Microvascular Research 101 (2015) 111–117

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Time-dependent effect of clonidine on microvascular permeability during endotoxemia

Karsten Schmidt [a,*], Jochen Frederick Hernekamp [b], Christoph Philipsenburg [a], Aleksandar R. Zivkovic [a], Thorsten Brenner [a], Stefan Hofer [a]

[a] Department of Anesthesiology, Heidelberg University Hospital, Heidelberg, Germany
[b] Department of Hand, Plastic and Reconstructive Surgery, Burn Center, BG Trauma Center Ludwigshafen, Ludwigshafen, University of Heidelberg, Germany

ARTICLE INFO

Article history:
Received 22 December 2014
Revised 17 June 2015
Accepted 7 July 2015
Available online 12 July 2015

Keywords:
Endotoxemia
Clonidine
Endothelial dysfunction
Microvascular permeability
Intravital microscopy

ABSTRACT

Background: Endothelial leakage with accompanying tissue edema and increased leukocyte adhesion are characteristics of the vascular inflammatory response. Tissue edema formation is a key mechanism in sepsis pathophysiology contributing to impaired tissue oxygenation and the development of shock. Sepsis mortality is directly associated with the severity of these microcirculatory alterations. Dysfunction of the sympathetic nervous system can have deleterious effects in generalized inflammation. This study evaluated the effect of the adrenergic alpha 2 agonist clonidine on microvascular permeability and leukocyte adhesion during endotoxemia.
Methods: Macromolecular leakage, leukocyte adhesion, and venular wall shear rate were examined in mesenteric postcapillary venules of rats by using intravital microscopy (IVM). Lipopolysaccharide (LPS) (4 mg/kg/h) or equivalent volumes of saline were continuously infused following baseline IVM at 0 min. IVM was repeated after 60 and 120 min in endotoxemic and nonendotoxemic animals. Clonidine (10 μg/kg) was applied as an i.v. bolus. Animals received either (i) saline alone, (ii) clonidine alone, (iii) clonidine 45 min prior to LPS, (iv) clonidine 10 min prior to LPS, (v) clonidine 30 min after LPS, or (vi) LPS alone. Due to nonparametric data distribution, Wilcoxon test and Dunn's multiple comparisons test were used for data analysis. Data were considered statistically significant at $p < 0.05$.
Results: LPS significantly increased microvascular permeability and leukocyte adhesion and decreased venular wall shear rate. Clonidine significantly reduced microvascular permeability when applied 45 min before or 30 min after LPS administration. Leukocyte adhesion and venular wall shear rate were not affected by clonidine during endotoxemia.
Conclusion: Clonidine reduces microvascular permeability in endotoxemic animals in a time-dependent manner. Adrenergic alpha 2 agonists might prove beneficial in stabilizing capillary leakage during inflammation.

Introduction

Sepsis continues to be a leading cause of morbidity and mortality in critically ill patients (Mayr et al., 2014). Microcirculatory alterations are key mechanisms in sepsis pathophysiology and their severity is directly associated with organ dysfunction and mortality (De Backer et al., 2014). Progressive tissue edema formation due to increased microvascular permeability reflects the breakdown of the endothelial barrier and is associated with impaired tissue oxygenation and the development of shock in sepsis. The central nervous system (CNS) and the immune system (IS) interact in response to pathogenic threats to provide host defense. The CNS responds to signals of injury or inflammation via the sympathetic (SNS) and the parasympathetic nervous system (PNS) (Bellinger and Lorton, 2014; Tracey, 2007).

The interest for the potentially deleterious effect of SNS overstimulation during sepsis resulted in several studies examining the effect of centrally acting α2 agonists such as clonidine in experimental sepsis (Geloen et al., 2013; Hofer et al., 2009). Clonidine is an established drug in critical care medicine as part of sedation regimes and for the management of conditions with a high sympathetic tone (Pichot et al., 2011). The pre-emptive administration of clonidine in a murine sepsis model of caecal ligation and puncture (CLP) resulted in a significant improvement of survival accompanied by a reduction of pro-inflammatory mediators (Hofer et al., 2009). A beneficial effect of anti-inflammatory cholinergic and PNS-associated mechanisms on endothelial dysfunction and leukocyte–endothelial interactions could already be demonstrated in experimental sepsis models (Peter et al., 2010; Saeed et al., 2005). α2-Agonists such as clonidine cause sympathetic inhibition combined with parasympathetic activation (Toader et al., 2008). Endothelial cells and leukocytes express adrenergic and cholinergic receptors, making them therapeutic targets for the treatment of

* Corresponding author at: Department of Anesthesiology, Heidelberg University Hospital, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany.
E-mail address: karsten.schmidt@med.uni-heidelberg.de (K. Schmidt).

http://dx.doi.org/10.1016/j.mvr.2015.07.002
0026-2862/

sepsis-induced microcirculatory alterations. This study aimed to determine the effects of clonidine on microcirculatory alterations during experimental endotoxemia focusing on microvascular permeability and leukocyte–endothelial interactions.

Materials and methods

Anesthesia and animal preparation

The general aspects of the materials and methods have been described in detail in previous publications (Peter et al., 2010; Walther et al., 2004). All experimental procedures and protocols used in this investigation were reviewed and approved by the Governmental Animal Protection Committee (project license number 35–9185.81/G-153/11). Male Wistar rats (n = 59, randomized to study groups, 250–300 g body weight; Janvier; St Berthevin, France) were maintained in an animal facility with a 12-h light–dark cycle and housed in stainless steel cages in a temperature- and humidity-controlled room. Standard diet and water were available ad libitum.

Anesthesia was induced with 60 mg/kg pentobarbital i.p. (Narcoren®, Merial GmbH, Hallbergmoos, Germany). Repeated pentobarbital injections were performed slowly under continuous hemodynamic monitoring to maintain anesthesia and cardiovascular stability. Animals received up to 10 mg/kg/h sodium pentobarbital intravenously (Walther et al., 2004). Anesthesia maintenance was regularly monitored by reflex tests (the pedal withdrawal reflex of the hind limb and the corneal reflex) combined with the monitoring of heart rate, blood pressure and respiration rate. Rectal temperature was measured with a thermistor probe and maintained at 37 °C with a heating lamp. The right jugular vein was cannulated for administrating test substances. The left carotid artery was cannulated for computerized monitoring and recording of mean arterial pressure (MAP) using a non-commercial small animal monitoring system (Small Animal Monitoring Vs. 1.2.6.3, Exp. Chirurgie, Heidelberg 2012).

A segment of the ileum was gently exteriorized through a mid-line abdominal incision and draped over a clear glass pedestal for viewing the mesentery by intravital microscopy. The exposed tissues were superfused continuously with buffered (pH = 7.4) and thermostat-controlled Ringer solution maintained at 37 °C.

A stock solution of LPS (Lipopolysaccharide, *Escherichia coli* 026:B6, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was prepared by dissolving LPS in saline to a concentration of 5 mg/ml. The solution was stored in a glass container at 5 °C. For experiments the stock solution was diluted in saline to the appropriate concentration for each rat.

Infusions of LPS (4 mg/kg/h) or equivalent volumes of saline solution were administered via the jugular vein. The LPS dosage of 4 mg/kg/h was identified in pilot experiments as the LPS dosage that induced significant inflammatory endothelial activation (macromolecular leakage, leukocyte–endothelial interactions) compared to nonendotoxemic animals. The inflammatory effect of this dosage is in line with previous studies from our group using 2 mg/kg/h of LPS (Peter et al., 2010; Walther et al., 2000, 2004) .

Clonidine (Catapressan®, Boehringer Ingelheim, Ingelheim am Rhein, Germany) was diluted in saline to the appropriate concentration for each rat and was injected i.v. according to the experimental protocol (Fig. 1). The dosage of 10 μg/kg clonidine was chosen based on the studies of Dabire et al. on the systemic and regional hemodynamic effects of cumulative dosages of clonidine (1, 3, 10, 30 and 100 μg/kg) in pentobarbital-anesthetized Wistar rats (Dabire and Richer, 1991) and on the study of Birnbaum et al., who used 10 μg/kg clonidine during endotoxemia in pentobarbital-anesthetized Wistar rats to assess sympathetic modulations of intestinal microvascular blood flow oscillations (Birnbaum et al., 2003).

For measurement of erythrocyte velocity fluorescent-labeled erythrocytes from donor rats were injected 10 min before baseline

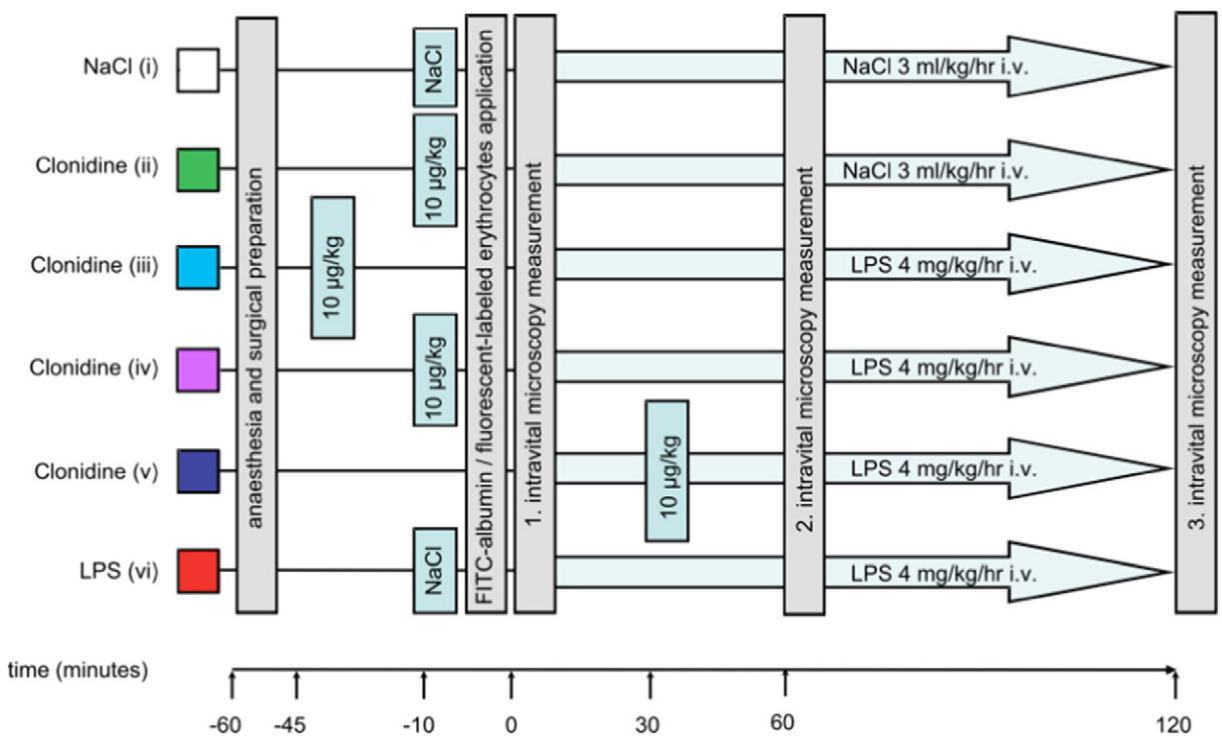

Fig. 1. Experimental protocol. Intravital microscopic measurements (IVM) were performed at 0, 60, and 120 min in endotoxemic and nonendotoxemic animals following a stabilization period after surgical preparation. For IVM fluorescein isothiocyanate-labeled bovine (FITC-albumin) albumin and fluorescent-labeled erythrocytes were injected 10 min before baseline measurements. LPS (4 mg/kg/h) or an equivalent volume of saline was continuously infused starting directly after baseline IVM at 0 min. Clonidine (10 μg/kg) was applied as an i.v. bolus in treatment groups. All administered fluids were calculated to guarantee that all animals received equal amounts of intravenous fluids. The color and number code of the experimental groups introduced in this figure is used in all other figures and tables.