin Receptor 2 Reduces Increase in Pulmonary Vascular Resistance in Ovine Sepsis Model

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Sepsis is a major public health problem in the U.S. affecting more than 700,000 people every year.

(Angus DC. J Am Med Assoc, 2010; Dellinger RP. Intens Care Med, 2013; Lagu et al., Crit Care Med, 2012)

The overall mortality of sepsis is around 30%.

(Strehlow et al., Ann Emerg Med, 2006; Lagu et al., Crit Care Med, 2012)

The mortality rate has not decreased in the past two decades, despite the major advances have been made in the management of sepsis.

(Esteban et al., Crit Care Med, 2007; Alberti et al., Intens Care Med, 2002)

In sepsis, vascular leakage and pulmonary vascular resistance are associated with poor prognosis and no treatment is available to date.

(Farand et al., Can J Anest, 2006; Cinel & Dellinger. Curr Opin Infect Dis, 2007)
Sepsis and Vascular Leakage: Pathophysiology and Management

- Microbial components
- Inflammatory response
- Vascular smooth muscle
- Vasodilation
- Plasma extravasation
- Interstitial space & third space fluid accumulation
- Increased fluid requirement
- Refractory hypotension
- Vasopressor requirement
- Ischemia & tissue injury
- ORGAN DYSFUNCTION

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TRANSLATIONAL
Intensive Care Unit
Arginine Vasopressin (AVP) as Treatment for Sepsis

- In 2008, a large clinical trial was performed to compare the therapeutic effect of AVP vs. norepinephrine.
- AVP failed to decrease the mortality of septic patients compared to norepinephrine.
- However, an improvement in survival rate was obtained in patients that received a lower dose of AVP.

(Russell et al., N Eng J Med, 2008; Vincent et al., Clin Anaes, 2008)
AVP Receptors

- **V1R**
  - Vascular smooth muscle cells
  - Vasoconstriction

- **V2R**
  - Renal collecting duct cells
  - Increase water reabsorption
  - Endothelial cells
  - Release of von Willebrand factor (vWF)

- **V3R**
  - Anterior pituitary gland
  - Release of adrenocorticotropic hormone (ACTH)
  - Myometrium
  - Uterine contractions

**Oxytocin receptor**

*Source: UTMB Health*
Septic Sheep Treated with $V_1R$ Agonist Accumulated Less Fluid Than Sheep Treated with $V_1R/V_2R$ Agonist (AVP)

Control (placebo)

Vasopressin (AVP)

$V_1R$ agonist (long acting)

Control

$V_1R/V_2R$ agonist

$V_1R$ agonist

MRSA sepsis, Saline, n=6

MRSA sepsis, AVP 0.01 U/min, n=6

MRSA sepsis, Phe2-Orn8-Vasotocin (POV), 0.01 U/min, n=6

*P <0.05 vs. BL
†P<0.05 vs. Control
‡P<0.05 vs. AVP

(Rehberg et al., AJP-Heart Circ Physiol, 2012)
V₂R Agonist Abolishes V₁aR Agonist’s Effect on Vascular Leakage

Cumulative Fluid Balance (mL/kg)

Time (h)

-50 0 50 100 150 200 250

Vasopressin (n=9-10)
FE 202158 (n=9)
FE 202158+V₂ AGONIST (n=6)

% P<0.05 Vasopressin vs. FE 202158
† P<0.05 FE 202158+V₂ AGONIST vs. FE 202158

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Goal:
To elucidate the role of V$_2$R receptor during methicillin-resistant *Staphylococcus aureus* (MRSA) sepsis and to test the safety and efficacy of the V$_2$R antagonist, tolvaptan (TLVP) as a potential treatment for septic patients.

Hypothesis:
Blockage of V$_2$R activation attenuates pulmonary and systemic vascular hyperpermeability and pulmonary vascular resistance during MRSA sepsis.
Materials & Methods: Conscious Ovine MRSA Sepsis Model

Female sheep weighing ~35kg were surgically prepared for chronic study at least 5-7 days before the experiment: pulmonary artery (Swan Ganz) (1), left atrium (2), and femoral artery (3) catheters were inserted for continuous monitoring of hemodynamics and intermittent blood sampling.

(Enkhbaatar et al., Shock, 2008)
Materials & Methods: Conscious Ovine MRSA Sepsis Model

Female sheep 30 - 40 kg

Smoke inhalation injury

Bronchoscope directed instillation of MRSA

~2.5X10^{11} CFU

24 hrs of monitoring under mechanical ventilation

(Enkhbaatar et al., Shock, 2008)
Materials & Methods:
Conscious Ovine MRSA Sepsis Model

(Enkhbaatar et al., Shock, 2008)
Materials & Methods:
Conscious Ovine MRSA Sepsis Model

24h/7 days Monitoring

(Enkhbaatar et al., Shock, 2008)
Animal Groups and Treatments

- Sham, n = 6, No injury + saline
- Control, n = 7, Injury + saline
- DDAVP (V₂R agonist, desmopressin), n = 6, Injury + DDAVP 38 ng/kg/h. 23 hrs infusion
- TLVP (V₂R antagonist, tolvaptan), n = 6, Injury + TLVP 417 mcg/kg/h. 23 hrs infusion

A total of 25 sheep were randomized into four groups.
- Continuous i.v. infusion, as a treatment, was given one hour after the injury.
- Fluid resuscitation was adjusted to maintain hematocrit ± 3% from baseline (BL).
TLVP Treatment Prevented the Systemic Accumulation of Fluid

Accumulated Fluid Net Balance

Time (hrs) 0 3 6 9 12 15 18 21 24

Fold Changes from BL

Sham, n=6
Control, n=7
DDAVP (V₂R agonist), n=6
TLVP (V₂R antagonist), n=6

Two-way ANOVA
**, p < 0.01 vs. Control
§, p < 0.05 vs. Sham
#, p < 0.05 TLVP vs. DDAVP
TLVP Treatment Reduced the Fluid Requirements

**Sham, n=6**  
**Control, n=7**  
**DDAVP (V₂R agonist), n=6**  
**TLVP (V₂R antagonist), n=6**

Two-way ANOVA
- *, p < 0.05 vs. Control
- §§, p < 0.05 vs. Sham
- #, p < 0.05 TLVP vs. DDAVP
TLVP Treatment Decreased the Pulmonary Artery Pressure (PAP)

Pulmonary Artery Pressure (PAP)

Two-way ANOVA

\* \( p < 0.01 \) vs. Control

§ \( p < 0.05 \) vs. Sham

\n
\n
Sham, n=6
Control, n=7
DDAVP (V₂R agonist), n=6
TLVP (V₂R antagonist), n=6

Two-way ANOVA

\* \( p < 0.01 \) vs. Control

§ \( p < 0.05 \) vs. Sham
Pulmonary Microvascular Capillary Pressure (Pc) and Water Content Were Decreased

Two-way ANOVA

\[ \text{Pc} = 0.6 \text{PCWP} + 0.4 \text{PAP} \]

- Sham, n=6
- Control, n=7
- DDAVP (V$_2$R agonist), n=6
- TLVP (V$_2$R antagonist), n=6

\[ \text{*, p < 0.05 vs. Control} \]
\[ \text{§, p < 0.05 vs. Sham} \]
TLVP Treatment Prevented the Increase of Left Atrium Pressure (LAP) and Brain Natriuretic Peptide (BNP)

Two-way ANOVA
* , p < 0.05 vs. Control
§ , p < 0.05 vs. Sham

- Sham, n=6
- Control, n=7
- DDAVP (V₂R agonist), n=6
- TLVP (V₂R antagonist), n=6
TLVP Treatment at 10 mg/kg/day Increased the Sodium Retention

**Plasma Sodium**

- **Sham, n=6**
- **Control, n=7**
- **DDAVP (V₂R agonist), n=6**
- **TLVP (V₂R antagonist), n=6**

**Urine Osmolality**

- **Sham, n=6**
- **Control, n=7**
- **DDAVP (V₂R agonist), n=6**
- **TLVP (V₂R antagonist), n=6**

*Two-way ANOVA*

*, p < 0.05 vs. Control

§, p < 0.05 vs. Sham
Lower Dose of TLVP Prevented the Accumulation of Fluids with Less Sodium Retention

Accumulated Fluid Net Balance

Plasma Sodium

Time (hrs)

Fluid balance (mL * Kg)

mmol/L

0 3 6 9 12 15 18 21 24

0 50 100 150 200

Time (Hrs)

0 3 6 9 12 15 18 21 24

130 140 150 160 170 180

Sham, n=6

Control, n=5

TLVP (400 mcg/kg/h. 8 hrs infusion), n=3

Two-way ANOVA

*, p < 0.05 vs. Control

**, p < 0.01 vs. Control

§, p < 0.05 vs. Sham
1) Blockage of V$_2$R with TLVP (10 mg/kg/day):

- Reduced fluid requirement
- Reduced fluid retention
- Reduced lung water content
- Attenuated heart muscle overstretch
- Improved heart performance
- Decreased pulmonary vascular resistance

2) V$_2$R agonist DDAVP did not affect the above variables

3) TLVP caused moderate and transient increase in plasma sodium
Conclusion

- The data indicate that the activation of arginine vasopressin $V_2$ receptor plays a critical role in the pathophysiology of vascular hyper-permeability and cardiopulmonary hemodynamic changes during sepsis.

- $V_2$R antagonist TLVP should be considered as a therapeutic tool in septic patients, particularly those with severe fluid retention and tissue edema.

Future Directions

- Investigate the $V_2$R mediated downstream mechanisms with \textit{in vitro} cell-based assays.

- Find the optimal therapeutic dose of TLVP.

- Test the combination of TLVP with $V_1$R agonist in our model.
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