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AF-16 in peritonitis induced sepsis

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ABSTRACT

Sepsis is a life-threatening condition due to a dysregulated immunological response to infection. Apart from source control and broad-spectrum antibiotics, management is based on fluid resuscitation and vasoactive drugs. Fluid resuscitation implicates the risk of volume overload, which in turn is associated with longer stay in intensive care, prolonged use of mechanical ventilation and increased mortality.

Antisecretory factor (AF), an endogenous protein, is detectable in most tissues and in plasma. The biologically active site of the protein is located inan 8-peptide sequence, contained in a synthetic 16-peptide fragment, named AF-16. The protein as well as the peptide AF-16 has multiple modulatory effects on abnormal fluid transport and edema formation/resolution as well as in a variety of inflammatory conditions. Apart from its' anti-secretory and anti-inflammatory characteristics, AF is an inhibitor of capillary leakage in intestine. It is not known whether the protein AF or the peptide AF-16 can ameliorate symptoms in sepsis. We hypothesized that AF-16 decreases the degree of hemodynamic instability, the need of fluid resuscitation, vasopressor dose and tissue edema in fecal peritonitis.

To test the hypothesis, we induced peritonitis and sepsis by injecting autologous fecal solution into abdominal cavity of anesthetized pigs, and randomized (in a blind manner) the animals to intervention (AF-16, n=8) or control (saline, n=8) group. After onset of hemodynamic instability (defined as mean arterial pressure < 60 mmHg maintained for > 5 minutes), resuscitation was initiated by an infusion of AF-16 or saline. We recorded respiratory and hemodynamic parameters hourly for twenty hours and collected post mortem tissue samples at the end of the experiment.

No differences between the groups were observed regarding hemodynamics, fluid balance, lung mechanics, gas exchange or histology. This experimental study suggests that AF-16 does not modulate sepsis symptoms in peritonitis induced sepsis.

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GUIDELINES

National Institute of Health guide for the care and use of Laboratory animals (NIH publications No 8023, revised 1978).

MATERIALS TEXT

Pigs (*Sus scrofa domesticus*) weight 30 +/- 5 kg.

Zoletil Forte (tiletamine and zolazepam) 50 mg/ml + 50 mg/ml

Rompun (xylazine) 20 mg/ml

Fentanyl 50 µg/ml

Ketamin 50 mg/ml

Midazolam 5 mg/ml

Esmeron 10 mg/ml

Glucose 2,5%

Glucose 10%

Ringers' Acetate

NaCl 0.9%

Novorapid 100 E/ml

KCl, saturated.

Piperacillin/Tazobactam 4 g/0,5 g.

AF-16.

Peripeheral venous catheters, pulmonary artery catheter, triple lumen central venous catheter, arterial catheter, PiCCO

(pulse contour cardiac output) catheter, large-bore intra-peritoneal drain.

8 mm internal diameter tube.

Sutures: Prolene 3-0, Vicryl 3-0.

Sterile cloth.

ABG syringes.

1 The animal is weighed. Premedication with Zoletil Forte (Tiletamin/Zolazepam) 6 mg/kg + Rompun (Xylazinklorid) 2.2 mg/kg i.m in neck muscle. Establishment of a peripheral venous line in ear vein. Infusion Ringers' Acetate 30 ml/kg/h first hour, second hour until induction of peritonitis infusion with Ringers' Acetate 10 ml/kg/h.

2 Animal placed supine. Induction with Fentanyl 5-10 µg/kg.Tracheostomi. Maintenance with Ketamin 30 mg/kg/h, midazolam 0.1-0.4 mg/kg/h and fentanyl 4 µg/kg/h, dissolved in glucose 2.5%. Rocuronium (Esmeron) 2.5 mg/kg/h, when anesthesia is established (assured by absence of reaction to painful stimulation between the front hooves).

3 Volume controlled ventilation with the following settings: tidal volume (V_T) 8 ml/kg, respiratory rate (RR) 25/min, inspiratory/expiratory time (I:E) 1:2, inspired oxygen concentration (F_IO_2) 0.3 and positive end-expiratory pressure (PEEP) 8 cmH_2O; V_T, I:E and PEEP were maintained constant throughout the protocol. F_IO_2 adjusted with aim at PaO_2 >10 kPa. Respiratory rate to adjust $PaCO_2$ <6,5 kPa.

4 Instrumentation: Arterial catheter in the right carotid artery, pulmonary artery catheter and a triple lumen central venous catheter via the right jugular vein. A PiCCO catheter inserted in the right femoral artery.
Midline laparotomy and catheterization of the urinary bladder. Small incision in caecum to collect feces, close incision. Insert a large-bore intra-peritoneal drain in the peritoneum and close the abdominal incision.
Feces 2 g/kg BW to be dissolved in 200 ml 5% glucose solution, store in **38 °C** until instillation in the peritoneum.

5 Preparation followed by at least 30 min of stabilization.

6 Baseline measurements: arterial and mixed venous blood gas analyses, hemodynamic parameters (systemic and pulmonary pressures, cardiac output, heart rate), respiratory parameters (F_IO_2, SaO_2, $ETCO_2$, plateau pressure, dynamic and static compliance) urinary output. Extra vascular lung water (EVLW) and stroke volume variation (SVV).

7 Induction of fecal peritonitis. Remove intraperitoneal drain and close abdominal wall. With induction of fecal peritonitis the infusion of Ringer's Acetate is discontinued.

8 Randomization: AF-16 (n=8) and control group (n=8), (block randomization: 4x4 sealed, opaque envelopes). The research team blinded for group allocation.

9 Onset of hemodynamic instability (individual time of peritonitis) (defined as MAP <60 mmHg for >5 min). Parameters (see point 6: arterial blood gas analyses, hemodynamic parameters (systemic and pulmonary pressures, cardiac output, heart rate), respiratory parameters (F_IO_2, SaO_2, $ETCO_2$, plateau pressure, dynamic and static compliance) and urine output measured hourly from this timepoint). SVV measured continuosly. EVLW and mixed venous blood gas analyses performed now and every three hours for the rest of the protocol).
After parameters: intervention group receives an initial bolus of AF-16, 20 mg/kg (50 mg/ml in 0.9% saline), over duration of 10 minutes, followed by an infusion of 40 mg/kg over 50 minutes. The control group receives equal volumes

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of vehicle (0.9% saline) instead. After four and eight hours bolus dose is repeated (AF-16 or vehicle).
Piperacillin/Tazobactam 2 gram every 8 hours i.v. with start after onset of hemodynamic instability.
Initiate protocolized resuscitation in both intervention and control groups.

10 Protocolized resuscitation:
Aiming at MAP > 60 mmHg. Start fluid resuscitation with Ringer's Acetate 10 ml/kg/h.
If signs of hypovolemia (SVV > 15% maintained for 10 min) a bolus of 150 ml Ringer's Acetate was administered. Fluid boluses are repeated until SVV is stable < 15%. When SVV decreases to < 13% with MAP >60 mmHg, infusion down to 5 ml/kg/h, and if the animal stable and SVV maintained < 13%, stop infusion. If signs of hypovolemia again appear, start infusion with 5 ml/kg/h, then 10 ml/kg/h, then administer boluses of 150 ml.
In case of hypotension (MAP < 60 mmHg) without increased SVV, start infusion of norepinephrine 5 ml/h (40 µg/ml) following a bolus of 1 ml (40 µg/ml), increase stepwise by 5 ml/h.
Administer glucose 30 % infusion, aiming at blood glucose 5-10 mmol/L, starting with 0.5 ml/kg/h. If b-glucose > 10 mmol/L start an insulin infusion 1E/ml with 1 ml/h.

11 After 20 hours after onset of hemodynamic instability. The animals are euthanized with 100 mmol KCl i.v. under deep anesthesia. If animals present with refractory shock and die before finishing protocol give 100 mmol Kcl i.v. when animal is terminal.

12 Animal is weighed.

13 Samples are taken from lungs from following regions: apical-medial, medial-medial, caudal-dorsal, caudal-medial and caudal-ventral. Samples are also taken from heart, liver, kidney, intestine and skin. The samples are immediately immersed in 10% buffered formalin for histology and for wet-to-dry ratio.

14 Samples for wet-to-dry ratio are measured in the above mentioned lung regions from the right lung, and from other mentioned organs. Samples are weighed, dried in an oven, at 50° C, until weight do not differ between two measurements.

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