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Cecal inoculum peritonitis: An alternative model for sepsis vascular dysfunction study

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ABSTRACT

Aims: Sepsis is a life threatening condition that is characterized by the loss of vascular reactivity. The factor (s) responsible for the diminished vascular function seen in sepsis are not well understood. The purpose of this study was to characterize the vascular dysfunction from the rat cecal inoculum (CI) sepsis model using cecal ligation and puncture (CLP), and lipopolysaccharide (LPS) sepsis as reference models.
Materials and methods: Experiments were performed on isolated aorta from CI, CLP and LPS treated rats using a combination of pharmacological approaches.
Key findings: Phenylephrine (PE)-induced aortic contraction was significantly decreased in each model ($p < 0.05$) and not normalized by L-NAME or indomethacin. The vascular response elicited in the CI model for acetylcholine (Ach) was more similar to that seen in the CLP than the LPS model. The removal of the endothelial layer increased sensitivity to L-NAME ($p < 0.05$) in aortae from CI group. Inhibition of the large conductance Ca^{2+}/voltage sensitive K^+ (BK_{Ca}) channel did not normalize PE hyporesponsiveness but did abolish sepsis-induced contractile oscillation. Inhibition of the voltage dependent $Kv_{1.5}$ channel was not able to reverse the vascular hyporesponsiveness, however, inhibition of the ATP dependent (K_{ATP}) channel inhibition partially restored the contractile response ($p < 0.05$). Elevation of VCAM expression and aortic structural alternation were observed in each model.
Significance: These results suggest that the CI model may be an additional tool that could be used to investigate the mechanisms of vascular hyporesponsiveness in sepsis.

1. Introduction

Sepsis is a complicated, potentially life threatening public health concern that affects over 750,000 Americans per year [1]. The underlying etiology of sepsis is incompletely understood, however it is thought that vascular dysfunction characterized by hypotension plays a key role in mediating patient survivability [2–4]. The two most commonly used models to investigate sepsis in the laboratory are the endotoxemia model using lipopolysaccharides (LPS) and the polymicrobial sepsis cecal ligation and puncture (CLP). The LPS model seems to be a favorable model for some types of vascular related studies given its severe inflammatory characteristics; however, it is unclear how well this procedure mimics the human condition [5]. The CLP model, unlike the LPS platform, uses a polymicrobial insult that is more applicable to clinical sepsis [6] but it appears to suffer from intra- and inter-laboratory variability [7]. Recent studies using the newer and less characterized cecal inoculum (CI) polymicrobial sepsis model have suggested that this model may produce a septic response that is more suitable than the CLP procedure in certain experiments [8–10]. Whether the mechanism(s) responsible for producing the hypotension observed in the CI model [9,11] are similar to that which mediate the loss of vascular reactivity seen in other the septic models has to our knowledge not been investigated.

Recent studies have identified several factors that might contribute to the vascular dysfunction seen with sepsis including alterations in nitric oxide (NO) production [12–14], increased vascular inflammation [15–17], endothelial dysfunction [18], and the dysregulation of potassium ion channel function [19–21]. Whether CI-induced peritonitis is characterized by 1) elevated vascular NO production, 2) vascular hyporesponsiveness, or 3) alterations in ion channel regulation has yet to be documented. The purpose, therefore, of this study was to characterize

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Table 1
Comparison of PE dose response.

	Reference	Log EC50 (Con)	Log EC50 (L-NAME)	Delta body weight (%)	Mortality (%)
Sham Con (CI)	This study	−7.13 ± 0.06	−7.37 ± 0.06*	1.5 ± 0.29	0
CI	This study	−6.79 ± 0.15**	−7.10 ± 0.09	−2.4 ± 0.29**	91
Sham Con (CLP)	This study	−6.98 ± 0.07	−7.24 ± 0.06*	1.1 ± 0.51	0
CLP	This study	−6.65 ± 0.16	−6.92 ± 0.12	−1.2 ± 0.38**	67
Con (LPS)	This study	−6.85 ± 0.10	−7.33 ± 0.12*	−0.025 ± 0.26	0
LPS	This study	−6.71 ± 0.27	−6.72 ± 0.14	−8.0 ± 1.1**	47
Sham Con (CLP)	Heesen BJ et al.	−7.0 (−6.6 to −7.3)	–	–	–
CLP	Heesen BJ et al.	−6.8 (−6.4 to −7.3)	–	–	–
Sham (CLP) f	McKennaTM	−6.92 ± 0.10	–	–	–
CLP f	McKennaTM	6.89 ± 0.17	–	–	–
Con (LPS) f	Julou-Schaeffer et al.	−7.15 ± 0.1	−8.02 ± 0.15*	–	–
LPS (20 mg/kg)f	Julou-Schaeffer et al.	−7.14 ± 0.09	−7.63 ± 0.09	–	–

f: Norepinephrine dose response data [53].
* $p < 0.05$ corresponding control vs. L-NAME.
** $p < 0.05$ corresponding control vs. sepsis or LPS.

the CI induced vascular dysfunction and if applicable, compare and contrast the degree of the vascular hyporesponsiveness produced by the different preclinical sepsis models. Our data suggest that the CI model could be an additional polymicrobial study model for the investigation of sepsis vascular dysfunction.

2. Materials and methods

2.1. Animal

Ten week old male Sprague Dawley rats were purchased from Hilltop Laboratories (Scottdale, PA) and housed for 2 weeks to allow acclimation before the initiation of any experiments. Animals were housed two to a cage with 12–12 dark–light cycles at 25 °C. Food and water were provided ad libitum. All animal care and experimental procedures were performed after approval by the Marshall University Institutional Animal Care and Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals [22].

2.2. Sepsis/endotoxemia procedures

2.2.1. Cecal inoculum (CI) model

Animals were anesthetized using isoflurane and fecal peritonitis was induced by making a small abdominal incision followed by cecal inoculum and closure of the incision as described previously [9]. The cecal material was prepared by mixing cecal contents obtained from fresh donor rats with 5% dextrose water to yield a concentration of 600 mg cecal material in 5 ml/kg. Sham control animals underwent an identical surgery to induce fecal peritonitis, but received sterile dextrose water (5 ml/kg, i.p.) only.

2.2.2. Cecal ligation puncture (CLP) model

Animals were anesthetized using isoflurane and fecal peritonitis was induced by CLP as described previously [6]. Briefly, a small incision was made to expose the cecum and fecal material was carefully pushed to the end of the cecum to maintain the same amount of fecal material. Thereafter, the cecum was ligated 4 cm from the end of the cecum with a 3/0 silk ligature and two punctures were made on the cecum with a 22 G needle. After the puncture, the cecum was placed back to the peritoneal cavity and the abdominal incision was closed using suture. Animals in the sham group underwent a laparotomy and cecal manipulation but no ligation or puncture.

2.2.3. LPS model

Endotoxemia was induced by i.p. injection of LPS (Sigma, St. Louis, MO, Lipopolysaccharides from *Escherichia coli* 055:B5) 40 mg/kg in 5 ml/kg of sterile saline (0.9% NaCl) to conscious animals. Control animals received vehicle (0.9% NaCl) i.p. injection.

2.3. Isometric tension recording

Animals were sacrificed at 18 h for CLP and CI models or at 24 h for the LPS model. These time points were chosen on the basis of pilot studies examining the mortality rate exhibited by the different sepsis models and corresponded to the earliest time of death observed in each of the different models. Aortic contractile function was measured as previously described with slight modifications [23,24]. Briefly, the aorta was removed and carefully cleaned of perivascular fat in ice cold HEPES buffered physiological saline solution containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES and 5 Tris; pH 7.4. Two ~3 mm wide lengths of the mid-thoracic aorta were used for contractile measurements while the rest of aortae was flash frozen in liquid N_2 and saved for biochemical analysis. Aortic rings were mounted on 2 stainless pins in glass organ baths containing bicarbonate buffered physiological saline solution (PSS) containing (mM) 130 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 14.9 $NaHCO_3$, 5.5 Glucose, 0.026 EDTA, and 1.6 $CaCl_2$. The bath was oxygenated with 95% O_2 plus 5% CO_2 to maintain pH 7.4 and temperature was maintained at 37 °C. The contents of the baths were changed every ~30 min through the experiments and 3–5 washes were performed between the dose response experiments. Isometric tension was continuously recorded using a force displacement transducer (FTD3, Grass Technologies, Warwick, RI) to a Grass polygraph and the data were digitized at 100 Hz using a Polyview 16 A/D converter (Grass Technologies, Warwick, RI).

Aortic rings were stretched in a stepwise manner to reach an optimal tension (1.4–1.6 g:separately determined by 80 mM KCl response in 200 mg passive tension each step). After a 2 h equilibration period, vessel viability was determined by iso potassium PSS (KPSS: 80 mM KCl equimolar NaCl replacement of PSS). Endothelial function was confirmed by acetylcholine (Ach: 10^{-6} M) after phenylephrine (PE: 10^{-6} M) induced contraction and Ach (10^{-6} M) response was calculated as a percentage of PE. The vascular contraction response was assessed by measuring the tension developed in response to the cumulative addition of phenylephrine (PE: 10^{-9} to 10^{-5} M) in the absence/presence of NG-nitro-L-arginine methyl ester (L-NAME: 10^{-4} M) and indomethacin (Indo: 10^{-5} M). In experiments using denuded aortic rings, the endothelium was removed by gentle rubbing using a small cotton-tip. The loss of endothelial cells was confirmed by the lack of responsiveness to Ach after pre-contracting the aorta with PE. The area under the curve was calculated from before and after the addition of L-NAME from each ring and was expressed as arbiter units.

In the separate experiments for the K^+ channel assessment, aortic rings were adjusted to optimal length in a stepwise manner and vessel