Resuscitation (2008) **76**, 120—128

available at www.sciencedirect.com

ScienceDirect

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

EXPERIMENTAL PAPER

Volume therapy with colloid solutions preserves intestinal microvascular perfusion in endotoxaemia☆

Jörn Schäper[a,*], Raees Ahmed[b], Tobias Schäfer[b], Antje Elster[b], Fabian Enigk[b], Helmut Habazettl[b,c], Shaaban Mousa[a], Michael Schäfer[a], Martin Welte[d]

[a] *Department of Anesthesiology and Critical Care Medicine, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12203 Berlin, Germany*
[b] *Insititute of Physiology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Arnimallee 22, 14095 Berlin, Germany*
[c] *Department of Anaesthesiology, Deutsches Herzzentrum Berlin, Augustenburger Platz 1, 13353 Berlin, Germany*
[d] *Department of Anaesthesiology and Critical Care Medicine, Klinikum Darmstadt, Grafenstrasse 9, 64283 Darmstadt, Germany*

Received 7 February 2007; received in revised form 20 June 2007; accepted 27 June 2007

KEYWORDS
Crystalloid;
Hydroxyethyl starch;
Gelatin;
Lipopolysaccharide;
Intravital microscopy;
Microcirculation;
Functional capillary density;
Lactate/pyruvate ratio

Summary Colloid solutions have been suggested to improve microvascular perfusion due to their anti-inflammatory properties. Whether this also applies for the gut, an important immunological organ vulnerable to hypoperfusion is unknown. This study investigated intestinal microcirculation of endotoxaemic rats after volume therapy with colloid solutions such as hydroxyethyl starch (HES) and gelatin or isotonic saline (NaCl). In addition intestinal oxygenation and morphology as well as mesenteric leukocyte—endothelium interaction were quantified. Rats were anaesthetised with urethane and ketamine, mechanically ventilated, and monitored haemodynamically. Normotensive endotoxaemia was induced by a continuous intravenous infusion of *Escherichia coli* lipopolysaccharide (LPS, 1.5 mg kg^{-1} h^{-1}). After 1 h of LPS infusion either 6% HES (16 ml kg^{-1}), 4% gelatin (16 ml kg^{-1}) or 0.9% NaCl (64 ml kg^{-1}) were infused for 1 h. Using intravital microscopy, functional capillary density (FCD) and red blood cell velocity (RBCV) were measured in the mucosa of the terminal ileum at baseline and 3 h after volume therapy. In another set of animals, mesenteric leukocyte—endothelium interaction was determined 3 h after volume therapy. In all animals intestinal lactate/pyruvate ratio and intestinal morphology were assessed. Three hours after volume therapy, FCD decreased in NaCl (808 [749/843] cm^{-1};

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at 10.1016/j.resuscitation.2007.06.026.

* Corresponding author. Tel.: +49 30 8445 2731; fax: +49 30 8445 4469.
E-mail address: joern.schaeper@charite.de (J. Schäper).

0300-9572/$ — see front matter
doi:10.1016/j.resuscitation.2007.06.026

median [quartiles] $P < 0.05$ versus baseline) but not in HES (995 [945/1036] cm^{-1}) and gelatin (988 [867/1193] cm^{-1}) groups. RBCV, lactate/pyruvate ratio and intestinal morphology did not differ among groups. Also mesenteric leukocyte—endothelium interaction was not significantly influenced by either treatment.

In conclusion, early volume therapy with HES or gelatin, but not with NaCl, preserved gut microvascular perfusion during endotoxaemia but did not have a significant effect on tissue oxygenation nor morphological appearance in this experimental model. An anti-inflammatory effect of colloid solutions was not seen and fails to explain the changes in intestinal microcirculation.

Introduction

Impairment of the microcirculation occurs early in the course of sepsis[1] and represents a hallmark of septic shock and organ failure.[2,3] Particularly, impairment of the intestinal microcirculation, which leads to decreased tissue oxygenation, mucosal injury and subsequent gut barrier dysfunction has been suggested to contribute to the development of septic shock and multi-organ dysfunction syndrome.[4] During systemic inflammation early volume therapy is crucial for the preservation of systemic haemodynamics, microvascular perfusion, and organ function and has been demonstrated to reduce the mortality of sepsis.[5] The goal of volume therapy is to maintain an adequate intravascular volume. In pigs with severe sepsis and capillary leak syndrome, artificial colloids such as hydroxyethyl starch (HES) and gelatin maintained plasma volume better when compared to Ringer's solution suggesting a positive effect of these colloids on the patency of microvessels.[6] Additionally, colloid solutions have been shown to have anti-inflammatory properties,[7–10] which may positively influence organ perfusion. In endotoxaemic hamsters beneficial effects of a new HES solution (mean molecular weight: 130 kDa, degree of substitution: 0.4) on microvascular perfusion and macromolecular leakage in the skin muscle were associated with an attenuation of leukocyte adhesion.[11] Although the gut is supposed to play an important role in systemic inflammation, there are no data about the effects of various resuscitation fluids on splanchnic organs during systemic inflammation. Therefore, the aim of this study was to investigate the effects of different resuscitation fluids on LPS-induced organ injury and immune response. Using intravital microscopy the effects of two clinically used colloids (6% HES [mean molecular weight 130 kDa, degree of substitution of 0.4] and 4% modified fluid gelatin) and a standard crystalloid (isotonic saline) on intestinal microvascular perfusion and mesenteric leukocyte—endothelium interaction were investigated in endotoxaemic rats.[12] Furthermore intestinal oxygenation and histology were assessed.

Materials and methods

Animals

Male Sprague—Dawley rats weighing 323 ± 50 g were used in the experiments. Animals were housed in our animal facility and had free access to standard rat chow and water until the experiment. All animals were handled according to the National Institutes of Health guidelines on the use of experimental animals with approval by the local animal care committee.

Experimental protocols

Anaesthesia was achieved by subcutaneous injections of urethane (Urethan 99% (N), Sigma—Aldrich Chemie GmbH, Deisenhofen, Germany, 1.5 g kg^{-1}) and ketamine (Ketavet, Pharmacia & Upjohn GmbH, Erlangen, Germany, 50 mg kg^{-1}) with supplementation of ketamine (15—30 mg kg^{-1}) approximately every 2 h. After tracheostomy (PE 205, Portex, Hythe, UK) and cannulation of the left carotid artery and both external jugular veins (PE 50, Portex), animals were mechanically ventilated with room air (Harvard Apparatus, Edenbridge, UK). Exspiratory CO_2 was analysed and ventilation was adjusted to keep values constant at 35 mmHg (TSE Technical & Scientific Equipment GmbH, Bad Homburg, Germany). Arterial and central venous pressures were monitored (transducer, model p23 ID, Gould-Statham, Hato Rey, Puerto Rico) and arterial blood samples (100 μl each) were analysed for blood gases, acid—base status and electrolytes (Rapidlab™ 348, Chiron Diagnostics GmbH, Fernwald, Germany) as well as hematocrit, white blood cell and platelet counts (Coulter Ac Tdiff, Coulter Corporation, Miami, USA). Body temperature was kept above 36 °C throughout the experiment using a warming pad.

Two protocols were chosen to investigate the effects of volume therapy during normotensive endotoxaemia (Figure 1). Protocol A investigated the effect of volume therapy on microvascular perfusion of the terminal ileum. A segment of the distal ileum was prepared for intravital microscopy and rats were allowed to stabilise for 30 min. After baseline recordings a continuous infusion of *Escherichia coli* LPS (1.5 mg kg^{-1} h^{-1}, Serotype 026: B6, Sigma—Aldrich Chemie GmbH, Deisenhofen, Germany) was started. This dose has been shown to reduce intestinal villus blood flow to 75% of baseline values after 1 h of continuous infusion.[13] After 1 h of LPS infusion animals were resuscitated for 1 h using either 16 ml kg^{-1} 6% HES 130/0.4 (Voluven®, Fresenius Kabi, Bad Homburg, Germany, $n = 7$), 16 ml kg^{-1} 4% succinylated gelatin (Gelafundin®, B.Braun, Melsungen, Germany, $n = 5$) or 64 ml kg^{-1} isotonic saline (Boehringer Ingelheim Delta Pharma, Pfullingen, Germany, $n = 7$) as these doses have been shown to result in comparable macrohaemodynamics in small rodents.[11] The effects on intestinal microcirculation were investigated 3 h after the end of volume therapy. In order to investigate potential mechanisms for the differences seen at 3 h after the end of volume therapy, we conducted a second protocol