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Desmopressin improves intestinal functional capillary density and decreases leukocyte activation in experimental endotoxemia

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ABSTRACT

Background: Blood flow to the intestine is decreased in sepsis in favor of vital organs resulting in ischemic damage of the gut mucosa. Once the mucosa is damaged, increased translocation of intestinal bacteria to the systemic circulation may occur. This in turn aggravates the inflammatory response contributing to the development of multi-organ failure. Desmopressin is a synthetic analog of vasopressin, an anti-diuretic hormone which has been shown to induce vasodilation and is thought to be implicated in immunomodulation. In this study, we investigate the effects of desmopressin on the intestinal microcirculation during sepsis in an experimental endotoxemia model in rats using intravital microscopy. In addition, we investigate the effects of desmopressin on systemic inflammation.

Methods: Forty Lewis rats were subdivided into four groups, where rats received intravenous saline (control), desmopressin (1 μg/kg/ml), lipopolysaccharide (5 mg/kg) or lipopolysaccharide followed by desmopressin. Inflammatory response was assessed by quantifying the number of temporary and firmly adherent leukocytes in submucosal venules. Capillary perfusion was determined by assessing the number of functional, non-functional and dysfunctional capillaries in the intestinal wall layers (muscularis longitudinalis, muscularis circularis and mucosa). Additionally, inflammatory cytokine levels were determined by multiplex assays.

Results: The number of firmly adhering leukocytes in V1 venules of rats receiving lipopolysaccharide and treated with desmopressin was significantly reduced compared to lipopolysaccharide only group (LPS: 259 ± 25.7 vs. LPS + DDAVP: 203 ± 17.2; n/mm^2; $p < 0.05$). Additionally, desmopressin treatment improved impaired intestinal microcirculation by improving functional capillary density following lipopolysaccharide administration in all examined layers of the intestinal wall. We also observed a significant decrease in TNF-α levels in rats which received desmopressin in endotoxemia compared to untreated rats (LPS: 383 ± 64.2; LPS + DDAVP: 261.3 ± 22; pg/ml; $p < 0.05$).

Conclusion: Desmopressin administration improved intestinal capillary perfusion and reduced inflammatory response in rat endotoxemia.

Introduction

Sepsis affects all aspects of the microcirculation including blood, endothelial, and smooth muscle cells (Ince, 2005). Microcirculatory dysfunction in sepsis has serious clinical implications. If left untreated it may result in multiorgan dysfunction (Deitch, 1992). Specifically during sepsis, blood flow to the intestine is decreased in favor of vital organs. The blood flow prioritization results in ischemic damage of the epithelial mucosa layer which serves as a barrier between the circulation and the bacterial flora allowing the translocation of bacteria from the gut to the systemic circulation. This phenomenon is known as the "second hit", eventually causing multi-system organ failure. Improvement of the intestinal microcirculation would increase oxygen delivery and decrease risk of mortality due to the "second hit" phenomenon (Deitch, 1992).

Desmopressin (DDAVP, 1-deamino-8-D-arginine vasopressin) is a synthetic analog of the nonapeptide antidiuretic pituitary hormone, vasopressin (Favory et al., 2009; Trigg et al., 2012). While vasopressin mediates its effects mainly via the vasopressin 1, 2 and 3 receptors (V1R, V2R, V3R), desmopressin only acts on the V2R (Birnbaumer, 2000). The V2R is mainly expressed in the kidney collecting duct and in endothelial cells. Activation of the V2R receptor by DDAVP in endothelial cells causes the release of von Willebrand factor (VWF) and

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tissue-type plasminogen activator (tPA) (Jochberger et al., 2009; Landry and Oliver, 2001). In addition, DDAVP increases plasma levels of coagulation factor VIII and increases the adhesion of platelets to vascular walls (Franchini, 2007). DDAVP is known to have strong vasodilator effects thought to be attributed to the cAMP-dependent activation of endothelial NO synthase (eNOS) (Kaufmann et al., 2003). cAMP signaling has also been associated with immune suppression specifically attenuating LPS and TNF-α mediated immune response implicating DDAVP and other cAMP activating compounds in attenuating the inflammatory response (Chassin et al., 2007).

In this study, we investigate the effects of DDAVP on the intestinal microcirculation in experimental endotoxemia in rats. Due to the important role, which the intestinal microcirculation plays in sepsis pathophysiology, it would be clinically advantageous to improve the microcirculation of the gut in patients with sepsis. Given that DDAVP is a known antidiuretic substance with vasodilator, anti-inflammatory and hemostatic effects, we believe that the administration of this compound in sepsis may improve intestinal microcirculation and improve survival (Birnbaumer, 2000). To our knowledge, this is the first study to determine the effects of DDAVP on the intestinal microcirculation in a model of sepsis. Here, we study the effects of DDAVP on leukocyte recruitment and functional capillary density in the intestinal microvasculature by intravital microscopy. In addition, we investigate the levels of inflammatory cytokines in response to DDAVP administration in experimental endotoxemia in rats.

Materials and methods

Animals

Forty male Lewis rats (body weight 280 ± 70 g; Charles River Laboratories, Sulzfeld, Germany) were housed in chip-bedded cages and kept on a 12 h light/dark cycle with ad lib food (Rat chow, Altromin, Lage, Germany) and water. Housing conditions were maintained at 22 °C with a 55–60% humidity environment. All animal procedures were performed according to the guidelines set by the German animal safety legislation and were approved by the institutional Animal Care Committee. Upon completion of experiments, rats were sacrificed by receiving an overdose of pentobarbital by intravenous administration and bilateral pneumothorax.

Anesthesia and surgical preparation

Anesthesia was induced by intraperitoneal administration of pentobarbital (60 mg/kg, Pentobarbital Natrium, Fargon, Barsbüttel, Germany). Anesthesia was maintained by intravenous pentobarbital injections (5 mg/kg) when required. The animals were positioned in a supine position and the neck was shaved and disinfected. To secure the airways, rats received a tracheostomy and were allowed to breathe room air. Polyethylene catheters (vein: inner diameter 0.28 mm, outer diameter 0.61 mm; artery: inner diameter 0.58 mm, outer diameter 0.96 mm; Smiths Medical, Kent, UK) were inserted in the left external jugular vein and common carotid artery for the administration of fluids, endotoxin and fluorescent dyes. The rats were placed on a hot plate (LHG hotplate HAS 01/AL, Harry Gestigkeit GmbH, Düsseldorf, Germany) to maintain a body temperature of 37 ± 0.5 °C and the temperature was monitored by using a rectal thermometer. Finally, the abdominal area was shaved and disinfected and a median laparotomy was performed from the xiphoid process to the symphysis 30 min prior to intravital microscopy.

Experimental protocol

Animals were randomly assigned to one of four groups (n = 10 per group): control (saline), DDAVP (desmopressin, 1 μg/kg/ml intravenously, [deamino-Cys1, D-Arg8]-Vasopressinacetat; Minirin®, Ferring Arzneimittel GmbH, Kiel, Germany), LPS (lipopolysaccharide, 5 mg/kg intravenously from *Escherichia coli*, serotype O26:B6; Sigma, Steinheim, Germany) and LPS + DDAVP (animals treated with DDAVP 1 μg/kg/ml intravenously 15 min after LPS administration). Intravenous administration of compounds (saline, DDAVP, LPS or LPS + DDAVP) was initiated 15 min after the insertion of the vein and artery catheters to allow the animals to recover. The laparotomy was performed 90 min post compound administration and was followed by intravital fluorescence microscopy procedure.

Intravital microscopy

Intravital microscopy was performed using an epifluorescent microscope (Axiotech Vario, Carl Zeiss, Jena, Germany) with a light source (HBO 50, Carl Zeiss, Jena, Germany), ocular (10×) and lens (20×/0.5 Achroplan, Carl Zeiss, Jena, Germany). The microscope contained both type #20 and #10 filters (Carl Zeiss, Jena, Germany) for the visualization of rhodamine 6G and fluorescein isothiocyanate albumin, respectively. These fluorescent dyes were used to visualize leukocytes and vessels, 2 h after starting experiments, on a segment of the terminal ileum 1 cm proximal from the ileocecal valve. To facilitate the microscopic evaluation of approximately 1 cm^2 of the intestinal surface a 76 × 26 mm transparent glass cover slip (Menzel, Braunschweig, Germany) was used. To avoid dehydration due to exposure to ambient air, areas of the intestine not being used were covered with gauze and continuously superfused with 37 °C isotonic saline. In addition, to avoid any mechanical damage to areas of the gut which were not in direct contact with the cover slip the "hanging drop" technique (Pavlovic et al., 2006) was used and the tissue was warmed to 37 °C. To minimize phototoxic effects due to light exposure, repeated observation by intravital fluorescence microscopy on the same area of the intestine was avoided. Video images were captured by a black-and-white CCD video camera (BC-12, AVT-Horn, Aalen, Germany) connected to a black-and-white monitor (PM-159, Ikegami Electronics, Neuss, Germany) and recorded by a Panasonic NV-SV120EG-S recorder (Matsushita Audio Video, Tokyo, Japan). Videos were reviewed at a later date for data analysis.

Fifteen minutes before intravital fluorescence microscopy was initiated rats received 200 μl of a 0.05% rhodamine 6G solution (Sigma) to visualize all leukocyte subpopulations and 200 μl of a 5% fluorescein isothiocyanate albumin dissolved in saline (sigma) to distinguish blood cells from plasma. The animal was then placed on a specialized heated microscope stage and the microscope was focused on the submucosa of the prepared intestinal section. Fields containing non-branching collecting venules of at least 300 μm in length and postcapillary venules were recorded for 30 s per field. Additionally, video recordings 30 s long were completed of random fields of the capillaries within each of the longitudinal and circular muscle layers. To capture video recordings of the mucosa a section of the intestinal lumen approximately 2 cm in length (antimesenteric) was opened using a microcautery knife. The intestine was then flushed with warm isotonic saline (37 °C), placed on a supporting device and six videos 30 s in length of randomly chosen mucosa sections were recorded. To ensure that cauterization did not influence the results video capture of the mucosa was only conducted on mucosa directly bordering the mesentery. All video analysis was blinded and took place offline on a video monitor.

Analyzed parameters included the following: number of adherent leukocytes (number of leukocytes during an observation period which remained firmly attached to the intestinal endothelial for at least 30 s), number of rolling leukocytes (the number of leukocytes which during an observation period of 30 s pass in a rolling motion through) and functional capillary density. Functional capillary density is a measure of the microcirculatory perfusion of the tissue. Functional capillary density determination was completed as described previously (Al-Banna et al., 2013). In brief, three layers of the intestinal wall were examined; muscularis longitudinalis, muscularis circularis and the mucosa. Capillaries were defined to be either functional, dysfunctional

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